CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 8 **CX/PR 17/49/11**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON PESTICIDE RESIDUES

49th Session

Beijing, China, 24-29 April 2017

Draft Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food

Comments at Step 6 (Replies to CL 2016/27-PR)

Comments of Albania, Australia, Canada, China, Colombia, Costa Rica, Cuba, Egypt, European Union, Haiti, IAEA, India, Mexico, New Zealand, Thailand, Uruguay, United States of America

Background

1. This document compiles comments received through the Codex Online Commenting System (OCS) in response to CL 2016/27-PR issued in July 2016 **(Annex I)**. Under the OCS, comments are compiled in the following order: general comments are listed first, followed by comments on specific paragraphs.

Guidance for interpreting Compiled comments table

2. The comments submitted through the OCS have been compiled in the Compiled comments table, hereby attached as **Annex I**.

3. Under the OCS, each paragraph of the **draft standard** is assigned a number (i.e. the title, section, subsections, texts, footnotes and in case of tables each grid).

4. For ease of reference, the draft standard has been reproduced with automatic paragraph numbers as assigned by the OCS and is hereby attached as **Annex II**.

5. Columns under **Annex I** are headed as follows:

- **"Para"** refers to the paragraph number assigned to the draft standard by the OCS (the paragraph number can be found in Annex II).
- **"Text"** refers to the text of the paragraph on which a proposed change or comment has been made. This text can be either the original text (if only a comment has been made), or the proposed text (if a textual modification has also been suggested).
- **"T"** refers to the comments classification. **C** is when users provide only a comment, while **P** is when they also suggest a proposed change. In the first case, the original text with an explanation has been inserted in the system; in the second case, the revised text with or without an explanation has been inserted.
- **"Comment"** includes the comment category, the author and the full text of the comment.

6. It is recommended that the Compiled comments table (Annex I) is read side by side or in conjunction with Annex II.

Annex I

Compiled comments table for Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food

Para	Text	T	Comment
G	(General Comment)	C	**Comment by Mexico** *Category: EDITORIAL* **Mexico** ITEM 3.1.1 Acetochlor (280) The available trials utilised three applications compared to critical GAP which is two postemergent applications, each at 1.7 kg ai/ha with the last prior to full flowering (R2 growth stage). The 2015 JMPR considered trials with three applications could be considered for use of the proportionality approach if the initial pre-emergent application did not contribute to the final residue. However, pre-plant and pre-emergence applications give rise to residues in soya beans at harvest as noted above. In a rotational crop study residues in soya bean follow crops were planted 253-425 days after application to a primary maize crop at 2.2 kg ai/ha, residues in grain ranged from < 0.02 to 0.1 mg/kg suggesting the pre-plant application might contribute < 0.02 to 0.05 mg/kg to the terminal residue. OBSERVATION The coefficient of variation of 0.02 to 0.10 mg / kg of pesticide residue in grain is close to 100%. Which shows a significant difference. SUGGESTION For greater study control, it is suggested to use smaller CV. ITEM 3.1.3 Flonicamid (282) Fruit vegetables, cucurbitaceae. The label from the USA allows foliar or soil/growth media applications to greenhouse cucumbers. Based on the supervised residue trials on greenhouse cucumbers reviewed by the 2015 Meeting, the foliar application was determined to be the method wich resulted in the highest residues (0.54 mg/kg). Due to there being only four trials matching the critical GAP of the USA, the Meeting considered these trials insufficient to recommend a maximum residue level for greenhouse cucumbers. The Meeting confirms its previous recommendation of a máximum residue level of 0.2 mg/kg and an STMR of 0.04 mg/kg for Fruiting Vegetables, Curcubits. OBSERVATION The MRL is considered high (0.2 mg / kg).

			SUGGESTION Cucurbitaceae are widely consumed internationally and in many cases not only one of these foods is part of a person's diet on the same day, which would affect the Acceptable Daily Dose (ADI). ITEM 3.2.2 Picoxystrobin (258) Picoxystrobin was evaluated as a new compound by the 2012 JMPR for toxicology and residues. The 2012 JMPR established an ADI of 0-0.09 mg/kg bw for picoxystrobin and an ARfD of 0.09 mg/kg bw. The 2012 JMPR proposed a residue definition for enforcement of picoxystrobin and estimated a number of maximum residue levels. However, the 2012 JMPR was unable to conclude on the toxicological relevance of two metabolites IN-H8612 and 2-(2-formylphenyl)-2-oxoacetic acid tentatively identified in plant metabolism studies, for which IEDIs were above the threshold of toxicological concern of 0.15 µg/person/day for compounds with alerts for genotoxicity. As a result, it was not possible to propose a residue definition for dietary risk assessment or calculate dietary intakes, and maximum residue levels were not recommended. The 2013 JMPR received additional toxicological data (a mouse micronucleus study) for INH8612 which showed no evidence of genotoxicity. Conservative estimates for chronic and acute exposure to IN-H8612 were both below the relevant TTC values for Cramer class III compounds with no evidence of genotoxicity. The 2013 JMPR concluded that there was no concern for dietary exposure to IN-H8612. However, no toxicological data were submitted for 2-(2formylphenyl)-2-oxoacetic acid, as the compound was unable to be synthesised in sufficient amounts. Although argument was provided that levels in soya beans were likely to be extremely low, the 2013 JMPR concluded that genotoxicity data or additional residues information would be required to allow further evaluation of 2-(2 formylphenyl)-2-oxoacetic acid. OBSERVATION Insufficient studies to ensure its use in agriculture. SUGGESTION In the absence of sufficient evidence on the genotoxicity of picoxystrobin and its metabolites, it is suggested that its use should be considered to be banned until the pertinent tests establishing the safety of mankind are carried out.

		C	**Comment by USA** *Category: EDITORIAL*
			USA to the definitions section, add: Residue Definition: The analytes that are identified and/or quantified to determine the pesticide residue. The residue definition may include the parent, isomers, metabolites, degradants and/or reaction products. 12. After validation, the method documentation should provide, in addition to performance specifications (data quality objectives), the following information: a. identity of the analytes, including all components of the residue definition In definitions section: Identification: Process of unambiguously determining the chemical identity of all components of the residue definition. In definition section: Recovery: Amount measured as a percentage of the amount of analyte(s) (as per the residue definition) originally added to a sample of the appropriate matrix, which contains either no detectable level of the analytes or a known detectable level. Recovery experiments provide information on both precision and trueness and thereby the accuracy of the method.
		C	**Comment by Albania** *Category: EDITORIAL*
			Albania ok.
		C	**Comment by Egypt** *Category: EDITORIAL*
			Egypt We would like to inform you that,Egypt approves the draft
		C	**Comment by Costa Rica** *Category: EDITORIAL*
			Costa Rica Costa Rica thanks the eWG for the work done; in that sense, we would like to comment that we have reviewed the document and since all the comments that were sent at the time were taken into account, this time we have no comments.
		C	**Comment by Cuba** *Category: EDITORIAL* Cuba agrees with the document, with no further criteria to be added.

		C	**Comment by New Zealand** *Category: EDITORIAL*
			New Zealand On reviewing the document, New Zealand considers the variation in undefined terminology is difficult to follow. It is important for these types of documents that they are unambiguous, especially given it's translation to other languages, and the technical nature of the content. An example is the use of performance criteria, performance parameters, performance acceptability criteria, performance characteristics, performance requirements, performance of a method, on-going performance, established performance limits, method performance validation/verification etc. In a number of cases, these terms are meaning the same, but with slight different wording. Therefore, New Zealand suggests the document is revised to remove such ambiguity.
G	(General Comment)	C	**Comment by Colombia** *Category: TECHNICAL*
			Colombia Leave the limit of Detection and limit of quantification under letter H of the table.
		C	**Comment by Uruguay** *Category: TECHNICAL*
			Uruguay Uruguay requests that consideration be given to incorporating into the document a table of representative matrices within the food groups based on analytical considerations to enable laboratories to select the validation matrices according to their chemical composition. This would guide the arduous task of laboratory validation. As a background, document CAC/GL 40-1993, Guidelines on Good Laboratory Practices in Pesticide Residue Analysis, on page 30 refers to a table with similar characteristics called "Representative commodities/samples for validation of analytical procedures for pesticide residues". This document of the year 1993 already took into account this tool so it is considered a step backwards not to include it in the new Guideline.
18	A. Applicability	P	**Proposed Change by European Union** *Category: SUBSTANTIVE* A. ~~Applicability~~ **Method Documentation**
			European Union
		C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union The title "Applicability" does not reflect the content of the paragraph.

55	1. The purpose of this guidance document is to define and describe the performance criteria, which should be met by methods to analyse pesticide residues in foods. It addresses the characteristics/parameters to provide scientifically acceptable confidence in the analytical method that is fit for the intended use and may be used to reliably evaluate pesticide residues for either domestic monitoring and/or international trade.	**C**	**Comment by Thailand** *Category: EDITORIAL* **Thailand** We would like to propose additional clause as a footnote in para 1 to be as follow; "1. The purpose of this guidance document is to define and describe the performance criteria, which should be met by methods to analyse pesticide residues in foods*. …." * Term of "foods" in this Guideline includes foods and feeds. Rationale: There are both CXLs of pesticides residue in foods and feeds. We are of the view that the scope of this draft Guidance should cover the performance criteria of methods of analysis for determination of pesticide residues in feeds as well.
56	2. This document is applicable to both single residue methods and multi-residue methods (MRMs) that analyse target compounds in all food commodities, including parent pesticide *[mother compound]* residues and/or their metabolites and degradants in food commodities per the residue definition.	**P**	**Proposed Change by Colombia** *Category: TRANSLATION* 2. This document is applicable to both single residue methods and multi-residue methods (MRMs) that analyse target compounds in all food commodities, including parent pesticide ~~(mother compound)~~**(parental pesticide)** residues and/or their metabolites and degradants in food commodities per the residue definition. **Colombia**
61	5. In regulatory applications, the maximum residue limit (MRL) is expressed in terms of the "residue definition," which may include the parent compound, a major metabolite, a sum of parent and/or metabolites, or a reaction product formed from the residues during analysis. Residue analytical methods should be able to measure all components of the residue definition.	**P**	**Proposed Change by India** *Category: EDITORIAL* 5. In regulatory applications, the maximum residue limit (MRL) is expressed in terms of the "residue definition," which may include the parent compound, ~~a~~ major ~~metabolite~~**metabolites**, a sum of parent and/or metabolites, or a reaction product formed from the residues during analysis. Residue analytical methods should be able to measure all components of the residue definition. **India**
62	6. *Fitness-for-purpose* is the extent to which the performance of a method meets the end-user's needs, and matches the criteria (data quality objectives) agreed between the laboratory and the end-user (or client) of the data, within technical and resource constraints. *Fitness-for-purpose* criteria could be based on some of the characteristics described in this document, but ultimately will be expressed in terms of acceptable combined uncertainty[1].	**C**	**Comment by Australia** *Category: EDITORIAL* **Australia** The IUPAC Guidelines (Footnote 1) were adopted as CAC/GL 49-2003, so the footnote might be better shown as CAC/GL 49-2003 'Harmonised IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis'.

70	b. participate in appropriate proficiency testing schemes for food analysis which conform to the requirement laid out in “The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories;” and	C	**Comment by China** *Category: EDITORIAL* **China** please indicate the original literature for “The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories
72	9. The analytical methods should be used within the internationally accepted, approved, and recognized laboratory Quality Management System[4] to be consistent with the principles in the document for quality assurance (QA) and quality control (QC) referenced above. The on-going performance is monitored through the Quality Management System in place in the laboratory.	C	**Comment by China** *Category: EDITORIAL* **China** Paragraph 9 and Paragraph 10: The last sentence of these paragraphs refer to the same meaning, and we recommend to combine them
73	General requirements for the competence of testing and calibration laboratories, ISO/IEC 17025	C	**Comment by Colombia** *Category: EDITORIAL* **Colombia** Include current version.

