Main changes introduced in Document Nº SANTE/11312/2021 with respect to the previous version (Document Nº SANTE 12682/2019)

1. Amendments which are concerned with editorial improvements throughout the document.

2. **E4-E5** The paragraphs are rewritten to clarify the terms of recovery correction and bias reduction. "Recovery correction" is applied when the analytical result obtained is reported after multiplying by a recovery factor (100/Mean Recovery). This means that by using standard addition, procedural calibration or ILIS we do not apply any "recovery correction". However, the result will be compensated for lower extraction and cleanup yields (reduction of bias) by applying these approaches.
   Note that the term "recovery" in this document is related to the relative bias of the method: Relative bias (%) = Recovery (%) – 100.

3. **New Appendix E** with an overview of options for bias reduction and recovery correction has been added in the document.

4. **E6** The guidance of "Results ≥10 mg/kg may be rounded to three significant figures or to a whole number" can lead to different decisions, therefore "or to a whole number" has been deleted. The guidance of rounding up of the uncertainty has been added in Appendix D.

5. **E14** Derived from the empiric studies, single residue methods (SRMs) have around 25 % robust standard deviation in general, therefore 50% default MU can be applied for SRMs. The last sentence regarding the possibility to apply lower expanded MU has been deleted.

6. **Table 3** of identification requirements. Techniques as FT ICR MS and sector MS are deleted as they are not used in pesticide residue methods routinely.

7. **Table 4 and Glossary** Example (=formula) based on calculation of matrix effect has been added in the text and in the Glossary, as well the addition of the formula for the relative matrix effect using a generic matrix (instead of solvent)

8. **Glossary** addition of definitions of "Recovery", "Standard additon" and "Procedural calibration"

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED SANTE 11312/2021

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Coordinators:

Tuija Pihlström	Swedish Food Agency, SFA, Uppsala, Sweden
Amadeo R. Fernández-Alba	EURL-FV, University of Almería, Almería, Spain
Carmen Ferrer Amate	EURL-FV, University of Almería, Almería, Spain
Mette Erecius Poulsen	EURL-CF, DTU National Food Institute, Lyngby, Denmark
Björn Hardebusch	EURL-AO, CVUA Freiburg, Freiburg, Germany
Michelangelo Anastassiades	EURL-SRM, CVUA Stuttgart, Fellbach, Germany

Advisory Board:

Ralf Lippold	EURL-AO, CVUA Freiburg, Freiburg, Germany
Luis Carrasco Cabrera	European Food Safety Authority, EFSA, Parma, Italy
André de Kok	Formerly Wageningen Food Safety Research, Wageningen, The Netherlands
Finbarr O´Regan	Pesticide Registration Division, DAFM, Kildare, Ireland
Patrizia Pelosi	National Institute of Health, ISS, Rome, Italy
Antonio Valverde	University of Almería, Almería, Spain
Sonja Masselter	AGES, Institute for Food Safety, Innsbruck, Austria
Hans Mol	Wageningen Food Safety Research, Wageningen, The Netherlands
Magnus Jezussek	LGL, Erlangen, Germany
Octavio Malato	EURL-FV, University of Almería, Almería, Spain
Radim Štěpán	Czech Agriculture and Food Inspection Authority, Prague, Czech Republic

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ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

A. Introduction and legal background

A1 The guidance in this document is intended for laboratories involved in the official control of pesticide residues in food and feed across the European Union (EU). This document describes the method validation and analytical quality control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues, including monitoring data sent to the European Food Safety Authority (EFSA), and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure.

The key objectives are:

- to provide a harmonized, cost-effective quality assurance and quality control system across the EU
- to ensure the quality and comparability of analytical results
- to ensure that acceptable accuracy is achieved
- to ensure that false positives or false negatives are avoided
- to support compliance with, and specific implementation of ISO/IEC 17025 (accreditation standard)

A2 The glossary (Appendix F) should be consulted for definitions and explanation of terms used in the text.

A3 This document is complementary and integral to the requirements in ISO/IEC 17025. It should thus be consulted during audits and accreditations of official pesticide residue laboratories according to ISO/IEC 17025.

In accordance with Article 37 of Regulation (EU) No. 625/2017, laboratories designated for official control of pesticide residues must be accredited to ISO/IEC 17025. According to Article 34 of Regulation (EU) No. 625/2017, analytical methods used in the context of official controls shall comply with relevant European Union rules or with internationally recognised rules or protocols or, in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols. Where the above does not apply, validation of analytical methods may further take place within a single laboratory according to an internationally accepted protocol.

According to Article 34 (6) of Regulation (EU) No. 625/2017, technical guidelines dealing with the specific validation criteria and quality control procedures in relation to analytical methods for the determination of pesticide residues may be adopted in accordance with the procedure referred to in Article 116 (1) of Regulation (EU) No. 625/2017. The present document includes mutually acceptable scientific rules for official pesticide residue analysis within the EU as agreed by all Member States of the European Union and constitutes a technical guideline in the sense of article 34 (6) of Regulation (EU) No. 625/2017.

B. Sampling, transport, traceability and storage of laboratory samples

Sampling

B1 Food samples should be taken in accordance with Directive 2002/63/EC or superseding legislation. For feed, the regulations are laid down in Annex I of Regulation (EC) No. 152/2009 or superseding legislation. Where it is impractical to take primary samples randomly within a lot, the method of sampling must be recorded. Samples taken according to Directive 2002/63/EC or Regulation (EC) No. *152/2009* should be considered as legal, official laboratory samples, representative for the lot or consignment from which they are taken. Therefore, the contribution of the sampling variability to the variability in measurement uncertainty of residue analytical results is not dealt with in this document.

Transport

B2 Samples must be transported under appropriate conditions to the laboratory in clean containers and robust packaging. Polythene or polypropylene bags, ventilated if appropriate, are acceptable for most samples but low-permeability bags (e.g. nylon film) should 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 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.

B3 Rapid transport 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 which would be acceptable to a discerning purchaser, otherwise samples should be considered as unfit for analysis.

Traceability

B4 Samples must be identified clearly and indelibly, in a way to ensure traceability. 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.

B5 On receipt, each laboratory sample must be allocated a unique code by the laboratory.

Storage

B6 Laboratory samples which are not analysed immediately should be stored under conditions that minimise decay. Fresh products should be stored in the refrigerator, but typically no longer than 5 days. Dried products may be stored at room temperature, but if storage time is expected to exceed two weeks, they should be sub-sampled and stored in the freezer.

C. Sample analysis

C1 All sample preparation and processing procedures should be undertaken within the shortest time practicable to minimise sample decay and pesticide losses. Analyses for residues of very labile or volatile pesticides should be started, and the procedures which could lead to loss of analyte should be completed as soon as possible, preferably on the day of sample receipt.

Sample preparation and processing

C2 Sample preparation, sample processing and sub-sampling to obtain portions should take place before any visible deterioration occurs. The parts of the commodity that should be analysed are stipulated in Regulation (EC) No 396/2005 Annex 1.

C3 Sample processing and storage procedures should have been demonstrated to have no significant effect on the residues present in the sample (see Directive 2002/63/EC). Where there is evidence that comminution (cutting and homogenisation) at ambient temperature has a significant influence on the degradation of certain pesticide residues, it is recommended that the samples are homogenised at low temperature (e.g. frozen and/or 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. For all other analyses, the whole laboratory sample needs to be comminuted. To improve the extraction efficiency of low moisture commodities (e.g. cereals, spices, dried herbs), it is recommended that small particle sizes, preferably less than 1 mm, are obtained. Milling should be performed in a way that avoids extensive heating of the samples, as heat can cause losses of certain pesticides.

C4 Sample comminution should ensure that the sample is homogeneous enough to ensure that sub-sampling variability is acceptable. If this is not achievable, the use of larger test portions or replicate portions should be considered in order to be able to obtain a better estimate of the true value. Upon homogenization or milling, samples may separate into different fractions, e.g. pulp and peel in the case of fruits, and husks and endosperm in the case of cereals. This fractionation can occur because of differences in size, shape and density. Because pesticides can be heterogeneously distributed between the different fractions, it is important to ensure that the fractions in the analytical test portion are in the same ratio as in the original laboratory sample. It is advisable to store in a freezer a sufficient number of sub-samples or analytical test portions for the number of analyses/repeated analyses that are likely to be required.

Pooling of samples

C5 Pooling of individual samples or sample extracts may be considered as an option for the analyses of commodities with a low frequency of pesticide residues (e.g. organic or animal products), provided that the detection system is sensitive enough. For example, when pooling 5 samples, the limit of quantification (LOQ) or screening detection limit (SDL) must be at least 5 times lower than the reporting limit (RL).

C6 Pooling of sub-samples before extraction will reduce the number of analyses required, but in some cases additional mixing or homogenisation of the pooled sub-samples, before withdrawing the analytical portion, may be necessary. Alternatively, sample extracts can be pooled before injection. The original samples or the extracts must be re-analysed in cases of pesticide residue findings at relevant levels.

Extraction

Extraction conditions and efficiency

C7 The recovery of incurred residues can be lower than the percentage recovery obtained from the analysis of spiked samples.[1] Where practicable, samples containing incurred residues can be analysed using varying extraction conditions to obtain further information on extraction efficiency. A number of parameters such as sample processing, temperature, pH, time, etc., can affect extraction efficiency and analyte stability. To improve the extraction efficiency of low moisture commodities (cereals, dried fruits), addition of water to the samples prior to extraction is recommended. The impact of the shaking time on analyte losses should be checked to avoid unacceptable losses. Where the MRL residue definition of a pesticide includes salts, it is important that the salts are dissociated by the analytical method used. This is typically achieved by the addition of water before, or during, the extraction process. A change of pH may also be necessary. Where the residue definition includes esters or conjugates that cannot be analysed directly, the analytical method should involve a hydrolysis step.

Clean-up, concentration/reconstitution and storage of extracts

C8 A clean-up or dilution step may be necessary to reduce matrix interferences and reduce contamination of the instrument system leading to an improved selectivity and robustness. Clean-up techniques take advantage of the difference in physicochemical properties (e.g. polarity, solubility, molecular size) between the pesticides and the matrix components. However, the use of a clean-up step in a multi-residue method can cause losses of some pesticides.

C9 Concentration of sample extracts can cause precipitation of matrix-components and, in some cases, losses of pesticides. Similarly, dilution of the extract with a solvent of a different polarity can also result in pesticide losses because of decreased solubility (e.g. dilution of methanol or acetonitrile extracts with water).

C10 To avoid losses during evaporation steps, the temperature should be kept as low as is practicable. A small volume of a high boiling point solvent may be used as a "keeper". Foaming 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 the introduction of water and other possible contaminants.

C11 Analyte stability in extracts should be evaluated during method validation. Storage of extracts in a refrigerator or freezer will minimise degradation. Losses of pesticides in extracts at room temperature can occur, e.g. in vials in an instrument´s auto sampler rack.

Chromatographic separation and determination

C12 Sample extracts are normally analysed using capillary gas chromatography (GC) and/or high performance or ultra performance liquid chromatography (HPLC or UPLC) coupled to mass spectrometry (MS) for the identification and quantification of pesticides in food and feed samples. Various MS detection systems can be used, such as a single or triple quadrupole, ion trap, time of flight or orbitrap. Typical ionisation techniques are: electron ionisation (EI), chemical ionisation (CI), atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). Different acquisition modes may be used such as full-scan, selected ion monitoring (SIM), selected reaction monitoring (SRM) and multiple reaction monitoring (MRM).

[1] Information on the evaluation of the extraction efficiency is available in SANTE/2017/10632 in its latest version.

C13 Nowadays, selective detectors for GC (ECD, FPD, PFPD, NPD) and LC (DAD, fluorescence) are less widely used as they offer only limited specificity. Their use, even in combination with different polarity columns, does not provide unambiguous identification. These limitations may be acceptable for frequently found pesticides, especially if some results are also confirmed using a more specific detection technique. In any case, such limitations in the degree of identification should be acknowledged when reporting the results.

Calibration for quantification

General requirements

C14 The lowest calibration level (LCL) must be equal to, or lower than, the calibration level corresponding to the RL. The RL must not be lower than the LOQ.

C15 Bracketing calibration must be used unless the determination system has been shown to be free from significant drift, e.g. by monitoring the response of an internal standard. The calibration standards should be injected at least at the start and end of a sample sequence. If the drift between two bracketing injections of the same calibration standard exceeds 30% (taking the higher response as 100%) the bracketed samples containing pesticide residues should be re-analysed. Results for those samples that do not contain any of those analytes showing unacceptable drift can be accepted provided that the response at the calibration level corresponding to the RL remained measurable throughout the batch, to minimise the possibility of false negatives. If required, priming of the GC or LC system should be performed immediately prior to the first series of calibration standard solutions in a batch of analyses.

C16 The detector response from the analytes in the sample extract should lie within the range of responses from the calibration standard solutions injected. Where necessary extracts containing high-level residues above the calibrated range must be diluted and re-injected. If the calibration standard solutions are matrix-matched (paragraph C21-23) the matrix concentration in the calibration standard should also be diluted proportionately.

C17 Multi-level calibration (three or more concentrations) is preferred. An appropriate calibration function must be used (e.g. linear, quadratic, with or without weighing). The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region should not be more than ±20%.

C18 Calibration by interpolation between two levels is acceptable providing the difference between the 2 levels is not greater than a factor of 10 and providing the response factors of the bracketing calibration standards are within acceptable limits. The response factor of bracketing calibration standards at each level should not differ by more than 20% (taking the higher response as 100%).

