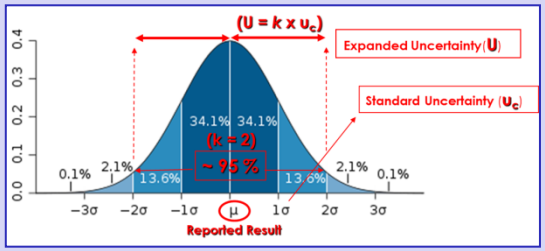
Document «**AQC** EU-SANTE»

***A**nalytical **Q**uality **C**ontrol and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*

Document SANTE/11312/2021



Measurement Uncertainty (MU) in the AQC EU-SANTE Document

Part II

Guidelines on Reporting and Interpreting Results Qualified with Measurement Uncertainty

(SANTE/11312/2021: Section E – Reporting Results)

Antonio Valverde & Carmen Ferrer
Pesticide Residues Research Group & EURL-FV
University of Almería, Spain

This tutorial has been prepared on behalf of the EURL-FV

«Uncertainty» in the Document SANTE/11312/2021

- A.- Introduction and legal background
- B.- Sampling, transport, traceability and storage of samples
- C.- Sample Analysis
- D.- Identification of analyte[illegible]
- **E.- Reporting results**
- F.- Pesticide standards, sto[illegible]
- G.- Analytical method validation and performance criteria
- H.- Additional recommendations

Correction for METHOD BIAS — **E4 – E5**

Qualifying results with UNCERTAINTY — **E7 – E10**

INTERPRETATION of results — **E11 – E14**

- Annex A *(Commodity groups and representative matrices)*
- Appendix A *(Method validation procedure: outline and example approc[illegible])*
- Appendix B *(Examples of conversion factors)*
- **Appendix C *(Examples for the estimation of measurement uncertainty)***
- Appendix D *(Example of rounding, reporting and interpreting results)*
- Appendix E *(Glossary)*

Already discussed in Part I

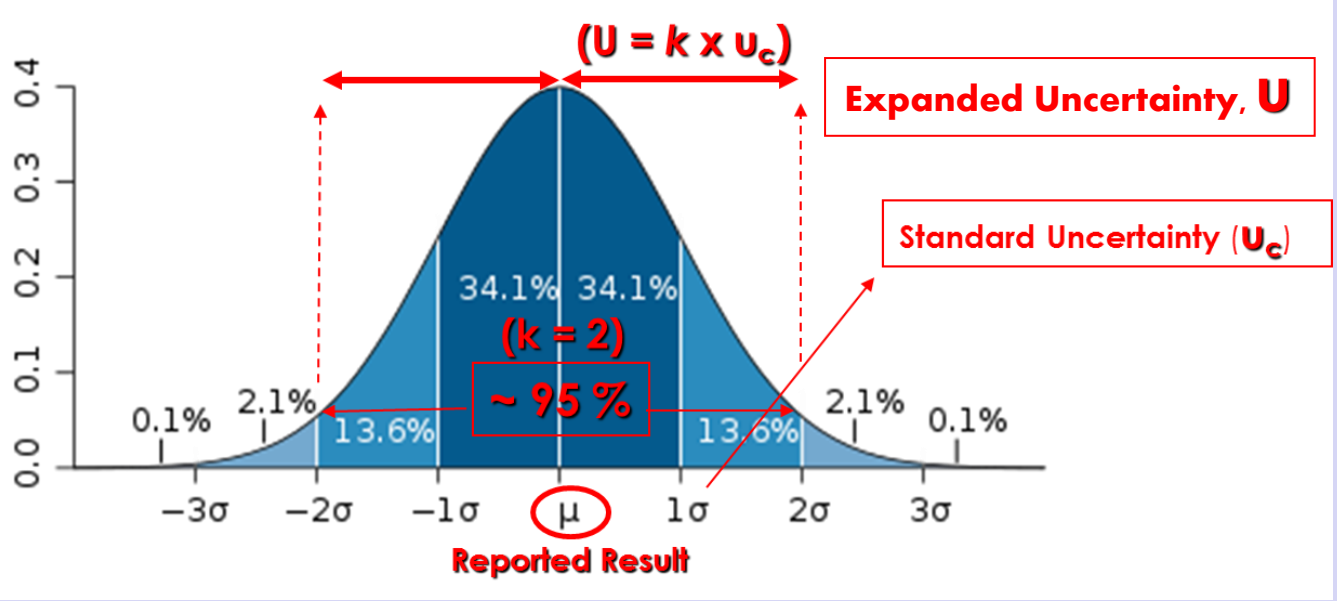
Document Nº SANTE/12682/2019

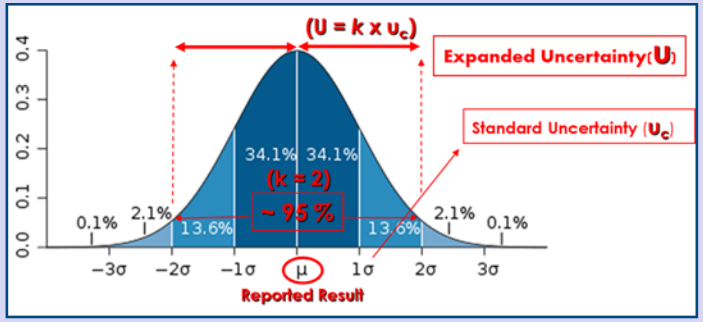
Measurement Uncertainty (MU)

