EU-China-Safe training event

Measurement Uncertainty

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Agenda

- What is measurement uncertainty and why do we need it?
  - Definitions
  - Sources of measurement uncertainty
  - Available guidance
- Uncertainty evaluation
  - Key rules
  - Converting and combining uncertainties
  - ‘Top-down’ approaches
  - Bioanalytical aspects
- Interpretation of uncertainty
  - Assessing compliance
- Sources of further information, training and guidance

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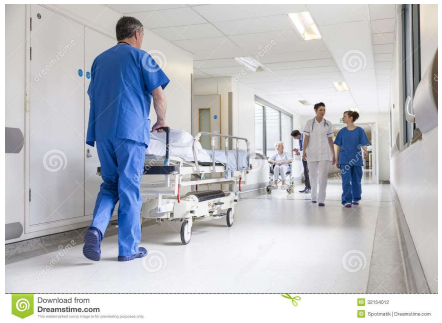
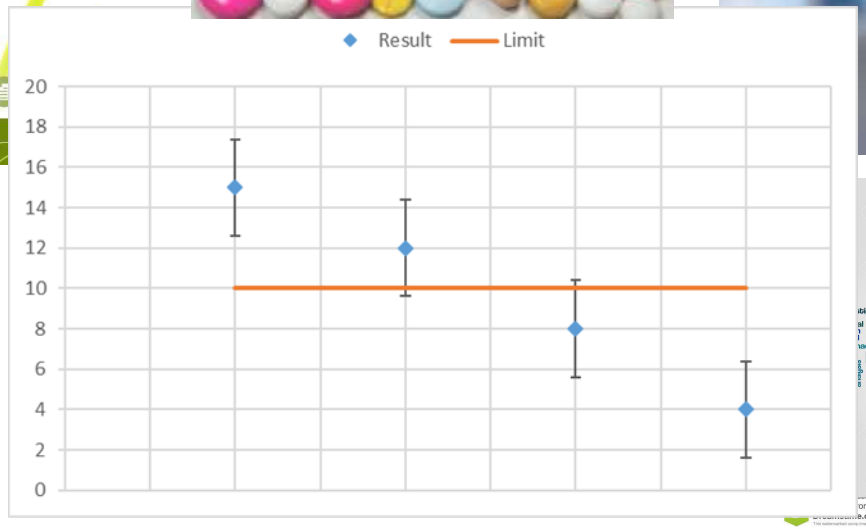
The importance of analytical results





https://affidiajournal.com/en/mycotoxins-eu-regulations-are-the-limits-too-strict-too-weak-or-just-fine

What is measurement uncertainty?

'GUM' definition

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

'Measurand' is *a particular quantity subject to measurement*

The part of the result after the **±**

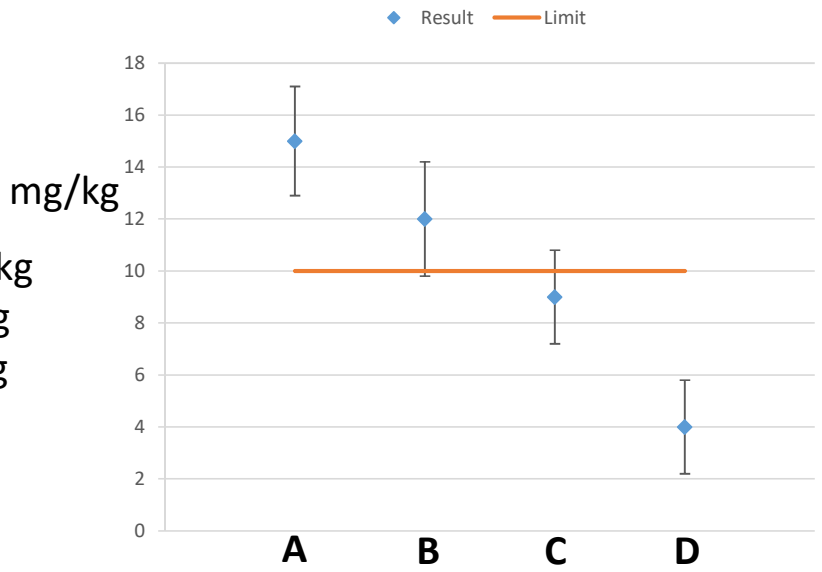
A range containing the 'true' value

GUM: Guide to the expression of uncertainty in measurement, JCGM 100:2008 (Joint Committee for Guides in Metrology) https://www.bipm.org/en/committees/jc/jcgm/publications

What is measurement uncertainty?

The part of the result after the **±**

A range containing the 'true' value



A: 15 ± 1.5 mg/kg, not less than 12.9 mg/kg, not more than 17.1 mg/kg
B: 12 ± 1.5 mg/kg, not less than 9.8 mg/kg, not more than 14.2 mg/kg
C: 9 ± 1.7 mg/kg, not less than 7.2 mg/kg, not more than 10.8 mg/kg
D: 4 ± 1.8 mg/kg, not less than 2.2 mg/kg, not more than 5.8 mg/kg

Which result(s) are/is over the limit of 10 mg/kg?

GUM: Guide to the expression of uncertainty in measurement, JCGM 100:2008 (Joint Committee for Guides in Metrology) https://www.bipm.org/en/committees/jc/jcgm/publications

How did we get here?

- Measurement uncertainty has been important in physical metrology for a long time (e.g. the physical constants, and engineering)
- Similar principles began to be applied in chemistry in the 20th century
- National Measurement Institutes & Designated Institutes compare their results regularly under the auspices of the BIPM (Bureau International des Poids et Mesures, International Bureau of Weights and Measures, an international organisation established by the Metre Convention, through which Member States act together on matters related to measurement science and measurement standards, https://www.bipm.org/en/home)
- Increasing global trade gave that activity more emphasis in the 1970s and it became apparent that methods of estimating uncertainty were far from harmonised.
- BIPM set up a working group that reported back in 1980 and the recommendations, concepts, definitions and method of implementation were collated and published as the GUM
- **GUM: Guide to the expression of uncertainty in measurement, JCGM 100:2008 (**Joint Committee for Guides in Metrology) https://www.bipm.org/en/committees/jc/jcgm/publications

Two approaches

‘GUM’* approach – “bottom-up”

- Write equation that completely describes the measurement system
- Evaluate the uncertainties associated with all parameters in the equation
  - Type A: statistical evaluation
  - Type B: any other data, e.g. certificates (of RMs, apparatus, …) instrument specifications
- Express all uncertainties as standard deviations
- Combine all uncertainties
- Apply a suitable coverage factor