75	10. The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, accurate and consistent results can be obtained within specified statistical limits for sample analysis. The validation should demonstrate the identity and concentration of the analyte, taking into account for matrix effects, provide a statistical characterization of recovery results, and indicate if the rates of false positives and negatives are acceptable. When the method is followed using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue testing laboratory. To ensure that validation of the method remains appropriate over time, the method should be continuously assessed using on-going proficiency testing and appropriate quality control samples (e.g. including recovery spikes).	**C**	**Comment by IAEA** *Category: EDITORIAL* **IAEA** Para 10 second last line: “Measures” could be used instead of “samples” (under “Appropriate quality control samples...”
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~10.~~ 10. The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, accurate and consistent results can be obtained within specified statistical limits for sample analysis. The validation should demonstrate the identity and concentration of the analyte, taking into account for matrix effects, provide a statistical characterization of recovery results, and indicate if the rates of false positives and negatives are acceptable. When the method is followed using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue testing laboratory. To ensure that validation of the method remains appropriate over time, the method should be continuously assessed using on-going ~~proficiency testing and appropriate quality control samples~~ **method validation** (e.g. ~~including~~ recovery spikes).
			European Union
		C	**Comment by European Union** *Category: SUBSTANTIVE* **European Union** A method cannot be continously assessed by proficiency testing since those only occur periodically.

75	10. The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, accurate and consistent results can be obtained within specified statistical limits for sample analysis. The validation should demonstrate the identity and concentration of the analyte, taking into account for matrix effects, provide a statistical characterization of recovery results, and indicate if the rates of false positives and negatives are acceptable. When the method is followed using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue testing laboratory. To ensure that validation of the method remains appropriate over time, the method should be continuously assessed using on-going proficiency testing and appropriate quality control samples (e.g. including recovery spikes).	**P**	**Proposed Change by Colombia** *Category: TECHNICAL* 10. The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, ~~accurate~~ **accurate, reliable** and consistent results can be obtained within specified statistical limits for sample analysis. The validation should demonstrate the identity and concentration of the analyte, taking into account for matrix effects, provide a statistical characterization of recovery results, and indicate if the rates of false positives and negatives are acceptable. When the method is followed using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue testing laboratory. To ensure that validation of the method remains appropriate over time, the method should be continuously assessed using on-going proficiency testing and appropriate quality control samples (e.g. including recovery spikes). **Colombia**
81	**a.** identity of the analytes, including isomers, metabolites and other components where appropriate (e.g. endosulfan I&II, spinosyn A&D);	**P**	**Proposed Change by India** *Category: TECHNICAL* **a.** identity of the analytes, including isomers, ~~metabolites~~ **metabolites, degradation products** and other components where appropriate (e.g. endosulfan I&II, spinosyn A&D); **India**
81	**a.** identity of the analytes, including isomers, metabolites and other components where appropriate (e.g. endosulfan I&II, spinosyn A&D);	**P**	**Proposed Change by Colombia** *Category: TRANSLATION* **a.** identity of the analytes, including isomers, metabolites and other components where appropriate (e.g. ~~endosulfun~~ **endosulfan** I&II, ~~spinosyn~~ **Spinosad.** A&D); **Colombia**

82	b. concentration range covered by the validation (e.g. “0.01-10 mg/kg”);	C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union This is an unrealistic range and thus confusing; therefore it is better to delete it.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~b.~~ b. concentration range covered by the validation ~~(e.g. “0.01-10 mg/kg”)~~;
			European Union **(16 Jan 2017 4:21 PM)**
83	c. range of sample matrices covered by the validation (e.g. “cucurbits, root vegetables, citrus”);	P	**Proposed Change by Colombia** *Category: TECHNICAL* c. range of sample matrices covered by the validation (e.g. ~~”cucurbits~~**”agricultural products based on their moisture content**, ~~root vegetables~~ **fat and sugar percentage**, ~~citrus”),~~ **pH)”); Justification of change: The latest developments in the world on methods for the determination of pesticide residues are based on the moisture content, fat percentage, sugar percentage and pH of the sample. Therefore, these conditions should be included in this item.**
			Colombia
85	e. if required, a quantitative result should be reported together with the expanded measurement uncertainty (MU).	C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union The measurement of the uncertainty should always be calculated during the validation of the method, although not necessarily reported.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* e. ~~if required,~~ a quantitative result ~~should be reported together with~~ **of** the expanded measurement uncertainty ~~(MU)~~**(MU) of the method has to be calculated in the validation procedure and reported, if required**.
			European Union
87	13. Ideally, selectivity should	P	**Proposed Change by New Zealand**

	be evaluated to demonstrate that no interferences occur which detrimentally affect the analysis. It is impractical to test the method against every potential interferant, but it is recommended that common interferences are checked by analysing a blank for every batch of samples and reagents. Background levels of plasticizers, septa bleed, cleaning agents, reagent impurities, laboratory contamination, carry-over, etc. tend to show up in reagent blanks and must be recognized by the analyst when they occur. Also, analyte-to-analyte interferences must be known by checking individual analytes in mixed standard solutions. Matrix interferences are evaluated by analyses of samples known to be free of the analytes.		*Category: TECHNICAL* 13. Ideally, selectivity should be evaluated to demonstrate that no interferences occur which detrimentally affect the analysis. It is impractical to test the method against every potential interferant, but it is ~~recommended~~ **required** that common interferences are checked by analysing a **reagent** blank for every batch of ~~samples and reagents.~~ **reagents** Background levels of plasticizers, septa bleed, cleaning agents, reagent impurities, laboratory contamination, carry-over, etc. tend to show up in reagent blanks and must be recognized by the analyst when they occur. Also, analyte-to-analyte interferences must be known by checking individual analytes in mixed standard solutions. Matrix interferences are evaluated by analyses of samples known to be free of the analytes.
			New Zealand There is a statement “it is recommended that common interferences are checked by analysing a blank for every batch of samples and reagents.” whereas later there is specific mention of a reagent blank.
88	14. As a general principle, selectivity should be such that interferences are inconsequential. The ultimate test of selectivity involves the rates of false positives and negatives in the analyses. To minimally estimate rates of false positives and negatives during method validation, an adequate number (suggested >5 each) of diverse matrix blanks (not from the same source) should be analysed along with spiked matrices at the analyte reporting level. Validations of screening methods (presence/absence analyses) are discussed in paragraphs 32-34.	**C**	**Comment by IAEA** *Category: SUBSTANTIVE*
			IAEA The terminology “inconsequential” requires explanation
		C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union It is not necessary to specify the number of blanks per matrix as the text indicates: “an adequate number”, which remains at the discretion of the analyst. Unnecessary in the context of the paragraph.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~14.~~ 14. As a general principle, selectivity should be such that interferences are inconsequential. The ultimate test of selectivity involves the rates of false positives and negatives in the analyses. To minimally estimate rates of false positives and negatives during method validation, an adequate number ~~(suggested >5 each)~~ of ~~diverse matrix~~ blanks **per matrix** (not from the same source) should be analysed along with spiked matrices at the analyte reporting level. ~~Validations of screening methods (presence/absence analyses) are discussed in paragraphs 32-34.~~
			European Union
90	15. With the exception of gross (also known as	**P**	**Proposed Change by IAEA** *Category: TECHNICAL*

	"spurious") errors in preparation of calibration materials, calibration errors are usually (but not always) a minor component of the total uncertainty, and can be safely assigned into other categories. For example, random errors resulting from calibration are part of the uncertainty, while systematic errors cause analytical bias, both of which are assessed as a whole during validation and on-going quality control. Nevertheless, there are some characteristics of calibration that are useful to know at the outset of method validation because they affect optimization of the final protocol. For example, it must be known in advance whether the calibration is linear or quadratic, passes through the origin, and is affected by the sample matrix or not. The described guidelines in this document relate more to validation, which may be more detailed than the calibration undertaken during routine analysis.		15. ~~With the exception of gross (also known as "spurious") errors in preparation of calibration materials, calibration errors are usually (but not always) a minor component of the total uncertainty, and can be safely assigned into other categories. For example, random errors resulting from calibration are part of the uncertainty, while systematic errors cause analytical bias, both of which are assessed as a whole during validation and on-going quality control. Nevertheless, there are some characteristics of calibration that are useful to know at the outset of method validation because they affect optimization of the final protocol. For example, it must be known in advance whether the calibration is linear or quadratic, passes through the origin, and is affected by the sample matrix or not. The described guidelines in this document relate more to validation, which may be more detailed than the calibration undertaken during routine analysis.~~ **IAEA** Section C (Calibration). This section, especially 15, requires rephrasing and clarification. For instance to distinguish instrument calibration from a calibration curve (which seems to be the subject of discussion)
93	**b.** the calibration standards should be evenly spaced over the concentration range of interest and the calibration range should encompass the entire concentration range likely to be encountered;	**P**	**Proposed Change by Colombia** *Category: TRANSLATION* **b.** the ~~calibration~~ **reference standards and reference material (RATIONALE: These terms are in the VIM (International Vocabulary of Metrology).** should be evenly spaced over the concentration range of interest and the calibration range should encompass the entire concentration range likely to be encountered; **Colombia**
94	**c.** the calibration standards should be dispersed over the	**P**	**Proposed Change by Colombia** *Category: TECHNICAL*

	whole sequence, or encompass the beginning and end of the run to demonstrate that calibration integrity is maintained over the entire sequence; and the fit of the calibration function must be plotted and inspected visually and/or by calculation of the residuals (differences between the actual and calculated concentrations of the standards), avoiding over-reliance on correlation coefficients. If individual residuals deviate by more than ±20%, statistical consideration of outliers should be made, possibly leading to re-analysis of the sequence if quality control criteria are not met.		c. the calibration standards should be dispersed over the whole sequence, or encompass the beginning and end of the run to demonstrate that calibration integrity is maintained over the entire sequence; and the fit of the calibration function must be plotted and inspected visually and/or by calculation of the residuals (differences between the actual and calculated concentrations of the standards), avoiding over-reliance on correlation coefficients. If ~~individual residuals~~ **residuals of the calibration curve (RATIONALE: Accuracy in terms, since it gives a parameter of acceptance or rejection of the calibration curve)** deviate by more than ±20%, statistical consideration of outliers should be made, possibly leading to re-analysis of the sequence if quality control criteria are not met.
			Colombia
		P	**Proposed Change by Colombia** *Category: TRANSLATION* c. the ~~calibration~~ **reference standards and reference material (RATIONALE: These terms are in the VIM (International Vocabulary of Metrology)** should be dispersed over the whole sequence, or encompass the beginning and end of the run to demonstrate that calibration integrity is maintained over the entire sequence; and the fit of the calibration function must be plotted and inspected visually and/or by calculation of the residuals (differences between the actual and calculated concentrations of the standards), avoiding over-reliance on correlation coefficients. If individual residuals deviate by more than ±20%, statistical consideration of outliers should be made, possibly leading to re-analysis of the sequence if quality control criteria are not met.
			Colombia
95	**D. Linearity and Intercept**	P	**Proposed Change by Colombia** *Category: TECHNICAL* **D. Linearity ~~and intercept~~**
			Colombia
96	17. Linearity can be tested by examination of a plot of residuals produced by linear regression of the responses	P	**Proposed Change by Colombia** *Category: TECHNICAL* 17. Linearity can be tested by examination of a plot of ~~residues~~ **residuals** produced by linear

	on the concentrations in an appropriate calibration set. Any curved pattern suggests a *lack of fit* due to a nonlinear calibration function. If this is the case, another function such as quadratic should be tested and applied, using at least five concentration levels. Despite its current widespread use as an indication of quality of fit, the coefficient of determination (R^2) may be misleading because it places greater significance on standards with higher concentrations. In this case, an appropriate weighting factor such as 1/x or $1/x^2$ should be considered.		regression of the responses on the concentrations in an appropriate calibration set. Any curved pattern suggests a *lack of fit* due to a nonlinear calibration function. If this is the case, another function such as quadratic should be tested and applied, using at least five concentration levels. Despite its current widespread use as an indication of quality of fit, the coefficient of determination (R^2) may be misleading because it places greater significance on standards with higher concentrations. In this case, an appropriate weighting factor such as 1/x or $1/x^2$ should be considered. **Colombia**
97	18. In general, the use of weighted-linear regression or weighted-quadratic function is recommended rather than linear regression for low part per billion (µg/kg) concentration determinations. The value of the intercept should be close to zero (e.g. <20% of the lowest calibration standard) to reduce errors in calculating residue concentrations at low levels.	**C**	**Comment by European Union** *Category: SUBSTANTIVE* **European Union** The text in brackets is not necessary and can create confusion.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* 18. In general, the use of weighted-linear regression or weighted-quadratic function is recommended rather than linear regression for low part per billion (µg/kg) concentration determinations. The value of the intercept should be close to zero ~~(e.g. <20% of the lowest calibration standard)~~ to reduce errors in calculating residue concentrations at low levels**, although the calibration curve should not be forced through the origin without justification**. **European Union**
97	18. In general, the use of weighted-linear regression or weighted-quadratic function is recommended rather than linear regression for low part per billion (µg/kg) concentration determinations. The value of the intercept should be close to zero (e.g. <20% of the lowest calibration standard) to reduce errors in calculating residue concentrations at low levels.	**C**	**Comment by Colombia** *Category: TECHNICAL* **Colombia** It is proposed to add at the end the following paragraph: Refer to statistical tools such as simple variance analysis, with linearity deviation calculation. RATIONALE: These tools are used to evaluate a linear model.)
99	19. Matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same type as	**P**	**Proposed Change by China** *Category: EDITORIAL* 19. Matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same **or similar** type as