GUM (BIPM, IEC, IFCC, ISO, IUPAC, OIML)
Guide to the Expression of Uncertainty in Measurement
(ISO, Geneva, 1993 - Reprint 1995 – ISO Guide 98-3, 2008)
ISBN: 92-67-10188-9

"A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributted to the measurand"

If the dispersion of the measured values is characterized by a NORMAL DISTRIBUTION:





The Key

The estimated UNCERTAINTY must be REALISTIC

- A standard deviation ***(combined standard uncertainty "u_c")***
- The width of a confidence interval ***(expanded uncertainty "U")***
- $U = k \, . \, u_c$ *(95% confidence with k = 2 for Normal Distribution)*

Result = Measurement ± expanded uncertainty (k = 2; 95%)

A Realistic Pesticide Residue Test Result

~~0.85 ± 0.01 mg/kg~~

0.85 ± 0.30 mg/kg *(k = 2; 95%)*

u_c = 0.15 mg/kg
U = 0.30 mg/kg
U´ = 35 %

from 0.55 to 1.15 mg/kg!

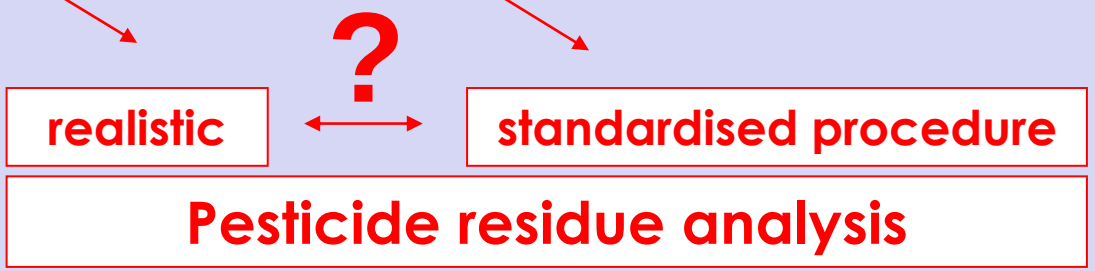
GUM Fundamentals

GUM (BIPM, IEC, IFCC, ISO, IUPAC, OIML)
Guide to the Expression of Uncertainty in Measurement
(ISO, Geneva, 1993 - Reprinted 1995 – Reissued as ISO Guide 98-3, 2008)

"A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributted to the measurand"

- A realistic uncertainty statement always improve the quality of the result

- Transparent and standardised procedure for evaluation / expression.



Uncertainty – Analytical Measurement Guidelines

EURACHEM / CITAC Guide CG 4 (QUAM:2012.P1)
Quantifying Uncertainty in Analytical Measurement (3rd Edition, 2012)
Example A4: Pesticide Multiresidue Analysis

NORDTEST Technical Report TR537 (Ed. 3.1)
Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories (2012)