C19 Single-level calibration may also provide accurate results if the detector response of the analyte in the sample extract is close to the response of the single-level calibration standard (within ±30%). Where a sample is spiked with an analyte for recovery determination purposes 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 may be determined in this way.

Analytes for calibration

C20 All targeted analytes must be injected in every batch of samples, at least at the level corresponding to the RL. Sufficient response at this level is required and should be checked to avoid false negatives.

Matrix-matched calibration

C21 Matrix effects are known to occur frequently in both GC and LC methods and should be assessed at the initial method validation stage. Matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same type as the sample, should be used for calibration. An alternative practical approach to compensate for matrix effects in GC-analyses is the use of analyte protectants that are added to both the sample extracts and the calibration standard solutions in order to equalise the response of pesticides in solvent calibrants and sample extracts. The most effective way to compensate for matrix effects is the use of standard addition or isotopically labelled internal standards.

C22 In GC, representative matrix calibration, using a single representative matrix or a mixture of matrices, can be used to calibrate a batch of samples containing different commodities. Although this is preferable to the use of calibration standards in solvent, compared to exact matrix matching, it is likely that the calibration will be less accurate. It is recommended that the relative matrix effects are assessed and the approach is modified accordingly.

C23 Compensation for matrix effects in LC-MS is more difficult to achieve because the matrix effects depend on the co-elution of each individual pesticide with co-extracted matrix components, which vary between different commodities. The use of matrix-matched calibration is, therefore, likely to be less effective compared to GC.

Standard addition

C24 Standard addition to analytical test portions (sample standard addition) is designed to compensate for matrix effects and losses during sample preparation. This technique assumes some knowledge of the likely residue level of the analyte in the sample (e.g. from a first analysis), so that the amount of added analyte is similar to that already present in the sample. In particular, it is recommended that standard addition is used for confirmatory quantitative analyses in cases of MRL exceedances and/or when no suitable blank material is available for the preparation of matrix-matched standard solutions. For standard addition a test sample is divided in three (or preferably more) test portions. One portion is analysed directly, and increasing amounts of the analyte are added to the other test portions immediately prior to extraction. The amount of analyte added to the test portion should be between one and five times the estimated amount of the analyte already present in the sample. The concentration of analyte present in the "unspiked" sample is calculated from the relative responses of the analyte in the sample and the spiked samples . In the standard addition approach the concentration of the analyte in the test sample is derived by extrapolation, thus a linear response in the appropriate concentration range is essential for achieving accurate results.

C25 Standard addition of at least two known quantities of analyte to aliquots of the sample extract, prior to injection (extract standard addition), is another form of standard addition. In this case adjustment is only for matrix effects, but not for recovery.

Effects of pesticide mixtures on calibration

C26 The detector response of individual pesticides in multi-pesticide calibration standards may be affected by one or more of the other pesticides in the same solution. Before use, multi-pesticide calibration standard solutions prepared in pure solvent should be checked against calibration standard solutions each containing a single pesticide (or a fewer number of pesticides) to confirm similarity of detector response. If the responses differ significantly, residues must be quantified using individual calibration standards in matrix, or better still, by standard addition.

Calibration for pesticides that are mixtures of isomers

C27 Quantification involving mixed isomer (or similar) calibration standard solutions, can be achieved by using either: summed peak areas, summed peak heights, or measurement of a single component, whichever is the most accurate.

Procedural Standard Calibration

C28 The use of procedural standards compensates for matrix effects and losses during extraction associated with certain pesticide/commodity combinations, especially where isotopically labelled standards are not available or are too costly. It is only applicable when a series of samples of the same type are to be processed within the same batch (e.g. products of animal origin, products with high fat content). Procedural standards are prepared by spiking a series of blank test portions with different amounts of analyte, prior to extraction. The procedural standards are then analysed in exactly the same way as the samples.

C29 Another application of procedural standard calibration is where pesticides need to be derivatised, but reference standards of the derivatives are not available or the derivatisation yield is low or highly matrix dependent. In such cases it is recommended to spike the standards to blank matrix extracts just prior to the derivatisation step. In this case the procedural standard calibration will also compensate for varying derivatisation yields.

Calibration using derivative standards or degradation products

C30 Where the pesticide is determined as a derivative or a degradation product, the calibration standard solutions should be prepared from a "pure" reference standard of the derivative or degradation product, if available.

Use of various internal standards

C31 An internal standard (IS) is a chemical compound added to the sample test portion or sample extract in a known quantity at a specified stage of the analysis, in order to check the correct execution of (part of) the analytical method. The IS should be chemically stable and/or typically show the same behaviour as of the target analyte.

C32 Depending on the stage of the analytical method in which the addition of IS takes place different terms are used. An injection internal standard (I-IS), also called instrument internal standard, is added to the final extracts, just prior to the determination step (i.e. at injection). It will allow a check and possible correction for variations in the injection volume. A procedural internal standard (P-IS) is an internal standard added at the beginning of the analytical method to account for various sources of errors throughout all stages in the method. An IS can also be added at a different stage of the analytical method to correct for both systematic and random errors that may have occurred during a specific stage of the analytical method. When selecting ISs it should be assured that they do not interfere with the analysis of the target analytes and that it is highly unlikely that they are present in the samples to be analysed.

C33 For multi-residue methods it is advisable to use more than one IS in case the recovery or detection of the primary IS is compromised. If only used to adjust for simple volumetric variations the ISs should exhibit minimal losses or matrix effects. When analysing a specific group of analytes with similar properties the IS can be chosen to exhibit similar properties and analytical behaviour to the analytes of interest. If the IS used for calculations has a significantly different behaviour (e.g. as to recovery or matrix effect) to one or more of the target analytes it will introduce an additional error in all quantifications.

C34 When the IS is added to each of the calibration standard solutions in a known concentration the detector response ratio of analyte and IS obtained from the injected calibration standard solutions are then plotted against their respective concentrations. The

concentration of analyte is then obtained by comparing the detector response ratio of analyte and IS of the sample extract, against the calibration curve.

C35 An isotopically labelled internal standard (IL-IS) is an internal standard with the same chemical structure and elemental composition as the target analyte, but one or more of the atoms of the molecule of the target analyte are substituted by isotopes (e.g. deuterium, ^{15}N, ^{13}C, ^{18}O). A prerequisite for the use of IL-ISs is the use of mass spectrometry, which allows the simultaneous detection of the co-eluting non-labelled analytes and the corresponding IL-ISs. IL-ISs can be used to accurately compensate for both analyte losses and volumetric variations during the procedure, as well as for matrix effects and response drift in the chromatography-detection system. Losses during extract storage (e.g. due to degradation) will also be corrected for by the IL-IS. Use of IL-ISs will not compensate for incomplete extraction of incurred residues.

C36 IL-ISs, can also be used to facilitate the identification of analytes because the retention time and peak shape of the target analyte and corresponding IL-IS should be the same.

C37 IL-ISs should be largely free of the native analyte to minimize the risk of false positive results. In the case of deuterated standards, an exchange of deuterium with hydrogen atoms, e.g. in solvents, can lead to false positives and/or adversely influence quantitative results.

Data processing

C38 Chromatograms must be examined by the analyst and the baseline fit checked and adjusted, as is necessary. Where interfering or tailing peaks are present, a consistent approach must be adopted for the positioning of the baseline. Peak area or peak height, whichever yields the more accurate results, may be used.

On-going method performance verification during routine analysis

Quantitative methods

Routine recovery check

C39 Where practicable, recoveries of all analytes in the scope should be measured within each batch of analyses. If this requires a disproportionately large number of recovery determinations, the number of analytes may be reduced. However, it should be in compliance with the minimum number specified in Table 1. This means, that at least 10% of the analytes (with a minimum of 5) should be included per detection system.

Table 1. *Minimum frequency of recovery checks (quantitative method performance verification)*

	Analytes for recovery check (minimum)	**All other analytes**
Number of analytes	At least 10 % of the scope per detection system covering all critical aspects of the method	Within a rolling programme to include all other analytes as well as representative commodities from different commodity groups
Minimum frequency *of recovery* checks	Every batch	At least every 12 months, preferably every 6 months
Level	RL	RL

C40 If at some point during the rolling programme (Table 1) the recovery of an analyte is outside of the acceptable range (see paragraph C43), then all of the results produced since the last satisfactory recovery must be considered to be potentially erroneous.

C41 The recovery of an analyte should normally be determined by spiking within a range corresponding to the RL and 2-10 x the RL, or at the MRL, or at a level of particular relevance to the samples being analysed. The spiking level may be changed to provide information on analytical performance over a range of concentrations. Recovery at levels corresponding to the RL 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, 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 extract should be determined from multiple test portions. If necessary, recoveries can be calculated using blank subtracted calibration, but the use of blank subtraction should be reported with the results. 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 RL.

C42 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.

Acceptance criteria for routine recoveries

C43 Acceptable limits for individual recovery results should normally be within the range of the mean recovery +/- 2x RSD. For each commodity group (see Annex A) the mean recovery results and RSDs may be taken from initial method validation or from on-going recovery results (within laboratory reproducibility, RSD_{wR}). A practical default range of 60-140 % may be used for individual recoveries in routine analysis. Recoveries outside the above mentioned range would normally require re-analysis of the batch, but the results may be acceptable in certain justified cases. For example, where the individual 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 recoveries or RSDs outside ± 20% must be investigated.

C44 Analysis of certified reference materials (CRMs) is the preferable option to provide evidence of method performance. As an alternative, in-house quality control samples may be analysed regularly instead. Where practicable, exchange of such materials between laboratories provides an additional, independent check of accuracy.

Screening methods

C45 Screening methods, especially those involving automated MS-based detection, offer laboratories a cost-effective means to extend their analytical scope to analytes which potentially have a low probability of being present in the samples. Analytes that occur more frequently should continue to be sought and measured using validated quantitative multi-residue methods.

C46 For qualitative multi-residue methods targeting very large numbers of analytes, it may not be practicable to include all analytes from the scope in each batch of analyses. To verify overall method performance for each batch, at least 10 % of the analytes (from the validated scope) that cover all critical points of the method should be spiked to the matrix. In a rolling programme, the performance for all analytes from the validated scope should be verified as indicated in Table 2.

C47 When using a screening method, the calibration standard solution corresponding to the RL or SDL should be positioned, at least at the beginning and the end of the sample sequence

to ensure that the analytes remain detectable throughout the whole batch of samples in the sequence. When an analyte is detected, it can only be tentatively reported. A subsequent confirmatory analysis using a validated quantitative method, including an appropriate calibration procedure, must be applied before a reliable quantitative result may be reported. If an analyte is not detected, then the result is reported as <SDL mg/kg or <RL mg/kg.

Table 2. *Minimum frequency of the detectability checks (screening method performance verification).*

	Analytes for detectability check (minimum)	**All other analytes**
Number of analytes	At least 10 % of the scope per detection system covering all critical aspects of the method	All analytes from the validated qualitative scope
Minimum frequency *of detectability* checks	Every batch	At least every 12 months, preferably every 6 months
Level	SDL or RL see paragraph G8	SDL or RL
Criterion	All analytes detectable	All (validated) analytes detectable

Proficiency testing

C48 For all official control laboratories it is mandatory to participate regularly in proficiency test schemes, particularly those organised by the EURLs. When false positive(s) or negative(s) are reported, or the accuracy (z scores) achieved in any of the proficiency tests is questionable or unacceptable, the problem(s) should be investigated. False positive(s), negative(s) and, or unacceptable performance, have to be rectified before proceeding with further determinations of the analyte/matrix combinations involved.

D. Identification of analytes and confirmation of results

Identification

Mass spectrometry coupled to chromatography

D1 Mass spectrometry coupled to a chromatographic separation system is a very powerful combination for identification of an analyte in the sample extract. It simultaneously provides retention time, mass/charge ratios (*m/z*) and relative abundance (intensity) data.

Requirements for chromatography

D2 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 of the analyte in the extract should correspond to that of the calibration standard (may need to be matrix-matched) with a tolerance of ±0.1min, for both gas chromatography and liquid chromatography. Larger retention time deviations are acceptable where both retention time and peak shape of the analyte match with those of a suitable IL-IS, or evidence from validation studies is available. IL-IS can be particularly useful where the chromatographic procedure exhibits matrix induced retention time shifts or peak shape distortions. Overspiking with the analyte suspected to be present in the sample will also help to increase confidence in the identification.

Requirements for mass spectrometry (MS)

D3 MS detection can provide mass spectra, isotope patterns, and/or signals for selected ions. Although mass spectra can be highly specific for an analyte, match values differ depending on the particular software used which makes it impossible to set generic guidance on match values for identification. This means that laboratories that use spectral matching for identification need to set their own criteria and demonstrate these are fit-for-purpose. Guidance for identification based on MS spectra is limited to some recommendations whereas for identification based on selected ions more detailed criteria are provided.

Recommendations regarding identification using MS spectra

D4 Reference spectra for the analyte should be generated using the same instruments and conditions used for analysis of the samples. If major differences are evident between a published spectrum and the spectrum generated within the laboratory, the latter must be shown to be valid. To avoid distortion of ion ratios the concentration of the analyte ions must not overload the detector. The reference spectrum in the instrument software can originate from a previous injection (without matrix present), but is preferably obtained from the same analytical batch.

D5 In case of full scan measurement, careful subtraction of background spectra, either manual or automatic, by deconvolution or other algorithms, may be required to ensure that the resultant spectrum from the chromatographic peak is representative. Whenever background correction is used, this must be applied uniformly throughout the batch and should be clearly recorded.