	the sample, should be used for calibration. An alternative practical approach to compensate for matrix effects in gas chromatographic (GC) analyses is the use of chemical components (analyte protectants) that are added to both the sample extracts and the calibration solutions in order to (ideally) maximize equally the response of pesticides in calibrants in solvent and sample extracts. Alternative ways to compensate for matrix effects involve the use of standard addition, isotopically labeled internal standards (IS), or chemical analogues. However, these approaches are often difficult in MRMs because there are too many residues in different matrices at different levels to devise routine procedures, and the lack of isotopically-labelled standards for so many analytes. If solvent-only calibration is used, a measurement of matrix effects should be made to demonstrate equivalence of results by comparing responses of matrix-matched with solvent-only standards.		the sample, should be used for calibration. An alternative practical approach to compensate for matrix effects in gas chromatographic (GC) analyses is the use of chemical components (analyte protectants) that are added to both the sample extracts and the calibration solutions in order to (ideally) maximize equally the response of pesticides in calibrants in solvent and sample extracts. Alternative ways to compensate for matrix effects involve the use of standard addition, isotopically labeled internal standards (IS), or chemical analogues. However, these approaches are often difficult in MRMs because there are too many residues in different matrices at different levels to devise routine procedures, and the lack of isotopically-labelled standards for so many analytes. If solvent-only calibration is used, a measurement of matrix effects should be made to demonstrate equivalence of results by comparing responses of matrix-matched with solvent-only standards.
			China “Same type” is not easy to handle in practice testing, and “same or similar” is recommended for the wording.
		C	**Comment by IAEA** *Category: TECHNICAL*
			IAEA Section E (Matrix effects): Could guidance be provided on choice of internal standards (especially isotopically labelled) and how this influences matrix effects and recoveries? This is a challenge in multi residue methods
		C	**Comment by New Zealand** *Category: EDITORIAL*
			New Zealand There are various statements made throughout the document where the dangers of matrix effects are mentioned so New Zealand considers the use of solvent-only calibration solutions is fraught with the risk of false negatives e.g. Section 19 “Matrix-matched calibration is commonly used to compensate for matrix effects.”; Section 36 “The requirement to recover a range of different pesticide residues in one extraction increases the potential for compromised selectivity in MRMs compared to single residue methods. Using less selective extraction and clean-up procedures is likely to result in greater co-extracted matrix material in the final extract.”; Section 42 “when the mean recovery is within the range of 70-120%.”
		P	**Proposed Change by New Zealand** *Category: TECHNICAL* 19. 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 gas chromatographic (GC) analyses is the use of chemical components (analyte protectants) that are added to both the sample extracts and the calibration solutions in order to (ideally) maximize equally the response of pesticides in calibrants in solvent and sample extracts. Alternative ways to compensate for matrix effects involve the use of standard addition, isotopically labeled internal standards (IS), or chemical analogues. However, these approaches are often difficult in MRMs because there are too many residues in different matrices at different levels to devise routine procedures, and the lack of isotopically-labelled standards for so many analytes. If solvent-only calibration is used, a measurement of matrix effects ~~should~~ **must** be made to demonstrate equivalence of results by comparing responses of matrix-matched with solvent-only standards.
			New Zealand
101	20. Trueness is the closeness of agreement between a test result and the accepted reference value of the property being measured. Trueness is stated quantitatively in terms of "bias," with smaller bias indicating greater trueness. Bias is typically determined by comparing the response of the method to a certified (if available) reference material with a known value assigned to the material. Multi-laboratory testing is recommended ideally. Where the uncertainty in the reference value is not negligible, evaluation of the results should consider the reference material uncertainty as well as the statistical variability from analysing the reference material. In the absence of certified reference materials[1,5]guidelines recommend use of an available reference material that is well characterized for the purpose of the validation study.	**C**	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Section F (Trueness and Recovery): A short paragraph may be necessary to explain the choice of internal standards, and what recovery levels (ranges) are tolerable when an IS is used compared to when it is not used. There is also a need to consider and address the issue of cross talk
105	23. For single-laboratory validation, two types of precision sets of conditions are relevant: (a) repeatability, the variability of measurements within the	**C**	**Comment by Colombia** *Category: TECHNICAL* **Colombia** We propose changing "For single-laboratory validation, three types of precision sets of conditions are relevant (a). repeatability (b)

	same analytical sequence, and (b) within-laboratory reproducibility, the variability of results among multiple sets of the same sample. It is important that the precision values are representative of likely test conditions. First of all, the variation in conditions among the runs should represent what would normally happen in the laboratory during routine use of the method. This can be done by on-going method performance validation/verification. For instance, variations in reagent batches, analysts, and instruments should be measured in ongoing quality control. Secondly, the test material used should be typical, in terms of matrix and (ideally) the state of comminution, of the materials likely to be encountered in real applications.		intermediate precision and c) within-laboratory reproducibility". RATIONALE: Conditions for laboratory validation are clearer
108	**H. Limit of Quantification (LOQ)**	C	**Comment by Colombia** *Category: TECHNICAL* **Colombia** It is suggested to add under H the concept of Limit of detection LOD and Limit of quantification (LOQ), being LOD the concentration or lower amount of a compound that is possible to detect with some degree of certainty and that can be differentiated of the answer given by the reagent blank and that the average signal-to-noise ratio (S/N) is equal to 3.3 in the analysis. RATIONALE: It is requested to add the Detection Limit Concept (LOD). This concept is very useful and highly applicable in international food trade)
109	26. By long-standing definition among analytical chemists, the LOQ is the concentration at which the average signal/noise ratio (S/N) equals	C	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Section H: (LOQ), Would it also be appropriate to discuss limit of detection and how this is

	10 in the analysis. The LOQ in practice can only be estimated because precise determination of the actual LOQ requires many analyses of spiked samples and matrix blanks but the LOQ can change day-to-day due to the performance state of the instrument, among many other factors. Some validation guidelines require that the LOQ be verified to meet method performance criteria via spiking experiments at the LOQ, however day-to-day variations in LOQ tend to force the analyst to greatly over-estimate the actual method LOQ, which can be difficult to implement the strict definition of the LOQ (S/N = 10). Thus spiking at the Lowest Validated Level (LVL) is the more descriptive and proper approach. Furthermore, quantification of analytes should not be made below the lowest calibrated level (LCL) in the same analytical sequence. The S/N at the LCL must be ≥10 (conc. ≥ LOQ), which can be set as a system suitability check required for each analytical sequence. A quality control matrix spike can also be included in each sequence to verify that the reporting limit is achieved in the analysis (an action level that is typically greater than the LCL). In essence, the point of the validation is not to determine the LOQ, but to demonstrate that the lowest reported concentration is meeting the need for the analysis.		determined? This is because a screen detection limit has been discussed under screening methods
		C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union If the method is not validated at the LCL, results at that level cannot be reported. Therefore it is the LVL that needs to be checked and not the LCL.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~26.~~ 26. By long-standing definition among analytical chemists, the LOQ is the concentration at which the average signal/noise ratio (S/N) equals 10 in the analysis. The LOQ in practice can only be estimated because precise determination of the actual LOQ requires many analyses of spiked samples and matrix blanks but the LOQ can change day-to-day due to the performance state of the instrument, among many other factors. Some validation guidelines require that the LOQ be verified to meet method performance criteria via spiking experiments at the LOQ, however day-to-day variations in LOQ tend to force the analyst to greatly over-estimate the actual method LOQ, which can be difficult to implement the strict definition of the LOQ (S/N = 10). Thus spiking at the Lowest Validated Level (LVL) is the more descriptive and proper approach. Furthermore, quantification of analytes should not be made below the lowest ~~calibrated~~ **validated** level ~~(LCL)~~ **(LVL)** in the same analytical sequence. The S/N at the LCL must be ≥10 (conc. ≥ LOQ), which can be set as a system suitability check required for each analytical sequence. A quality control matrix spike can also be included in each sequence to verify that the reporting limit is achieved in the analysis (an action level that is typically ~~greater than~~ the ~~LCL)~~**LVL)**. In essence, the point of the validation is not to determine the LOQ, but to demonstrate that the lowest reported concentration is meeting the need for the analysis.
			European Union
111	27. The validated range is the interval of analyte concentration within which the method can be regarded as validated. The LVL is the	P	**Proposed Change by Thailand** *Category: TECHNICAL* 27. The validated range is the interval of analyte concentration within which the method can be regarded as validated. The LVL is the lowest

	lowest concentration assessed during validation that meets method performance criteria. It is important to realize that the validated range is not necessarily identical to the useful range of the calibration. While the calibration may cover a wide concentration range, the validated range (which is usually more important in terms of uncertainty) will typically cover a more restricted range. In practice, most methods will be validated for at least two levels of concentration. The validated range may be taken as a reasonable extrapolation between these points of concentration, but many laboratories choose to validate at a third level to demonstrate linearity. For monitoring residue concentrations with respect to Codex standards, the analytical method must be sensitive enough so that the LVL for each analyte is at or below the current Codex maximum residue limit (CXL). The validation range should cover the existing CXL. When a CXL does not exist, the lowest level may be MRLs established by a national regulatory authority. If no CXL or MRL exists for a given analyte/matrix pair, then 0.01 mg/kg generally serves as the desirable LVL. In MRMs, the typical analytical goal is to set the LVL (and reporting level) at 0.01 mg/kg in diverse, yet representative commodities.		concentration assessed during validation that meets method performance criteria. It is important to realize that the validated range is not necessarily identical to the useful range of the calibration. While the calibration may cover a wide concentration range, the validated range (which is usually more important in terms of uncertainty) will typically cover a more restricted range. In practice, most methods will be validated for at least two levels of concentration. The validated range may be taken as a reasonable extrapolation between these points of concentration, but many laboratories choose to validate at a third level to demonstrate linearity. For monitoring residue concentrations with respect to Codex standards, the analytical method must be sensitive enough so that the LVL for each analyte is at or below the current Codex maximum residue limit (CXL). The validation range should cover the existing CXL. When a CXL does not exist, the lowest level may be MRLs established by a national regulatory authority. If no CXL or MRL exists for a given analyte/matrix pair, then 0.01 mg/kg **or LOQ** generally serves as the desirable LVL. In MRMs, the typical analytical goal is to set the LVL (and reporting level) at 0.01 mg/kg in diverse, yet representative commodities.
			Thailand We would like to propose an amendment to the second last sentence of para 27 to be read as follow; “When a CXL does not exist, the lowest level may be MRLs established by a national regulatory authority. If no CXL or MRL exists for a given analyte/matrix pair, then 0.01 mg/kg or LOQ generally serves as the desirable LVL. In MRMs, the typical analytical goal is to set the LVL (and reporting level) at 0.01 mg/kg in diverse, yet representative commodities.” Rationale: Limit of Quantitation (LOQ) of some pesticide/commodity combination could not be at the 0.01 mg/kg. The desirable LVL of some pesticide/commodity combination, which there are no MRLs existing, can be LOQ
		C	**Comment by European Union** *Category: EDITORIAL*
			European Union For the sake of clarification.
		P	**Proposed Change by European Union** *Category: EDITORIAL* 27. The validated range is the interval of analyte concentration within which the method can be regarded as validated. The LVL is the lowest

			concentration assessed during validation that meets method performance criteria. It is important to realize that the validated range is not necessarily identical to the useful range of the **instrumental** calibration. While the calibration may cover a wideconcentration range, the validated range (which is usually more important in terms of uncertainty) will typically cover a more restricted range. In practice, most methods will be validated for at least two levels of concentration. The validated range may be taken as a reasonable extrapolation between these points of concentration, but many laboratories choose to validate at a third level to demonstrate linearity. For monitoring residue concentrations with respect to Codex standards, the analytical method must be sensitive enough so that the LVL for each analyte is at or below the current Codex maximum residue limit (CXL). The validation range should cover the existing CXL. When a CXL does not exist, the lowest level may be MRLs established by a national regulatory authority. If no CXL or MRL exists for a given analyte/matrix pair, then 0.01 mg/kg generally serves as the desirable LVL. In MRMs, the typical analytical goal is to set the LVL (and reporting level) at 0.01 mg/kg in diverse, yet representative commodities. **European Union**
114	29. Examples of the factors that a ruggedness test could address are: changes in the instrument, operator, or brand/lot of reagent; concentration of a reagent; pH of a solution; temperature of a reaction; time allowed for completion of a process, and/or other pertinent factors.	**C**	**Comment by European Union** *Category: EDITORIAL* **European Union** For the sake of clarity.
		P	**Proposed Change by European Union** *Category: EDITORIAL* 29. Examples of the factors that a ruggedness test could address are: **small** changes in the instrument~~, operator, or,~~ brand/lot of ~~reagent~~**reagent or changes in the operator**; concentration of a reagent; pH of a solution; temperature of a reaction; time allowed for completion of a process, and/or other pertinent factors. **European Union**
120	32. Screening methods are usually either qualitative or semi-quantitative in nature, with the objective being to discriminate samples which	**C**	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Para 32 (Screening): Is semi-quantitative a suitable terminology to use?

