Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed SANCO/10684/2009

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Outline of the presentation

(A) Goal and background of the guidelines

(B) History of the EU QC guidelines

(C) Procedure for the revision (Advisory group, Questionnaire, National Reference Laboratories, Workshop)

(D) Previous revisions

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The guidance in this document is intended

- For laboratories involved in the official monitoring of pesticide residues in food and feed in the European Union.

- The document describes the analytical quality control (AQC) requirements and the method validation

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Legal basis

The document entails mutually acceptable scientific rules for official pesticide residue analysis within the EU as agreed by all Member States of the European Union and constitutes a technical guideline in the sense of article 28 of Regulation 396/2005. It should thus be consulted in audits and accreditations of official pesticide residue laboratories according to ISO/IEC 17025.

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Requirements for official laboratories

1. Implementation of ”Method validation and quality control procedures for pesticide residues analysis in food an feed”

2. Accredited according to the ISO/IEC 17 025 standard

3. Participation in EU Proficiency tests

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Why do we need the guidelines?

- To harmonize cost effective AQC in the EU (to find an optimum between cost and output (efficiency/quality)
- To help monitoring laboratories achieve an acceptable standard
- The reported results are reliable and consistent with other similar results
- To support compliance with ISO/IEC 17025 accreditation standard

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Introduction - history of the guidelines

Harmonised guidelines were discussed first at the EU Workshop on Coordinated Analytical Quality Control 1997

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Reviews:

2. Doc. SANCO/3103/2000
   -discussed at 2nd EU AQC,1999, in Greece
3. Doc. SANCO/10476/2003
   -discussed at 3rd EU AQC, 2003 in UK
4. Doc. SANCO/10232/2006
   -discussed at 4th EU AQC, 2005 in Sweden
5. Doc. SANCO/3131/2007
   -discussed at 5th EU AQC, 2007 in Spain (EU RL)
6. Doc. SANCO 10684/2009
   -discussed at 6th EU AQC, 2009 in Copenhagen (EU RL)
7. Doc. SANCO xxxx/2011
   -will be discussed at 7th EU AQC, 2011 in Freiburg (EU RL)

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Advisory group-AVG

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• Miquel Gamón	EU RL-FV
• Amadeo Fernández R. Alba	EU RL-FV
• Ralf Lippold	EU RL-AO
• Michelangelo Anastassiades	EU RL-SRM
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• Sonja Messelter (2009)	A
• Hans Mol (2009)	NL
• Darinka Steinberger (2011)	SV
• Magnus Juzkec (2011)	D
• Luis Martin-Plaza	COM
• Tuija Pihlström (Coordinator)	SE

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Mechanism for updating the guidelines

- Selection of issues/topics in guidelines that need updating by the Advisory group
- Questionnaire to be sent to the NRLs/official laboratories
- Workshop –the draft document is discussed
- Publication of the revised document (COM)
- Since 2006 – EU Reference Laboratories are responsible for the revision

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Guidance in

- The whole analytical chain (from sampling to reporting results)-quality control
- To define minimum criteria for validation and analysis (allows free choice of method)

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Table of contents

- Accreditation
- Sampling, transport, processing and storage of samples
- Pesticide standards, calibration solutions, etc.
- Extraction and concentration
- Contamination and interference
- Analytical calibration, representative analytes
- Analytical methods and analytical performance
- Method validation
- Routine recovery determination
- Proficiency testing and analysis of reference materials
- Confirmation of results
- Reporting of results

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Revision in 2005:
Main topics in the previous revisions

Proposed and revised

- Confirmation by masspectrometry –more detailed guidance on both qualitative and quantitative aspects
- Measurement uncertainty – default figure for measurement uncertainty
- Correction for recoveries- to correct or not to correct

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Revision in 2007:
Main topics in the previous revisions

Proposed and revised

- Frequencies and minimum number of analytes for *calibration* Table 1
- Frequencies and minimum number of analytes for *routine recovery* Table 2
- Validation – inclusion of animal products and cereals
- Common interpretation of results in respect of the correction of results for recovery

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Revision in 2009:

Main topics in the previous revisions

Proposed and revised

- Mass spectrometry-requirement for identification
- New definitions-identification /confirmation
- Appendix A :The Validation procedure and examples
- Appendix B : Examples of conversion factors
- Annex 1: Selection of representative matrices
- Qualiatative screening methods
- Reporting results - correction for recoveries

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Main topics in the previous revisions
Measurement uncertainty

Purpose:

A general fixed figure for measurement uncertainty

In order to:

- unify the approaches of measurement uncertainty
- have onsistency in enforcement

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A default expanded uncertainty figure for enforcement authorities

- Based on the results of the first 7 EU proficiency test on fruit and vegetables
- As a result, for most pesticide/matrix combinations the inter-laboratory reproducibility is RSD wR<25%

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A default expanded uncertainty figure of 50%

- With 95% confidence within ±2SD (k=2), which leads to expanded uncertainty value of 50%
- An exceedence of the acute reference dose, an expanded uncertainty with a lower confidence level can be applied
- Provided that the laboratory proves its own calculated expanded uncertainty to be less than 50%.