Requirements for identification using selected ions

D6 Identification relies on the correct selection of ions. They must be sufficiently selective for the analyte in the matrix being analysed and in the relevant concentration range. Molecular ions, (de)protonated molecules or adduct ions are highly characteristic for the analyte and should be included in the measurement and identification procedure whenever possible. In general, and especially in single-stage MS, high *m/z* ions are more selective than low *m/z* ions

(e.g. *m*/z <100). However, high mass *m*/z ions arising from loss of water or loss of common moieties may be of little use. Although characteristic isotopic ions, especially Cl or Br clusters, may be particularly useful, the selected ions should not exclusively originate from the same part of the analyte molecule. The choice of ions for identification may change depending on background interferences. In high resolution MS, the selectivity of an ion of the analyte is determined by the narrowness of the mass extraction window (MEW) that is used to obtain the extracted ion chromatogram. The narrower the MEW, the higher the selectivity. However, the minimum MEW that can be used relates to mass resolution.

D7 Extracted ion chromatograms of sample extracts should have peaks of similar retention time, peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch. Chromatographic peaks from different selective ions for the analyte must fully overlap. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon for identification.

D8 Different types and modes of mass spectrometric detectors provide different degrees of selectivity , which relates to the confidence in identification. The requirements for identification are given in Table 3. They should be regarded as guidance criteria for identification, not as absolute criteria to prove the presence or absence of an analyte.

Table 3. *Identification requirements for different MS techniques.*[2]

MS detector/Characteristics		**Acquisition**	**Requirements for identification**	
Resolution	**Typical systems (examples)**		**minimum number of ions**	**additionally**
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited *m*/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap	full scan, limited *m*/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] <1 mDa for *m*/z <200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

D9 The relative intensities or ratios of selective ions, expressed as a ratio relative to the most intense ion, that are used for identification, should match with the reference ion ratio. The reference ion ratio is the average obtained from solvent standards measured in the same sequence and under the same conditions as the samples. Standards in matrix may be used

[2] For definition of terms relating to mass spectrometry see Murray et al. (2013) Pure Appl. Chem., 85:1515–1609.

instead of solvent standards as long as they have been demonstrated to be free of interferences for the ions used at the retention time of the analyte. For determination of the reference ion ratio, responses outside the linear range should be excluded.

D10 Larger tolerances may lead to a higher percentage of false positive results. Similarly, if the tolerances are decreased, then the likelihood of false negatives will increase. The tolerance given in Table 3 [3,4] should not be taken as an absolute limit and automated data interpretation based on the criteria without complementary interpretation by an experienced analyst is not recommended.

D11 As long as sufficient sensitivity and selectivity are obtained for both ions, and responses are within the linear range, ion ratios in unit mass resolution MS/MS have shown to be consistent[3] and should not deviate more than 30% (relative) from the reference value.

D12 For accurate mass measurement / high resolution mass spectrometry, the variability of ion ratios is not only affected by S/N of the peaks in the extracted ion chromatograms, but may also be affected by the way fragment ions are generated, and by matrix. For example, the range of precursor ions selected in a fragmentation scan event ('all ions', precursor ion range of 100 Da, 10 Da, or 1 Da) results in different populations of matrix ions in the collision cell which can affect fragmentation compared to solvent standards. Furthermore, the ratio of two ions generated in the same fragmentation scan event tends to yield more consistent ion ratios than the ratio of a precursor from a full scan event and a fragment ion from a fragmentation scan event. For this reason, no generic guidance value for ion ratio can be given. Due to the added value of accurate mass measurement, matching ion ratios are not necessary, however, they may provide additional support for identification.

D13 For a higher degree of confidence in identification, further evidence may be gained from additional mass spectrometric information. For example, evaluation of full scan spectra, isotope pattern, adduct ions, additional accurate mass fragment ions, additional product ions (in MS/MS), or accurate mass product ions.

D14 The chromatographic profile of the isomers of an analyte may also provide evidence. Additional evidence may be sought using a different chromatographic separation system and/or a different MS-ionisation technique.

Confirmation of results

D15 If the initial analysis does not provide unambiguous identification or does not meet the requirements for quantitative analysis, a confirmatory analysis is required. This may involve re-analysis of the extract or the sample. In cases where a MRL is exceeded, a confirmatory analysis of another analytical portion is always required. For unusual pesticide/matrix combinations, a confirmatory analysis is also recommended.

D16 The use of different determination techniques and/or confirmation of qualitative and/or quantitative results by an independent expert laboratory will provide further supporting evidence.

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[3] H.G.J. Mol, P. Zomer, M. García López, R.J. Fussell, J. Scholten, A. de Kok, A. Wolheim, M. Anastassiades, A. Lozano, A. Fernandez Alba. Analytica Chimica Acta 873 (2015) 1–13

[4] S.J. Lehotay,Y. Sapozhnikova, H.G.J. Mol, Trends in Analytical Chemistry 69 (2015) 62–75.

E. Reporting results

Expression of results

E1 The results from the individual analytes measured must always be reported and their concentrations expressed in mg/kg. Where the residue definition includes more than one analyte (see examples, Appendix B), the respective sum of analytes must be calculated as stated in the residue definition and must be used for checking compliance with the MRL. If the analytical capabilities of a laboratory do not allow quantification of the full sum of a residue as stated in the residue definition, a part of the sum may be calculated but this should be clearly indicated in the report. In case of electronic submission of results for samples that are part of a monitoring programme, concentrations of all individual analytes and their LOQs must be submitted.

E2 For quantitative methods, residues of individual analytes below the RL must be reported as <RL mg/kg. Where screening methods are used and a pesticide is not detected, the result must be reported as <SDL mg/kg.

Calculation of results

E3 Where the same homogenised sample is analysed by two techniques, the result should be that obtained using the technique which is considered to be the most accurate. Where two results are obtained by two equally accurate techniques or by replicate measurement(s) of an analytical test portion of the homogenised sample using the same technique, the mean of the result should be reported.

In case there are two replicates the relative difference of the individual results should not exceed 30% of the mean. Close to the RL, the variation may be higher and additional caution is required in deciding whether or not this limit has been exceeded.

Correction for method bias

E4 As a practical approach, residues results do not have to be adjusted for method bias when the mean bias is less than 20% and the default expanded measurement uncertainty of 50% is not exceed.

In case the bias exceeds 20%, the result can be mathematically corrected using a recovery factor. In this case, the initial result obtained for the applicable pesticide after analysis is multiplied with the recovery factor [100%/recovery%]. Regarding the recovery% to be used for correction for recovery, there are multiple options. These include the mean recovery obtained during initial validation, the mean recovery obtained during on-going validation, or the (mean) recovery obtained for spiked samples concurrently analysed with the samples. The most appropriate option depends on the recovery data available for a method for the various pesticides and matrices, and may therefore differ for different laboratories.

Aspects to take into consideration in choosing between the recovery correction options include the reliability and consistency of the recovery of a pesticide for a certain matrix or group of matrices over time, and dependency of the recovery on concentration. On-going validation data covering multiple matrices from a commodity group (see Annex A) over a longer period of time provides valuable information to make an informed decision and to what extent recoveries from different matrices can be averaged.

E5 In case of lack of a suitable recovery factor to be used for recovery correction, alternative approaches to reduce method bias may be considered to avoid the need for recovery correction, e.g. the use of standard addition before sample extraction (C24), addition of an

isotopically labelled internal standard (IL-IS, C35) before sample extraction, or the use of procedural calibration (C28).

An overview of the options to account for method bias and use of recovery correction factors is provided in Appendix E, Table 1 and 2.

Rounding of data

E6 It is essential to maintain uniformity in reporting results for pesticide residues. In general, results at or above the RL but <10 mg/kg should be rounded to two significant figures. Results ≥10 mg/kg should be rounded to three significant figures. The RL should be rounded to 1 significant figure at <10 mg/kg and two significant figures at ≥10 mg/kg. These rounding rules do not necessarily reflect the uncertainty associated with the reported data. Additional significant figures may be recorded for the purpose of statistical analysis and when reporting results for proficiency tests. In some cases the rounding may be specified by, or agreed with the customer/stakeholder of the control or monitoring programme. Rounding to significant figures should be done after the calculation of the result. See Appendix D.

Qualifying results with measurement uncertainty

E7 It is a requirement under ISO/IEC 17025 that laboratories determine and make available the (expanded) measurement uncertainty (MU), expressed as U', associated with analytical results. Laboratories should have sufficient repeatability/reproducibility data from method validation/verification, inter-laboratory studies (e.g. proficiency tests), and in-house quality control tests, which can be used to estimate the MU[5].
The MU describes the range around a reported or experimental result within which the true value is expected to lie within a defined probability (confidence level). MU ranges must take into consideration all potential sources of error.

E8 MU data[6] should be applied cautiously to avoid creating a false sense of certainty about the true value. Estimates of typical MU that are based on previous data may not reflect the MU associated with the analysis of a current sample. Typical MU may be estimated using an ISO (Anonymous 1995, 'Guide to the expression of uncertainty in measurement' ISBN 92-67-10188-9) or Eurachem[7] approach. Reproducibility RSD (or repeatability RSD if reproducibility data are not available) may be used, but the contribution of additional uncertainty sources (e.g. heterogeneity of the laboratory sample from which the test portion has been withdrawn) due to differences in the procedures used for sample preparation, sample processing and sub-sampling should also be included. Extraction efficiency and differences in standard concentrations should also be taken into account. MU data relate primarily to the analyte and matrix used and should only be extrapolated to other analyte/matrix combinations with extreme caution. MU tends to increase at lower residue levels, especially as the LOQ is approached. It may therefore be necessary to generate MU data over a range of residue levels to reflect those typically found during routine analysis.

E9 Two approaches for estimation of MU with example calculations are provided in Appendix C. One is based on the use of intra-laboratory QC data for individual pesticides in a commodity group. The second deals with an approach that derives a generic MU for the laboratory's multi-residue methods based on an overall combination of intra-laboratory precision and PT-derived bias.

E10 A practical approach for a laboratory to verify its MU estimation, based on its own within-laboratory data, is by evaluating its performance in recent proficiency tests (see Appendix C).

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[5] Codex Alimentarius Commission Guideline CAC/GL 59-2006, Guidelines on estimation of uncertainty of results.
[6] L. Alder *et al*., Estimation of measurement uncertainty in pesticide residue analysis. J. AOAC Intern., 84 (2001) 1569-1577.
[7] EURACHEM/CITAC Guide, Quantifying uncertainty in analytical measurement, 3rd Edition, 2012, http://www.eurachem.org/images/stories/guides/pdf/QUAM2012_P1.pdf

Proficiency test results can provide an important indication of the contribution of the inter-laboratory bias to the MU of an individual laboratory. Replicate analyses of a specific sample, combined with concurrent recovery determinations, can improve the accuracy of a single laboratory result and improve the estimate of MU. These uncertainty data will include the repeatability of sub-sampling and analysis, but not any interlaboratory bias. This practice will be typically applied when the analytical results are extremely important (e.g. an MRL compliance check).

Interpretation of results for enforcement purposes

E11 Assessment of whether a sample contains a residue which is an MRL exceedance 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 MU. The possibility of residue loss or cross-contamination having occurred before, during, or after sampling, must also be considered.

E12 A default expanded MU of 50% (corresponding to a 95% confidence level and a coverage factor of 2) has been calculated from EU proficiency tests. In general, this 50 % value 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). A prerequisite for the use of the 50% default expanded MU is that the laboratory must demonstrate that its own expanded MU is less than 50%. For further risk management evaluations, in specific and justified cases, laboratories may report to regulatory authorities their own estimated lower expanded MU value if supported by sufficient intra- and inter-laboratory evidence.

E13 If laboratories experience individual cases of unacceptably high repeatability, or within-laboratory reproducibility-RSD_{wR} (e.g. at very low concentration levels), or unsatisfactory z-scores during proficiency tests, the use of a correspondingly higher MU figure must be considered.

E14 If required, the result should be reported together with the expanded MU as follows: Result = x ± U (units), with x representing the measured value. For official food control by regulatory authorities, compliance with the MRL must be checked by assuming that the MRL is exceeded if the measured value exceeds the MRL by more than the expanded uncertainty (x – U > MRL). With this decision rule, the value of the measurand should be above the MRL with at least 97.5% confidence.[8] Thus, the sample is considered non-compliant if x-U > MRL. E.g., in case the MRL = 1, the result x = 2.2 and U=50%, then x-U = 2.2 – 1.1 (= 50% of 2.2)=1.1, which is >MRL.

[8] EURACHEM/CITAC Guide, Use of uncertainty information in compliance assessment, 1st Edition, 2007.

F. Pesticide standards, stock solutions and calibration standard solutions

Identity, purity, and storage of reference standards (neat substance)

F1 Reference standards of analytes should be of known purity and must be assigned with a unique identification code and recorded in a way that ensures full traceability (including source of supply, badge number, date of receipt and place of storage). 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. This way the reference standard may be retained and a new expiry date may be allocated, providing that it is checked by the appropriate date and its purity is shown to remain acceptable. Ideally, the chemical identity of a freshly acquired reference standard should be checked if the analyte is new to the laboratory. For screening purposes only, the reference standards and derived solutions may be used after the expiry date, providing that the RL can be achieved. If the pesticide has been detected, a new or certified reference standard and calibration standard solution made thereof has to be used for quantification..

Preparation and storage of stock standards

F2 When preparing stock standards (solutions, dispersions or gaseous dilutions) of reference standards (analytes and internal standards) documentation should be such as to ensure full traceability. The date of preparation, the identity and mass (or volume, for highly volatile analytes) of the reference standard and the identity and volume of the solvent (or other diluents) must be recorded. The solvent(s) must be appropriate to the analyte (solubility, no chemical reactions) and method of analysis. Moisture must be excluded during equilibration of the reference standard to room temperature before use, and concentrations must be corrected for the purity of the reference standard.