	contain no residues above a threshold value (“negatives”) from those which may contain residues above that value (“indicated positives”). The validation strategy therefore focuses on establishing a threshold concentration above which results are “potentially positive,” determining a statistically based rate for both “false positive” and “false negative” results, testing for interferences and establishing appropriate conditions of use. The screening concept offers laboratories an 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 monitored using validated quantitative MRMs. As in quantitative methods, screening methods should also be checked in terms of selectivity and sensitivity. In some applications, commercial test kits may be useful, but current techniques have rarely met multi-residue screening needs economically in practice. Selectivity and analytical scope are often improved when chromatography or other form of separation is used prior to detection. Another approach is to use screening methods that involve mass spectrometry (MS)-based detection, which is able to distinguish particular chemicals from each other.	C	**Comment by European Union** *Category: SUBSTANTIVE* **European Union** In screening methods the terms “false positive” and “false negative” are not correct, as the results have not been identified.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~32.~~ 32. Screening methods are usually either qualitative or semi-quantitative in nature, with the objective being to discriminate samples which contain no residues above a threshold value (“negatives”) from those which may contain residues above that value (“indicated positives”). The validation strategy therefore focuses on establishing a threshold concentration above which results are “potentially positive,” determining a statistically based rate for ~~both “false positive” and “false negative” results~~**false detects (positives or negatives)**, testing for interferences and establishing appropriate conditions of use. The screening concept offers laboratories an 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 monitored using validated quantitative MRMs. As in quantitative methods, screening methods should also be checked in terms of selectivity and sensitivity. In some applications, commercial test kits may be useful, but current techniques have rarely met multi-residue screening needs economically in practice. Selectivity and analytical scope are often improved when chromatography or other form of separation is used prior to detection. Another approach is to use screening methods that involve mass spectrometry (MS)-based detection, which is able to distinguish particular chemicals from each other.
			European Union
121	33. The selectivity of screening methods should be adequate and must be able to distinguish the presence of the target compound, or group	C	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Para 33. Clarification may be necessary for the word “adequate” and what it entails: so is the

	of compounds, from other substances that may be present in the sample material. Selectivity of screening methods is normally not as great as that of aquantitative method. Screening methods often take advantage of a structural feature common to a group or class of compounds and may be based on immunoassays or spectrophotometric responses which may not unambiguously identify a compound.		phrase “normally not as great as that of quantitative method”
		C	**Comment by European Union** *Category: EDITORIAL*
			European Union For the sake of clarification.
		P	**Proposed Change by European Union** *Category: EDITORIAL* 33. The selectivity of screening methods should be adequate and must be able to distinguish the presence of the target compound, or group of compounds, from other substances that may be present in the sample material. Selectivity of screening methods is normally not as great as that of aquantitative method. Screening methods ~~often~~ **can** take advantage of a structural feature common to a group or class of compounds and may be based on immunoassays or spectrophotometric responses which may not unambiguously identify a compound.
			European Union
122	34. The validation of a screening method based on a screening detection limit (SDL) can be focused on detectability. For each	P	**Proposed Change by Thailand** *Category: TECHNICAL* 34. The validation of a screening method based on a screening detection limit (SDL) can be focused on detectability. For each

	representative type of matrix, a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL. The samples and at least 5 matrix blanks from different sources (more replicates of greater diversity provides better validation) with a minimum of two different samples for each type of matrix should be suitable for the intended scope of the laboratory. Additional validation data can be collected from on-going QC-data and method performance verification during routine analysis. 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 (e.g. an acceptable false-negative rate of 5%).		representative ~~type of matrix~~**type of matrix**, a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL. The samples and at least 5 matrix blanks from different sources (more replicates of greater diversity provides better validation) with a minimum of two different samples for each type of matrix should be suitable for the intended scope of the laboratory. Additional validation data can be collected from on-going QC-data and method performance verification during routine analysis. The SDL of the qualitative screeningmethod 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 (e.g. an acceptable false-negative rate of 5%).
			Thailand We would like to seek the clarification on the meaning of “type of matrix” in second line of this para. We are not sure whether this term means “Commodity group” or “Commodity class”. For more clarification, the term “type of matrix” should be amended to be “Commodity group” or “Commodity class” depending on the meaning of this term. This is because the terms “Commodity group” and “Commodity class” are usually used in the pesticide residue analysis. Moreover, there is the commodity grouping set in the Codex STAN CAC/GL 40-1993 as “Table 5. Representative commodities/samples for validation of analytical procedures for pesticide residues”. We, therefore, propose to add the content of Table 5 in this document. The insertion can be putting the content of Table 5 into this para, putting the content of Table 5 as Annex of this Guideline or referring to the Table 5 of CAC/GL 40-1993.
		C	**Comment by IAEA** *Category: TECHNICAL*
			IAEA Para 34: Clarification is also required for “different sources”
122	34. The validation of a screening method based on a screening detection limit (SDL) can be focused on detectability. For each	P	**Proposed Change by Colombia** *Category: TECHNICAL* 34. The validation of a screening method based on a screening detection limit (SDL) ***[from the acronym in English]*** can be focused on

	representative type of matrix, a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL. The samples and at least 5 matrix blanks from different sources (more replicates of greater diversity provides better validation) with a minimum of two different samples for each type of matrix should be suitable for the intended scope of the laboratory. Additional validation data can be collected from on-going QC-data and method performance verification during routine analysis. 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 (e.g. an acceptable false-negative rate of 5%).		detectability. For each representative type of matrix, a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL. The samples and at least 5 matrix blanks from different sources (more replicates of greater diversity provides better validation) with a minimum of two different samples for each type of matrix should be suitable for the intended scope of the laboratory. Additional validation data can be collected from on-going QC-data and method performance verification during routine analysis. 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 (e.g. an acceptable false-negative rate of 5%). **Colombia**
125	36. The requirement to recover a range of different pesticide residues in one extraction increases the potential for compromised selectivity in MRMs compared to single residue methods. Using less selective extraction and clean-up procedures is likely to result in greater co-extracted matrix material in the final extract. The nature and quantities of such co-extracted material can vary markedly based on the matrix method analytes of interest. Care is therefore required when setting criteria for the precision and trueness of MRMs to ensure that quantification will not be affected by chemical interferences.	**P**	**Proposed Change by Australia** *Category: EDITORIAL* 36. The requirement to recover a range of different pesticide residues in one extraction increases the potential for compromised selectivity in MRMs compared to single residue methods. Using less selective extraction and clean-up procedures is likely to result in greater co-extracted matrix material in the final extract. The nature and quantities of such co-extracted material can vary markedly based on the ~~matrix~~ **matrix,** method **and** analytes of interest. Care is therefore required when setting criteria for the precision and trueness of MRMs to ensure that quantification will not be affected by chemical interferences. **Australia**
126	37. In addition to the selectivity of a method, the ability of the method to provide a reliable quantitative result must be demonstrated	**C**	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Para 37: Ideally the SD should be less than 30%. Could reference to this be provided?

	(i.e. trueness - see section F and precision – see section G). Ideally, the relative standard deviation between the original sample and replicates will be less than 30 percent.	C	**Comment by European Union** *Category: SUBSTANTIVE* **European Union** For consistency with paragraph 39 of this document (Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20%).
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~37.~~ 37. In addition to the selectivity of a method, the ability of the method to provide a reliable quantitative result must be demonstrated (i.e. trueness - see section F and precision – see section G). Ideally, the relative standard deviation between the ~~original sample and~~ **concentration of the** replicates will be less than ~~30~~ **20** percent. **European Union**
127	38. Acceptability criteria for a quantitative analytical method should be demonstrated at both initial and on-going validation stages, as being capable of providing acceptable mean recovery values at each spiking level. For validation, a minimum of 5 replicates is required (to check the recovery and precision) at the targeted LVL, LOQ, or reporting limit of the method, and at least one additional higher level, for example, 2-10x the LVL or the MRL. If a method is being used for compliance testing (i.e. if a commodity is complaint with an established MRL) the MRL (or CXL) must be one of the spiking levels. When the residue definition includes two or more analytes, the method should be validated for all analytes.	P	**Proposed Change by Australia** *Category: EDITORIAL* 38. Acceptability criteria for a quantitative analytical method should be demonstrated at both initial and on-going validation stages, as being capable of providing acceptable mean recovery values at each spiking level. For validation, **it is recommended that** a minimum of 5 ~~replicates is required~~ **replicate tests be performed** (to check the recovery and precision) at the targeted LVL, LOQ, or reporting limit of the method, and at least one additional higher level, for example, 2-10x the LVL or the MRL. If a method is being used for compliance testing (i.e. if a commodity is ~~complaint~~ **compliant** with an established MRL) the MRL (or CXL) ~~must~~ **should** be one of the spiking levels. When the residue definition includes two or more analytes, the method should be validated for all analytes. **Australia**
128	39. The trueness of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another	P	**Proposed Change by Thailand** *Category: TECHNICAL* 39. The trueness of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another method for which the

	method for which the performance parameters have previously been rigorously established (typically a collaboratively studied method), or by determination of the recovery of analyte fortified into known blank sample material. Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20%. In certain cases (typically with MRMs), recoveries outside this range may be acceptable, such as when recovery is lower but consistent (e.g. demonstrating good precision). This is more justifiable if the reason for the systematic low bias is well established by chemistry (e.g. known analyte distribution between phases in a partitioning step). However, a more accurate method should be used, if practicable. Recoveries >120% are likely to be attributable to a positive interference or bias that should be investigated.		performance parameters have previously been rigorously established (typically a collaboratively studied method), or by determination of the recovery of analyte fortified into known blank sample material. Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20%. **For the concentration ≤ 0.01 mg/kg, acceptable mean recoveries range from 60-120% with a RSD ≤ 30%.** In certain cases (typically with MRMs), recoveries outside this range may be acceptable, such as when recovery is lower but consistent (e.g. demonstrating good precision). This is more justifiable if the reason for the systematic low bias is well established by chemistry (e.g. known analyte distribution between phases in a partitioning step). However, a more accurate method should be used, if practicable. Recoveries >120% are likely to be attributable to a positive interference or bias that should be investigated.
			Thailand We recognize that the content of the second sentence (line 4- 5) of this para (started from Acceptable means recovery …) is similar to the content of Table 3 in GL 40/1993: Guidelines on Good Laboratory Practice in Pesticides Residue Analysis. To ensure consistency among Codex documents, we would like to propose the addition of this sentence to read as follows; “Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20%. For the concentration ≤ 0.01 mg/kg, acceptable mean recoveries range from 60-120% with a RSD ≤30%.”
		C	**Commen by China** *Category: EDITORIAL*
			China “Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20% “. For residue analysis, at different fortified levels, the RSD and recovery range may vary, and may have different requirements. From our experience, this is much important in pesticide residue analysis. So requirements in CAC GL 40 on method validation parameters are recommended.
		C	**Comment by IAEA** *Category: SUBSTANTIVE*
			IAEA Para 39: Clarification needs to be made that rigorous and collaborative are not the same. There is also need to consider incurred