*The GUM is also published as ISO/IEC Guide 98 part 3

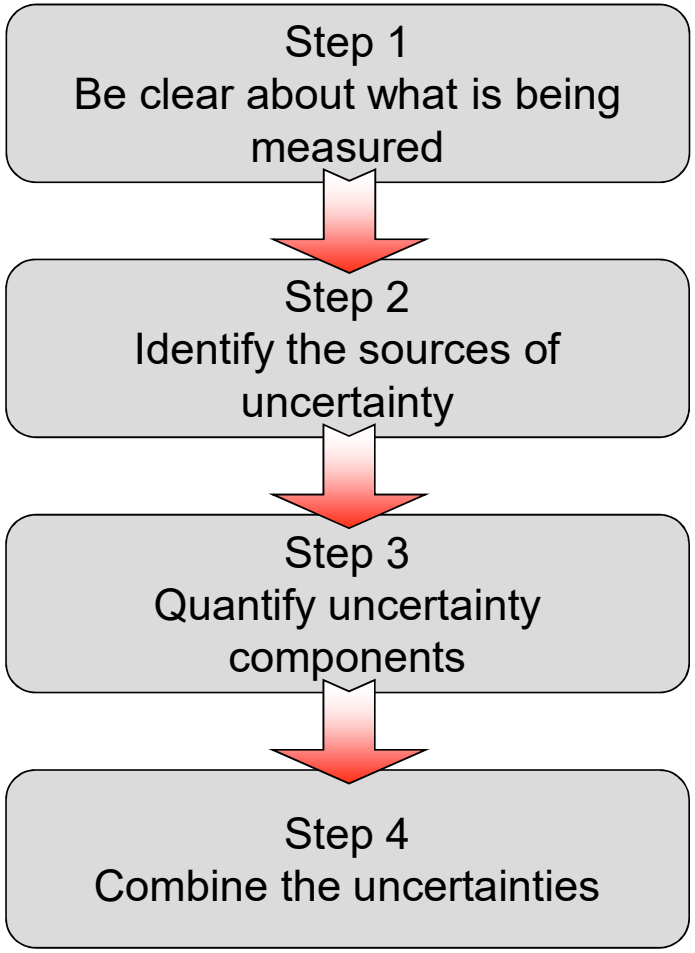
“Top-down” approach

- Use method performance data
  - validation data on precision and bias
  - ongoing QC data
- Capture the effect of a number of sources of uncertainty
- Look at the variation in method ***outputs*** rather than method ***inputs***
- Cover method scope
  - matrix, analyte concentration …
- Combine all uncertainties
- Apply a suitable coverage factor

Sources of measurement uncertainty

- Physical
  - mass, volume, temperature, pressure …
- Chemical / manipulation
  - extraction, clean up, concentration or dilution, derivatisation …
- Instrument
  - operating conditions, electrical supply, calibration…….
- Analyst
  - Individual analyst's interpretation of the method, rigour of adherence to the SOP
- Doesn't include gross errors (mistakes, e.g. loss of sample continuity, transcription errors, adding the wrong reagent …)

Evaluating uncertainty



Write down equation used to calculate result.

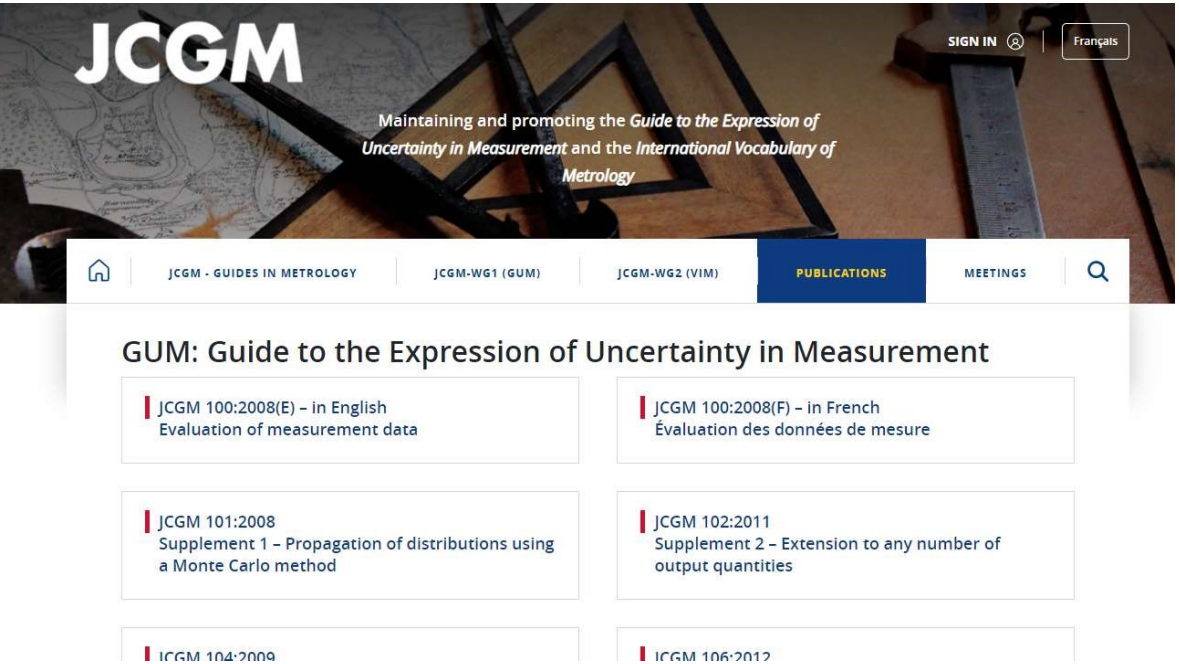
Parameters appearing in the equation will contribute to the uncertainty. What other factors will influence the result?

Estimate the size of each uncertainty component (the effect it will have on the result). Convert all estimates to the same form (standard uncertainty, *u*).

Combine using rules for combination of variances.

$$u_c = \sqrt{u_1^2 + u_2^2 + u_3^2 + ...}$$

Available guidance



https://www.bipm.org/en/committees/jc/jcgm/publications

Evaluation of measurement data — Guide to the expression of uncertainty in measurement GUM

Evaluation of measurement data — An introduction to the “Guide to the expression of uncertainty in measurement” and related documents

Guide to the expression of uncertainty in measurement — Part 6: Developing and using measurement models

International vocabulary of metrology – Basic and general concepts and associated terms (VIM)

Evaluation of measurement data – The role of measurement uncertainty in conformity assessment

Available guidance

https://www.eurachem.org/index.php

Translations available

Quality assurance, accreditation and terminology

Guide to Quality in Analytical Chemistry: An Aid to Accreditation (2016)

Quality Assurance for Research and Development and Non-routine Analysis (1998)

Terminology in Analytical Measurement: Introduction to VIM 3 (2011)

Measurement uncertainty

Quantifying Uncertainty in Analytical Measurement, 3rd Edition (2012)

Measurement uncertainty arising from sampling, 2nd edition (2019)

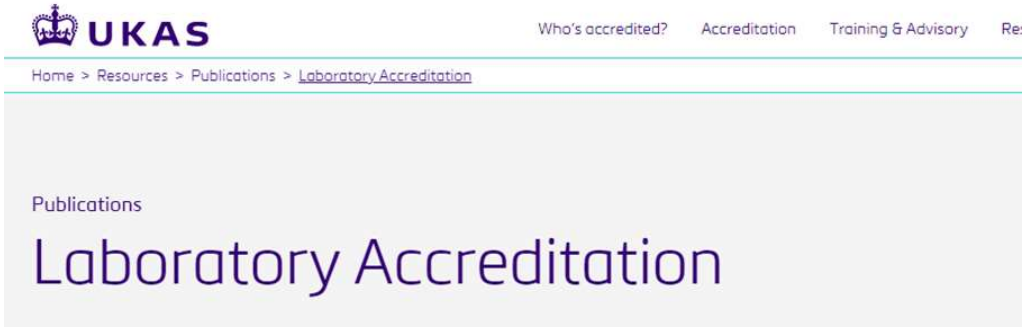
Use of uncertainty information in compliance assessment (2021)

Setting target measurement uncertainty (2015)