F3 For the preparation of stock standards not less than 10 mg of the "reference" standard should be weighed using a 5 decimal place balance. The ambient temperature should be corresponding to that, at which the glassware has been calibrated, otherwise preparation of the stock and working standard should be based on mass measurement. Volatile liquid analytes should be dispensed by volume or weight (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 any reactive metals).

F4 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. Following equilibration to room temperature, solutions must be re-mixed and a check made to ensure that the analyte remains completely dissolved, especially where solubility at low temperatures is limited. The use of a different solvent, different storage conditions or the preparation of stock solutions with lower concentration can help to overcome this problem. The stability of pesticides may depend on the solvent used. Currently available data show that stock standards solutions of the large majority of pesticides, when stored adequatley, are sufficiently stable for several years when prepared in organic solvents such as toluene, acetone, acetonitrile, methanol or ethyl acetate.

F5 For suspensions (e.g. dithiocarbamates) and solutions (or gaseous dilutions) of highly volatile fumigants that should be prepared freshly, the concentration of the analyte solution should be compared with a second solution made independently at the same time.

Preparation, use and storage of working standards

F6 When preparing working standards, a record must be kept of the identity and amount of all solutions and solvents employed. As for stock solutions, the solvent(s) must be appropriate to the analyte (solubility, no chemical reactions) 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 potential 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 the analyte has remained in solution, especially where solubility at low temperatures is limited.

F7 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 any impurity or artefact. If degradation of the analyte occurs during extraction, clean-up or separation, and the degradation product is commonly found in samples, but excluded from the residue definition, then the results must be confirmed using an alternative technique that avoids this problem.

Testing and replacement of standards

F8 The stability of an existing and possibly expired "reference" standard may be checked by preparing a new stock standard and comparing the detector responses. The comparison should be undertaken using appropriate dilutions of individual standards or mixtures of standards. Inexplicable differences in apparent concentrations between old and new standards must be investigated. Discrepancies between the concentrations of new and old solutions may be due to a number of factors other than simply analyte degradation (e.g. analyte precipitation, solvent evaporation, differences in the purities between the old and new reference standards, errors in weighing, or errors in the instrumental analysis).

F9 The means from at least five replicate measurements for each of two solutions (old and new) should not normally differ by more than ±10%. The mean value from the new solution is taken to be 100% and is also used as a basis for the calculation of the percentage-difference. Where the difference of the means exceeds ±10% from the new standard, then storage time or conditions may have to be adjusted. Both old and new solution should be checked against another new solution that is prepared independently from the first two.

F10 The variability of (at least 5) replicate injections (expressed as repeatability-RSD_r) should also be taken into account. Efforts towards low variability should be pursued to minimize the uncertainty of the calculated concentration difference between the new and the old solution. An internal standard may be used to reduce measurement variation. It is furthermore recommended to inject the old and new standards in alternating order to reduce any bias caused by signal drift.

F11 Where sufficient evidence exists (data from ≥2 other labs) that a certain pesticide is stable using specified storage conditions (time, solvent, temperature etc.) then other laboratories reproducing these storage conditions can reduce their own stability checks accordingly. However, possible solvent evaporation must be checked gravimetrically on a regular basis. In some cases certain additives (e.g. acids) may have to be added to stock solutions to prevent degradation of the analytes.

G. Analytical method validation and performance criteria

Quantitative methods

G1 Within-laboratory method validation should be performed to provide evidence that a method is fit for the intended purpose. Method validation is a requirement of accreditation bodies, and must be supported and extended by method performance verification during routine analysis (analytical quality control and on-going method validation). Where practicable, all procedures (steps) that are undertaken in a method should be validated.

G2 Representative matrices may be used to validate multi-residue and single-residue methods. As a minimum, one representative commodity from each commodity group as described in Annex A must be validated, depending on the intended scope of the method. When the method is applied to a wider variety of matrices, complementary validation data should be acquired, e.g. from on-going QC during routine analyses. An example of a practical approach to the validation procedure is presented in Appendix A.

G3 The method must be tested to assess sensitivity/linearity, mean recovery (as a measure of trueness or bias), precision (as repeatability RSD_r) and LOQ. Besides quantitative validation aspects, also the identification parameters must be assessed e.g. ion ratio and retention time. A minimum of 5 replicates is required (to check the recovery and precision) at the targeted LOQ or RL of the method, and at least one other higher level, for example, 2-10x the targeted LOQ or the MRL. Where the residue definition includes two or more analytes, then wherever possible, the method should be validated for all analytes included in the residue definition.

G4 If the analytical method does not permit determination of recovery (for example, direct analysis of liquid samples, SPME, or headspace analysis), then only the precision (not the trueness) is determined from repeat analyses of calibration standards. The bias is usually assumed to be zero, although this is not necessarily the case. In SPME and headspace analyses the trueness and precision of calibration may depend on the extent to which the analyte has equilibrated with respect to the sample matrix. Where methods depend upon equilibrium, this must be demonstrated during method validation.

G5 Where results are expressed on the basis of fat content or dry weight, the method used to determine the dry weight or fat content should be validated using a widely recognised method. For feeding stuffs the methods listed in Appendix III of Regulation (EC) No 152/2009 are obligatory.

Method performance acceptability criteria

G6 A quantitative analytical method should be demonstrated at both initial and extended validation stages, as being capable of providing acceptable mean recovery values at each spiking level and for at least one representative commodity from each of the relevant commodity groups (see Annex A and Table 4). Mean recoveries from initial validation should be within the range 70–120%, with an associated repeatability RSDr ≤ 20%, for all analytes within the scope of a method. In exceptional cases, mean recovery rates outside the range of 70-120% can be accepted if they are consistent (RSD ≤ 20%) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step), but the mean recovery must not be lower than 30 % or above 140 %. Within-laboratory reproducibility (RSDwR), which may be determined from on-going QC-data in routine analyses, should be ≤ 20%, excluding any contribution due to sample heterogeneity. The LOQ is the lowest spike level of the validation meeting these method performance acceptability criteria.

G7 The validation must also be used to verify the ability of the method to identify the analyte according to the requirements specified in section D. In justified cases, the validation data

may be used to set performance-based criteria, for individual analytes, rather than applying the generic criterion given in Table 4.

Table 4. *Validation parameters and criteria.*

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Deviation of back-calculated concentration from true concentration ≤± 20 %	C14-C19
Matrix effect	Difference of response from standard in matrix extract and standard in solvent	*	C21-C29 Glossary
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	≤ MRL	G6
Specificity	Response in reagent blank and blank control samples	≤ 30 % of RL	C41
Recovery	Average recovery for each spike level tested	70-120 %	G3,G6
Precision (RSD_r)	Repeatability RSD_r for each spike level tested	≤ 20 %	G3, G6
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20 %	G3, G6
Robustness	Average recovery and RSD_{wR}, derived from on-going method validation / verification	See above	G6, C39-C44
Ion ratio	Check compliance with identification requirements for MS techniques	Table 3	Section D
Retention time		± 0.1 min.	D2

* in case of more than 20% signal suppression or enhancement, matrix effects need to be addressed in calibration (C22-C30)

Screening methods

G8 For screening methods the confidence of detection of an analyte at a certain concentration level should be established. This can be achieved using screening methods based on the RL from the validation of a quantitative method or screening methods based on the SDL from the validation of a qualitative method.

G9 The validation of a screening method based on an SDL can be focused on detectability. For each commodity group (see Annex A), a basic validation should involve analysis of at least 20 samples spiked at the estimated SDL. The samples selected should represent multiple commodities from the same commodity group, with a minimum of two samples for each individual commodity included and will be representative for the intended scope of the

laboratory. Additional validation data can be collected from on-going AQC-data and method performance verification during routine analysis.

Method performance acceptability criteria

G10 When the screening method is only intended to be used as a qualitative method, there are no requirements with regard to recovery of the analytes. In order to determine the selectivity, the possible presence of false detects should be checked using non-spiked (preferably "blank") samples. Provided the analytes that are tentatively detected by the screening method are identified and confirmed by a second analysis of the sample using an appropriate confirmatory method, there is no need for a strict criterion for the number of false positive detects. The SDL of the qualitative screening method is the lowest level at which an analyte has been detected (not necessarily meeting the MS-identification criteria) in at least 95% of the samples (i.e. an acceptable false-negative rate of 5%).

G11 For analytes that have not been included in the initial or on-going method validation, the confidence level of detection at a certain residue level will not be known. Consequently analytes outside of the scope of validation can be detected using the method, but no SDL can be specified.

G12 When using a qualitative screening method, only analytes that have been validated can be added to the routine scope of the laboratory.

H. Additional recommendations

Contamination

H1 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 residues, or with volatile analytes. Samples known, or thought, to have such residues should be doubly sealed in polythene or nylon bags and transported and processed separately.

H2 Volumetric equipment, such as flasks, pipettes and syringes must be cleaned scrupulously, especially before re-use. As far as practicable, separate glassware, etc., should be allocated to standards and sample extracts, in order to avoid cross-contamination. The use of excessively scratched or etched glassware should be avoided. Solvents used for fumigant residues analysis should be checked to ensure that they do not contain target analyte(s).

H3 Where an internal standard is used, unintended contamination of extracts or analyte solutions with the internal standard, or vice versa, must be avoided.

H4 Where the analyte occurs naturally, or as a contaminant, or is produced during the analysis (e.g. biphenyl in herbs, inorganic bromide in all commodities, sulphur from soil, or carbon disulfide from the Brassicaceae), low-level residues from pesticide use cannot be distinguished from background levels. Natural occurrence of these analytes must be considered in the interpretation of results. Dithiocarbamates, precursors of carbon disulfide, ethylenethiourea or diphenylamine can occur in certain types of rubber articles and this source of contamination must be avoided.

Interference

H5 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, and wash bottles), polishes and lubricants are frequent sources of interferences. Vial seals should be PTFE-lined. Extracts should be kept out of contact with seals, especially after piercing, for example, by keeping vials upright. Vial seals may have to 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.

H6 Matrix effects or matrix interferences from natural constituents of samples are 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. Matrix effects in terms of suppression or enhancement of the detection system response is dealt with in paragraph C21. If it is not practicable to eliminate matrix effects or to compensate for such effects by matrix-matched calibration, the overall accuracy of analysis should nonetheless comply with the criteria in paragraph G6.

Annex A. Commodity groups and representative commodities[9]

Vegetable and fruits, cereals and food of animal origin

Commodity groups	*Typical commodity categories wthin the group*	*Typical representative commodities within the category*
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches,
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumbers, melons
	Brassica vegetables	Cauliflowers, Brussels-sprouts, cabbages, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Fresh legume vegetables	Fresh peas with pods, peas, mange tout, broad beans, runner beans, French beans
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables	Sugar beet, carrots, potatoes, sweet potatoes
2. High acid content and high water content[10]	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberries, blueberries, raspberries, black currants, red currants, white currants, grapes
3. High sugar and low water content[11]	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts, chestnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahina, hazelnut paste
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field beans, dried broad beans, dried haricot beans (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grains; maize, rice wholemeal bread, white bread, crackers, breakfast cereals, pasta, flour.
6. "Difficult or unique commodities"[12]		Hops Cocoa beans and products thereof, coffee, tea Spices

[9] SANCO/12574/2014
On the basis of OECD Environment, Health and safety Publications, Series on Testing and Assessment, No72 and Series of Pesticides No39

[10] If samples are pH-adjusted during the extraction step, by adding buffers or large amounts of acids or bases, then the commodity Group 2 can be merged with commodity Group 1.

[11] Where commodities of Group 3 are mixed with water prior to extraction to achieve a water content of >70%, this commodity group may be merged with Group 1. The RLs should be adjusted to account for smaller sample portions (e.g. if 10g portions are used for commodities of Group 1 and 5g for Group 3, the RL of Group 3 should be twice the RL of Group 1 unless a commodity belonging to Group 3 is successfully validated at a lower level).

[12] "Difficult commodities" should only be fully validated if they are frequently analysed. If they are only analysed occasionally, validation may be reduced to just checking the reporting limits using spiked blank extracts.

Commodity groups	*Typical commodity categories wthin the group*	*Typical representative commodities within the category*
7. Meat (muscle) and Seafood	Red muscle	Beef, pork, lamb, game, horse
	White muscle	Chicken, duck, turkey
	Offal	Liver, kidney
	Fish	Cod, haddock, salmon, trout
8. Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow and goat cheese
	Dairy products	Yogurt, cream
9. Eggs	Eggs	Chicken, duck, quail and goose eggs
10. Fat from food of animal origin	Fat from meat	Kidney fat, lard
	Milk fat[13]	Butter

Feed

Commodity groups	*Typical commodity categories within the group[14]*	*Typical representative commodities within the category*
1. High water content	Forage crops Brassica vegetables Leaves of root and tuber vegetables Root and tuber Silage	Grasses, Alfalfa, Clover, Rape Kale/Cabbage Sugar beet leaves and tops Sugar beet and fodder beet roots, carrots, potatoes Maize, clover, grasses By-products and food waste such as apple pomace, tomato pomace, potato peels, flakes and pulp, sugar beet pulp, molasses[15]
2. High acid content and high water content		By-products and food waste such as Citrus pomace [10,15]
3. High oil/fat content and very low water content	Oil seeds, oil fruits, their products and by products Fat/oil of vegetable and animal origin	Cottonseed, linseed, rapeseed, sesame seed, sunflower seed, seed, soybeans Palm oil, rapeseed oil, soya bean oil, fish oil, fatty acid distillate Compound feed with high lipid content
4. Intermediate oil content and low water content	Oil seed cake and meal	Olive, rape, sunflower, cotton-seed, soybeans cake or meal
5. High starch and/or protein content and low water and fat content [14]	Cereal grains, their products, by-products and food waste Legume seeds By-products and food waste	Barley, oat, maize, rice, rye, spelt, triticale and wheat kernels, flakes, middlings, hulls and bran. Bread, brewers' and distillers' grains Cereal based composite feed Dried beans, peas, lentils Seed hulls

[13] If methods to determine non-polar pesticides in commodities of Group 7 are based on extracted fat, these commodities can be merged with Group 10.