			samples. The possible impact of using internal standards need to stated; Also the phrasing "acceptable mean recoveries for enforcement purposes should be from…" seems prescriptive. Methods with recoveries below this range may still be (with good precision) suitable for enforcement
		P	**Proposed Change (11) by Australia on 14 Nov 2016 5:02 AM** *Category: EDITORIAL* 39. The trueness of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another method for which the performance parameters have previously been rigorously established (typically a collaboratively studied method), or by determination of the recovery of analyte fortified into known blank sample material. Acceptable mean recoveries for enforcement purposes should **normally** range from 70-120% with a RSD ≤20%. In certain cases (typically with MRMs), recoveries outside this range may be acceptable, such as when recovery is lower but consistent (e.g. demonstrating good precision). This is more justifiable if the reason for the systematic low bias is well established by chemistry (e.g. known analyte distribution between phases in a partitioning step). However, a more accurate method should be used, if practicable. Recoveries >120% are likely to be attributable to a positive interference or bias that should be investigated.
			Australia
129	40. Analysis of incurred matrix to support method validation is encouraged. For interpreting recoveries, it is necessary to recognize that analyte spiked into a test sample may not behave in the same manner as the	P	**Proposed Change by Colombia** *Category: TRANSLATION* 40. Analysis of incurred or ~~accumulated~~ **fortified** matrix to support method validation is encouraged. For interpreting recoveries, it is necessary to recognize that analyte spiked into a test sample may not behave in the same manner as the biologically incurred analyte

	biologically incurred analyte (pesticide residue). In many situations, the amount of an extracted incurred residue is less than the total incurred residues actually present. This may be due to losses during extraction, intra-cellular binding of residues, the presence of conjugates, or other factors that are not fully represented by recovery experiments using analyte-fortified blank matrices.		(pesticide residue). In many situations, the amount of an extracted incurred residue is less than the total incurred residues actually present. This may be due to losses during extraction, intra-cellular binding of residues, the presence of conjugates, or other factors that are not fully represented by recovery experiments using analyte-fortified blank matrices
			Colombia (Feb 28th 2017 9:17 PM)
131	42. In general, residues data do not have to be adjusted for recovery when the mean recovery is within the range of 70-120%. Recovery corrections should be made consistent with the guidance provided by the CAC/GL 37-2001[7]. It is of over-riding importance that all data, when reported, should (a) be clearly identified as to whether or not a recovery correction has been applied and (b) include the amount of the correction and the method by which it was derived, if a recovery correction has been applied. This will promote direct comparability of data sets. Correction functions should be established on the basis of appropriate statistical considerations, and documented, archived and made available to the client.	C	**Comment by China** *Category: EDITORIAL*
			China "Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20% ". For residue analysis, at different fortified levels, the RSD and recovery range may vary, and may have different requirements. From our experience, this is much important in pesticide residue analysis. So requirements in CAC GL 40 on method validation parameters are recommended.
		P	**Proposed Change by Australia** *Category: EDITORIAL* 42. ~~In general, residues data do not have to be adjusted for recovery when the mean recovery is within the range of 70-120%.~~ Recovery corrections should be made consistent with the guidance provided by the CAC/GL 37-2001[7]. ~~It is of over~~ **This will promote direct comparability of data sets. Correction functions should be established on the basis of appropriate statistical considerations, and documented, archived and made available to the client.** ~~-riding importance that all data, when reported, should (a) be clearly identified as to whether or not a recovery correction has been applied and (b) include the amount of the correction and the method by which it was derived, if a recovery correction has been applied. This will promote direct comparability of data sets. Correction functions should be established on the basis of appropriate statistical considerations, and documented, archived and made available to the client.~~
			Australia
		C	**Comment by New Zealand** *Category: TECHNICAL*
			New Zealand In the first sentence how can mean recovery be assessed if there is no recovery curve?
135	44. By far, gross errors (spurious mistakes made	C	**Comment by IAEA** *Category: SUBSTANTIVE*

	during sample preparation) are the greatest source of misidentifications in MS-based methods. For this reason, all regulatory enforcement actions (above an MRL or for those with no MRL on that commodity) require confirmation of the result via re-extraction of a replicate test portion of the original sample and re-analysis, ideally using different chemistries of sample preparation and/or analysis.		**IAEA** Para 44: The phrasing " reanalysis, ideally using different chemistries...." may be removed or reworded. Sounds prescriptive to the analyst and authorities
136	45. Selectivity is the primary consideration for methods of identification. The method should be sufficiently selective to provide unambiguous identification. MS coupled to a chromatographic separation method is a very powerful combination for identification of an analyte in the sample extract. This method provides information about the structure of the analyte that is not obtainable with chromatography alone. GC-MS and LC-MS tools (full-scan, selected ion mode, high-resolution, tandem MS/MS, hybrid systems, among other advanced techniques) provide many measurable parameters, such as retention times, chromatographic peak shapes, ion intensities and relative abundances/ratios, mass accuracies, and other useful aspects to help make analyte identifications.	**C**	**Comment by IAEA** *Category: TECHNICAL* **IAEA** Para 45: Selectivity seems to be prescribing MS only here
139	47. Current practices in qualitative (and quantitative) analysis of pesticide residues commonly involve chromatography + selected ion monitoring (SIM) or MS/MS techniques. Full-spectral (full-scan or time-of-flight) MS is also an	**C**	**Comment by China** *Category: EDITORIAL* **China** Line3, about "matching factor': we recommend to give definition of this term, and it may be different for different workstations from various companies. For ins, NIST database may use 0-999 as indicator, and Agilent Chemstation may give percentages.

	acceptable tool that uses spectral library matching factors and/or relative abundances of major ions within the full spectra. The latter case can be treated as ion ratios in the criteria given below using at least 3 ions. In the former case, matching factors should be ≥900 (≥90% match) for regulatory identification purposes, and the library reference spectra should be obtained from background-subtracted high purity standards on the same instrument using the same conditions as in the sample analysis. The following identification criteria should be met:	C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union Delete the brackets. Full spectral is clear enough. Besides, full scan and time of flight refer to different things (acquisition mode and analyser) and it´s possible to work in full scan using a time of flight instrument but also a single quadrupole or orbitrap. Matching factors depend on the specific software used, so it´s not correct to use the same threshold for all of them. Furthermore it is not clear the scientific basis applied.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* 47. Current practices in qualitative ~~(and quantitative)~~ **and quantitative** analysis of pesticide residues commonly involve chromatography + selected ion monitoring (SIM) or MS/MS techniques. Full-spectral ~~(full-scan or time-of-flight)~~ MS is also an acceptable tool that uses spectral library matching factors and/or relative abundances of major ions within the full spectra. The latter case can be treated as ion ratios in the criteria given below using at least 3 ions. In the former case, ~~matching factors should be ≥900 (≥90% match) for regulatory identification purposes, and~~ the library reference spectra should be obtained from background-subtracted high purity standards on the same instrument using the same conditions as in the sample analysis. The following identification criteria should be met:
			European Union
140	a. Analyte retention time reference values must be determined from contemporaneously analysed (within the same batch) high concentration calibration standards in solvent-based solutions (matrix-matched calibration standards may be used if it is known that no interferences are present).	C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union It is preferable to determine the reference values in the same matrix or from the same commodity group than the samples to be analysed.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~a.~~ **a.** Analyte retention time reference values must be determined from contemporaneously analysed (within the same batch) high concentration **matrix-matched** calibration standards ~~in solvent-based solutions (matrix-matched calibration standards may be used~~ if it is known that no interferences are ~~present)~~**present otherwise using solvent**

			based solutions.
			European Union
		P	**Proposed Change by Australia** *Category: EDITORIAL* **a.** Analyte retention time reference values ~~must~~ **should** be determined from contemporaneously analysed (within the same batch) high concentration calibration standards in solvent-based solutions (matrix-matched calibration standards may be used if it is known that no interferences are present).
			Australia
141	b. Ion ratio reference values are to be set in the same way as in paragraph 47 a. The different ions used for identification must co-elute and have similar peak shapes. The ion from the calibration standard with the higher average intensity is to be used as the denominator in the ion ratio, expressed in percentage (due to signal fluctuations, matrix effects, etc.… deviations of ion ratios up to 30% are acceptable).	C	**Comment by China** *Category: EDITORIAL*
			China "30%"is not indicated clearly, and what the basis for setting this value, proper literature may be listed.
142	c. The signal to noise ratios for measured peaks must be greater than 3 and/or the signal must exceed the threshold intensity level as compared to the signal of a suitable calibration standard or control encompassing the level of interest.	P	**Proposed Change by Australia** *Category: EDITORIAL* **c.** The signal to noise ratios for measured peaks must be greater than 3 and/or the signal ~~must~~ **should** exceed the threshold intensity level as compared to the signal of a suitable calibration standard or control encompassing the level of interest.
			Australia
144	e. All measured reagent and matrix blank samples should be shown to be free of carry-over, contamination, and/or interferences above 20% of the LOQ.	C	**Comment by IAEA** *Category: TECHNICAL*
			IAEA Number € para 47: needs clarification (and/or interference above 20% of the LOQ)
144	e. All measured reagent and matrix blank samples should be shown to be free of carry-over, contamination, and/or interferences above 20% of the LOQ.	C	**Comment by Colombia** *Category: TECHNICAL*
			Colombia Include literal f. The use of ions with a mass (M/Z) (mass/charge ratio) (greater than 100) is recommended since ions with an (M/Z) tend to be less selective.
145	48. The minimum acceptable retention time for the analyte(s) should be at least twice the retention time corresponding to the void	C	**Comment by European Union** *Category: TECHNICAL*
			European Union For a better harmonisation with SANTE/11945/2015 and performance of the

	volume of the column. The retention time of the analyte in the extract should correspond to that of the reference value (47a) within ±0.2 min or 0.2% relative retention time, for both gas chromatography and liquid chromatography.		new LC and GC instruments.
		P	**Proposed Change by European Union** *Category: TECHNICAL* 48. The minimum acceptable retention time for the analyte(s) 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 reference value (47a) within ±0.2 min or 0.2% relative retention time, for both gas chromatography and liquid ~~chromatography~~**chromatography (preferably ±0.1 min if possible).**
			European Union (16 Jan 2017 4:21 PM)
145	48. The minimum acceptable retention time for the analyte(s) 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 reference value (47a) within ±0.2 min or 0.2% relative retention time, for both gas chromatography and liquid chromatography.	C	**Comment by Colombia** *Category: TECHNICAL*
			Colombia The reference to the minimum acceptable retention time for the analytes should be clarified.
		P	**Proposed Change by Colombia** *Category: TRANSLATION* 48. The minimum acceptable retention time for the analyte(s) should be at least twice the retention time corresponding to the ~~void~~ **dead** volume of the column. The retention time of the analyte in the extract should correspond to that of the reference value (47a) within ±0.2 min or 0.2% relative retention time, for both gas chromatography and liquid chromatography.
			Colombia
148	50. 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 CXL/MRL is exceeded, a confirmatory analysis of another test portion is always required. For unusual pesticide/matrix combinations, a confirmatory analysis is also	C	**Comment by IAEA** *Category: EDITORIAL*
			IAEA Para 51: Clarification is also needed on "confirmatory analysis of another test portion is always required". Is it referring to another portion of the same samples?

	recommended.		
149	51. If the initial confirmatory method is not based on an MS technique, the confirmatory methods should involve MS-based analyte identification. Moreover, the confirmatory methods should use an independent approaches based on different chemical mechanisms (such as LC and GC separations). In some situations, confirmation by independent laboratories may be appropriate. Examples of analytical techniques that may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 2.	C	**Comment by IAEA** *Category: TECHNICAL*
			IAEA Para 51: "In some cases….." needs rewording. It also presumes that MS is the solution or the only one; Technology should also not be the driver
		C	**Comment by IAEA** *Category: EDITORIAL*
			IAEA Para 51: a typo on "an independent approaches"
150	**Table 1. Identification criteria for different MS techniques**	C	**Comment by New Zealand** *Category: EDITORIAL*
			New Zealand New Zealand considers that Table 1 should be aligned with 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, or there should at least be a discussion why they are departing from this widely accepted directive.
150	**Table 1. Identification criteria for different MS techniques**	C	**Comment by Colombia** *Category: EDITORIAL*
			Colombia Letters d and f need to be placed
199	d) ≤10 ppm	P	**Proposed Change by Canada** *Category: SUBSTANTIVE* d) **mass accuracy ≤≤5** ~~10~~ ppm
			Canada
		C	**Comment by European Union** *Category: TECHNICAL*
			European Union New HRMS instruments have typically mass errors <5 ppm in MS2.
		P	**Proposed Change by European Union** *Category: TECHNICAL* d) ~~≤10 ppm~~
			European Union
201	f) if the precursor mass accuracy is less than 5 ppm and the product ion mass accuracy is less than 10 ppm, ion ratio tolerances is optional	C	**Comment by Canada** *Category: EDITORIAL*
			Canada Rationale: Changes in Table 1 footnote d) and f) provide criteria, which allow using either mass accuracy or ion ration for identification.