Qualitative analysis

Assessment of performance and uncertainty in qualitative chemical analysis (2021)

Available guidance



https://www.ukas.com/resources/publications/laboratory-accreditation/

UKAS: LAB 12, The Expression of Uncertainty in Testing (Edition 3, November 2019)

UKAS: M3003, The Expression of Uncertainty and Confidence in Measurement (Edition 4, October 2019)

https://ilac.org/

ILAC-G17:01/2021, ILAC Guidelines for Measurement Uncertainty in Testing

Uncertainty evaluation

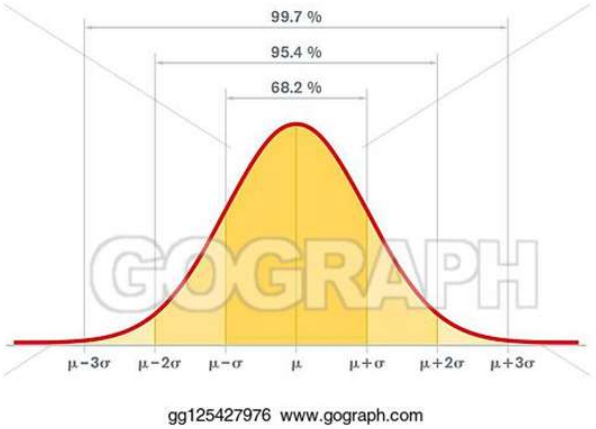
Topics we will discuss

- Key rules
- Converting and combining uncertainties
- ‘Top-down’ approaches
- Bioanalytical aspects

Gathering data

- Experimental studies
- Manufacturers’ specifications
- Calibration certificates
- Method validation data
  - In house
  - Collaborative studies
- Quality control data
- Literature data
- Experience
  - A feeling for what is normal or abnormal
- Calculation

Uncertainty components



- All components must be converted to a standard form:
  - **The standard uncertainty, *u***

a standard uncertainty is an uncertainty expressed as standard deviation

- Uncertainty information comes in different forms:
  - Standard deviation
  - 95% confidence interval
  - Expanded uncertainty
  - Stated range (values equally likely across range – a rectangular distribution)
  - Stated range (values close to mean more likely than values at the extremes of the range, a triangular distribution)
- Standard deviations can be combined in a rigorous way
- But we need rules to convert to **the standard uncertainty, *u***

Standard deviation

$$s = \sqrt{\frac{\sum(xi - \bar{x})\text{^}2}{(n-1)}} \qquad \text{... (1)}$$

$$\text{Standard error of the mean} = \frac{s}{\sqrt{n}} \qquad \text{... (2)}$$

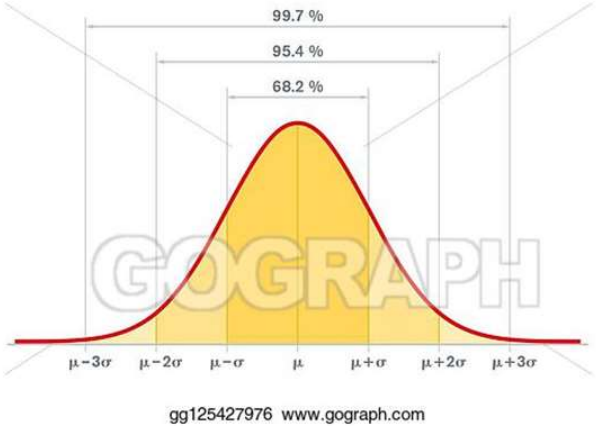
Where

s = *standard deviation*

x_i = individual result

$\bar{x}$ = the mean

n = number of measurements



A confidence interval

Confidence interval = $\bar{x} \pm \frac{t \times s}{\sqrt{n}}$ … (3)

Consider a result given with a confidence interval, e.g.

Concentration = 120 mg kg^{-1} ± 3 mg kg^{-1} with a level of confidence of not less than 95%

A confidence interval is calculated from equation (3)

Where, $\bar{x}$ = the mean, n = number of measurements, t is the students t value for a given level of confidence and s is the standard deviation (Note $s/\sqrt{n}$ is the standard error of the mean)

It is rare to know what n the number of measurements was,

Hence not possible to look up t in statistical tables from the degrees of freedom

Use the 'large sample' value of 1.96 for 95% CI hence the standard error (of the mean) is 3/1.96 = 1.5 mg kg^{-1}

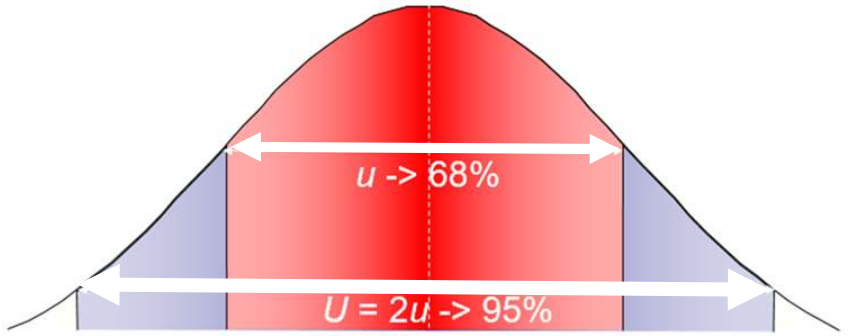
(The value '1.96' is often rounded to 2)

Expanded uncertainty

Similarly if a result is given as:

Concentration = 120 mg kg^{-1} ± 3 mg kg^{-1}, with an expanded uncertainty with a coverage factor of $k = 2$

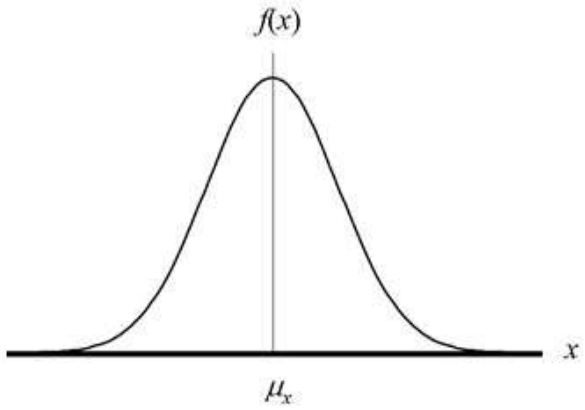
The standard uncertainty is 3/2 = 1.5 mg kg^{-1}

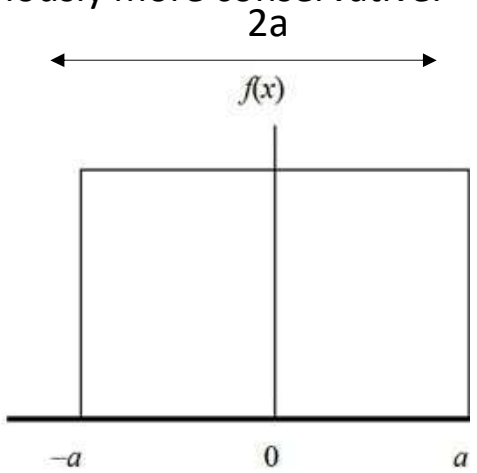


$U = k.u$

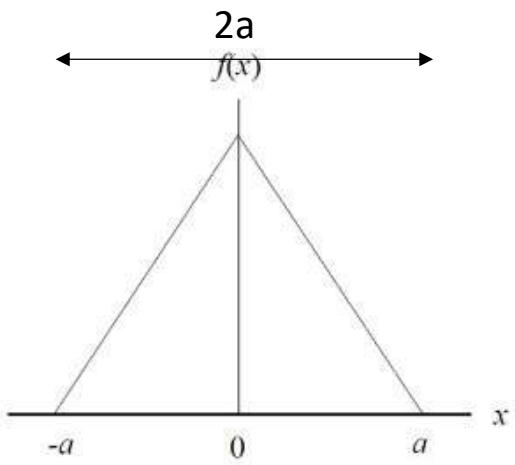
Rectangular and triangular distributions

A range ±*a* is given without an estimate of the distribution of confidence level (e.g. manufacturing tolerance such as a volumetric flask), no information on the likely distribution, a rectangular distribution can be assumed. If the actual value is more likely to be close to the nominal value rather than the extreme a triangular distribution may be more appropriate. The former is obviously more conservative.