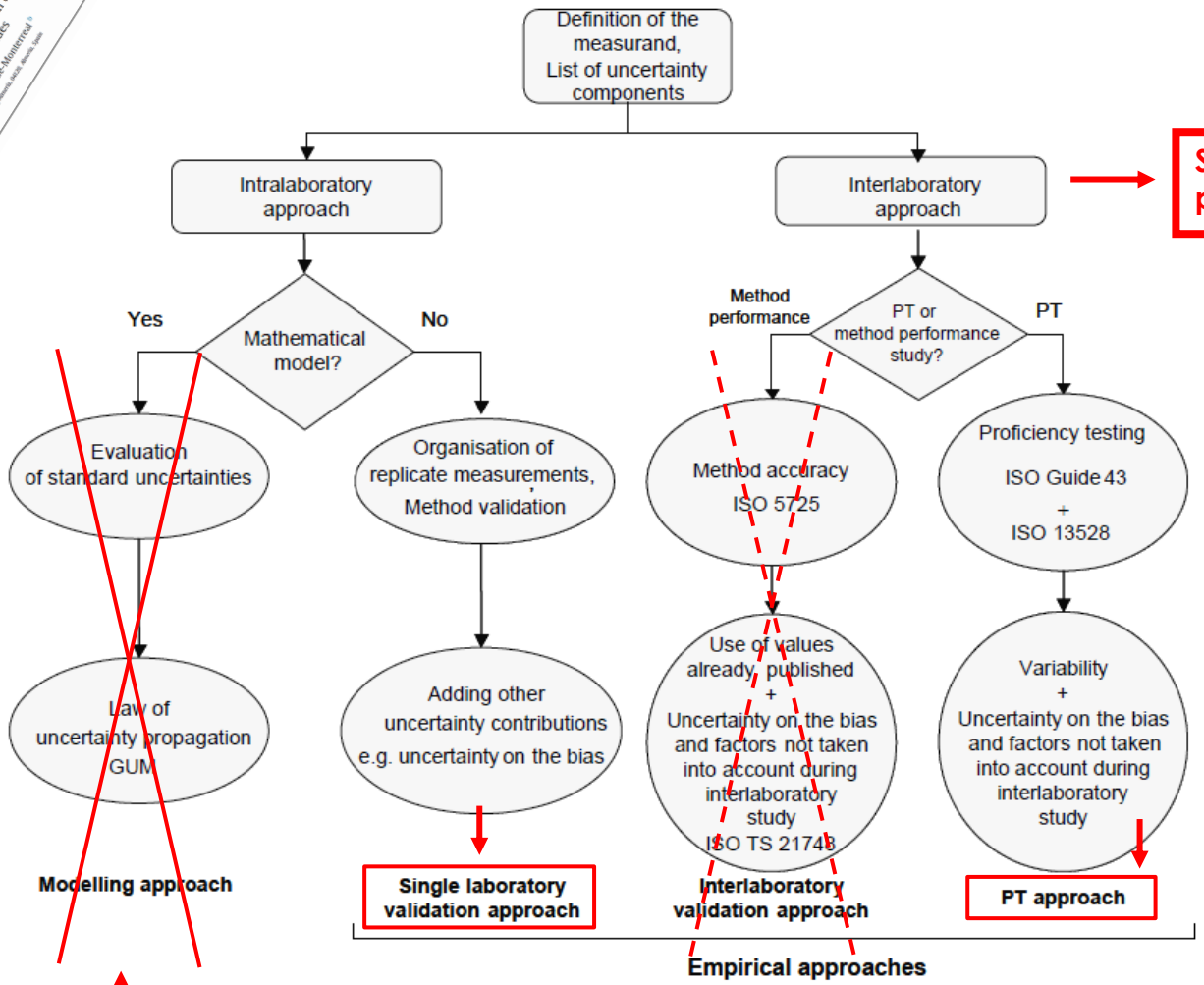
EUROLAB Technical Report No. 1/2006
Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results (2006)

EUROLAB Technical Report No. 1/2007
Measurement Uncertainty Revisited: Alternative Approaches to Uncertainty Evaluation (2007)

Codex Guidelines - Uncertainty - Pesticide Residues

CAC/GL 59-2006 *(Amendment 2011)*
Guidelines on Estimation of Uncertainty of Results

EUROLAB road map for uncertainty estimation approaches
(modified for Multiresidue Analysis of Pesticides)



Default-Fixed Fit-For-Pourpose Value

Scientific judgements previous experience

Horwitz Equation concentration -dependent

William Horwitz (J. AOAC Int. 86, 109-111. 2003): ***«This absurd and budget-busting approach (for analytical chemistry) arose from metrological chemists taking over in entire the concepts developed by metrologists for physical processes …»***

ISO/IEC 17025: 2017
7. Process Requirements

7.6. Evaluation of mesaurement uncertainty

7.6.3 «A laboratory performing testing shall evaluate measurement uncertainty. Where the test method precludes rigorous evaluation of measurement uncertainty, an estimation shall be made based on an understanding of the theoretical principles or practical experience of the performance of the method.»

No doubt, it refers to Pesticide Residue Analysis …

… and most of the analytical test methods!!!

«Uncertainty» in the Document SANTE/11312/2021

- A.- Introduction and legal background
- B.- Sampling, transport, traceability and storage of samples
- C.- Sample Analysis
- D.- Identification of analyte[illegible]
- **E.- Reporting results**
- F.- Pesticide standards, sto[illegible]
- G.- Analytical method validation and performance criteria
- H.- Additional recommendations

Correction for METHOD BIAS

Qualifying results with UNCERTAINTY

INTERPRETATION of results

- Annex A *(Commodity groups and representative matrices)*
- Appendix A *(Method validation procedure: outline and example approaches)*
- Appendix B *(Examples of conversion factors)*
- **Appendix C *(Examples for the estimation of measurement uncertainty)***
- Appendix D *(Example of rounding, reporting and interpreting results)*
- Appendix E *(Glossary)*

Qualifying Results with UNCERTAINTY & **INTERPRETATION of Results**

E12 A **default expanded MU of 50%** … is recommended to be used by regulatory authorities in cases of enforcement decisions (MRL- exceedances) …