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Definitions

Ex. MRL =1 mg/kg

1) Found residue = 1.0 mg/kg

-> no exceedence of the MRL

2) Found residue = 1.1 mg/kg

-> exceedence of the MRL no enforcement

3) Found residue = 2.2mg/kg

-> exceedence of the MRL and enforcement taking account 50 % measurement uncertainty

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Main topics in the previous revisions
Confirmation of results

Regarding paragraphs “confirmation by mass spectrometry” needs updating and expanding. More detailed guidance is required, particularly there are needs to provide guidance on both qualitative (detection/confirmation) and quantitative (determination) aspects.

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"Confirmation by mass spectrometry"

Requirements for mass spectrometry

1) *Identification*

- Identification relies on proper selection of diagnostic ions
- Different types and modes of mass spectrometric detectors provide different degrees of selectivity, which relates to the confidence in identification. The requirements for identification are given in
- Table 3. Identification requirements for different types of mass spectrometers.

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Table 3. Identification requirements for different types of mass spectrometers

MS mode:	Single MS (standard mass resolution)	Single MS (high resolution/high mass accuracy)	MS/MS
Typical systems (examples)	quadrupole, ion trap, time-of-flight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole ion trap, hybride MS (e.g. Q-TOF, Q-trap)
Acquisition:	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for identification:	≥ 3 diagnostic ions, (preferably including quasi molecular ion)	≥ 2 diagnostic ions (preferably including the quasi molecular ion). Mass accuracy < 5 ppm. At least one fragment ion.	≥ 2 product ions
Ion ratio(s):	according to Table 5		

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"Confirmation by mass spectrometry"

2) *Determination* (= quantitative result according to the AQC criteria)

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

- i) retention time;
- ii) ion mass/charge ratio; and
- iii) abundance data

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New definitions in the Glossary

Table 5 **Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques**

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

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“Confirmation by mass spectrometry”

3) Confirmation (= two or more analysis)

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Main topics in the previous revisions

Common interpretation of results in respect of the correction of results for recovery

Purpose and an intended effect of the correction:

1. Correction should improve the results- closer to the "true value"
2. The result can be compensated for the incomplete extraction of the analyte from sample
3. Common interpretation in regulation of contaminants/VMPs takes account correction for recovery

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Main topics in the previous revisions

Common interpretation of results in respect of the correction of results for recovery

SANCO 10232/2006

§83 Residue data exceeding an MRL must be corrected for recovery. The adjustment should be stated based either using the mean value from three recoveries performed in same matrix and analysed in the same batch or using two standard additions e.g. at two and five times the residue in the sample. In general, residues below MRLs are not to be adjusted for recovery, when the batch recoveries fall within the acceptable range. If residue data are adjusted for recovery this should be done as described above and must be stated.

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Main topics in the previous revisions

Common interpretation of results in respect of the correction of results for recovery

How to measure "recovery":

1. The mean value of 3 recoveries analysed in the same matrix/batch as proposed. Too laborious and not normal praxis with 3 recovery tests in the same matrix.
2. At which level should the recoveries be measured?
3. Normal praxis one single (or two) recovery
4.

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Main topics in the previous revisions

Common interpretation of results in respect of the correction of results for recovery

4. What is criteria for acceptable recovery? From validation?
5. Residue data to establish MRL are not corrected for recovery
6. To correct residues >MRL but not <MRL?

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Reporting results

§83 In general, residues data do not have to be adjusted for recovery, when the mean recovery is in the range of 70-120% . If they are adjusted for recovery, then this must be stated.

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Reporting results

66. Data on numerical exceedences of the MRL residues must be supported by individual recovery results in the same batch within the range of the mean recovery (70-120 %) ± 2 x RSD, at least for the confirmatory analyses. If recovery within this range cannot be achieved, enforcement action is not necessarily precluded, but the risk of relatively poor accuracy must be taken into account. It is recommended to correct for recovery preferably by using standardaddition according to paragraph 47 or isotopically labelled standards in all cases of violation

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2007 Analysis of regular samples

Calibration of the detection system **QC**

The detection system should be *calibrated (=checked) with all analytes for every batch of analyses.

*at one level =calibration

Purpose of the calibration:

To avoid false negatives

To test sensitivity of the detection system

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How to select representative analytes for calibration?