$u(x) = a/\sqrt{3}$



$u(x) = a/\sqrt{6}$

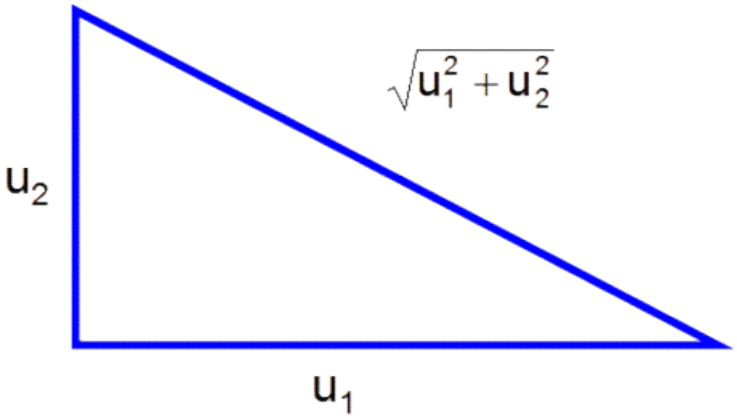
https://www.isobudgets.com/probability-distributions-for-measurement-uncertainty/

Converting data summary

Uncertainty components must be expressed as standard deviations before they can be combined

Data expressed as:	**Conversion rule:**
Standard deviation	No conversion required
Expanded uncertainty	Divide by stated coverage factor, k
95% confidence interval	Divide by 2
Stated range (values equally likely across range)	Assume a rectangular distribution, divide by $\sqrt{3}$
Stated range (values close to mean more likely than values at the extremes of the range)	Assume a triangular distribution, divide by $\sqrt{6}$

Combining uncertainties



- Calculation of final result involves addition or subtraction

  $y = a + b + c + \ldots$

- Uncertainties combined as standard deviations

$$u(y) = \sqrt{u(a)^2 + u(b)^2 + u(c)^2 + \ldots}$$

Combining uncertainties

- Calculation of final result involves multiplication or division
- Uncertainties combined as relative standard deviations

$$y = \frac{a \times b}{c}$$

$$\frac{\mathrm{u}(y)}{y} = \sqrt{\left(\frac{\mathrm{u}(a)}{a}\right)^2 + \left(\frac{\mathrm{u}(b)}{b}\right)^2 + \left(\frac{\mathrm{u}(c)}{c}\right)^2}$$

- Where the terms are multiplied or divided the uncertainties are expressed as relative standard deviations before being squared
- This leads to the uncertainty in *y* also being expressed as a relative standard deviation, from which, knowing the value of *y* we can calculate u(*y*)

Top-down approach

More suitable for a chemistry laboratory

Why is ‘bottom-up’ approach difficult for chemical methods?

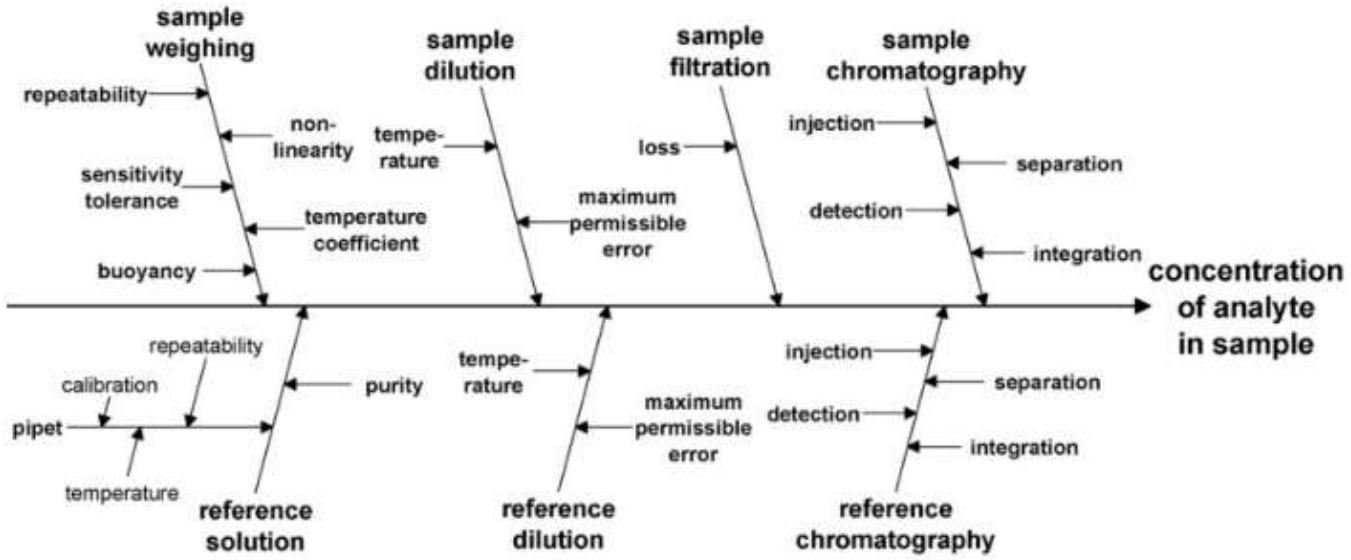


Fig. 4. The Ishikawa diagram with the uncertainty sources of the measurement uncertainty of the analysis shown in Fig. 3.

Meyer, V.R., 2007. Measurement uncertainty. Journal of Chromatography A, 1158(1-2), pp.15-24.