		P	**Proposed Change by Canada** *Category: SUBSTANTIVE* f) if the ~~precursor~~ mass accuracy ~~is less than 5 ppm~~ **of both a precursor** and ~~the~~ **its** product ion ~~mass accuracy~~ is ~~less~~ **greater** than ~~10 ppm~~**5ppm**, **identification should be based on** ion ~~ratio tolerances is optional~~**ratio"**
			Canada
215	Derivatization	C	**Comment by European Union** *Category: SUBSTANTIVE*
			European Union Derivatization is not a detection method.
		P	**Proposed Change by European Union** *Category: SUBSTANTIVE* ~~Derivatization~~
			European Union
217	LC-immunogram	C	**Comment by European Union** *Category: TECHNICAL*
			European Union Immunogram is the result, not the detection method.
		P	**Proposed Change by European Union** *Category: TECHNICAL* ~~LC-immunogram~~**LC-immunoaffinity**
			European Union
222	**ANNEX**	C	**Comment by China** *Category: EDITORIAL*
			China The following terms were not quoted in the text: "Analytical quality controls, multi-class method ， sample processing". So proper quotations may be added accordingly.
223	**DEFINITIONS**	P	**Proposed Change by India** *Category: TECHNICAL* **DEFINITIONS** **Kindly include the following two definitions** **Metabolite: Component of a pesticide residue occcurring in a commodity as a result of biotic transformation (metabolism) of a pesticide in a biological system (e.g. plant, animal)** **Degradation product: Component of a pesticide residue occurring in commodity as a result of abiotic transformation of pesticide in/on plant, animal (e.g.impact of heat, light, moisture, pH change etc.)**
			India
		C	**Comment by IAEA** *Category: EDITORIAL*
			IAEA Could a list of acronyms be added? e.g. to capture SIM, TOF, Q-TOF etc? If SDL is considered, then perhaps LOD should also be mentioned and defined.

243	**Matrix:** The material or component sampled for pesticide residue studies.	C	**Comment by Haiti** *Category: EDITORIAL*

Annex II

[1]DRAFT GUIDELINES ON PERFORMANCE CRITERIA FOR METHODS OF ANALYSIS FOR THE DETERMINATION OF PESTICIDE RESIDUES IN FOOD

[2]**(At Step 6)**

[3]TABLE OF CONTENTS

[54]OBJECTIVE

1. [55]The purpose of this guidance document is to define and describe the performance criteria, which should be met by methods to analyse pesticide residues in foods. It addresses the characteristics/parameters to provide scientifically acceptable confidence in the analytical method that is fit for the intended use and may be used to reliably evaluate pesticide residues for either domestic monitoring and/or international trade.

2. [56]This document is applicable to both single residue methods and multi-residue methods (MRMs) that analyse target compounds in all food commodities, including parent pesticide residues and/or their metabolites and degradants in food commodities per the residue definition.

3. [57]This guidance covers qualitative and quantitative analyses, each having their own method performance requirements. Performance acceptability criteria of methods for analyte identification and confirmation are also addressed.

[58]PRINCIPLES FOR THE SELECTION AND VALIDATION OF METHODS

A. [59]Defining the Purpose of the Method and Scope

4. [60]The intended purpose of the method is usually described in a statement of scope, which defines the analytes (residues), the matrices, and the concentration ranges. It also states whether the method is intended for screening, quantification, identification, and/or confirmation of results.

5. [61]In regulatory applications, the maximum residue limit (MRL) is expressed in terms of the “residue definition,” which may include the parent compound, a major metabolite, a sum of parent and/or metabolites, or a reaction product formed from the residues during analysis. Residue analytical methods should be able to measure all components of the residue definition.

6. [62]*Fitness-for-purpose* is the extent to which the performance of a method meets the end-user's needs, and matches the criteria (data quality objectives) agreed between the laboratory and the end-user (or client) of the data, within technical and resource constraints. *Fitness-for-purpose* criteria could be based on some of the characteristics described in this document, but ultimately will be expressed in terms of acceptable combined uncertainty[1].

7. [64]Selection of methods is based on analytes and the intended purpose of the analyses[2].

B. [66]**Supplementing other Codex Alimentarius Commission Guidelines**

8. [67]The Codex Alimentarius Commission (CAC) has issued a guideline[3] for laboratories involved in the testing of foods for import/export which recommends that such laboratories should:

a. [69]use internal quality control procedures, such as those described in the "Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories;"

b. [70]participate in appropriate proficiency testing schemes for food analysis which conform to the requirement laid out in "The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories;" and

c. [71]whenever available, use methods which have been validated according to principles provided by the CAC.

9. [72]The analytical methods should be used within the internationally accepted, approved, and recognized laboratory Quality Management System[4] to be consistent with the principles in the document for quality assurance (QA) and quality control (QC) referenced above. The on-going performance is monitored through the Quality Management System in place in the laboratory.

C. [74]**Method Validation**

10. [75]The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that when a test is performed by a properly trained analyst using the specified equipment and materials and exactly following the method protocol, accurate and consistent results can be obtained within specified statistical limits for sample analysis. The validation should demonstrate the identity and concentration of the analyte, taking into account for matrix effects, provide a statistical characterization of recovery results, and indicate if the rates of false positives and negatives are acceptable. When the method is followed using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue testing laboratory. To ensure that validation of the method remains appropriate over time, the method should be continuously assessed using on-going proficiency testing and appropriate quality control samples (e.g. including recovery spikes).

[76]**PERFORMANCE PARAMETERS FOR ANALYTICAL METHODS**

11. [77]The general requirements for the individual performance characteristics of a method are summarized below[1, 5]

[79]**A. Applicability**

12. [80]After validation, the method documentation should provide, in addition to performance specifications (data quality objectives), the following information:

a. [81]identity of the analytes, including isomers, metabolites and other components where appropriate (e.g. endosulfan I&II, spinosyn A&D);

b. [82]concentration range covered by the validation (e.g. "0.01-10 mg/kg");

c. [83]range of sample matrices covered by the validation (e.g. "cucurbits, root vegetables, citrus");

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[63][1] IUPAC Harmonized Guidelines For Single-Laboratory Validation of Methods of Analysis, Pure & Appl. Chem., 74(5), 2002; 835 – 855

[65][2] OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO (2007)17

[68][3] Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food, CAC/GL 27-1997

[73][4] General requirements for the competence of testing and calibration laboratories, ISO/IEC 17025

[78][5] OECD Guidance Document for Single Laboratory Validation of Quantitative Analytical Method-Guidance used in support of pre-and post-registration data requirements for plant protection and biocidal products ENV/JM/MONO(2014)20

d. [84]protocol describing the equipment, reagents, detailed step-by-step procedure including permissible variations (e.g. “heat at 100 ± 5 °C for 30 ± 5 min”), calibration and quality procedures, special safety precautions required, and intended application and critical uncertainty requirements;

e. [85]if required, a quantitative result should be reported together with the expanded measurement uncertainty (MU).

[86]B. Selectivity

13. [87]Ideally, selectivity should be evaluated to demonstrate that no interferences occur which detrimentally affect the analysis. It is impractical to test the method against every potential interferant, but it is recommended that common interferences are checked by analysing a blank for every batch of samples and reagents. Background levels of plasticizers, septa bleed, cleaning agents, reagent impurities, laboratory contamination, carry-over, etc. tend to show up in reagent blanks and must be recognized by the analyst when they occur. Also, analyte-to-analyte interferences must be known by checking individual analytes in mixed standard solutions. Matrix interferences are evaluated by analyses of samples known to be free of the analytes.

14. [88]As a general principle, selectivity should be such that interferences are inconsequential. The ultimate test of selectivity involves the rates of false positives and negatives in the analyses. To minimally estimate rates of false positives and negatives during method validation, an adequate number (suggested >5 each) of diverse matrix blanks (not from the same source) should be analysed along with spiked matrices at the analyte reporting level. Validations of screening methods (presence/absence analyses) are discussed in paragraphs 32-34.

[89]C. Calibration

15. [90]With the exception of gross (also known as “spurious”) errors in preparation of calibration materials, calibration errors are usually (but not always) a minor component of the total uncertainty, and can be safely assigned into other categories. For example, random errors resulting from calibration are part of the uncertainty, while systematic errors cause analytical bias, both of which are assessed as a whole during validation and on-going quality control. Nevertheless, there are some characteristics of calibration that are useful to know at the outset of method validation because they affect optimization of the final protocol. For example, it must be known in advance whether the calibration is linear or quadratic, passes through the origin, and is affected by the sample matrix or not. The described guidelines in this document relate more to validation, which may be more detailed than the calibration undertaken during routine analysis.

16. [91]Replicate measurements are needed to provide an empirical estimate of uncertainty. The following calibration procedures are recommended for the initial method validation:

a. [92]determinations at five or more concentrations should be performed;

b. [93]the calibration standards should be evenly spaced over the concentration range of interest and the calibration range should encompass the entire concentration range likely to be encountered;

c. [94]the calibration standards should be dispersed over the whole sequence, or encompass the beginning and end of the run to demonstrate that calibration integrity is maintained over the entire sequence; and the fit of the calibration function must be plotted and inspected visually and/or by calculation of the residuals (differences between the actual and calculated concentrations of the standards), avoiding over-reliance on correlation coefficients. If individual residuals deviate by more than ±20%, statistical consideration of outliers should be made, possibly leading to re-analysis of the sequence if quality control criteria are not met.

[95]D. Linearity and Intercept

17. [96]Linearity can be tested by examination of a plot of residuals produced by linear regression of the responses on the concentrations in an appropriate calibration set. Any curved pattern suggests a *lack of fit* due to a nonlinear calibration function. If this is the case, another function such as quadratic should be tested and applied, using at least five concentration levels. Despite its current widespread use as an indication of quality of fit, the coefficient of determination (R^2) may be misleading because it places greater significance on standards with higher concentrations. In this case, an appropriate weighting factor such as $1/x$ or $1/x^2$ should be considered.

18. [97]In general, the use of weighted-linear regression or weighted-quadratic function is recommended rather than linear regression for low part per billion (µg/kg) concentration determinations. The value of the intercept should be close to zero (e.g. <20% of the lowest calibration standard) to reduce errors in calculating residue concentrations at low levels.

[98]**E. Matrix Effects**

19. [99]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 gas chromatographic (GC) analyses is the use of chemical components (analyte protectants) that are added to both the sample extracts and the calibration solutions in order to (ideally) maximize equally the response of pesticides in calibrants in solvent and sample extracts. Alternative ways to compensate for matrix effects involve the use of standard addition, isotopically labeled internal standards (IS), or chemical analogues. However, these approaches are often difficult in MRMs because there are too many residues in different matrices at different levels to devise routine procedures, and the lack of isotopically-labelled standards for so many analytes. If solvent-only calibration is used, a measurement of matrix effects should be made to demonstrate equivalence of results by comparing responses of matrix-matched with solvent-only standards.

[100]**F. Trueness and Recovery**

20. [101]Trueness is the closeness of agreement between a test result and the accepted reference value of the property being measured. Trueness is stated quantitatively in terms of “bias,” with smaller bias indicating greater trueness. Bias is typically determined by comparing the response of the method to a certified (if available) reference material with a known value assigned to the material. Multi-laboratory testing is recommended ideally. Where the uncertainty in the reference value is not negligible, evaluation of the results should consider the reference material uncertainty as well as the statistical variability from analysing the reference material. In the absence of certified reference materials[1,5] guidelines recommend use of an available reference material that is well characterized for the purpose of the validation study.

21. [102]Recovery refers to the proportion of analyte determined in the final result compared with the amount added (usually to a blank) sample prior to extraction, generally expressed as a percentage. Errors in measurement will lead to biased recovery figures that will deviate from the actual recovery in the final extract. Routine recovery refers to the determination(s) performed in quality control spikes in the analysis of each batch of samples.