[14] Where a commodity is common to both food and feed e.g cereals, only one validation is required.

[15] Sample size to water ratio must be optimised for the individual commodities, by adding water before extraction to simulate the raw product.

Commodity groups	***Typical commodity categories within the group[14]***	***Typical representative commodities within the category***
6. "Difficult or unique commodities"[12]	Straw Hay Premixtures	Barley, oat, maize, rice, rye and wheat straw Grasses By-products and food waste such as potato protein and fatty acid distillate
7. Meat and Seafood	Animal origin based composite feed	Fish meal
8. Milk and milk products	Milk	Milk replacer By-products and food waste such as whey [15)]

Appendix A. Method validation procedure: outline and example approaches

Validation is undertaken following the completion of the method development or before a method that has not been previously used is to be introduced for routine analysis. We distinguish between initial validation of a quantitative analytical method to be applied in the laboratory for the first time and the extension of the scope of an existing validated method for new analytes and matrices.

Quantitative analysis

1. Initial full validation

Validation needs to be performed

- for all analytes within the scope of the method
- for at least 1 commodity from each of the commodity groups (as far as they are within the claimed scope of the method or as far as applicable to samples analysed in the laboratory)

Experimental:

A typical example of the experimental set up of a validation is:

Sample set (sub-samples from 1 homogenised sample):

- Reagent blank
- 1 blank (non-spiked) sample
- 5 spiked samples at target LOQ
- 5 spiked samples at 2-10x target LOQ

Instrumental sample sequence:

- Conditioning blanks in GC
- Calibration standards
- Reagent blank
- Blank sample
- 5 spiked samples at target LOQ
- 5 spiked samples at 2-10 x target LOQ
- Calibration standards

Spiking of commodities is a critical point in validation procedures
In general the spiking procedure should reflect as much as possible the techniques used during routine application of the method. If for example, samples are milled cryogenically and extracted in frozen condition spiking must be done on frozen analytical test portions of blank material and extracted immediately. Where samples are milled at room temperature and extracted on average after 20 min, spiking should be done on blank test portions at room temperature. In general, spiking of samples will not simulate incurred residues even if the spiked sample is left standing for a certain time. To study the relative extractability of incurred residues agriculturally treated samples should be taken.

Data evaluation:
Inject the sample sequence, calibrate and quantify as is described in this AQC document.

Evaluate the parameters from Table 4 and verify them against the criteria.

2. Extension of the scope of the method: new analytes

New analytes that are to be added to a previously validated method need to be validated using the same procedure as outlined above for initial validation.

Alternatively, the validation of new analytes can be integrated in the on-going quality control procedure. As an example: with each batch of routine samples, one or more commodities from the applicable commodity category are spiked at the LOQ and one other higher level. Determine the recovery and occurrence of any interference in the corresponding unspiked sample. When for both levels, 5 recovery values have been collected, the average recovery and within-laboratory reproducibility (RSD_{wR}) can be determined and tested against the criteria in Table 4.

3. Extension of the scope of the method: new matrices

A pragmatic way of validation of the applicability of the method to other matrices from the same commodity group is to perform using the on-going quality control performed concurrently with analysis of the samples (see below).

4. On going validation / performance verification

The purpose of on-going method validation is to:

- demonstrate robustness through evaluation of mean recovery and within-laboratory reproducibility (RSD_{wR})
- demonstrate that minor adjustments made to the method over time do not unacceptably affect method performance
- demonstrate applicability to other commodities from the same commodity category (see also Annex 1)
- determine acceptable limits for individual recovery results during routine analysis

Experimental:

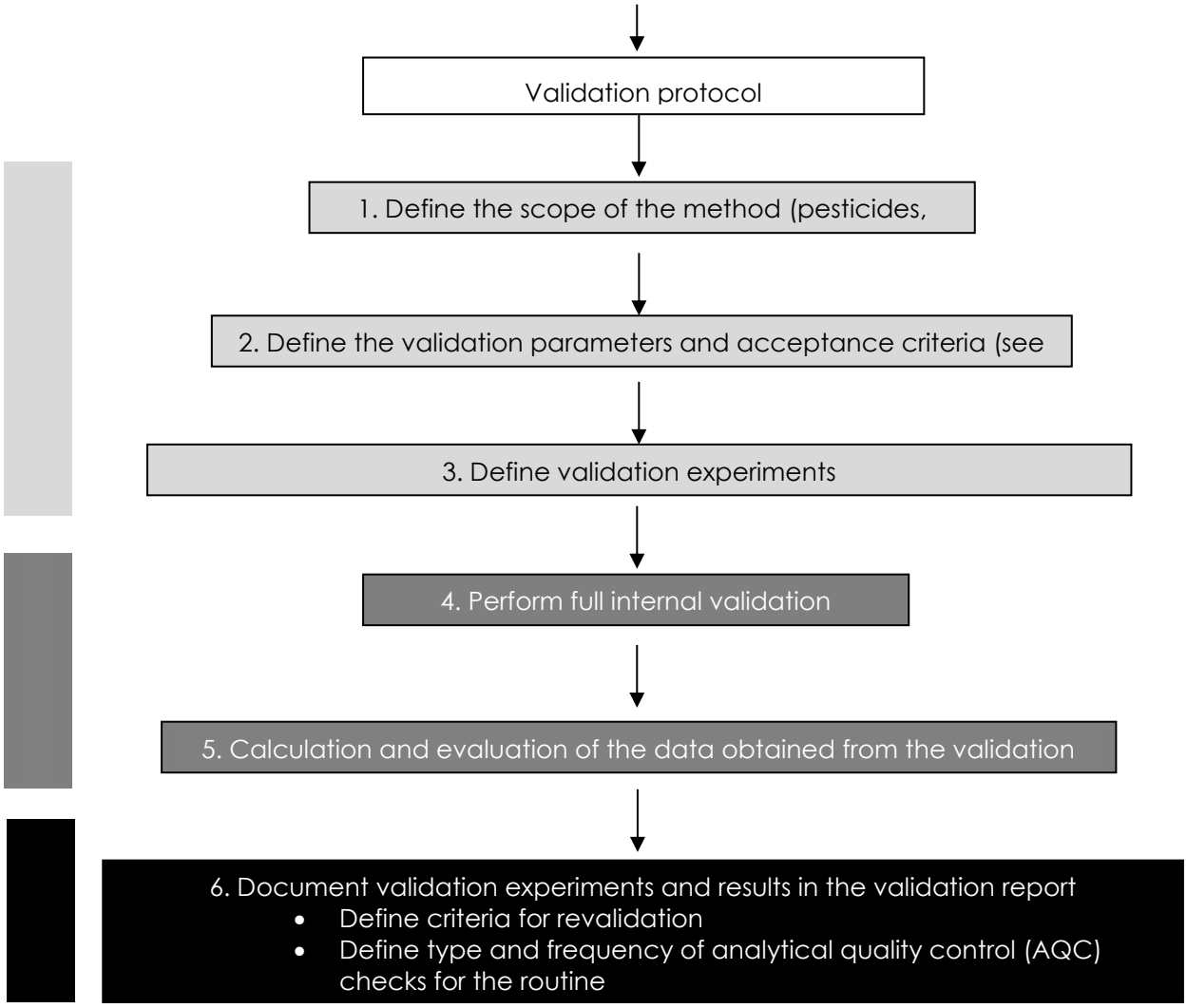
Typically, with each batch of samples routinely analysed, one or more samples of different commodities from the applicable commodity category are spiked with the analytes and analysed concurrently with the samples.

Data evaluation:

Determine for each analyte the recovery from the spiked sample and occurrence of any interference in the corresponding unspiked sample. Periodically (e.g. annually) determine the average recovery and reproducibility (RSD_{wR}) and verify the data obtained against the criteria from Table 4. These data can also be used to set or update limits for acceptability of individual recovery determinations as outlined in paragraph G6 of the AQC document and estimation of the measurement uncertainty.

Identification criteria: retention time (see D2), MS criteria (see Table 3 and D12).

INITIAL VALIDATION PLAN FOR QUANTITATIVE METHODS



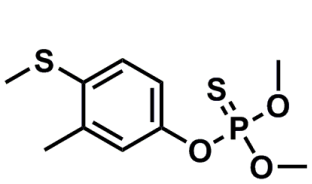
Appendix B. Examples of conversion factors.

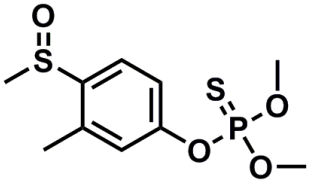
The MRL residue definitions for a number of pesticides include not only the parent pesticide, but also its metabolites or other transformation products.

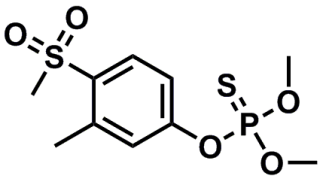
In Example 1, the sum of the components is expressed as fenthion, following adjustment for the different molecular weights (conversion factors). In Example 2, the sum of (*E*)-metaflumizone and (*Z*)-metaflumizone is expressed as their arithmetic sum (metaflumizone).

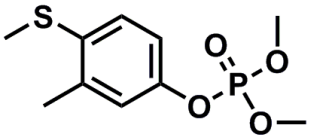
The following examples illustrate the two different types of summing that are required in order to meet the requirements of the residue definition.

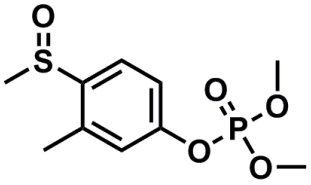
Example 1.
Fenthion, its sulfoxide and sulfone, and their oxygen analogues (oxons), all appear in the residue definition and all should be included in the analysis.

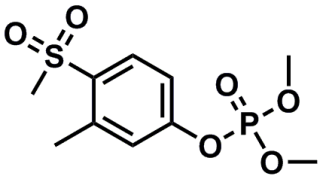

Fenthion


Fenthion-sulfoxide


Fenthion sulfone


Fenthion-oxon


Fenthion-oxonsulfoxide


Fenthion-oxonsulfone

Example of calculating the conversion factor (Cf)

$C_{FenthionSO\ to\ Fenthion} = (Mw_{Fenthion}/Mw_{FenthionSO}) \times C_{Fenthion\ SO} = (278.3/294.3) \times C_{Fenthion\ SO} = 0.946 \times C_{FenthionSO}$

Compound			Mw	Cf
Fenthion	RR´S	P=S	278,3	1,00
Fenthion sulfoxide	RR´SO	P=S	294,3	0,946
Fenthion sulfone	RR´SO_2	P=S	310,3	0,897
Fenthion oxon	RR´S	P=O	262,3	1,06
Fenthion oxon sulfoxide	RR´SO	P=O	278,3	1,00
Fenthion oxon sulfone	R´SO_2	P=O	294,3	0,946

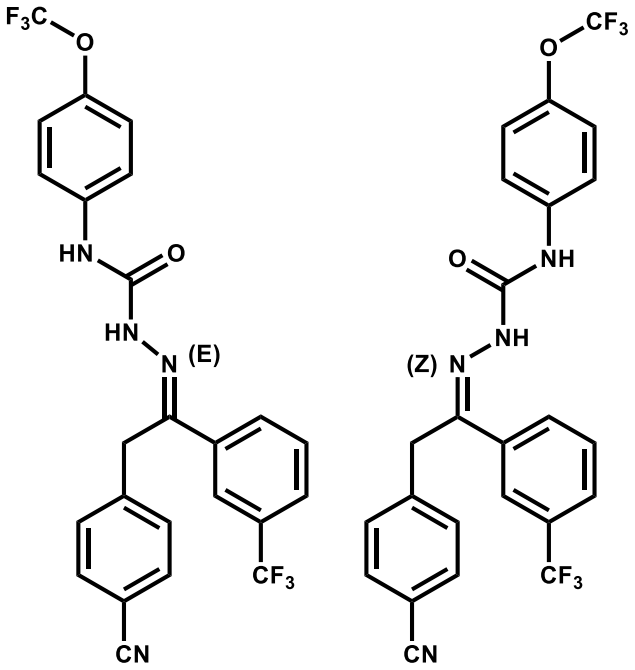
Residue Definition: Fenthion (fenthion and its oxygen analogue, their sulfoxides and sulfones expressed as parent)

Where the residue is defined as the sum of the parent and transformation products, the concentrations of the transformation products should be adjusted according to their molecular weight being added to the total residue concentration.

$C_{FenthionSum} = 1.00 \times C_{Fenthion} + 0.946 \times C_{Fenthion\ SO} + 0.897 \times C_{Fenthion\ SO2} + 1.06 \times C_{Fenthionoxon} + 1.00 \times C_{Fenthionoxon\ SO} + 0.946 \times C_{Fenthionoxon\ SO2}$

Example 2.