‘Top-down’ approach

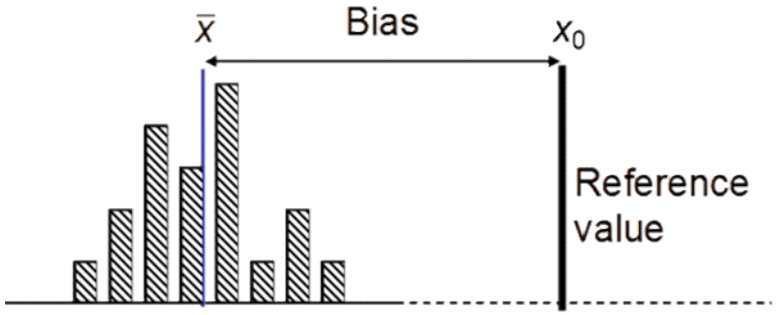
- Use method performance data
  - validation data on precision and bias
  - ongoing QC data
- Capture the effect of a number of sources of uncertainty
- Look at the variation in method *outputs* rather than method *inputs*
- Cover method scope
  - matrix, analyte concentration

‘Top-down’ requirements

- The best available estimate of precision
  - from validation studies (including collaborative inter-laboratory studies) or ongoing QC
  - a parameter varied representatively during a precision experiment requires no further study
- The best available estimate of bias and its uncertainty
  - includes method bias and laboratory bias
- Other significant effects evaluated
  - by experiment, or from existing data

Contributions to uncertainty

Method bias	Inherent bias in method, will affect all results from the method
Laboratory bias	Local implementation of the method
Repeatability	Random variation in results obtained in same run
Intermediate precision	Random variation in results over different runs, days, analysts …
Uncertainty in the result	Value should include everything above



OR Reproducibility, additional labs – collaborative study

Evaluating precision

- Aim to cover as many sources of variation in the results as possible
  - For example, extended time period, different analysts, different calibration standards, different environmental conditions …
- A parameter varied representatively during a precision study (repeatability, intermediate precision) requires no further evaluation
- Types of data
  - Data obtained during method validation
  - Quality control data, repeat analysis of QC materials (control charts)
  - Collaborative study data (reproducibility standard deviation)
    - If a lab can demonstrate satisfactory implementation of the method

Method performance data – combining uncertainty estimates

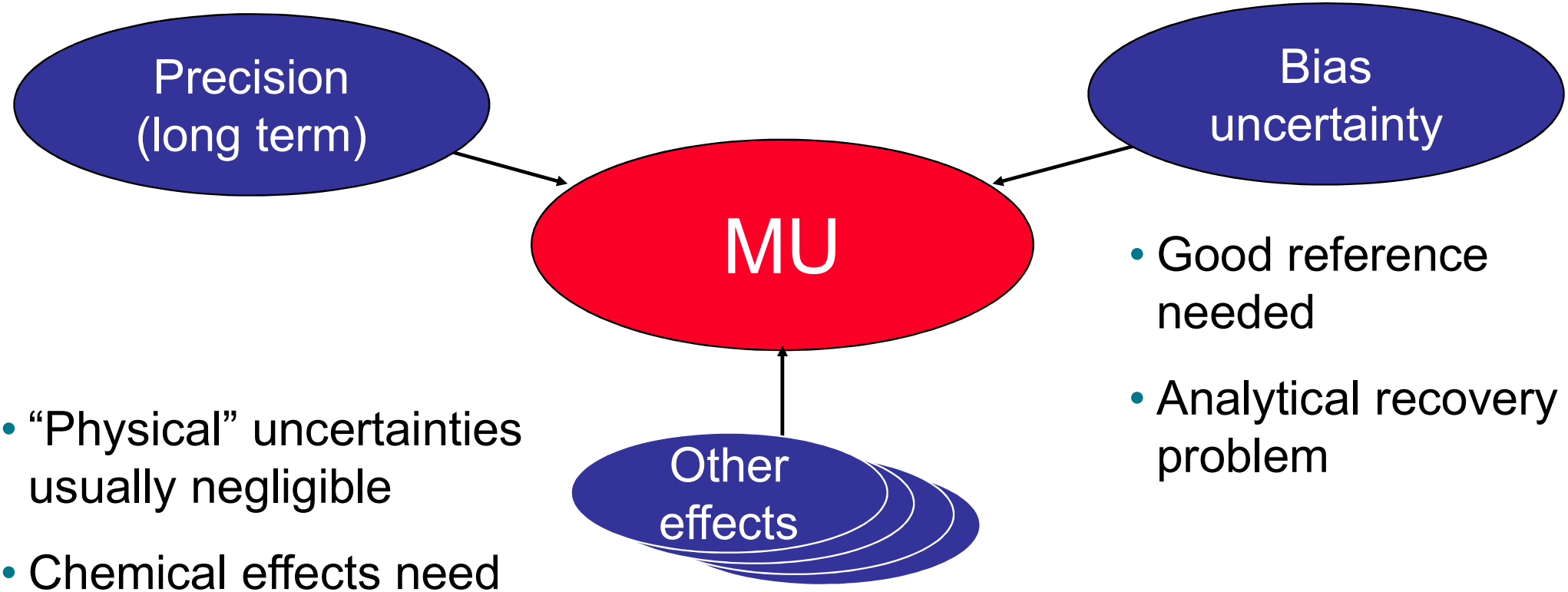
$$C_{corr} = \frac{C_{obs}}{Rm \times Rs} \times f_e \qquad \text{... (4)}$$

$$\frac{u(C_{corr})}{C_{corr}} = \sqrt{\left\{\frac{u(C_{obs})}{C_{obs}}\right\}^2 + \left\{\frac{u(Rm)}{Rm}\right\}^2 + \left\{\frac{u(Rs)}{Rs}\right\}^2 + \left\{\frac{s_{obs}}{C_{obs}}\right\}^2} \qquad \text{... (5)}$$

Where

- C_{corr} : measurement result corrected for recovery; $u(C_{corr})$: combined uncertainty in measurement result
- C_{obs} : measurement result before correction; $u(C_{obs})$: uncertainty in measurement result other than precision and recovery
- f_e: 'correction factor for precision (=1);
- *Rm*: method recovery; *u*(*Rm*): uncertainty in method recovery
- *Rs*: correction factor for variation in recovery with sample type; *u*(*Rs*): variation in recovery with sample type