[103]**G. Precision**

22. [104]Precision is the closeness of agreement between independent (replicate) test results obtained under stipulated conditions. It is usually specified in terms of standard deviation (SD) or relative standard deviation (RSD), also known as coefficient of variation (CV). The distinction between precision and bias depends on the level at which the analytical system is viewed. Thus, from the viewpoint of a single determination, any deviation affecting the calibration used in the analysis would be seen as a bias. From the point of view of the analyst reviewing a year’s work, the analytical bias will be different every day and should act like a random variable with an associated precision, incorporating any stipulated conditions for the estimation of this precision.

23. [105]For single-laboratory validation, two types of precision sets of conditions are relevant: (a) repeatability, the variability of measurements within the same analytical sequence, and (b) within-laboratory reproducibility, the variability of results among multiple sets of the same sample. It is important that the precision values are representative of likely test conditions. First of all, the variation in conditions among the runs should represent what would normally happen in the laboratory during routine use of the method. This can be done by on-going method performance validation/verification. For instance, variations in reagent batches, analysts, and instruments should be measured in ongoing quality control. Secondly, the test material used should be typical, in terms of matrix and (ideally) the state of comminution, of the materials likely to be encountered in real applications.

24. [106]In single-laboratory validations, precision often varies with analyte concentration. Typical assumptions are that: (a) there is no change in precision with analyte level, or (b) that the standard deviation is proportional to, or linearly dependent on, analyte level. In both cases, the assumption needs to be checked if the analyte level is expected to vary substantially.

25. [107]Precision data may be obtained for a wide variety of different sets of conditions in addition to the minimum of repeatability and between-run conditions indicated here, and it may be appropriate to acquire additional information. For example, it may be useful to the assessment of results, or for improving the measurement, to have an indication of separate operator and run effects between- or within- day, or to have an indication of the precision attainable using one or several instruments. A range of different designs and statistical analysis techniques is available, and careful experimental design is strongly recommended in all such studies. The initial validation should be conducted at the targeted limit of quantification (LOQ) or reporting limit of the method, and at least one other higher level, for example, 2-10x the targeted LOQ or the MRL.

[108]**H. Limit of Quantification (LOQ)**

26. [109]By long-standing definition among analytical chemists, the LOQ is the concentration at which the average signal/noise ratio (S/N) equals 10 in the analysis. The LOQ in practice can only be estimated because precise determination of the actual LOQ requires many analyses of spiked samples and matrix blanks but the LOQ can change day-to-day due to the performance state of the instrument, among many other factors. Some validation guidelines require that the LOQ be verified to meet method performance criteria via spiking experiments at the LOQ, however day-to-day variations in LOQ tend to force the analyst to greatly over-estimate the actual method LOQ, which can be difficult to implement the strict definition of the LOQ (S/N = 10). Thus spiking at the Lowest Validated Level (LVL) is the more descriptive and proper approach. Furthermore, quantification of analytes should not be made below the lowest calibrated level (LCL) in the same analytical sequence. The S/N at the LCL must be ≥10 (conc. ≥ LOQ), which can be set as a system suitability check required for each analytical sequence. A quality control matrix spike can also be included in each sequence to verify that the reporting limit is achieved in the analysis (an action level that is typically greater than the LCL). In essence, the point of the validation is not to determine the LOQ, but to demonstrate that the lowest reported concentration is meeting the need for the analysis.

[110]**I. Analytical Range**

27. [111]The validated range is the interval of analyte concentration within which the method can be regarded as validated. The LVL is the lowest concentration assessed during validation that meets method performance criteria. It is important to realize that the validated range is not necessarily identical to the useful range of the calibration. While the calibration may cover a wide concentration range, the validated range (which is usually more important in terms of uncertainty) will typically cover a more restricted range. In practice, most methods will be validated for at least two levels of concentration. The validated range may be taken as a reasonable extrapolation between these points of concentration, but many laboratories choose to validate at a third level to demonstrate linearity. For monitoring residue concentrations with respect to Codex standards, the analytical method must be sensitive enough so that the LVL for each analyte is at or below the current Codex maximum residue limit (CXL). The validation range should cover the existing CXL. When a CXL does not exist, the lowest level may be MRLs established by a national regulatory authority. If no CXL or MRL exists for a given analyte/matrix pair, then 0.01 mg/kg generally serves as the desirable LVL. In MRMs, the typical analytical goal is to set the LVL (and reporting level) at 0.01 mg/kg in diverse, yet representative commodities.

[112]**J. Ruggedness**

28. [113]The ruggedness (often synonymous with robustness) of an analytical method is the resistance to change in the results produced by the analytical method when deviations are made from the experimental conditions described in the procedure. The limits for experimental parameters should be prescribed in the method protocol (although this has not always been done in the past), and such permissible deviations, separately or in any combination, should produce no meaningful change in the results produced. A “meaningful change” here would imply that the method would not meet the data quality objectives defined by the *fitness for purpose*. The aspects of the method that are likely to affect results should be identified, and their influence on method performance evaluated by using ruggedness tests.

29. [114]Examples of the factors that a ruggedness test could address are: changes in the instrument, operator, or brand/lot of reagent; concentration of a reagent; pH of a solution; temperature of a reaction; time allowed for completion of a process, and/or other pertinent factors.

[115]**K. Measurement Uncertainty (MU)**

30. [116]The formal approach to measurement uncertainty estimation is a calculated estimate from an equation or mathematical model, around which the true value can be expected to lie within a defined level of probability. The procedures described in method validation are designed to ensure that the equation used to *estimate the resul*t, with due allowance for random errors of all kinds, is a valid expression embodying all recognized and significant effects upon the result. Further considerations and description of the measurement uncertainty are provided in “Guidelines on Estimation of Uncertainty of Results”[6].

31. [118]It is preferable to express the uncertainty of measurement as a function of concentration and compare that function with a criterion of *fitness for purpose* agreed between the laboratory and the client or end-user of the data. One possibility is to calculate MU from proficiency test data[6].

[117][6] Estimation of Uncertainty of Results, CAC/GL 59-2006

[119]**PERFORMANCE ACCEPTABILITY CRITERIA OF SCREENING METHODS**

32. [120]Screening methods are usually either qualitative or semi-quantitative in nature, with the objective being to discriminate samples which contain no residues above a threshold value ("negatives") from those which may contain residues above that value ("indicated positives"). The validation strategy therefore focuses on establishing a threshold concentration above which results are "potentially positive," determining a statistically based rate for both "false positive" and "false negative" results, testing for interferences and establishing appropriate conditions of use. The screening concept offers laboratories an 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 monitored using validated quantitative MRMs. As in quantitative methods, screening methods should also be checked in terms of selectivity and sensitivity. In some applications, commercial test kits may be useful, but current techniques have rarely met multi-residue screening needs economically in practice. Selectivity and analytical scope are often improved when chromatography or other form of separation is used prior to detection. Another approach is to use screening methods that involve mass spectrometry (MS)-based detection, which is able to distinguish particular chemicals from each other.

33. [121]The selectivity of screening methods should be adequate and must be able to distinguish the presence of the target compound, or group of compounds, from other substances that may be present in the sample material. Selectivity of screening methods is normally not as great as that of a quantitative method. Screening methods often take advantage of a structural feature common to a group or class of compounds and may be based on immunoassays or spectrophotometric responses which may not unambiguously identify a compound.

34. [122]The validation of a screening method based on a screening detection limit (SDL) can be focused on detectability. For each representative type of matrix, a minimal validation should involve analysis of at least 5 samples spiked at the estimated SDL. The samples and at least 5 matrix blanks from different sources (more replicates of greater diversity provides better validation) with a minimum of two different samples for each type of matrix should be suitable for the intended scope of the laboratory. Additional validation data can be collected from on-going QC-data and method performance verification during routine analysis. 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 (e.g. an acceptable false-negative rate of 5%).

[123]**PERFORMANCE ACCEPTABILITY CRITERIA OF QUANTITATIVE METHODS**

35. [124]Selectivity is of particular importance in defining the performance characteristics of quantitative methods used in regulatory control programs for pesticide residues in foods. Ideally, the method needs to provide a signal response that is free from interferences from other analytes and matrix compounds that may be present in a sample or sample extract. Chromatographic analyses based on peaks, which are not fully resolved, provide less reliable quantitative results. Use of element-specific detectors or different detection wavelengths or MS-based detectors which are better able to distinguish a particular compound or structure, combined with chromatographic separation, improves the selectivity of quantitative methods.

36. [125]The requirement to recover a range of different pesticide residues in one extraction increases the potential for compromised selectivity in MRMs compared to single residue methods. Using less selective extraction and clean-up procedures is likely to result in greater co-extracted matrix material in the final extract. The nature and quantities of such co-extracted material can vary markedly based on the matrix method analytes of interest. Care is therefore required when setting criteria for the precision and trueness of MRMs to ensure that quantification will not be affected by chemical interferences.

37. [126]In addition to the selectivity of a method, the ability of the method to provide a reliable quantitative result must be demonstrated (i.e. trueness - see section F and precision – see section G). Ideally, the relative standard deviation between the original sample and replicates will be less than 30 percent.

38. [127]Acceptability criteria for a quantitative analytical method should be demonstrated at both initial and on-going validation stages, as being capable of providing acceptable mean recovery values at each spiking level. For validation, a minimum of 5 replicates is required (to check the recovery and precision) at the targeted LVL, LOQ, or reporting limit of the method, and at least one additional higher level, for example, 2-10x the LVL or the MRL. If a method is being used for compliance testing (i.e. if a commodity is complaint with an established MRL) the MRL (or CXL) must be one of the spiking levels. When the residue definition includes two or more analytes, the method should be validated for all analytes.

39. [128]The trueness of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another method for which the performance parameters have previously been rigorously established (typically a collaboratively studied method), or by determination of the recovery of analyte fortified into known blank sample material. Acceptable mean recoveries for enforcement purposes should range from 70-120% with a RSD ≤20%. In certain cases (typically with MRMs), recoveries outside this range may be acceptable, such as when recovery is lower but consistent (e.g. demonstrating good precision). This is more justifiable if the reason for the systematic low bias is well established by chemistry (e.g. known analyte distribution between phases in a partitioning step). However, a more accurate method should be used, if practicable. Recoveries >120% are likely to be attributable to a positive interference or bias that should be investigated.

40. [129]Analysis of incurred matrix to support method validation is encouraged. For interpreting recoveries, it is necessary to recognize that analyte spiked into a test sample may not behave in the same manner as the biologically incurred analyte (pesticide residue). In many situations, the amount of an extracted incurred residue is less than the total incurred residues actually present. This may be due to losses during extraction, intra-cellular binding of residues, the presence of conjugates, or other factors that are not fully represented by recovery experiments using analyte-fortified blank matrices.

41. [130]At relatively high concentrations, analytical recoveries are expected to approach one hundred percent. At lower concentrations, particularly with methods involving extensive extraction, isolation, and concentration steps, recoveries may be lower than at higher concentrations. Regardless of what average recoveries are observed, recovery with low variability is desirable so that a reliable correction for recovery can be made to the final result, when required.

42. [131]In general, residues data do not have to be adjusted for recovery when the mean recovery is within the range of 70-120%. Recovery corrections should be made consistent with the guidance provided by the CAC/GL 37-2001[7]. It is of over-riding importance that all data, when reported, should (a) be clearly identified as to whether or not a recovery correction has been applied and (b) include the amount of the correction and the method by which it was derived, if a recovery correction has been applied. This will promote direct comparability of data sets. Correction functions should be established on the basis of appropriate statistical considerations, and documented, archived and made available to the client.

43. [133]In accordance with ISO IEC17025[4], participation in a proficiency testing program should be done. Many proficiency testing schemes are available and affordable for laboratories worldwide that conduct pesticide residue monitoring. Inter-laboratory testing may also be performed.

[134]**PERFORMANCE ACCEPTABILITY CRITERIA OF METHODS FOR ANALYTE IDENTIFICATION AND CONFIRMATION**

44. [135]By far, gross errors (spurious mistakes made during sample preparation) are the greatest source of misidentifications in MS-based methods. For this reason, all regulatory enforcement actions (above an MRL or for those with no MRL on that commodity) require confirmation of the result via re-extraction of a replicate test portion of the original sample and re-analysis, ideally using different chemistries of sample preparation and/or analysis.