Residue Definition: Metaflumizone (sum of *E*- and *Z*- isomers))



$C_{Metaflumizone} = 1.00 \times C_{(E)\text{-}Metaflumizone} + 1.00 \times C_{(Z)\text{-}Metaflumizone}$

Appendix C. Examples for the estimation of measurement uncertainty of results

Establishment of the measurement uncertainty (MU) is a requirement under ISO/IEC 17025 (E5). It is also required to demonstrate that the laboratory's own MU is not exceeding the 50% default value used by regulatory authorities in cases of enforcement decisions (E10).

In order to estimate the MU of results for the determination of pesticide residues, several documents are recommended to be read that help to provide a better understanding of this topic, such as Eurachem,[16] Nordtest,[17] Eurolab,[18] Codex CAC/GL 59-2006[19] Guidelines and A. Valverde et al.[20]

In this appendix two appraoches for estimation of MU are described and example calculations provided. The first one of them deals with MU estimation based on intra-laboratory QC data for individual pesticides in a commodity group. The second one deals with an approach that derives a generic MU for the laboratory's multi-residue methods based on an overall combination of intra-laboratory precision and PT-derived bias.

In the examples only within-laboratory variability and bias are considered as these are typically the main contributors. However, other factors, such as heterogeneity of the laboratory sample and the tolerance in differences of standard solutions (F9) may contribute to the overall MU. Contributions are significant when their uncertainty is greater than one third of the magnitude of the largest contributer.

In both examples, an expanded coverage factor of k = 2 is assumed to calculate the expanded MU represented by U' from the relative standard uncertainty u' in equation 1.

$$U' = k \times u' \qquad \text{Equation 1}$$

Approach 1. Estimating MU based on intra-laboratory validation/QC data.

Here estimation is based on[16,17,19]:

$$u' = \sqrt{u'(bias)^2 + u'(precision)^2} \qquad \text{Equation 2}$$

with u' = measurement uncertaintly

u'(bias) = uncertainty component for the bias

u'(precision) = uncertainty component for the precision

In principle, the precision component should be estimated from experiments different than those used to estimate the bias component, and the latter should preferably be based on an external (independent) source such as CRM and PT reference values. The reality is that for the majority of the pesticide/matrix combinations only data from internal QC samples (spiked samples) are available and that bias and precision components can only be estimated from the same (on-going) validation experiments.

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[16] EURACHEM/CITAC Guide, Quantifying uncertainty in analytical measurement, 3rd Edition, 2012, http://www.eurachem.org/images/stories/guides/pdf/QUAM2012_P1.pdf

[17] NORDTEST NT TR 537 edition 4 2017:11 http://www.nordtest.info/images/documents/nt-technical-reports/NT_TR_537_edition4_English_Handbook_for_calculation_of_measurement_uncertainty_in_environmental_laboratories.pdf

[18] EUROLAB Technical Report 1/2007: Measurement uncertainty revised: alternative approaches to uncertainty evaluation, European Federation of National Associations of Measurement, Testing and Analytical Laboratories, www.eurolab.org, Paris, 2007

[19] Codex Alimentarius Commission ,CAC/GL 59-2006 (Amendment 1-2011) Guidelines on Estimation of Uncertainty of Results, www.codexalimentarius.net/download/standards/10692/cxg_059e.pdf , Rome 2006 and 2011

[20] A. Valverde, A. Aguilera, A. Valverde-Monterreal, Practical and valid guidelines for realistic estimation of measurement uncertainty in multi-residue analysis of pesticides, Food Control 71 (2017) 1-9.

A first estimate of u´(bias) and u´(precision) is usually obtained at the initial validation stage for each pesticide/representative matrix/level combination. However, a much more realistic estimation is calculated for each pesticide from a number (usually, ≥10) of long-term QC tests (spiked samples) for each pesticide for one or more matrices of the same commodity group.

Estimation of the u'(bias) component without correction for recovery

The bias is the difference between the measured value and the true value. In absence of CRM or PT assigned values, the true value is the spiked concentration, and the bias is the difference between the spiked and the measured concentration. The relative bias is given by:

$$relative\ bias = \frac{measured\ concentration - spiked\ concentration}{spiked\ concentration} \times 100\% \qquad \text{Equation 3}$$

u'(bias) can be calculated using the following equation:

$$u'(bias) = \sqrt{RMS'(bias)^2 + u'(Cref)^2} \qquad \text{Equation 4}$$

with RMS'(bias) = root mean square of the relative bias = $\sqrt{\frac{\sum bias_i^2}{N}} = \sqrt{mean_{bias}^2 + SD.P_{bias}^2}$

with $mean_{bias}$ = the mean of the relative bias

$SD.P_{bias}$ = the population standard deviation of the relative bias (stdev.p in Excel)

$u'(C_{ref})$ = uncertainty of the spiked concentration.

When certified analytical standards and calibrated/verified volumetric material/balances are used to prepare the spiked samples, it can be assumed that the uncertainty associated with the spiking level is negligible. Equation 4 then simplifies to:

$$u'(bias) = \sqrt{mean_{bias}^2 + SD.P_{bias}^2} \qquad \text{Equation 5}$$

Estimation of the u'(bias) component with correction for recovery

In case the analysis result is mathematically corrected for recovery using a recovery factor (see E4), then the u'(bias) can be calculated using the following equation:

$$u'(bias) = \sqrt{\left(\frac{RSD_{wR}}{\sqrt{N}}\right)^2 + u'(Cref)^2} \qquad \text{Equation 6}$$

with RSD_{wR} = within-laboratory reproducibility of the recovery

N = number of recovery tests

When certified analytical standards and calibrated/verified volumetric material/balances are used to prepare the spiked samples, it can be assumed that the uncertainty associated with the spiking level is negligible. Equation 6 then simplifies to:

$$u'(bias) = \frac{RSD_{rW}}{\sqrt{N}} \qquad \text{Equation 7}$$

Estimation of the u'(precision) component

As precision component the within-laboratory reproducibility (RSD_{rw}) of the pesticide is used:

u'(precision) = RSD_{rw} Equation 8

The RSD_{rw} is preferably derived from spiked samples from ≥10 sample batches over a longer period of time (on-going validation). When multiple matrices from a commodity group are analysed and one RSD_{rw} value is used for that group, the RSD_{rw} should be based on spiked samples of different matrices reflecting the scope of analysis in order to obtain a realistic estimate for the commodity group. It is recommended to periodically re-assess the RSD_{rw}, e.g. every year, or in case of low method application frequency every 20 results, and to consider updating of the RSD_{rw} (either using the entire data set, or only the more recent data).

If on-going validation data are not (yet) available, repeatability data from initial validation may be used. Note that especially when this represents only one matrix analysed on a single day, this is likely to result in an underestimation of the precision component.

Estimation of the combined measurement uncertainty

The combined measurement uncertainty is estimated by equation 2, and using equation 5 and 8 is:

$$u' = \sqrt{mean_{bias}^2 + SD.P_{bias}^2 + RSD_{rW}^2}$$ Equation 9

When analysis results are mathematically corrected for recovery using a recovery factor, the combined measurement uncertainty is estimated by equation 2, using equation 7 and 8:

$$u' = \sqrt{\left(\frac{RSD_{WR}}{\sqrt{N}}\right)^2 + RSD_{rW}^2}$$ Equation 10

Note: when N≥9, u' is approximately RSD_{rw}^2

Example calculations.

Example A. This example applies to all situations where results are not corrected for recovery. A laboratory analyses pesticide X in fruit and vegetables (commodity group 1, Annex A). With each batch of samples, a sample spiked at 0.050 mg/kg is included in the batch. A different matrix is chosen each time to take the variability of matrices from this commodity group into account. In the example, the measurement uncertainty is based on the QC data obtained after nine batches of analysis (Table I).

Table I. Example A, pesticide X (low bias, good within-lab reproducibility)

Date	QC samples spiked @0.05 mg/kg	measured (mg/kg)	rel. bias (%) [equation 3]
10/Jan	Apple	0.051	2
26/Jan	Pear	0.045	-10
04/Feb	Lettuce	0.050	0
08/Feb	cauliflower	0.056	12
22/Feb	Cherries	0.052	4
28/Feb	Onion	0.046	-8
05/Mar	French beans	0.048	-4
06/Mar	Carrots	0.045	-10
22/Mar	Leek	0.037	-26
	N	9	
	mean	0.0478	-4.44

SD.Pbias (stdev.p) (%)		10.232
standard dev. measured (mg/kg) (stdev.s)	0.00543	
RSDwR (%)	11.357	
u'(bias) (%) [equation 5]		11.1555
u'(precision) = RSDwR (%) [equation 8]	11.357	
u' combined (%) [equation 2 and 9]	15.920	
U' (expanded MU) (%) [equation 1]	31.839	

The estimated expanded measurement uncertainty is 32%. For pesticide X the laboratory has demonstrated that the expanded MU is not exceeding the 50% default value (E14). The regulatory authorities can use the 50% default value for enforcement decisions.

Example B. This example is similar to example B, but for this pesticide a relatively high bias is observed. As can be seen from the calculation in Table II, while the RSD_{rw} is the same as in example A, the higher bias results in an expanded MU of 63%.

Table II. Example B, pesticide Y (high bias, good within-lab reproducibility)

Date	QC sample spiked @0.05 mg/kg	measured (mg/kg)	rel. bias (%)
10/Jan	Apple	0.038	-24
26/Jan	Pear	0.034	-32
04/Feb	Lettuce	0.037	-26
08/Feb	cauliflower	0.042	-16
22/Feb	Cherries	0.039	-22
28/Feb	Onion	0.034	-32
05/Mar	French beans	0.036	-28
06/Mar	Carrots	0.034	-32
22/Mar	Leek	0.028	-44
	N	9	
	Mean	0.0358	-28.4
	SD.Pbias (stdev.p) (%)		7.470
	standard dev. measured (mg/kg) (stdev.s)	0.00396	
	RSDwR (%)	11.073	
	u'(bias) (%) [equation 5]		29.4090
	u'(precision) = RSDwR (%) [equation 8]	11.073	
	u' combined (%) [equation 2 and 9]	31.424	
	U' (expanded MU) (%) [equation 1]	62.849	

For pesticide, Y the laboratory has demonstrated that the expanded MU is exceeding the 50% default value (E14) when results are not corrected for recovery. If, at the end of the analytical program, the results were corrected for the average recovery achieved over the 3 month period, then the u'(bias) need only to reflect the uncertainty associated with the mean recovery[19] and equation 7 applies. The average recovery in example B is [100%-bias%]=71.6%. The RSD_{rw} of this recovery is the same as the RSD_{rw} of the measured concentrations (11.073%).

With that, the u'(bias) according to equation 7 is 3.691%, resulting in a combined u' of 11.672% and an expanded MU of 23%.

Approach 2. Estimating a generic MU using PT data.

It is recognised that for multi-residue methods applied to a wide range of matrices, calculation of individual MUs may not always be possible because it requires substantial efforts and bias data may not be available for all pesticides in a sufficient number of matrices. As an alternative to approach 1, the expanded MU may be calculated using the within-laboratory reproducibility relative standard deviation combined with estimates of the method and the laboratory bias using PT data applying equation 11.

$$u' = \sqrt{u'(RSD_{wR})^2 + u'(bias)^2} \qquad \text{Equation 11}$$

In equation 12:

u´	is the combined standard uncertainty
u´(RSD_{wR})	is the within-laboratory reproducibility
u´(bias)	is the uncertainty component arising from method and laboratory bias, estimated from PT data.

To calculate u´(RSD_{wR}) preferably long-term quality control (QC) recovery data should be used although recoveries coming from validation data can be included too.

Note: within-laboratory variability coming from calibration is considered to be included in the long-term quality control recovery variability[15].

The standard deviation of all the recoveries percentage taken into account is calculated.

For the example presented here, validation recoveries are taken for all pesticides that have been validated in the same multi residue method (MRM) and for which the laboratory is used to take part in the PTs. Also the long-term QC recovery data in the range of 60%-140% are included for two different levels and for the fruit and vegetables matrices normally analysed in the laboratory. A minimum of 31 results must be taken into account[18]. For two methods: one for LC with 93 pesticides and the other for GC with 66 pesticides, the standard deviation of all the recovery percentages is 0.15. The u´(RSD_{wR}) is therefore 0.15.

The u´(bias) component is calculated from the performance of the laboratory in PT studies as stated in many guidelines[15-18]. Participation of EU official laboratories in the EUPTs is mandatory. Therefore taking results from at least 2 EUPT-FV will provide enough data (above 31 results) to conduct this approach.

For this example, the 2 EUPT-FV results reported are in total 39 pesticide results. From these two PTs the information that needs to be used is the assigned value or median, the real dispersion of results reported by the laboratories for each of the pesticides present in the sample (the Qn or robust standard deviation) and the number of laboratories reporting quantitative results for those pesticides.

Table I shows the number of the EUPT-FV wherein the lab has participated (column A), the pesticides reported (column B), the pesticide concentration reported (column C), the assigned value or median (column D), the square of the bias (column E) which is [(column C – column D) / (column D)]^2, then the dispersion of the data from the participants or Qn (column F), then the number of laboratories reporting results for each of the pesticides (column G), then the square root of column G (column H) and then the coefficient between column F and column H (column I).