- Regularly found pesticides
- Occasionally found pesticides

- ”Difficult” pesticides (unstable, most volatile, most polar, non-polar)

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Analysis QC

Representative analytes

"The representative analytes to be calibrated in each batch must be at least 15 analytes plus 25% of the total number of analytes included in the analytical scope of each determination system."

For example, if the analytical scope of an instrument method covers 40 analytes, the determination system must be calibrated with at least 25 representative analytes. If the scope of analysis in determination system is 20 or less, then all analytes should be calibrated."

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Analysis of regular samples
Table 1. Minimum frequencies for calibration

	Representative analytes		All other analytes
Minimum frequency of calibration	Calibration in each batch of analyses. At least at the level corresponding to the reporting limit.		A rolling programme at least every third month At least at the level corresponding to the reporting limit

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Analysis of regular samples QC

Frequency for routine recovery

Purpose:

Acceptable screening and method at the time of analysis for all analytes searched

In a perfect world - recovery of all analytes measured with each batch.

If not possible the minimum of acceptable frequency of recovery and number of analytes is given.

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Table 2. Frequency for routine recovery and performance verification

	Representative analytes	All other analytes
Minimum frequency of recovery	10% of representative analytes (at least 5 per detection system) in each batch of analyses	Within rolling programme to include all other analytes at least every 12 months, but preferably every 6 months
	Within a rolling program covering all representative analytes as well as different types of commodities, at different concentration levels including the level corresponding to the reporting limit	At least at the level corresponding to the reporting limit.

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Appendix B.
Examples of conversion factors

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

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Appendix B: Examples of calculation of conversiong factors for residue definition

To types of ”SUM”

EX 1: Aldicarb (sum of aldicarb + aldicarb sulfoxide + aldicarb sulfone expressed as aldicarb)

EX 2: Triadimefon and Triadimenol (sum of triadimefon and triadimenol

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Residue Definition

Fenthion, its sulfoxides and sulfones, and their oxygen analogues (oxons), all appear in the residue definition and all should be included in the analysis.

$$C_{\text{Fenthion Sum}} = 1.00 \times C_{\text{Fenthion}} + 0.946 \times C_{\text{Fenthion SO}} + 0.897 \times C_{\text{Fenthion SO2}} + 1.06 \times C_{\text{Fenthion oxon}} + 1.00 \times C_{\text{Fenthion oxon SO}} + 0.946 \times C_{\text{Fenthion oxon SO2}}$$

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The validation procedure
Representative matrices

Validation needs to be performed:

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

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The validation procedure
Representative matrices

Selection of representative matrices according to their biological or "analytical" properties

(water, sugar, lipid, pH)

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Example of representative commodities

Commodity Categories	Commodities included in this category	Typical representative commodities
High water content	Pome fruit Stone fruit	Apples, pears Apricots, cherries, peaches,
High acid content and high water content	Citrus fruit Berries Currants	Lemon, mandarin, orange Strawberry, blueberry

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Example of representative commodities

Commodity Categories	Commodities included in this category	Typical representative commodities
Meat	Read meet White meat Fish Offal	Beef, Pork, game, Chicken, duck Cod, salmon Liver , kidney
Milk and milk products	Milk Cheese Butter	Cow, buffalo, coat
Eggs	Eggs	Cicken, dick,quail
Honey	Honey	

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The validation procedure

We distinguish between initial validation of a quantitative analysis method to be applied in the laboratory for the first time and to extension of the scope of an existing validated method for new analytes and matrices.

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Multi residue method (MRM)
Procedure of validation

Quantitative Initial full validation

Method fully validated

Quantitative extension of the scope

Extension of the scope to **new analytes/matrices**

Full validation for new analytes

Simplified validation for new matrices

Quantitative on going method verification (QC data)

Recovery study for other matrices which results will be reported

One level/one replicate

Qualitative validation of screening methods

Future topic?

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Examples of validation (NFA) in MRM

Initial full validation		
Recoveries	Level	Two levels 0.01 and 0.05 mg/kg
	Replicates	5 /level
	Matrix	One from each group (mainly analysed)
	Quantification	Matrix matched
Repeatability	RSD_r	1) Calculate RSD_r at each level/matrix
		2) Overall RSD_{wR} n=30 (all matrices/levels) at each level

Validation of qualitative screening methods

- Focus on detectability
- The detection at the lowest at least in 95% of the samples (i.e. a false-negative rate of 5% is accepted)
- There are no requirements with regard to linearity and recovery.

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Conclusions

- When the document is practical, flexible and general it makes it easier to apply by many more laboratories (=better for all)
- Strict and general -more general guidelines are preferred since too specific requirements will lead to disagreements
- Minimum criteria define which allows laboratories free choice of methods
- Reviewed every second year keeping abreast with technical developments

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http://europa.eu.int/comm/food/plant/protection/resources/qualcontrol_en.pdf

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Any questions?

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