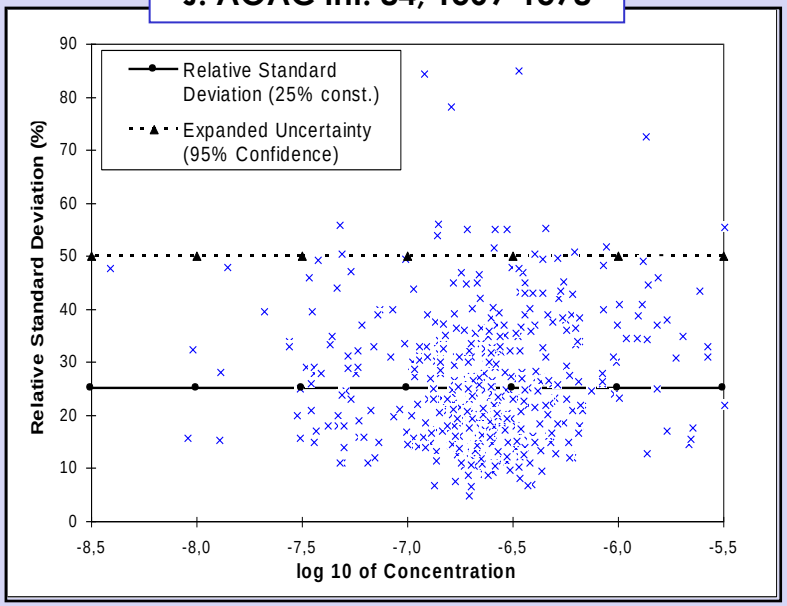
Document SANTE/11312/2021

Qualifying Results with UNCERTAINTY & INTERPRETATION of Results

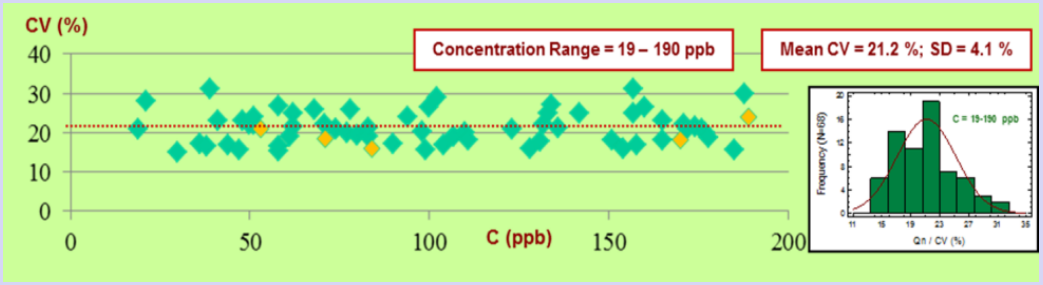
E12 A **default expanded MU of 50%** … is recommended to be used by regulatory authorities in cases of enforcement decisions (MRL- exceedances) …

Document SANTE/11312/2021

Alder et al. (2001)
J. AOAC Int. 84, 1569-1578



EUPT-FV



Qualifying Results with UNCERTAINTY & **INTERPRETATION of Results**

E12	A **default expanded MU of 50%** … is recommended to be used by regulatory authorities in cases of enforcement decisions (MRL- exceedances) …
E12	… A prerequisite for the use of 50% default expanded MU is that the laboratory must demosntrate that its **own expanded MU** is less than 50%...

Document SANTE/11312/2021

Qualifying Results with UNCERTAINTY & INTERPRETATION of Results

E12 … A prerequisite for the use of 50% default expanded MU is that the laboratory must demosntrate that its **own expanded MU** is less than 50%...

Document SANTE/11312/2021

Tutorial – Part I

Appendix C (SANTE/11312/2021) - Approach 1

Intra-Laboratory Validation/QC

$$u' = (u'_{bias}{}^2 + u'_{precision}{}^2)^{1/2}$$

(RSD_{wR})

(N) Recovery Tests

- Recovery (%) = (found level/spiking level)*100
- Relative bias (bias´)(%) = (recovery – 100)
- Mean-recovery (%)
- Mean relative bias (Mean-bias´) (%)
- Standard Deviation of recovery (SD-recovery) (%)
- Standard Deviation of relative bias (SD-bias´) (%)
- Relative Standard Deviation of recovery (RSD_{wR}) (%)