Then equation 12 is used:

$$u' = \sqrt{RMS'_{bias}{}^2 + u'(c_{ref})^2} \qquad \text{Equation 12}$$

Where:

- RMS´$_{bias}$ is the Root Mean Square of the sum of the squared bias [(sum of column E) divided by the number of results taken from the PTs (m =39)] as indicated in equation 13.

$$RMS'_{bias} = \sqrt{\sum \frac{\left(bias_i'\right)^2}{m}} = \sqrt{\frac{1.999}{39}} = 0.2263 \qquad \text{Equation 13}$$

- u´(C_{ref}) is an estimation of an average over several PTs. It is calculated as the sum of the Qn divided by the square root of the number of results reported by the laboratories for each of the pesticides in the scope (column I), then divided by the number of results (m) taken from the PTs (39) and multiplied by a factor of 1.253 according to ISO 13528[21]. This ISO states that u´(C_{ref}) must be multiplied by this factor, whenever the assigned value in PTs is the median. Is calculated following equation 14.

$$u'\left(c_{ref}\right) = \frac{\sum_i \frac{Qn}{\sqrt{No.}}}{m} \times 1.253 = \frac{0.9326}{39} \times 1.253 = 0.02996 \qquad \text{Equation 14}$$

When entering the results from equation 13 and 14 into equation 12, we get the u´(bias):

$$u'\left(bias\right) = \sqrt{RMS_{bias}'^{\,2} + u'\left(c_{ref}\right)^2} = \sqrt{0.2263^2 + 0.02996^2} = 0.2284$$

Note: the u´(bias) can be calculated from the participation of the laboratory in other PTs.

Now, back to equation 11 and entering the u´(RSD_{wR}) = 0.15 and the u´(bias):

$$u' = \sqrt{u'\left(RSD_{wR}\right)^2 + u'\left(bias\right)^2} = \sqrt{0.15^2 + 0.2284^2} = 0.2732$$

So back to equation 1, u´ = 0.27 and the expanded measurement uncertainty is therefore:

U' = k × u' = 2 × 0.273 = 0.546 U'=54,6%

[20] ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparisons, International Standardisation Organisation

Table I

A	B	C	D	E	F	G	H	I
EUPT-FV	Pesticides	Lab Results	PT Assigned Values	$(bias'_i)^2$	Qn	No. Results	$\sqrt{No.}$	$\frac{Qn}{\sqrt{No.}}$
EUPT-FV-10 Carrot	Acetamiprid	0.337	0.419	0.0383	0.18	85	9.220	0.020
	Boscalid	0.139	0.238	0.1720	0.22	74	8.602	0.026
	Chlorpyrifos-methyl	0.056	0.078	0.0796	0.26	126	11.225	0.023
	Diazinon	0.412	0.603	0.1003	0.24	125	11.180	0.021
	Endosulfan Sulphate	0.062	0.102	0.1538	0.29	110	10.488	0.028
	Hexythiazox	0.396	0.509	0.0493	0.29	80	8.944	0.032
	Isofenphos-methyl	0.436	0.499	0.0159	0.17	69	8.307	0.020
	Kresoxim-methyl	0.028	0.050	0.1936	0.22	113	10.630	0.021
	Malathion	0.697	0.771	0.0091	0.32	124	11.136	0.029
	Methamidophos	0.245	0.342	0.0798	0.37	103	10.149	0.036
	Methiocarb	0.096	0.157	0.1510	0.31	65	8.062	0.038
	Methomyl	0.538	0.739	0.0740	0.22	88	9.381	0.023
	Oxamyl	0.274	0.322	0.0222	0.19	84	9.165	0.021
	Pendimethalin	0.056	0.074	0.0592	0.21	96	9.798	0.021
	Phosmet	0.139	0.236	0.1689	0.28	95	9.747	0.029
	Quinoxyfen	0.244	0.298	0.0328	0.23	95	9.747	0.024
	Triadimenol	0.265	0.331	0.0398	0.27	103	10.149	0.027
	Vinclozolin	0.90	1.04	0.0181	0.24	124	11.136	0.022
EUPT-FV-11 Cauliflower	Aldicarb	0.679	0.658	0.0010	0.20	91	9.539	0.021
	Azinphos-methyl	0.349	0.355	0.0003	0.28	128	11.314	0.025
	Boscalid	0.373	0.414	0.0098	0.25	102	10.100	0.025
	Buprofezin	0.453	0.638	0.0841	0.30	118	10.863	0.028
	Cadusafos	0.810	0.611	0.1061	0.24	76	8.718	0.028
	Carbofuran	0.245	0.283	0.0180	0.20	107	10.344	0.019
	Deltamethrin	0.138	0.157	0.0146	0.25	130	11.402	0.022
	Diazinon	1.140	1.25	0.0077	0.26	144	12.000	0.022
	Isofenphos-methyl	0.498	0.54	0.0060	0.24	86	9.274	0.026
	Lambda-cyhalothrin	0.211	0.266	0.0428	0.24	138	11.747	0.020
	Metalaxyl	0.445	0.45	0.0001	0.21	122	11.045	0.019
	Methamidophos	0.341	0.4045	0.0246	0.33	109	10.440	0.032
	Methidathion	0.453	0.472	0.0016	0.24	136	11.662	0.021
	Methomyl	0.190	0.277	0.0986	0.18	84	9.165	0.020
	Monocrotophos	0.322	0.4375	0.0697	0.21	95	9.747	0.022
	Oxamyl	0.230	0.2485	0.0055	0.17	89	9.434	0.018
	Parathion-methyl	0.277	0.32	0.0181	0.24	129	11.358	0.021
	Phosalone	0.383	0.368	0.0017	0.30	136	11.662	0.026
	Procymidone	0.750	0.78	0.0015	0.20	136	11.662	0.017
	Thiacloprid	0.961	0.879	0.0087	0.15	82	9.055	0.017
	Triazophos	0.612	0.538	0.0189	0.30	132	11.489	0.026
			$\sum (bias'_i)^2$	1.999			$\sum \frac{Qn}{\sqrt{No.}}$	0.9326
			No. of Results (m) 39				No. of Results (m) 39	

Appendix D. Example of rounding, reporting and interpreting results

Rounding:

The following general rules are proposed for rounding the result of a pesticide residue concentration:

a) The result should be rounded to either two significant figures for results < 10 mg/kg or three significant figures for results ≥ 10 mg/kg (see paragraph E6).
b) If the digit following the digit to be rounded in the primary result is 0, 1, 2, 3 or 4, the digit will not change when the rounding is applied.
c) If the digit following the digit to be rounded in the primary result is 5, 6, 7, 8 or 9, the digit will increase by one unit when the rounding is applied.
d) The expanded measurement uncertainty will be estimated by using the final rounded result.
e) The value of the expanded uncertainty is always rounded up unless (after rounding of the second non-retained digit) the first non-retained digit would be 0. The value of the expanded uncertainty should be given with the same number of decimals as the rounded result.

1) NIST GLP 9; Good Laboratory Practice for Rounding Expanded Uncertainties and Calibration Values; (https://www.nist.gov/system/files/documents/2019/05/14/glp-9-rounding-20190506.pdf)
2) EUROPEAN COMMISSION, DIRECTORATE GENERAL, JOINT RESEARCH CENTRE Directorate F – Health, Consumers and Reference Materials, PR-D-00014: Property value assignment
3) German Standard: DIN 1333:1992

Example:

Primary result = 0.02454705 mg/kg

This result should be rounded to two significant figures (0.02**4**54705)

Result after rounding = 0.025 mg/kg (Final result; two significant figures)

Primary value for the Expanded Uncertainty (50% criteria) = 0.025/2 = 0.0125 mg/kg

Rounded value of the Expanded Uncertainty = 0.013 mg/kg

REPORTED RESULT = 0.025 mg/kg ± 0.013 mg/kg (k = 2; 95%)

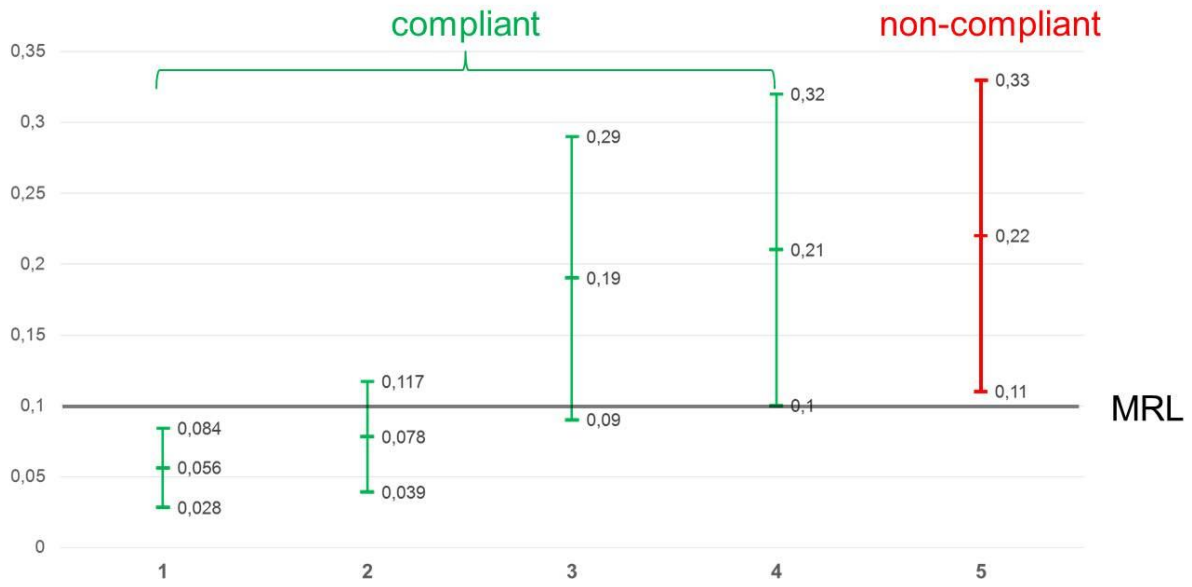
Examples for Rounding and Interpreting results:

In the following table , examples are given for rounding and interpreting results. In the columns Primary result and Primary value for the Expanded Uncertainty the digit to be rounded is marked bold. Interpretation of the results is according E14 where is given that a sample is considered non-compliant if x-U > MRL.

Table 1 *Examples for rounding and interpreting results.*

No.	Primary result	Rounded result	Primary value for the Expanded Uncertainty	Rounded value of the Expanded Uncertainty	**Reported result**	Result plus Expanded Uncertainty	Result minus Expanded Uncertainty	MRL	Interpretation
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg]	mg/kg	mg/kg	
1	0.055**5**97	0.056	± 0.02**8**	± 0.028	0.056 ± 0.028	0.084	0.028	0.1	Result plus expanded uncertainty <MRL; Compliant
2	0.07**8**43	0.078	± 0.03**9**	± 0.039	0.078 ± 0.039	0.117	0.039	0.1	Result < MRL; Compliant
3	0.1**9**43	0.19	± 0.0**9**5	± 0.10	0.19 ± 0.10	0.29	0.09	0.1	Result> MRL; Compliant due to the uncertainty interval
4	0.2**1**34	0.21	± 0.1**0**5	± 0.11	0.21 ± 0.11	0.32	0.10	0.1	Result > MRL; Compliant due to the uncertainty interval
5	0.2**1**68	0.22	± 0.1**1**0	± 0.11	0.22 ± 0.11	0.33	0.11	0.1	Result minus expanded uncertainty >MRL ; Non-compliant

Reported results with respect to their uncertainties:



Appendix E. An overview of the options to account for method bias and use of recovery correction factors

Table 1. *Analytical procedures to reduce method bias*

		Reduces bias due to				
Option	**Procedure**	losses during the extraction	cleanup losses	injection errors	matrix effects	cross reference
1. Matrix-matched calibration	calibration standards prepared in extract of blank sample of the same matrix	no	no	no	yes	C21-C23
2. Procedural calibration	calibration standards prepared in sub-portions of blank sample of the same matrix, analyte added before extraction	yes [1]	yes	no	yes	C28
3. Use of internal standard (IS) (other than the isotopic analogue of the analyte)	a. Internal standard added to the calibration standards, and to each sample before extraction (procedural internal standard)	possibly [1,2]	possibly [2]	possibly [2]	possibly [2]	C32-C34
	b. Internal standard added to the raw extract before cleanup (procedural internal standard)	no	possibly [2]	possibly [2]	possibly [2]	C32-C34
	c. Internal standard added to the calibration standards, and to the final extract of each sample (injection internal standard)	no	no	possibly [3]	possibly [2]	C32-C34
4. Use of isotopically labeled internal standard (ILIS) [4]	a. isotope analogue added to the calibration standards, and to each sample before extraction	yes [1]	yes	yes	yes	C35-C37
	b. Isotope analogue added to the raw extract before cleanup	no	yes	yes	yes	C35-C37
	c. isotope analogue added to the calibration standards, and to the final extract of each sample	no	no	yes	yes	C35-C37
5. Standard addition method	a. *Sample standard addition*: analyte standard added to test-portions of each sample before extraction	yes [1]	yes	no	yes	C24
	b. *Extract standard addition*: analyte standards added to aliquots of the final extract of each sample	no	no	no	yes	C25

[1] applies to spiked samples. May not compensate for incomplete extraction of incurred residues.
[2] an internal standard other than the isotopic analogue only reliably reduces bias when its properties and analytical behaviour are highly similar to the analyte of interest (C33).
[3] only when the internal standard is stable and not prone to matrix effects (C33), or when the matrix effect for the analyte in sample extract and calibrant are the same.
[4] the ILIS here is considered to be the analogue of the analyte.

Note: only the analytical procedures 2, 3a (possibly), 4a, and 5a can fully account for method bias. In all other cases one or more sources of bias have not been addressed, and correction for remaining method bias may be needed (see Table 2).