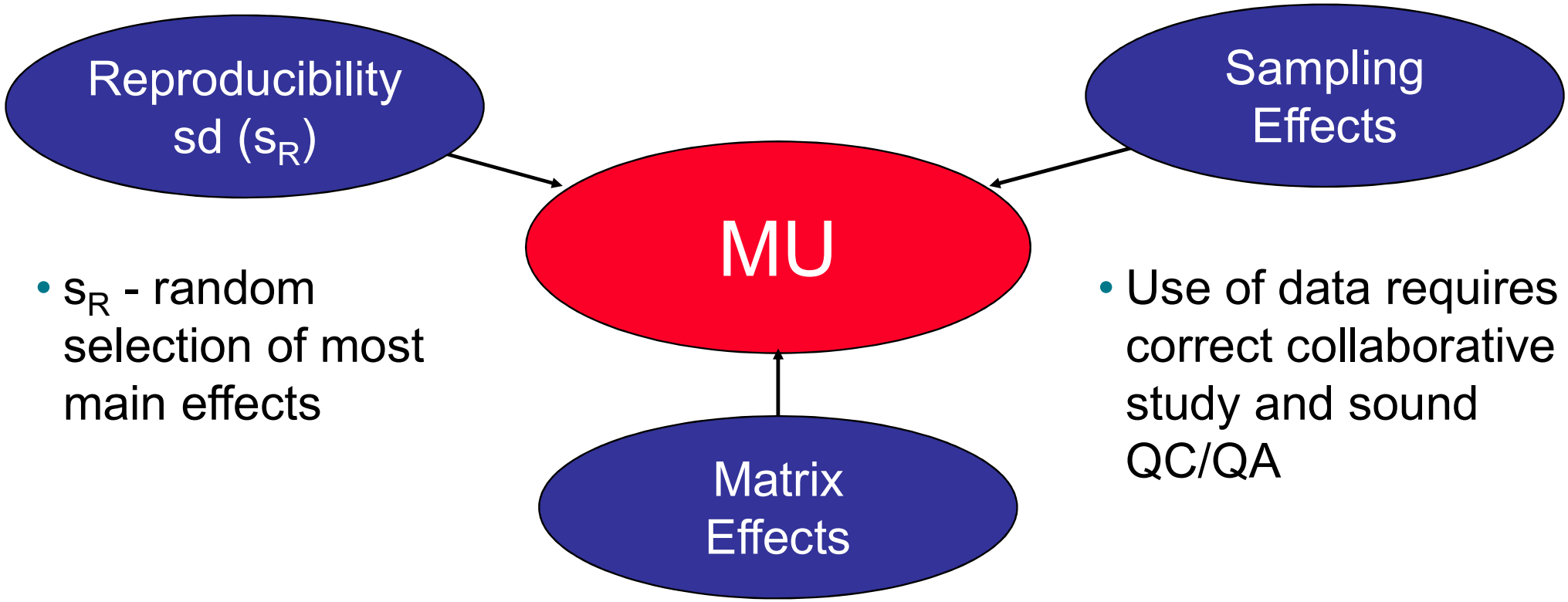
In-house validation data



- "Physical" uncertainties usually negligible
- Chemical effects need study

- Good reference needed
- Analytical recovery a problem

Collaborative study data
ISO 21748



- s_R - random selection of most main effects

- Use of data requires correct collaborative study and sound QC/QA

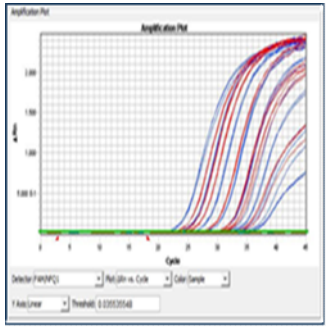
Using collaborative study data (ISO 21748:2017)

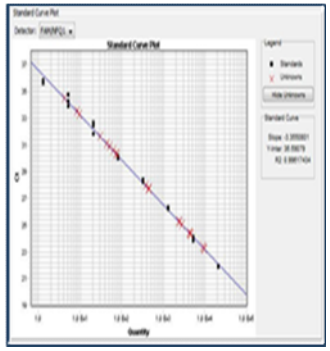
- ISO 21748:2017; Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty evaluation
  - does not describe the application of repeatability data in the absence of reproducibility data.
  - assumes that recognized, non-negligible systematic effects are corrected, either by applying a numerical correction as part of the method of measurement, or by investigation and removal of the cause of the effect.
- Obtain r, R and bias estimates from collaborative study
- Establish whether bias and precision are as expected
- Where bias and precision are under control, combine effects appropriately to form a combined uncertainty estimate
- Evaluation of measurement uncertainties using data obtained from studies conducted in accordance with ISO 5725-2, and comparison of collaborative study results with measurement uncertainty obtained using principles of uncertainty propagation
- ISO 5725-3 provides additional models for studies of intermediate precision

Summary

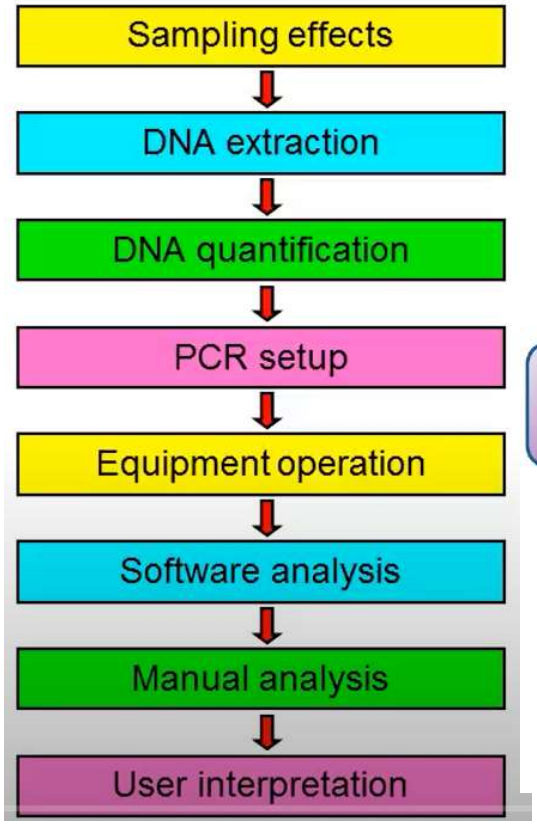
- The ‘bottom-up’ approach is impractical for many test methods
- The ‘top-down’ approach utilises method performance data
- ISO 21748:2017 provides an approach for using collaborative study data
  - Requires checks for consistency with study performance
  - Allows for changes in the test item type
  - Often reduces to a simple reproducibly standard deviation

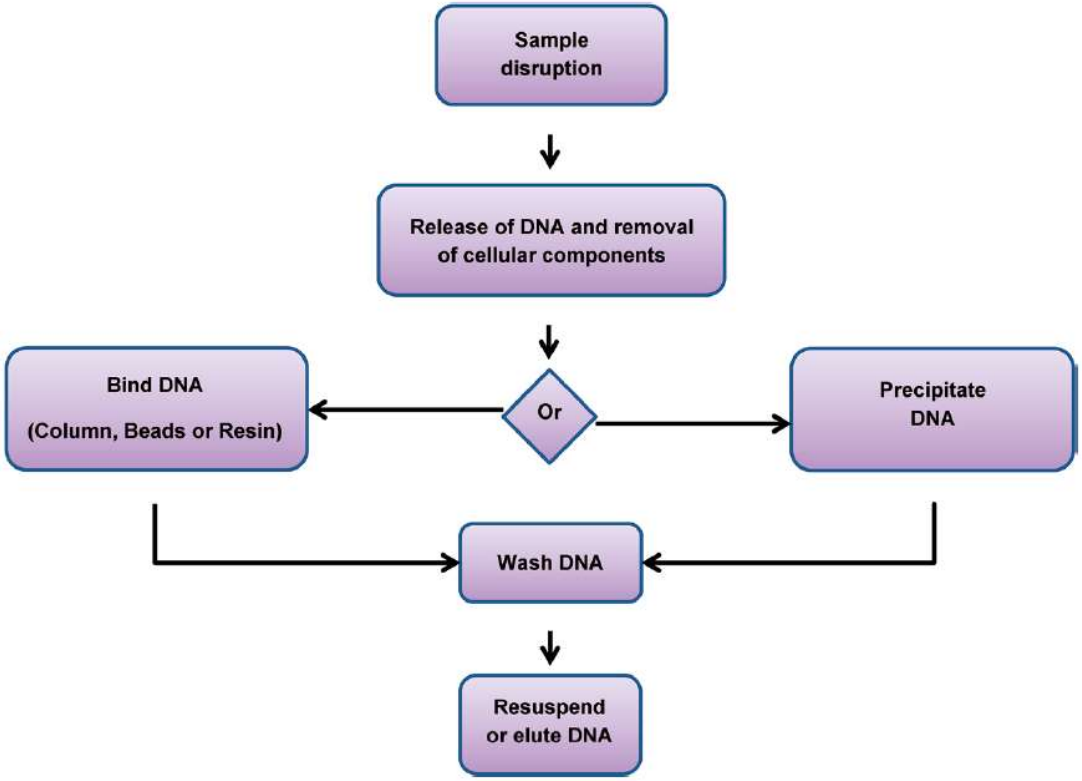
Bioanalytical aspects





Typical DNA analysis





Co-extracted:

Proteins
RNA
Polysaccharides
Polyphenols
Secondary metabolites

CTAB etc

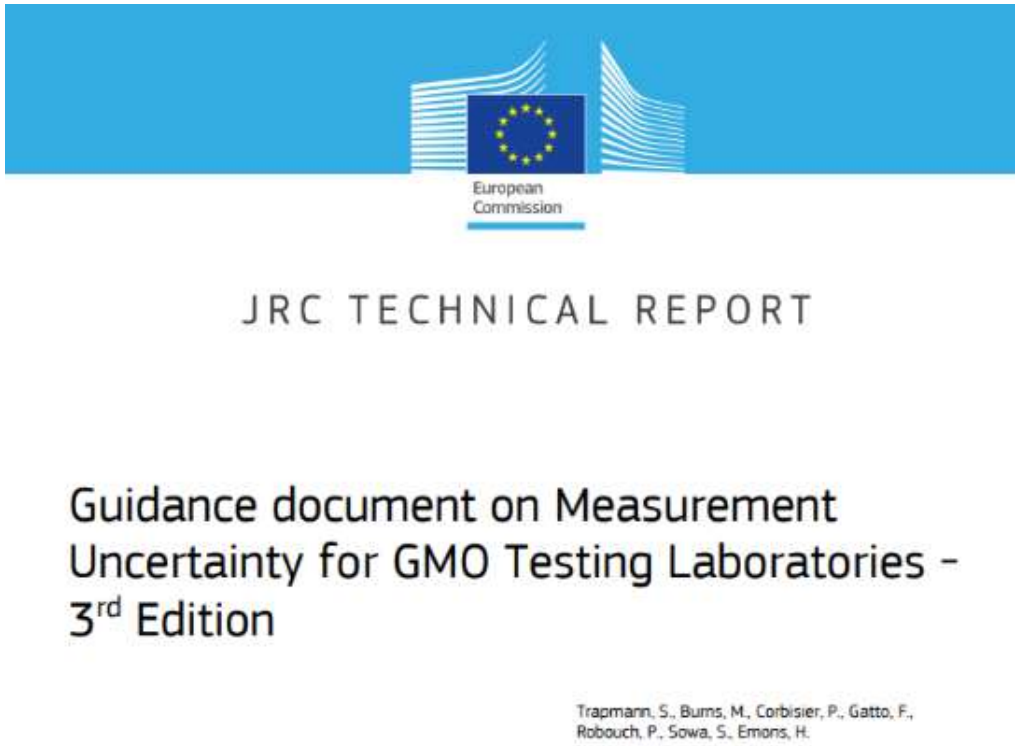
1 http://www.foodauthenticity.global/training

2 Timothy Wilkes, DNA Extraction from Food Matrices Ch 3 *in* DNA Techniques to Verify Food Authenticity, *Eds* Burns, Foster & Walker

Key aspects

- Both ‘bottom-up and ‘top-down’ approaches
- For ‘top-down’
  - Sufficient level of replication
    - Replicate samples, extractions, runs, reactions
- Precision of assay – repeatability and reproducibility
- Usual principles of measurement uncertainty evaluations apply
  - All uncertainty components expressed as standard deviations
  - Combined in the usual way
  - Appropriate coverage factor to give expanded uncertainty
- Reporting
  - Transparency about how measurement uncertainty was arrived at

Sources of information



- Guidance on how to estimate measurement uncertainty associated with quantitative GMO bioanalysis by real time PCR
- Developed by JRC at the request of The European Network of GMO Laboratories (ENGL), a consortium of official enforcement laboratories designated by the EU Member States plus Norway, Switzerland and Turkey.
- https://gmo-crl.jrc.ec.europa.eu/ENGLabs#inline-nav-engl-reports
- https://gmo-crl.jrc.ec.europa.eu/guidance-documents

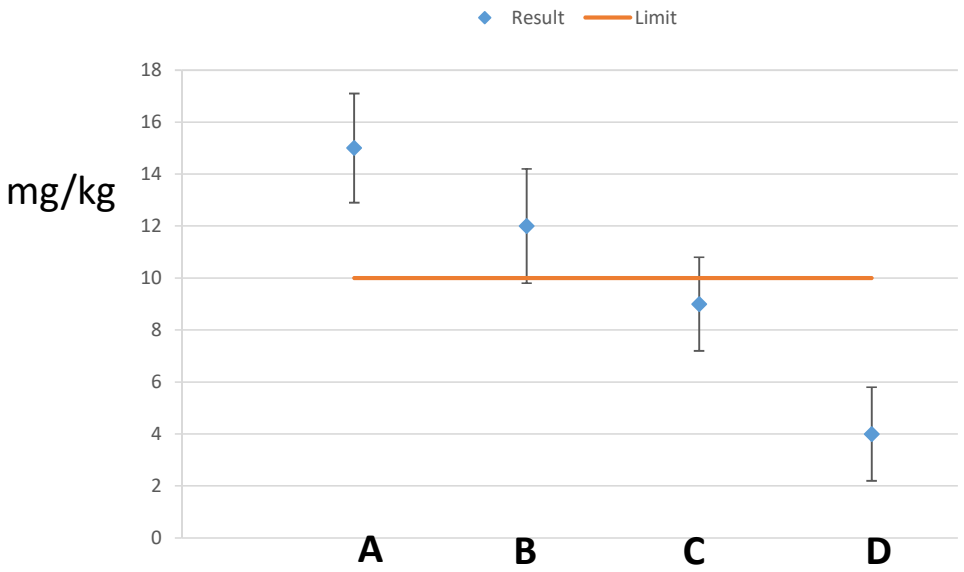
Interpretation of uncertainty
Assessing compliance

Recap – what is measurement uncertainty

- A number that characterises the distribution of possible values for the ‘true’ amount
- An expanded uncertainty is the uncertainty multiplied by a coverage factor for increased confidence
  - Some assumptions – ‘normality’, dispersion independent of ‘true’ concentration
- Based on the known performance of the method when carried out correctly

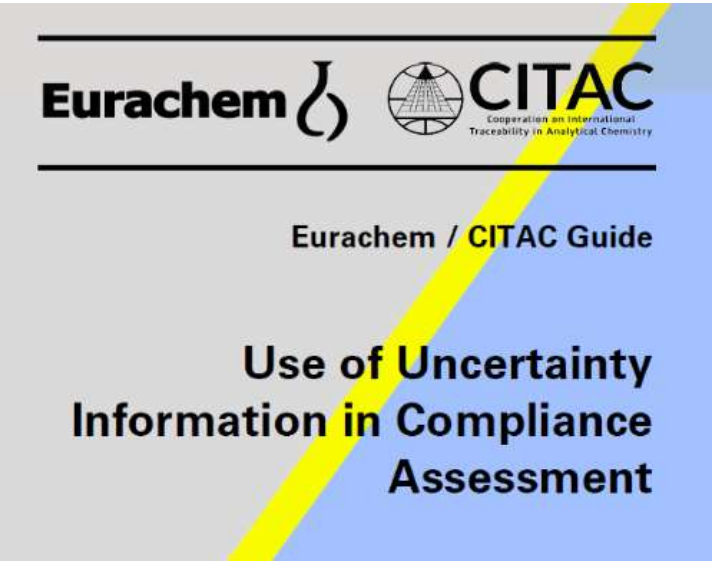
Assessing compliance - which result(s) are/is over the limit of 10 mg/kg?