$(\sum(bias')^2/N) = (Mean\text{-}bias')^2 + (SD\text{-}bias')^2$

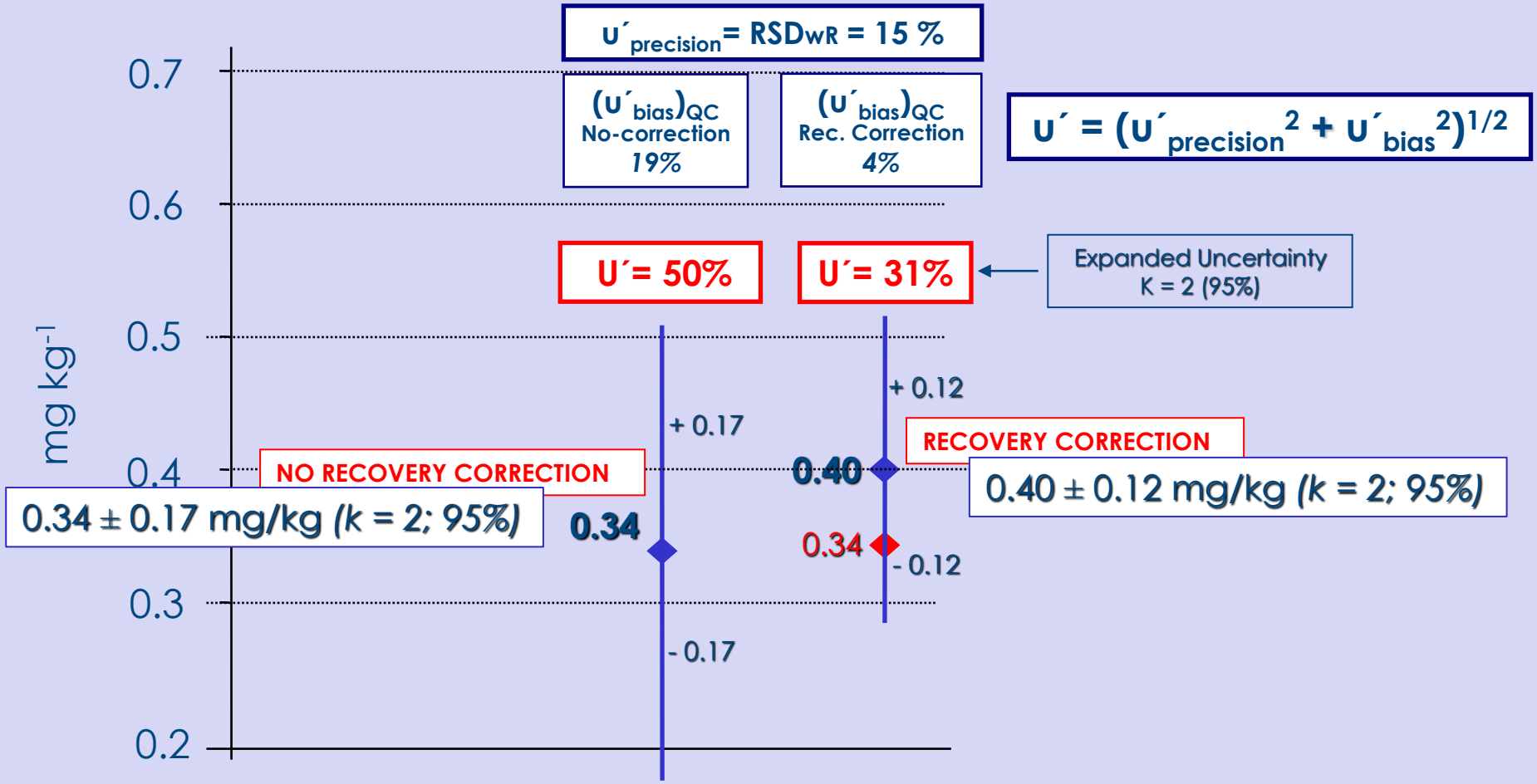
$$u'^2 = (Mean\text{-}bias')^2 + (SD\text{-}bias')^2 + (RSD_{wR})^2$$ NO RECOVERY CORRECTION

$$u'^2 = (RSD_{wR})^2/N + (RSD_{wR})^2$$ RECOVERY CORRECTION

Example in Part I of this Tutorial

Intra-laboratory QC Recovery data (3 months)
N = 14; Mean Rec = 86%; RSD_{wR} = 15%; *(Mean-bias´ = 14 %)*

A measurement result of 0.34 mg/kg reported with correction and no correction for recovery



Correction of results with recovery factors?

According to GUM, a measurement result should always be corrected if the bias is significant and based on reliable data such as Certified Reference Materials

We use spiked QC samples as "Certified Reference Materials"!!!!

Mean Recovery values resulting in significant bias
(Student´s t-test; 2-tailed; 95% confidence)

N	RSD_{wR}	
	10%	20%
5	Mean Rec < 88 %	Mean Rec < 75%
10	Mean Rec < 93%	Mean Rec < 86 %
20	Mean Rec < 95 %	Mean Rec < 91 %
50	Mean Rec < 97 %	Mean Rec < 95 %

N = 14 and RSD_{wR} = 15 %; Significant bias when Mean Rec < 91 % aprox

Mean Recovery = 86% → RECOVERY CORRECTION

Correction for METHOD BIAS

Document SANTE/11312/2021

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

E4 … In case the bias exceeds 20%, the result can be mathematically corrected using a **recovery factor … (100%/recovery%) …**

Correction for METHOD BIAS

Document SANTE/11312/2021

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

E4 … In case the bias exceeds 20%, the result can be mathematically corrected using a **recovery factor … (100%/recovery%) …**

E4 … Regarding the recovery % to be used for correction for recovery, there are multiple options. These include the mean recovery obtained during initial validation, the mean recovery obtained during on-going validation, or the (mean) recovery obtained for spiked samples concurrently analysed with the samples**.** The most appropriate option depends on the recovery data available for a method for the various pesticides and matrices, and may therefore differ for different laboratories.