Table 2. *Options to correct method bias (mathematically, recovery* correction)

		Corrects for bias due to				
Option	**Procedure**	losses during the extraction	cleanup losses	injection errors	matrix effects	cross reference
Recovery correction	mathematical correction for recovery = result obtained * 100%/recovery% [1]	yes [2]	yes	no	na [3]	E4

Recovery used for correction	**What/how**	**Pros**	**Cons**
1. Average recovery from on-going validation	Take the average recovery of spiked samples concurrently analysed with the samples over a longer period of time. Different concentrations and matrices from one commodity group can be combined when analyte behaviour is similar.	Correction based on multiple recoveries. Reflects variation in time. Representative for matrices within commodity group. Reflects multiple concentrations.	Especially for labs with limited sample numbers and/or high variability in sample matrices: Data may not be available, or not for all commodity groups.
2. Average recovery from initial validation	Take the average recovery across different concentrations. In case validation is done for more than one matrix from the commodity group and analyte behaviour is similar, the average of all data can be taken.	Correction based on multiple recoveries. Reflects multiple concentrations. May reflect several matrices from a commodity group.	Single time point, does not reflect variation in time. Only one (or few) matrices of the commodity group covered. Might not be fully up-to-date and representative for all matrices.
3. Recovery included in the batch	Take the recovery obtained from the spiked sample concurrently analysed with the samples. Optionally multiple recoveries can be included (concentrations and/or matrices for commodity group), then the average can be taken.	If the spiked matrix is the same as the sample(s): could better reflect recovery for that matrix (at that moment) in case the matrix/method behaves differently from the situtation in initial or on-going validation.	Correction is based on a single recovery value which may be less reliable than an average from (on-going) validation. When the batch contains multiple matrices: only valid when matrix is representative for all matrices analysed.

[1] recovery as defined in glossary. Recovery used for correction: either the average from initial validation, the average from on-going validation, or the batch recovery. The most appropriate option depends on available data in the lab, see E4.
[2] applies to spiked samples. May not compensate for incomplete extraction of incurred residues.
[3] na = not applicable (in the definition of recovery used in the glossary, the matrix effects (if significant) are taken into account in determination of the recovery value).

Note: in lack of a reliable recovery factor for correction, approaches 2, 3a (possibly), 4a, and 5a from Table 1 could be used to account for method bias.

Appendix F. Glossary

Accuracy	Closeness of agreement between an analytical result and the true or accepted reference value. When applied to a set of results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias) (ISO 5725-1).
Adduct ion	Ion formed by the interaction of a precursor ion with one or more atoms or molecules to form an ion containing all the constituent atoms of the precursor ion as well as the additional atoms from the associated atoms or molecules.
Analyte	The chemical species for which the concentration (or mass) is to be determined. For the purposes of these procedures: a pesticide or a metabolite, breakdown product or derivative of a pesticide or an internal standard.
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)	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.). A determination batch may incorporate more than one extraction batch. This document does not refer to "batch" in the IUPAC or Codex sense, which relates to manufacturing or agricultural production batches.
Bias	The difference between the (mean) measured value and the true value.
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. (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) from the target analyte in the sample extract and known quantities of the analyte prepared as standard solutions. 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(/MS)
Comminution	The process of reducing a solid sample to smaller fragments by blending, crushing, pulverising, grinding, etc.
Confirmation	Confirmation is the combination of two or more analyses that are in agreement with each other (ideally, using methods of orthogonal selectivity), at least one of which meets identification criteria. It is impossible to confirm the complete absence of residues. Adoption of an "RL" at the LCL avoids the unjustifiably high cost of confirming the presence or absence of residues at unnecessarily low levels. 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. Assays based on an ECD tend to demand confirmation. because of their lack of specificity. Mass spectrometric techniques are often the most practical and the least equivocal approach to confirmation. AQC procedures for confirmation should be rigorous.
Contamination	Unintended introduction of a target analyte into a sample. extract, internal standard solution etc., by any route and at any stage during sampling or analysis.
Determination/detection system	Any system used to detect and determine the concentration or mass of the analyte. For example, GC-MS(/MS) , GC-FPD, LC-MS/MS, LC-ToF, etc.
Deviation of back-calculated concentration	Deviation of calculated concentration of the calibration standards by the calibration function from the true concentrations Deviation of back-calculated concentration (%)= $(C_{measured} - C_{true})x100/C_{true}$

ECD	Electron-capture detector.
EI	Electron ionisation.
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 & PFPD	Flame-photometric detector and pulsed flame photometric detector (may be specific to sulphur or phosphorus detection).
Fragment ion	Product ion that results from the dissociation of a precursor ion.
GC	Gas chromatography.
Identification	Is a qualitative result from a method capable of providing structural information (e.g. using mass spectrometric (MS) detection) that meets acceptable criteria for the purpose of the analysis. The process of generating of sufficient evidence to ensure that a result for a specific sample is valid. Analytes must be identified correctly in order to be quantified. AQC procedures for identification should be rigorous.
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.
Internal quality control (IQC)	See AQC.
Internal standards	Definitions are given in the main body of text (C31-C37)
Laboratory sample	The sample sent to and received by the laboratory.
LC	Liquid chromatography (primarily high performance liquid chromatography, HPLC, and Ultra high performance liquid chromatography, UPLC).
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/MS	Liquid chromatographic separation coupled with tandem mass spectrometric detection.
Level	In this document, refers to concentration (e.g. mg/kg, µg/ml) or quantity (e.g. ng, pg).

LOD (as referred to in Reg. 396/2005)	Limit of determination (LOD) means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods; in this respect it can be regarded as the LOQ (see below).
LOQ	Limit of quantitation (quantification). The lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method and identification criteria. LOQ is preferable to LOD because it avoids possible confusion with "limit of detection". However, in Reg. 396/2005, MRLs that are set at the limit of quantification/determination are referred to as "LOD MRLs". not "LOQ MRLs".
Mass accuracy:	Mass accuracy is the deviation of the measured *accurate* mass from the calculated *exact* mass of an ion. It can be expressed as an absolute value in milliDaltons (mDa) or as a relative value in parts-per-million (ppm) error and is calculated as follows: (accurate mass – exact mass) Example: the experimentally measured mass = 239.15098. the theoretical exact mass of the ion *m/z* = 239.15028. The mass accuracy = (239.15098 – 239.15028) = 0.7 mDa or (accurate mass – exact mass) / exact mass * 10^6 Example: the experimentally measured mass = 239.15098. the theoretical exact mass of the ion *m/z* = 239.15028 The mass accuracy=(239.15098–239.15028)/239.15028 * 10^6=2.9 ppm
Mass extraction window (MEW)	**Width** of the mass range around the exact mass used to obtain the extraction ion chromatograms. e.g. exact mass ± 1 mDa or exact mass ± 5 ppm.
Mass resolution	**Mass resolution** (peak width definition. FWHM): $(m/z)/\Delta(m/z)$, where $\Delta(m/z)$ is the **F**ull **W**idth of the mass profile peak at **H**alf its **M**aximum (FWHM) height. The resolution of a mass spectrometry instrument is the ability to distinguish between two ions with similar *m/z* values (IUPAC definition[22]: the smallest mass difference between two equal magnitude peaks so that the valley between them is a specified fraction of the peak height).

[22] Murray et al. (2013) Pure Appl. Chem., 85:1515–1609

Mass resolving power	**Mass resolving power:** measure of the ability of a mass spectrometer to provide a specified value of mass resolution (so: an instrument specification) The resolving power, defined at full-width half maximum (FWHM), is m/Δm, where m is the *m/z* being measured and Δm the width of the mass peak at half peak height. Note 1: for magnetic sector instruments another definition is used ("10% valley"). Roughly the difference between the two definitions is a factor of 2 (i.e. 10.000 resolving power by the 10% valley method equals 20.000 resolving power by FWHM). Note 2: mass resolving power is often confused or interchangeably used with mass resolution (see definition above).
Matrix blank	See blank.
Matrix effect	An influence of one or more co extracted compounds from the sample on the measurement of the analyte concentration or mass. It may be observed as increased or decreased detector response compared with that produced by solvent solutions of the analyte. The presence or absence of such effects may be demonstrated by the difference of response from standard in matrix extract and standard in solvent $ME\ (in\ \%) = \left(\frac{\text{Rstd in matrix extract}}{\text{R std in solvent}} - 1\right)$ x100 R= Detector response
Matrix-matched /matrix-based calibration	Calibration using standards prepared from extracts of the same (matrix-matched) or any other (matrix-based) blank matrix.
May	MAY within this document means perhaps or possibly an option (the action is optional).
Method	A sequence of procedures or steps Wherever possible from receipt of a sample through to the calculation and reporting of results.
Method validation	The process of characterising the performance to be expected of a method in terms of its scope, specificity, accuracy, sensitivity, repeatability and within laboratory reproducibility. Some information on all characteristics, except within laboratory 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 should involve analysis of certified reference materials, participation in proficiency tests, or other inter-laboratory comparisons.
MRL	Maximum residue level. In Regulation 396/2005, MRLs are listed for pesticide/commodity combinations. An asterisk indicates that the MRL* is set at or about the LOQ with the LOQ being here a consensus figure rather than a measured value.

MRM	In pesticide residue analysis: multi-residue method.
MRM	In mass spectrometry: Application of selected reaction monitoring (SRM) to multiple product ions from one or more precursor ions.
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.
Must	MUST within this document means an absolute requirement (the action is mandatory). MUST NOT means an absolute no.
Non-compliant(or violative) residue	A residue that exceeds the MRL when expanded measurement uncertainty is subtracted
NPD	Nitrogen-phosphorus detector.
Performance verification	See analytical quality control (AQC).
Precision	The closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results the more precise the procedure. A measure of precision (or imprecision) is the standard deviation[21].
Precursor ion	Ion that reacts to form particular product ions or undergoes specified neutral losses. The reaction can be of different types including unimolecular dissociation, ion/molecule reaction, change in charge state, possibly preceded by isomerization.
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 calibration	Procedural standards are prepared by spiking a series of blank test portions with different amounts of analyte, prior to extraction. The procedural standards are then analysed in exactly the same way as the samples.
Product ion	Ion formed as the product of a reaction involving a particular precursor ion.
Reagent blank	See blank.
Recovery (of analyte through an analytical method)	Recovery (of analyte through an analytical method): Also referred to as 'extraction recovery', 'absolute recovery', or 'recovery factor'.[23] The proportion of analyte remaining at the point of the final determination following its addition (usually to a blank sample) prior to extraction. Usually expressed as a percentage. 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 accuracy 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.
"Reference" standard	A solid, liquid or gaseous compound that has been prepared in a largely purified form and packed appropriately to ensure stability and allow transportation and storage. The storage conditions, expiry date, and purity must be indicated as well as the hydratation water content and the isomer composition where this is relevant. Where standards are bought in solution they should be treated as secondary standards (i.e. as stock or working solutions).

[23] Burns DT, Danzer K, Tow A., IUPAC Recommendations 2002, Use of the terms "recovery" and "apparent recovery" in analytical procedures. Appl. Chem., 2002, 74(11), 2201-2205.

Repeatability (r)	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. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result. 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 (RL)	The lowest level at which residues will be reported as absolute numbers. It is equal to or higher than the LOQ. For EU monitoring purposes where samples for surveys are analysed over a 12-month period, the same RL should be achievable throughout the whole year.
Reproducibility (R)	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. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result. Within-lab-reproducibility ($RSDw_R$) is that produced in a single laboratory under these conditions. 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.

SDL (qualitative screening)	The screening detection limit of a qualitative screening method is the lowest concentration for which it has been demonstrated that a certain analyte can be detected (not necessarily meeting unequivocal identification criteria) in at least 95% of the samples (i.e. a false-negative rate of 5% is accepted).
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.
Should	SHOULD within this document means a recommendation that may be ignored but only in particular circumstances (because of valid reasons) and the full implications of ignoring the recommendation must be understood and carefully assessed before choosing a different course of action. SHOULD NOT means not recommended. although it may be acceptable in particular circumstances, but the full implications of ignoring the recommendation must be understood and carefully assessed.
Significant figures	Those digits in a number that are known with certainty, plus the first uncertain digit. Ex. 3 significant figures. 0.104. 1.04. 104. 1.04 x10^4 The 1 and the middle 0 are certain, and the 4 is uncertain, but significant. Note: Initial zeroes are never significant. Exponential number has no effect on the number of significant figures.
SIM	Selected ion monitoring. Operation of a mass spectrometer in which the abundance of several ions of specific *m/z* values are recorded rather than the entire mass spectrum.
S/N	Signal-to-noise ratio.
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.
Specificity	The ability of the detector (supported by the selectivity of the extraction, clean-up, derivatisation or separation, if necessary) to provide signals that 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.
SRM	Selected reaction monitoring. Measurement of specific product ions corresponding to *m/z* selected precursor ions recorded via two or more stages of mass spectrometry (MS^n).

Standard	A general term which may refer to a "pure" standard, stock standard, working standard or calibration standard.
Standard addition	a) "Sample standard addition" refers to standard addition to analytical test portions, prior to extraction b) "Extract standard addition" refers to standard addition to aliquots of the sample extract, prior to injection
Stock standard solution	The most concentrated solution (or solid dilution, etc.) of the "pure" standard or internal standard, from which aliquots are used to prepare working standard solutions or calibration standard solutions.
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. See also Directive 2002/63/EC.
Trueness	The measure of trueness is normally expressed as 'bias'. The closeness of agreement between the average value obtained from a series of test results (i.e. the mean recovery) an accepted reference or true value (ISO 5725-1).
Uncertainty (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.
Unit mass resolution	Mass resolution such that it is possible to clearly distinguish a peak corresponding to a singly charged ion from its neighbours 1 Dalton away, usually with no more than 5–10 % overlap.
Validation	See method validation.
Violative residue	A residue which exceeds the MRL or is unlawful for any other reason.
Within-laboratory reproducibility	See reproducibility.
Working standard solution	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 standard solutions.