A: 15 ± 1.5 mg/kg,
not less than 12.9 mg/kg, not more than 17.1 mg/kg
B: 12 ± 1.5 mg/kg,
not less than 9.8 mg/kg, not more than 14.2 mg/kg
C: 9 ± 1.7 mg/kg,
not less than 7.2 mg/kg, not more than 10.8 mg/kg
D: 4 ± 1.8 mg/kg,
not less than 2.2 mg/kg, not more than 5.8 mg/kg



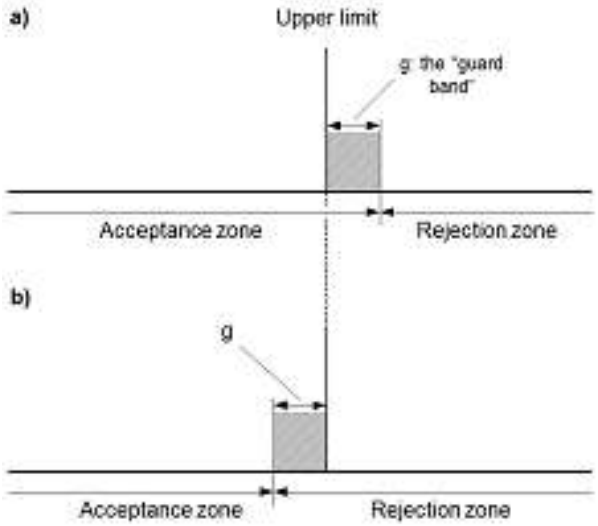
Interpretation is easier if a decision rule is agreed

Eurachem guidance (2021)



https://www.eurachem.org/index.php/publications/guides/uncertcompliance

- Provides guidance on how uncertainty may be taken into account in deciding compliance with a limit.
- Applicable to decisions on compliance with regulatory or manufacturing limits where a decision is made on the basis of a decision rule, together with a measurement value and the associated measurement uncertainty.



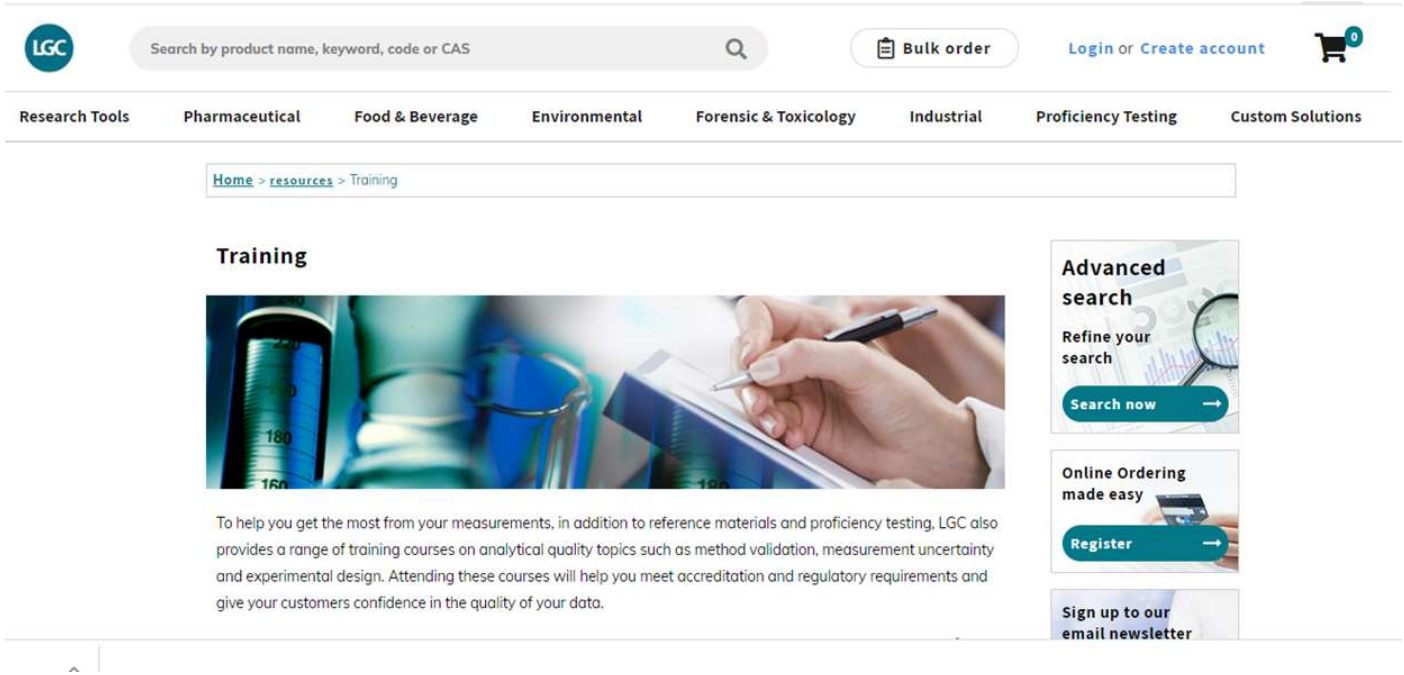
Sources of further information, training and guidance

Some further information

- **GUM: Guide to the expression of uncertainty in measurement, JCGM 100:2008 (**Joint Committee for Guides in Metrology) https://www.bipm.org/en/committees/jc/jcgm/publications
- JCGM – Joint Committee for Guides in Metrology (BIPM), https://www.bipm.org/en/committees/jc/jcgm/publications
- Eurachem, https://www.eurachem.org/index.php
- UKAS: LAB 12, The Expression of Uncertainty in Testing (Edition 3, November 2019)
- UKAS: M3003, The Expression of Uncertainty and Confidence in Measurement (Edition 4, October 2019)
- ILAC-G17:01/2021, ILAC Guidelines for Measurement Uncertainty in Testing
- JRC/ENGL Guidance on how to estimate measurement uncertainty associated with quantitative GMO bioanalysis by real time PCR, https://gmo-crl.jrc.ec.europa.eu/guidance-documents
- Statistics and Chemometrics for Analytical Chemistry James Miller, Jane C Miller, Robert D. Miller, Pearson, 2018

Training

- There are many organisations offering training in measurement uncertainty, the one I am most familiar with is LGC



https://www.lgcstandards.com/GB/en/resources/training

LGC's most popular training courses

Method validation:

- This three-day course introduces the statistics required for interpreting validation data and provides the tools to plan and carry out effective validation studies. **More information and booking instructions.**

Estimation of measurement uncertainty:

- This two-day course covers the most common approaches to evaluating measurement uncertainty, following the ISO principles and using method validation data. The course provides a practical approach to evaluating uncertainty in testing laboratories. **More information and booking instructions.**

Designing effective experiments:

- Modern analytical methods and production processes are complex, with many different factors affecting the outcome. In order to be competitive, companies need to minimize resources expended on development and maximize process performances. Design of Experiments (DoE) enables these complex situations to be understood, reducing the cost of gaining an in-depth knowledge of the process which can be translated into competitive advantage. **More information and booking instructions.**

Statistics for analytical scientists:

- This one-day course is aimed at analysts and covers the statistics most commonly applied to analytical data. It will allow analysts to answer questions such as, 'Which is the best way to summarise my data?', 'Is there a real difference between the results produced by different test methods?', 'How should I evaluate the results obtained from an instrument calibration experiment?'. **More information and booking instructions.**

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NML – National Measurement Laboratory in LGC