Correction for METHOD BIAS

Document SANTE/11312/2021

E5 … alternative approaches to reduce method bias may be considered to avoid the need for recovery correction, e.g. the use of standard addition before sample extraction, addition of an isotopically labelled internal standard (IL-IS) before sample extraction, or the use of procedural calibration.

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

Table 1. *Analytical procedures to reduce method bias*

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

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

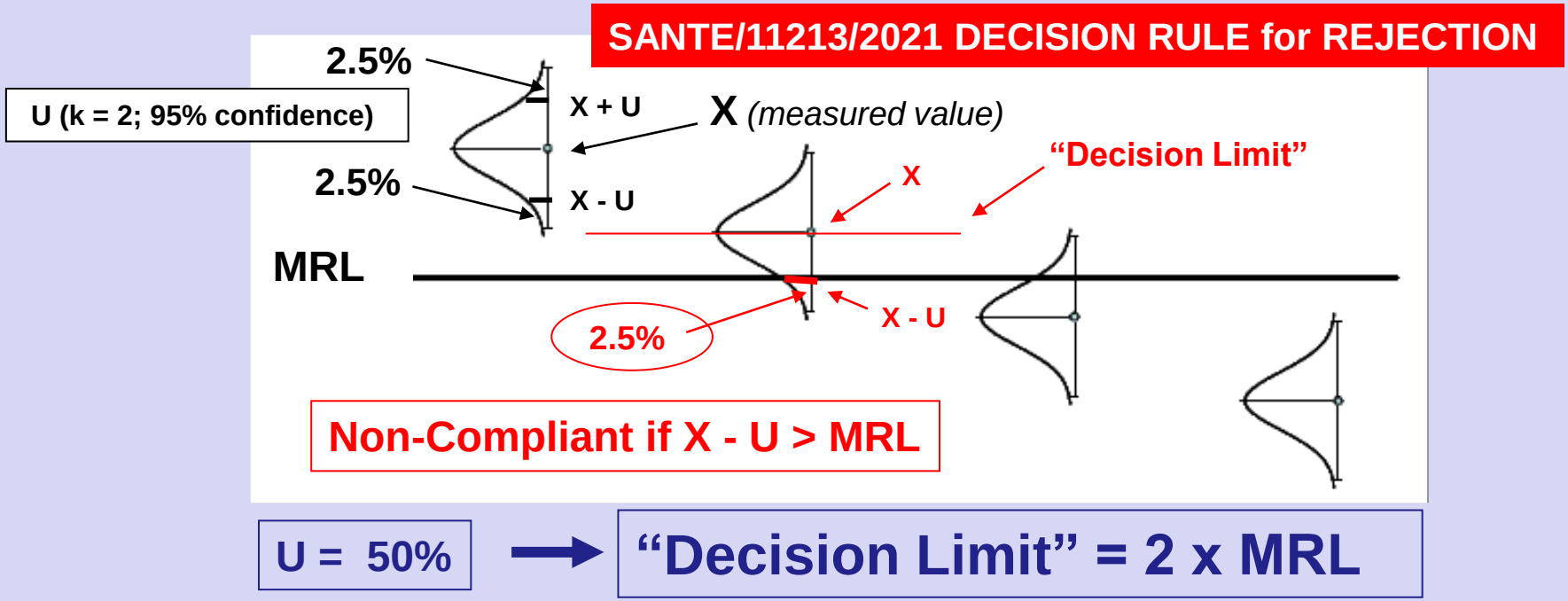
		Corrects for bias due to				
Option	**Procedure**	losses during the extraction	cleanup losses	injection errors	matrix effects	cross reference
Recovery correction	mathematical correction for recovery = result obtained * 100%/recovery% [1]	yes [2]	yes	no	na [3]	E4
Recovery used for correction	**What/how**	**Pros**		**Cons**		
1. Average recovery from on-going validation	Take the average recovery of spiked samples concurrently analysed with the samples over a longer period of time. Different concentrations and matrices from one commodity group can be combined when analyte behaviour is similar.	Correction based on multiple recoveries. Reflects variation in time. Representative for matrices within commodity group. Reflects multiple concentrations.		Especially for labs with limited sample numbers and/or high variability in sample matrices: Data may not be available, or not for all commodity groups.		
2. Average recovery from initial validation	Take the average recovery across different concentrations. In case validation is done for more than one matrix from the commodity group and analyte behaviour is similar, the average of all data can be taken.	Correction based on multiple recoveries. Reflects multiple concentrations. May reflect several matrices from a commodity group.		Single time point, does not reflect variation in time. Only one (or few) matrices of the commodity group covered. Might not be fully up-to-date and representative for all matrices.		
3. Recovery included in the batch	Take the recovery obtained from the spiked sample concurrently analysed with the samples. Optionally multiple recoveries can be included (concentrations and/or matrices for commodity group), then the average can be taken.	If the spiked matrix is the same as the sample(s): could better reflect recovery for that matrix (at that moment) in case the matrix/method behaves differently from the situtation in initial or on-going validation.		Correction is based on a single recovery value which may be less reliable than an average from (on-going) validation. When the batch contains multiple matrices: only valid when matrix is representative for all matrices analysed.		