45. [136]Selectivity is the primary consideration for methods of identification. The method should be sufficiently selective to provide unambiguous identification. MS coupled to a chromatographic separation method is a very powerful combination for identification of an analyte in the sample extract. This method provides information about the structure of the analyte that is not obtainable with chromatography alone. GC-MS and LC-MS tools (full-scan, selected ion mode, high-resolution, tandem MS/MS, hybrid systems, among other advanced techniques) provide many measurable parameters, such as retention times, chromatographic peak shapes, ion intensities and relative abundances/ratios, mass accuracies, and other useful aspects to help make analyte identifications.

[137]**A. MS-Based Identification**

46. [138]There are no universally accepted criteria for identification. Table 1 gives examples of criteria.

[132][7] Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement. Pure & Appl. Chem., 71,1999; 337 – 348. CAC/GL 37-2001

47. [139]Current practices in qualitative (and quantitative) analysis of pesticide residues commonly involve chromatography + selected ion monitoring (SIM) or MS/MS techniques. Full-spectral (full-scan or time-of-flight) MS is also an acceptable tool that uses spectral library matching factors and/or relative abundances of major ions within the full spectra. The latter case can be treated as ion ratios in the criteria given below using at least 3 ions. In the former case, matching factors should be ≥900 (≥90% match) for regulatory identification purposes, and the library reference spectra should be obtained from background-subtracted high purity standards on the same instrument using the same conditions as in the sample analysis. The following identification criteria should be met:

a. [140]Analyte retention time reference values must be determined from contemporaneously analysed (within the same batch) high concentration calibration standards in solvent-based solutions (matrix-matched calibration standards may be used if it is known that no interferences are present).

b. [141]Ion ratio reference values are to be set in the same way as in paragraph 47 a. The different ions used for identification must co-elute and have similar peak shapes. The ion from the calibration standard with the higher average intensity is to be used as the denominator in the ion ratio, expressed in percentage (due to signal fluctuations, matrix effects, etc.… deviations of ion ratios up to 30% are acceptable).

c. [142]The signal to noise ratios for measured peaks must be greater than 3 and/or the signal must exceed the threshold intensity level as compared to the signal of a suitable calibration standard or control encompassing the level of interest.

d. [143]The ion transitions chosen for identification purposes should make chemical/structural sense (be sure that the ions chosen do not originate from a degradant, impurity, or confusion with a different chemical than the analyte).

e. [144]All measured reagent and matrix blank samples should be shown to be free of carry-over, contamination, and/or interferences above 20% of the LOQ.

48. [145]The minimum acceptable retention time for the analyte(s) 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 reference value (47a) within ±0.2 min or 0.2% relative retention time, for both gas chromatography and liquid chromatography.

49. [146]Methods based on high-resolution mass spectrometry are considered to provide improved reliability through accurate measurement of the mass/charge of the ion than cannot otherwise be obtained using unit-resolution mass spectrometry techniques. Different types and models of mass spectrometric detectors provide different degrees of selectivity, which relates to the confidence in identification. The example criteria for identification provided in Table 1.should only be regarded as guidance criteria for identification, not as absolute criteria to prove presence or absence of a compound.

[147]**B. Confirmation**

50. [148]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 CXL/MRL is exceeded, a confirmatory analysis of another test portion is always required. For unusual pesticide/matrix combinations, a confirmatory analysis is also recommended.

51. [149]If the initial confirmatory method is not based on an MS technique, the confirmatory methods should involve MS-based analyte identification. Moreover, the confirmatory methods should use an independent approaches based on different chemical mechanisms (such as LC and GC separations). In some situations, confirmation by independent laboratories may be appropriate. Examples of analytical techniques that may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 2.

[150]**Table 1. Identification criteria for different MS techniques**

[151]**MS detector / characteristics**	[152]**Typical systems (examples)**	[153]**Acquisition**	[154]**Requirements for identification**	
			[158]**minimum number of ions**	[159]**other**
[160]Unit mass resolution	[161]quadrupole, [162]ion trap, TOF	[163]full scan, limited m/z range, SIM	[164]3 ions	[165]S/N ≥ 3[e)] [166] [167]Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within [168]**±30% (relative)** [169]of average [170]of calibration standards from same sequence[f]
[171]MS/MS	[172]triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	[173]selected or multiple reaction monitoring, mass resolution for precursor-ion isolation equal to or better than unit mass resolution	[174]2 product ions	
[176]Accurate mass [177]measurement	[178]High resolution MS: TOF or Q-TOF [179]Orbitrap or Q-Orbitrap [180]FT-ICR-MS [181]sector MS	[182]full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	[183]2 ions with [184]mass accuracy [185]≤ 5 ppm[a,b, c)]	
		[189]combined single stage MS and [190]MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	[191]2 ions: [192]1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm[a,c] [193]*plus* [194]1 MS/MS product ion[d)]	

[196][a)] preferably including the molecular ion, (de)protonated molecule or adduct ion

[197][b)] including at least one fragment ion

[198][c)] < 1 mDa for m/z < 200

[199][d)] ≤10 ppm

[200][e)] in case noise is absent, a signal should be present in at least 5 subsequent scans

[201][f)] if the precursor mass accuracy is less than 5 ppm and the product ion mass accuracy is less than 10 ppm, ion ratio tolerances is optional

[202]**Table 2. Examples of detection methods suitable for the confirmatory analysis of substances**

[203]**Detection method**	[204]**Criterion**
[205]LC or GC and MS	[206]If sufficient number of fragment ions are monitored
[207]LC-DAD	[208]If the UV spectrum is characteristic
[209]LC – fluorescence	[210]In combination with other techniques
[211]2-D TLC – (spectrophotometry)	[212]In combination with other techniques
[213]GC-ECD, NPD, FPD	[214]Only if combined with two or more separation techniques
[215]Derivatization	[216]If it was not the first choice method
[217]LC-immunogram	[218]In combination with other techniques
[219]LC-UV/VIS (single wavelength)	[220]In combination with other techniques

[221]

[222]**ANNEX**

[223]**DEFINITIONS**

[224]**Analyte:** The chemical substance sought or determined in a sample (CAC/GL 72-2009).

[225]**Analyte protectant:** Compounds that strongly interacts to fill active sites in the gas chromatographic system, thereby reducing the analyte interactions with those active sites and yielding less peak tailing or losses, thus a higher analyte response.

[226]**Analytical quality controls**: Calibration standards, blanks, spikes, reference sample, systems suitability sample, or similarly laboratory-generated analytical test designed to verify if the batch (sequence) of samples being analysed meet the specified performance characteristics (data quality objectives).

[227]**Applicability**: The analytes, matrixes, and concentrations for which an analytical method can be used satisfactorily (CAC/GL 72-2009).

[228]**Coefficient of Variation (CV):** Often referred to as the Relative Standard Deviation (RSD). This is a measure of precision in quantitative studies comparing the variability of sets with different means.

[229]**Confirmation**: The combination of two or more analyses that are in agreement with each other, at least one of which meets identification criteria.

[230]**Confirmatory method:** A method that is capable of providing complementary information in agreement with a previous result. Ideally, a different subsample is analysed with a method involving a different chemical mechanism than in the first analysis, and one of the methods meets analyte identification criteria with an acceptable degree of certainty at the level of interest.

[231]**False positive:** A result wrongly indicating that the analyte is present or exceeds a specified concentration (e.g. CXL/MRL or reporting level).

[232]**False negative:** A result wrongly indicating that the analyte is not present or does not exceed a specified concentration (e.g. CXL/MRL or reporting level).

[233]**Fortification**: Addition of analytes for the purposes of determining the recovery (also known as spiking).

[234]**Identification:** Process of unambiguously determining the chemical identity of an analyte or its metabolite(s) in an analysis.

[235]**Incurred residue:** Residue occurring in a commodity resulting from specific use of a pesticide or from consumption by an animal or environmental contamination in the field, as opposed to residues present due to laboratory fortification of samples.

[236]**Interference:** Intrinsic or extrinsic response unrelated to an analyte (e.g. noise) due to electronic, chemical, or other factors related to the instrumentation, environment, method, or sample.

[237]**Interferent**: A chemical or other factor causing an interference

[238]**Internal standard (IS):** A chemical added at a known amount to samples and/or standards in a chemical analysis, including the blank and calibration standards. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the concentrations. This ratio for the samples is then used to obtain the analyte concentrations. The internal standard used needs to provide a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable from each other.

[239]**Limit of quantification (LOQ):** The lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. In practice, this is typically the analyte concentration at which the average signal/noise is 10. [See also paragraph 26].

[240]**Linearity**: The ability of a method of analysis, within a certain range, to provide an instrumental response or results, proportional to the quantity of analyte to be determined in the laboratory sample (CAC/GL 72-2009).

[241]**Lowest Calibrated Level (LCL):** The lowest concentration (or mass), which the determination system is successfully calibrated, through the analysis batch.

[242]**Lowest Validated Level (LVL):** The lowest validated spiking level meeting the method performance acceptability criteria.

[243]**Matrix:** The material or component sampled for pesticide residue studies.

[244]**Matrix blank:** Sample material or sample portion containing no detectable concentration of the analytes of interest.

[245]**Matrix effect**: An influence of the one or more undetected components from the sample on the measurement of the analyte concentration or mass.

[246]**Matrix-matched standards:** Standard solutions prepared in final extracts of matrix blanks similar to that of the sample to be analysed which is intended to compensate for matrix effects and possible interferences during analysis.

[247]**Maximum residue level/limit (MRL/CXL):** Maximum concentration of a residue that is legally permitted or recognized as acceptable in, or on, food commodities as set by Codex (CXL) or a national regulatory authority (MRL). The term “tolerance” used in some countries is, in most instances, synonymous with MRL (normally expressed as mg/kg product weight).

[248]**Measurement uncertainty**: Parameter associated with the results of a measurement, characteristic of the dispersion of the values that could be reasonably attributed to what is measured.

[249]**Multi-class method**: Method which allows simultaneous measurement of 2 or more residue groups (or families).

[250]**Multiresidue method (MRM):** A method which can determine a large number of compounds typically from different chemical classes

[251]**Precision**: Degree of variability of a measurement around a mean.

[252]**Quantitative method**: A method capable of producing analyte concentration (determinative) results with trueness and precision that comply with established criteria.

[253]**Recovery**: Amount measured as a percentage of the amount of analyte(s) (active substance and relevant metabolites) originally added to a sample of the appropriate matrix, which contains either no detectable level of the analyte or a known detectable level. Recovery experiments provide information on both precision and trueness and thereby the accuracy of the method.

[254]**Relative Standard Deviation (RSD):** The standard deviation, divided by the absolute value of the arithmetic mean, expressed in percentage. It refers to the precision of the method (also known as coefficient of variation-CV).

[255]**Repeatability**: Precision usually expressed as RSD, obtained from the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time (CAC/GL 72-2009).

[256]**Reproducibility**: Precision (typically expressed as RSD) from observation conditions where independent test/measurements results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment (CAC/GL 72-2009).

[257]**Ruggedness**: A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate various in method parameters and provides an indication of its reliability during normal usage (CAC/GL 72-2009).

[258]**Sample preparation:** Involves the extraction of a test portion of the sample, its clean-up and other steps in the method that leads to a final extract for analysis.

[259]**Sample processing**: Procedure to yield a test portion for analysis that is representative of the collected sample and maintains the integrity of the analytes. This involves cutting, homogenization, comminution, blending, or other means using appropriate techniques and equipment depending on the sample type and sizes of the collected sample and test portions.

[260]**Screening Detection Limit (SDL):** Lowest level of fortification that has been shown to have certainty at a 95% confidence level.

[261]**Screening Method:** A method that meets predetermined criteria to detect the presence, or absence, of an analyte or class of analytes, at or above the minimum concentration of interest.

[262]**Selectivity:** The extent to which a method can determine particular analyte(s) in a mixture(s) or matrices(s) without interferences from other components of similar behaviour (CAC/GL 72-2009).

[263]**Sensitivity:** Quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured (CAC/GL 72-2009).

[264]**Single Residue Method**: A method which determines a single analyte or a small group of analytes with similar physico-chemical properties.

[265]**Standard addition**: The method of standard addition is a type of quantitative analysis approach sometimes used in analytical chemistry whereby a known quantity of analyte is added directly to the aliquots of final extracts.

[266]**Trueness**: The closeness of agreement between the average of an infinite number of replicate measured quantity value and a reference quantity value (CAC/GL 72-2009).

[267]**Uncertainty**: A parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurement.