Recovery/bias evaluation

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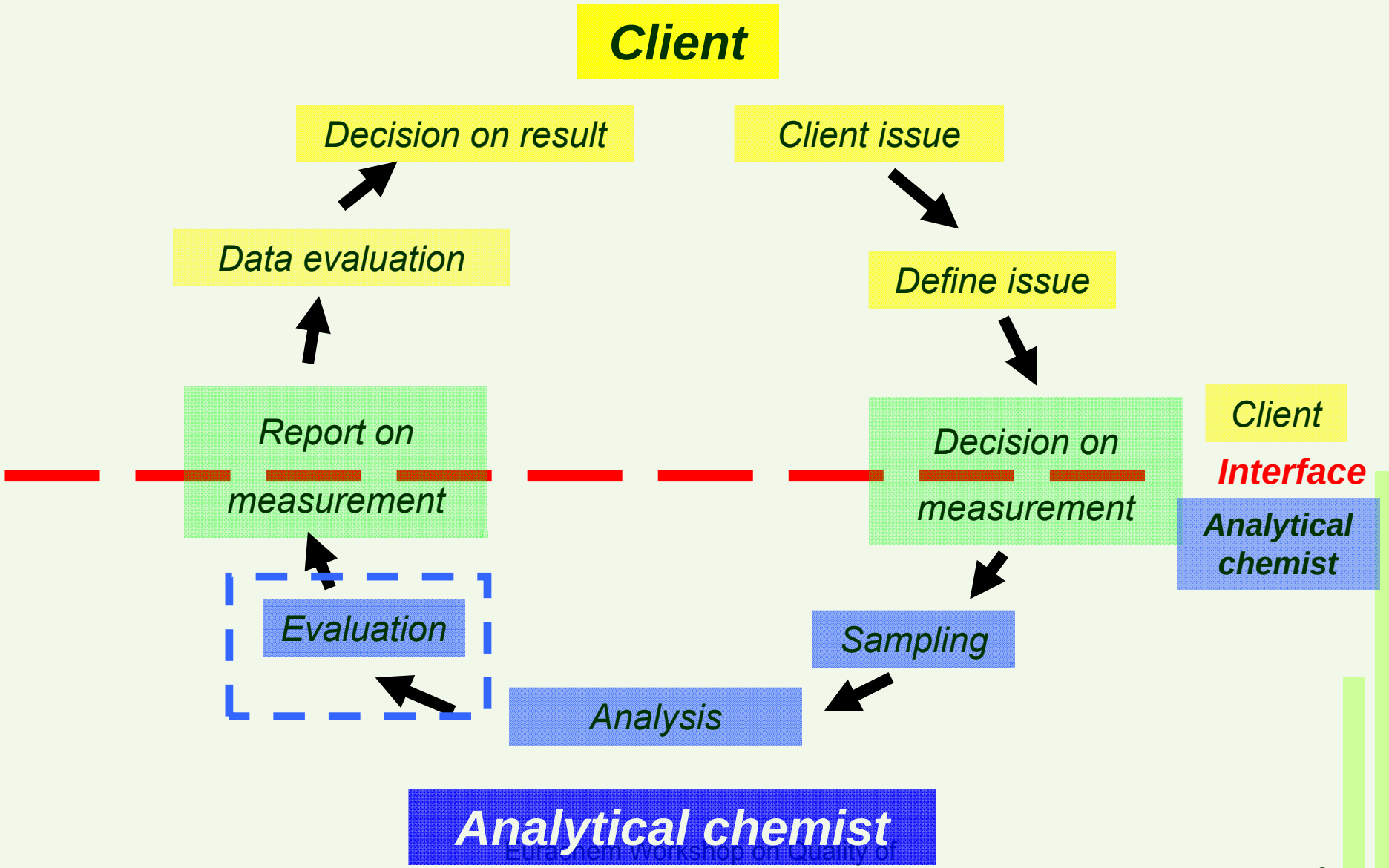
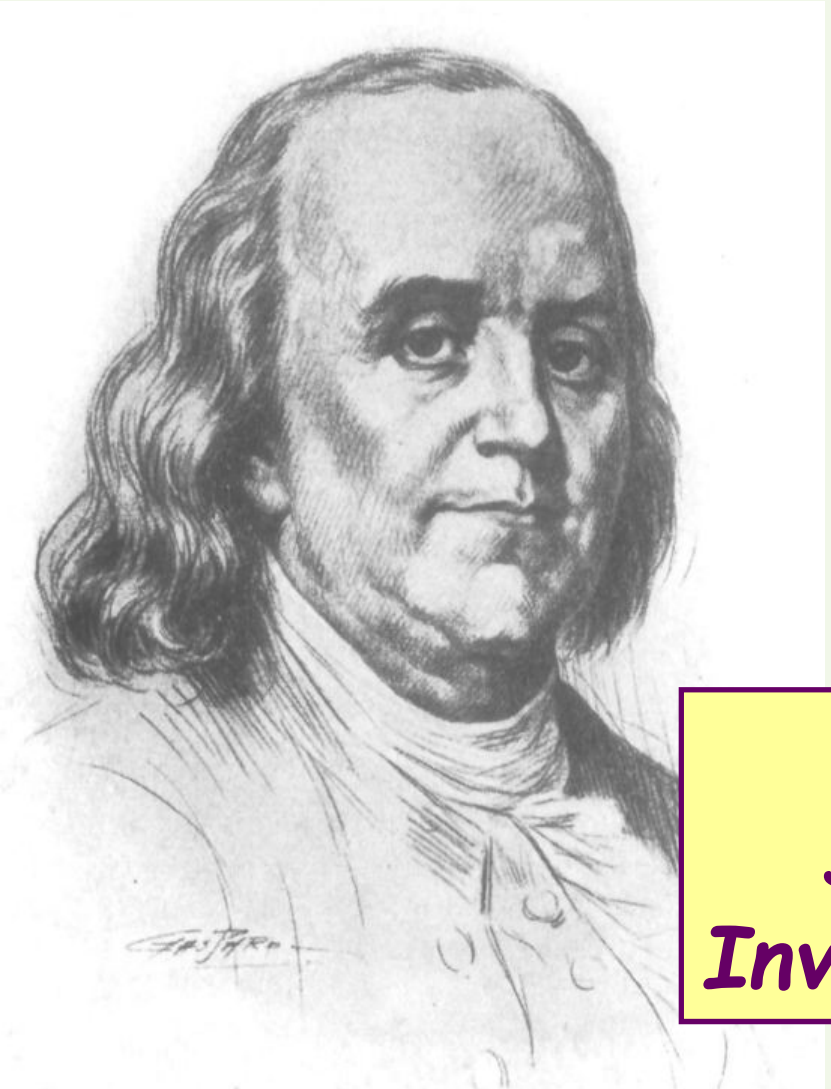
Overview

- What is bias?
- What are the main issues in bias evaluation?
  - Time frame
  - Random and systematic effects
  - Approaches for bias evaluation
- Some examples

The measurement cycle





Benjamin Franklin 1706-1790

Tell me and I forget.
Show me and I forget.
***Involve** me and I remember.*

What is bias?

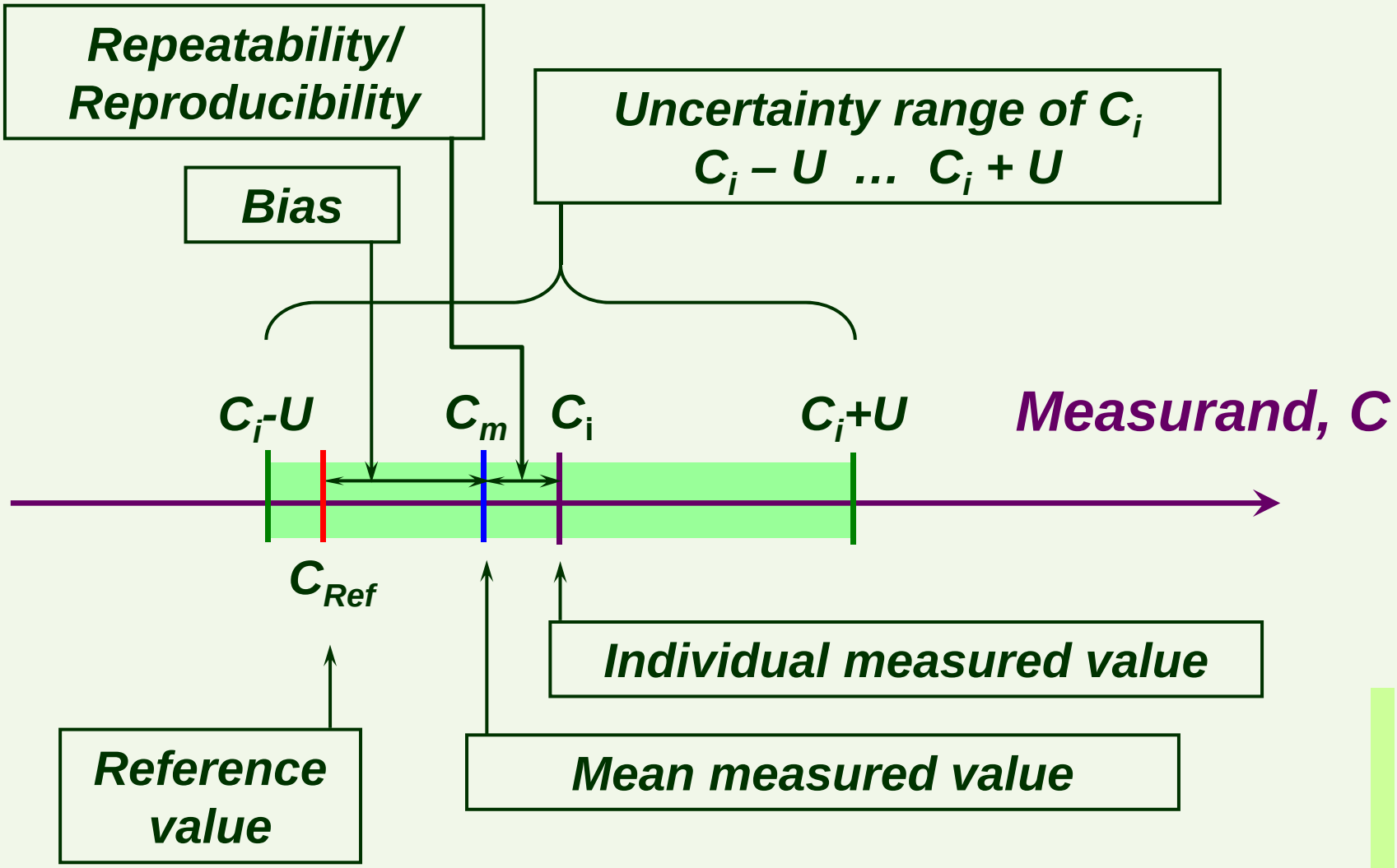
- Bias is …
  1. difference between the **measured value** and the **true value**
  2. Difference between the **measured value** and a **reference value**
  3. Difference between the **mean of a large number of replicate measured values** and the **true value**
  4. Difference between the **mean of a large number of replicate measured values** and a **reference value**

For eliminating the random effects

We never know the true value

VIM: Bias is an estimate of a systematic measurement error

Bias and related concepts



Which of these situations describe bias?

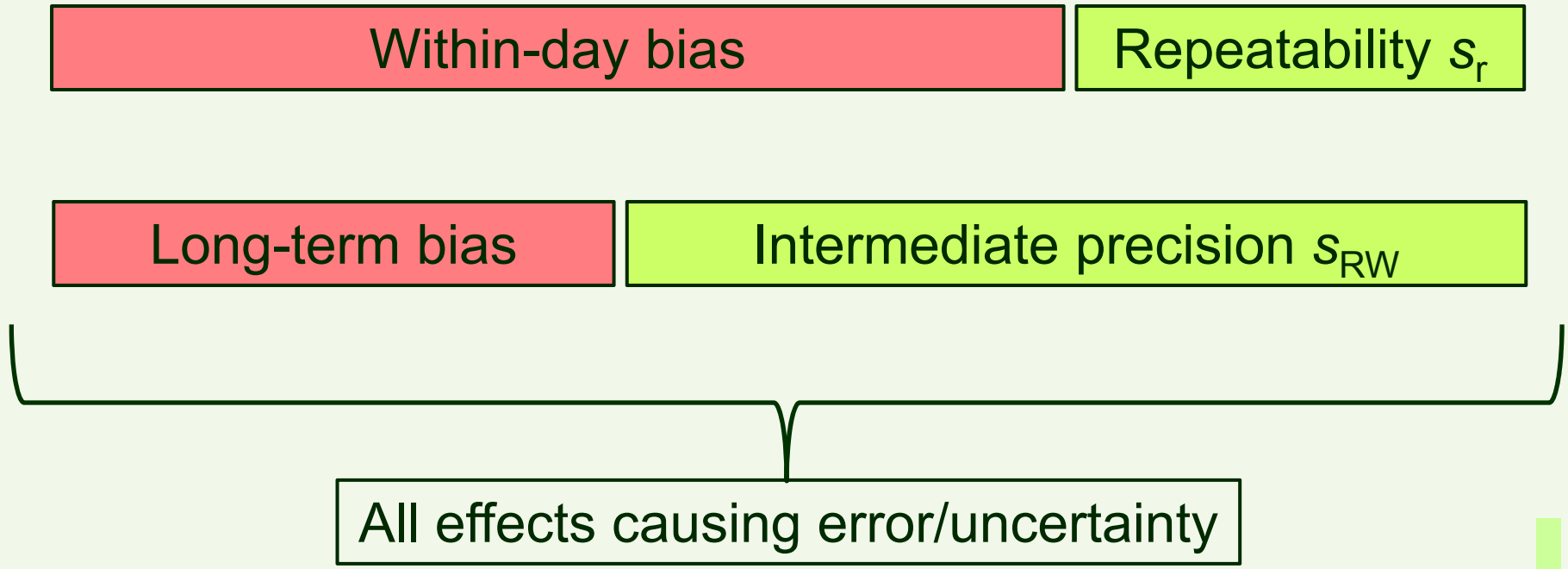
1. All the results of a specific day are systematically influenced by the calibration graph of that day
2. Delicate analyte partially decomposes during sample preparation leading to lowered results
3. The titrant concentration determined on a particular day is slightly lower or higher than the true concentration
4. Because of the specifics of the used sample preparation procedure the sample is digested incompletely, leading to lowered values

Does bias depend on the time frame?

1. Yes, bias determined within a single day is different from one determined on different days (and averaged)
2. No

Systematic and random effects

- Random and systematic effects can be grouped differently:



The longer is the time frame the more effects change their „status“:
systematic ⟶ random

Why is lab/method bias more useful than within-day bias?

- Within-day bias should be redetermined every day
  - Long-term bias can be determined less frequently
- It is useful to work with the lowest possible bias
  - **s_{RW} can be determined more reliably than bias**
  - It is good if most of the uncertainty sources are included into the random component s_{RW}

From now on in this session we only address the long-term bias (lab/method bias)

Example: LC-MS determination of a delicate bioactive compound in blood plasma

Effect	Systematic within day	Systematic in long term
Calibration graph of a specific day	Y	(N)
Injection volume of autosampler is 5% higher than nominal	N	N
Delicate analyte partially decomposes at room temperature before samples are loaded into cooled autosampler	(N)	(N)
Repeatability of peak integration	N	N
Ionization suppression in the ESI source by a co-eluting compound	Y	Y
Baseline noise	(N)	(N)

Which are important issues in determining bias?

Issue	*Bias*	s_{RW}
Sufficient number of replicates	Y	Y
Sufficiently long timeframe	Y	Y
Homogeneous sample	Y	Y
Matrix match	Y	Y
Concentration range match	Y	Y
Reliable reference value	Y	N
Determination of one can be hindered by the other	Y	N

Which are the best approaches for determining bias?

Approach	How good?
Analysing spiked blank matrix	
Replicate measurements of a routine sample	
Using a PT sample and consensus value as reference value	
Analysing a CRM	
Analysing a routine sample with a reference procedure	

How to calculate/express bias?

Way of expressing	Formula	When to use?
Absolute bias	$bias = C_{lab\_mean} - C_{ref}$	If bias is absolute or the *C* range is narrow
Relative bias	$bias = \frac{C_{lab\_mean} - C_{ref}}{C_{ref}}$	If bias is proportional
Recovery	$R = \frac{C_{\text{uncorrected}}}{C_{\text{Ref}}}$	Sample with ref value, bias is proportional
Recovery	$R = \frac{C_1 - C_0}{\Delta C}$	Spiking, bias is proportional

How to conduct a spiking experiment?

- *Two analysts determined meropenem (an antibiotic) in blood plasma. Both needed to determine the recovery of the procedure. They obtained blank plasma samples and did the following:*

- ***Analyst 1** took 500 µl of the blank plasma and added 400 µl of methanol for protein precipitation. He separated the precipitated proteins by centrifugation and transferred the supernatant into an HPLC vial. 100 µl of meropenem standard solution with suitable concentration was added to the supernatant and the resulting solution was injected into the HPLC system for analysis.*

- ***Analyst 2** took 500 µl of the blank plasma, added 100 µl of meropenem standard solution and mixed well. She then added 500 µl of methanol for protein precipitation. She separated the precipitated proteins by centrifugation and injected the resulting supernatant into the HPLC system for analysis.*

Which analyst did it more correctly? Why?

Analyte has to be added at as early stage as possible!

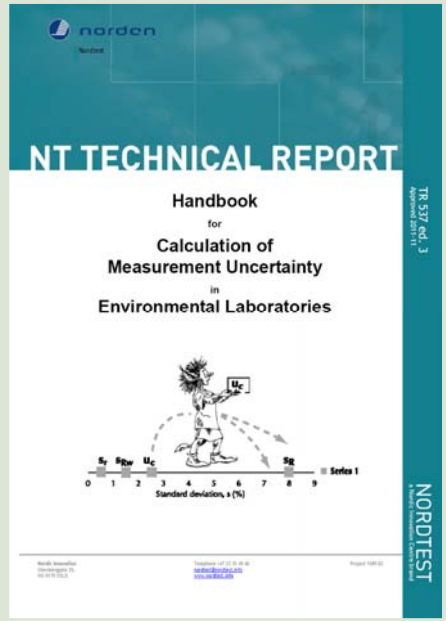
How to evaluate uncertainty due to bias?

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} \qquad u(Cref) = \sqrt{\frac{\sum u(Cref_i)^2}{n}}$$

$$u(bias) = \sqrt{RMS_{\text{bias}}{}^2 + u(Cref)^2}$$

This component accounts for the average bias of the laboratory results from the C_{ref}

This component accounts for the average uncertainty of the reference values C_{ref}



ESTIMATION OF MEASUREMENT UNCERTAINTY IN CHEMICAL ANALYSIS

Search

5.3. SOURCES OF UNCERTAINTY

Brief summary: The overview of possible uncertainty sources, relevant to pesticide analysis, is presented. Most of the uncertainty sources are linked to specific steps in the analysis procedure. It is stressed that sample preparation is usually the biggest contributor to measurement uncertainty. When performing chemical analysis then every care should be taken to minimize (preferably eliminate) the influence of the uncertainty sources, as far as possible. And what cannot be eliminated, has to be taken into account. It is not necessary to quantify every uncertainty source individually. Instead, it is often more practical to quantify several uncertainty sources jointly.

Measurement uncertainty sources

http://www.uttv.ee/naita?id=17587

http://sisu.ut.ee/measurement/

Thank you for your participation!

- The slides are available from:

  http://www.ut.ee/ams/eurachem-2014/

- More explanations and examples:

  http://sisu.ut.ee/measurement/

- You are always welcome to contact me:

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