Interpretation of results for enforcement purposes

Document SANTE/11312/2021

E14 … the MRL is exceeded **if the measured value exceeds the MRL by more than the expanded uncertainty (x – U > MRL).** With this decision rule, the value of the measurand is **above the MRL with at least 97.5% confidence**.

SANTE/11213/2021 DECISION RULE for REJECTION



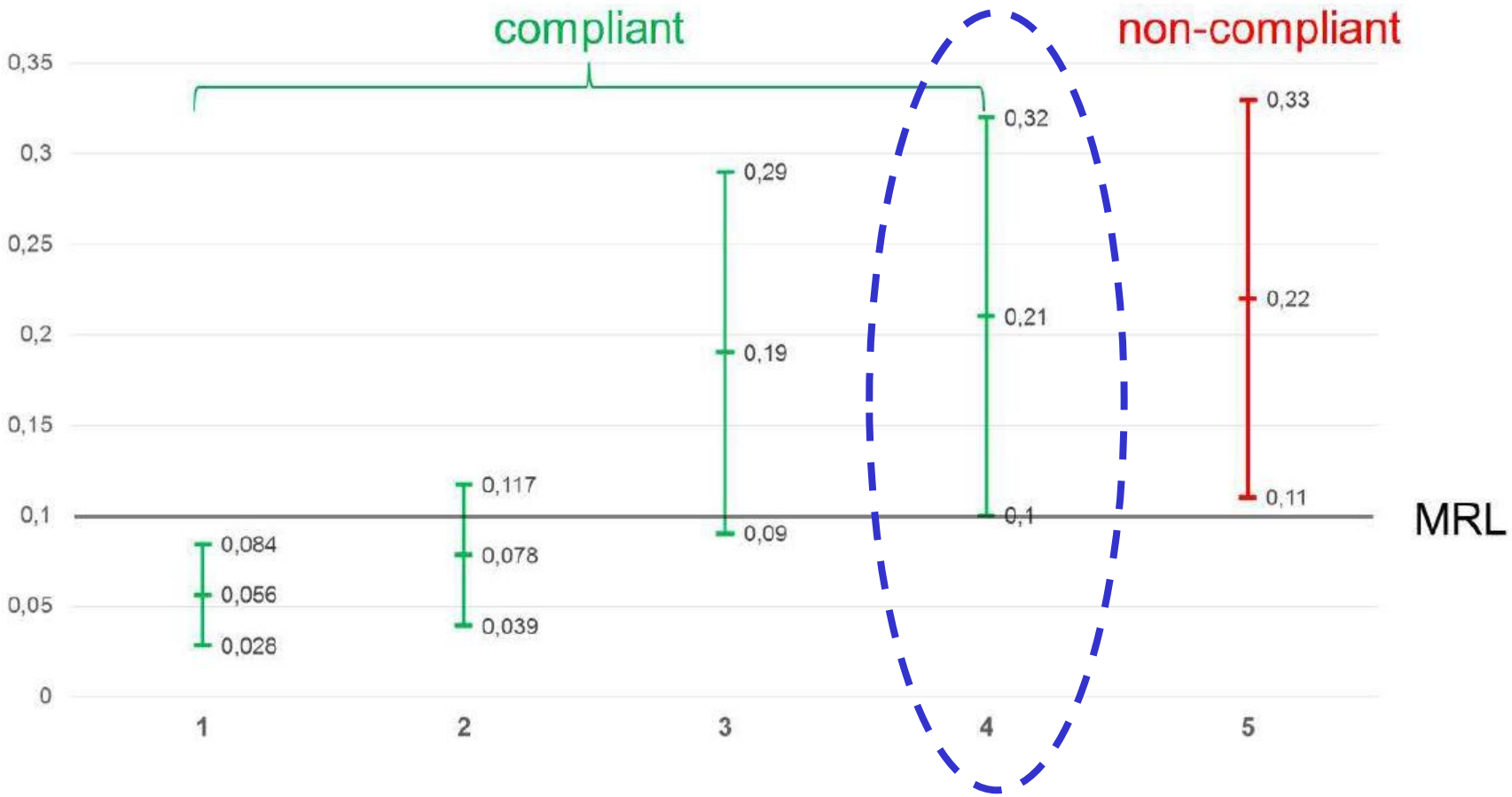
U = 50% → **"Decision Limit" = 2 x MRL**

EURACHEM / CITAC Guide: Use of Uncertainty Information in Compliance Assessment (1st Edition, 2007)

Interpretation of results for enforcement purposes

Document SANTE/11312/2021

Appendix D. Example of rounding, reporting and interpreting results



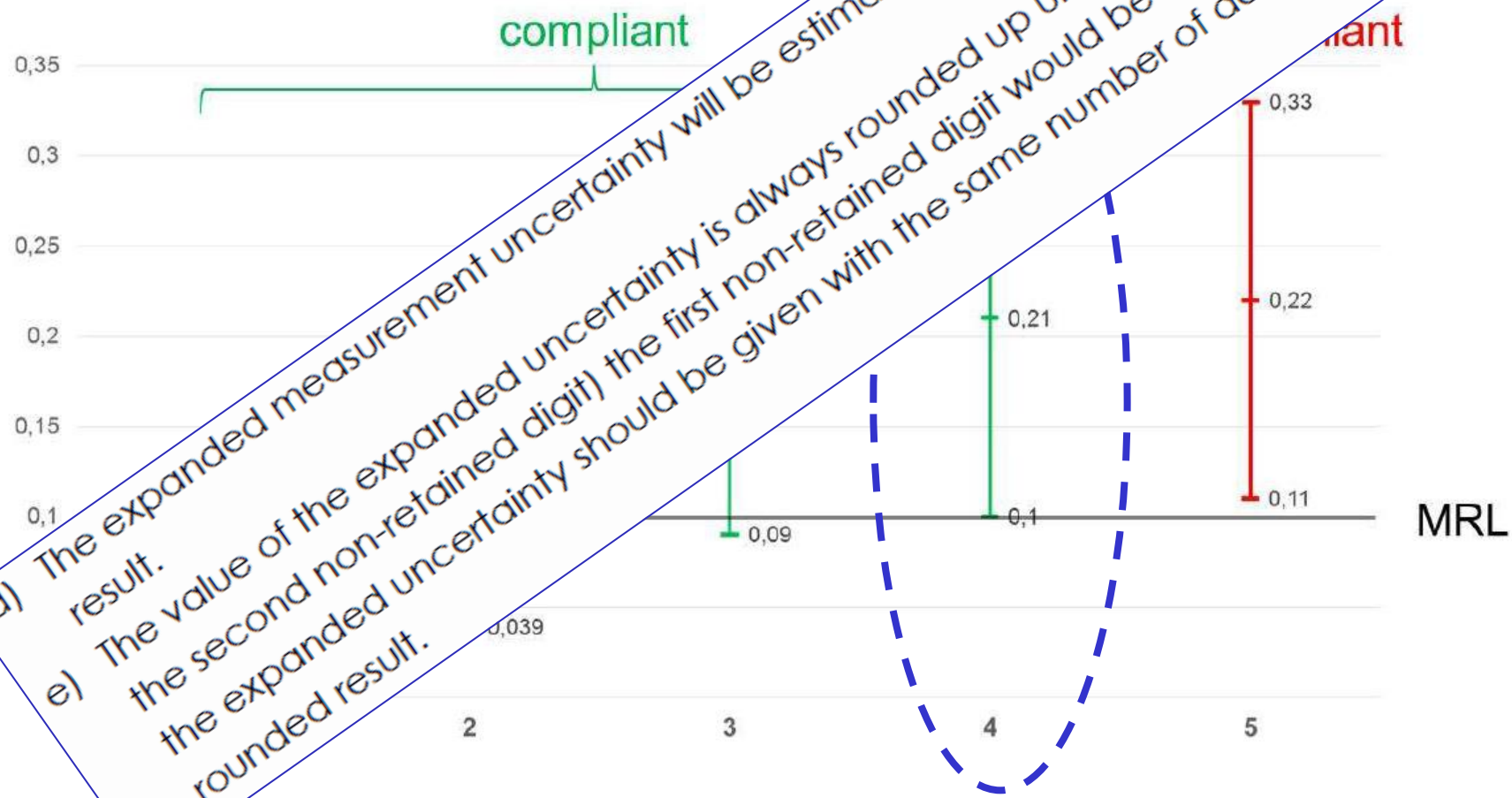
Interpretation of results for enforcement purposes

Document S… 1312/2021

Appendix D. Example of rounding, reporting …

d) The expanded measurement uncertainty will be estimated by using the final rounded result.

e) The value of the expanded uncertainty is always rounded up unless (after rounding of the second non-retained digit) the first non-retained digit would be 0. The value of the expanded uncertainty should be given with the same number of decimals as the rounded result.



Three important CONCLUSIONS useful to understand the AQC-EU-SANTE criteria on Measurement Uncertainty (MU)

The nature of the test methods used in pesticide residue analysis precludes a rigorous, and statistically valid, calculation of MU
(supported by the accreditation standard ISO/IEC 17025)

In multi-residue analysis of pesticides, it is not the goal to obtain very accurate MU estimates for one specific pesticide in a particular matrix. It is more important to obtain an overall and realistic estimate for a wide variety of materials and analyte levels covered by the validated scope
(supported by the EURACHEM / CITAC Guide CG 4)

EUPTs results support the use a default value of 50% relative expanded MU, if the test method has been validated and controlled according to the AQC-EU-SANTE criteria

Document «**AQC** EU-SANTE»

***A**nalytical **Q**uality **C**ontrol and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*

Document SANTE/11312/2021

Many thanks for your attention!!!