HBM4EU

science and policy
for a healthy future

Estimation of measurement uncertainty

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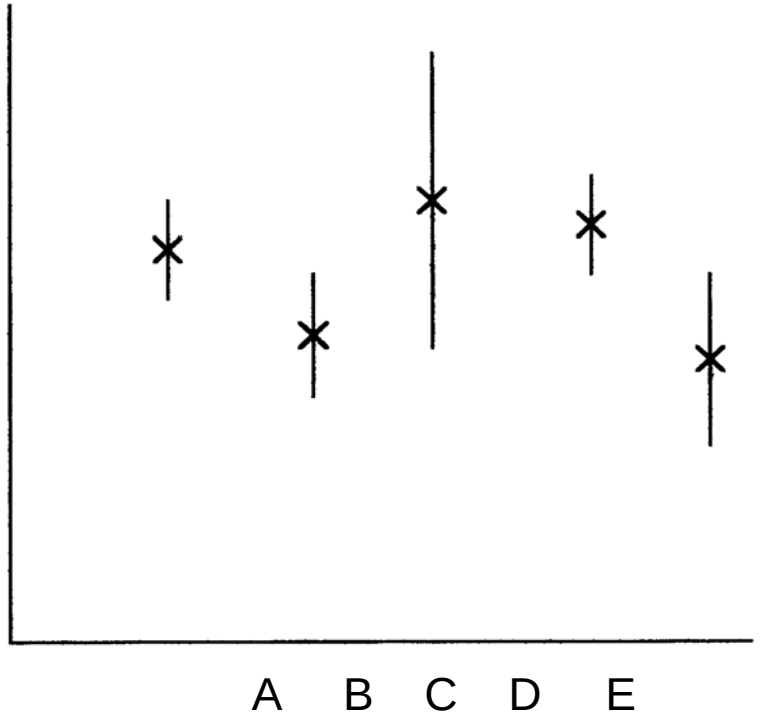
1st HBM4EU Training School 2018

What is Uncertainty?

- A parameter, closely linked to a measurement result. It describes the range of values that the analyst believes could reasonably be attributed to the measurand

- It is FUNDAMENTAL PROPERTY of a result

- requirement of the standard SIST EN ISO/IEC 17025



Definition of uncertainty

"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…. concentration of an analyte

NOTE 1: The parameter may be, for example, a **standard deviation** (or a given multiple of it), or the width of a confidence interval.

NOTE 2: Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the *statistical distribution of the results of series of measurements* and can be characterised by standard deviations. The other components, which also can be characterised by standard deviations, are evaluated from *assumed probability distributions* based on experience or other information. The ISO Guide refers to these different cases as **Type A** and **Type B** estimations respectively.

Uncertainty components

Type A

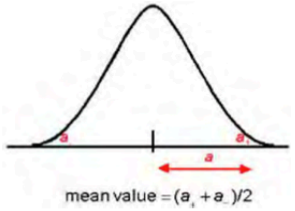
Evaluated from the statistical distribution of the results of series of measurements and characterised by standard deviations

- Derived from repeated measurements
- Quality control material
- Duplicate analysis of samples

Standard uncertainty = **standard deviation**

In nuclear measurements:

Standard uncertainty = $\sqrt{\text{Peak area}}$



Normal distribution

Type B

Characterised by standard deviations, evaluated from assumed probability distributions based upon

- Previous measurement data
- Professional opinion
- Manufacturer's data
- Uncertainties assigned to reference material

Both approaches will be demonstrated during this training!!

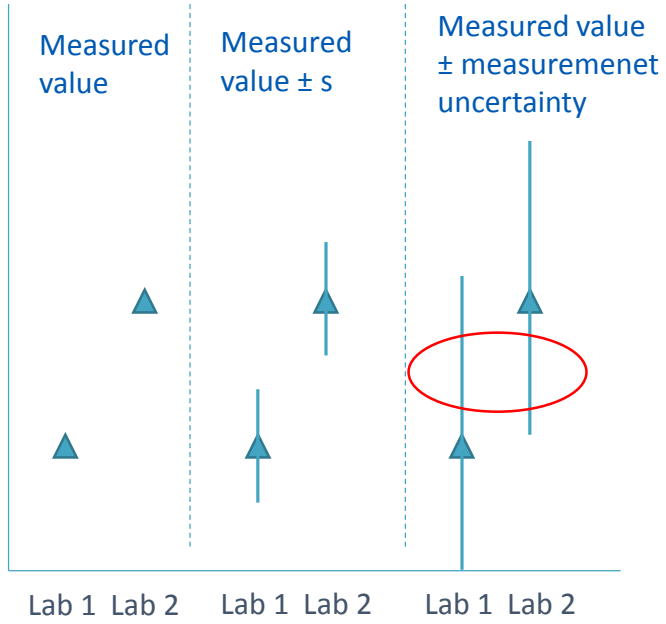
Why is uncertainty important?

Result of a measurement itself is an **estimation** of a true value.

to assess the **reliability** of the result

to know the **confidence** that can be placed in any decisions based on its use

in order to **compare** measurement results



Compliance against limits

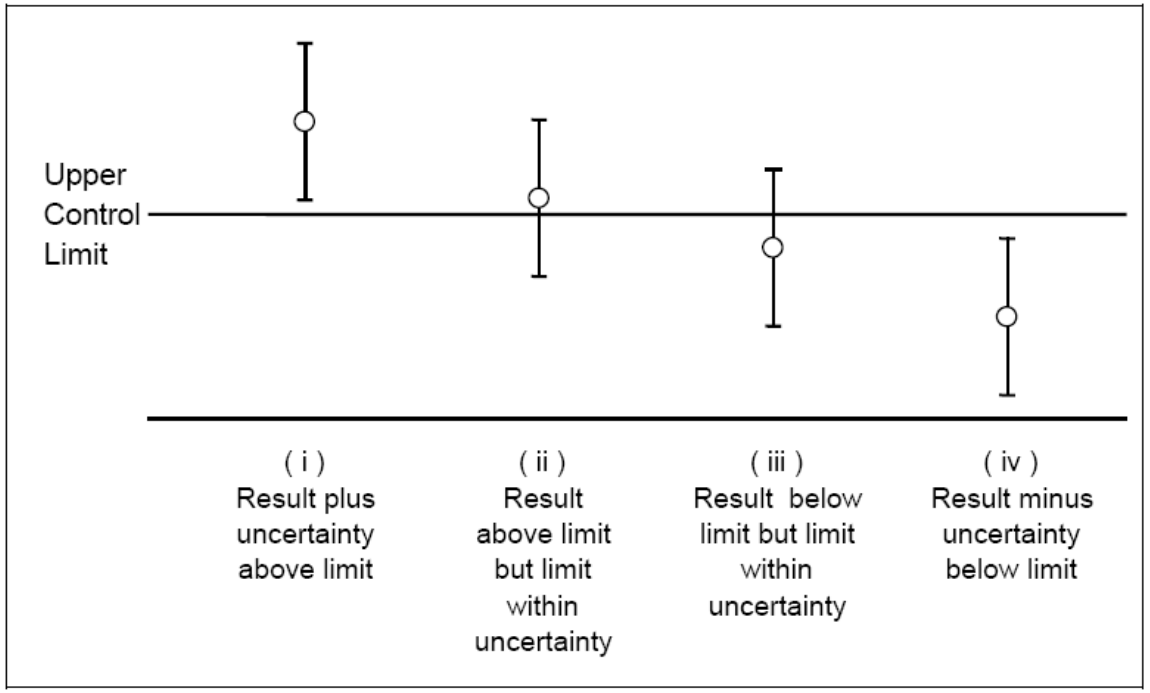


Figure 2: Uncertainty and compliance limits

Source: EURACHEM guide

Uncertainty sources

- incomplete definition
- sampling
- matrix effects and interferences
- environmental conditions
- uncertainties of weights and volumetric equipment
- uncertainties of reference values
- approximations and assumptions incorporated in the measurement method and procedure
- random variation

How to estimate uncertainty of measurement results

General strategy

- Guide to the expression of uncertainty in measurement [ISO GUM]
- **MODEL EQUATION** (empirical and statistical approach)
- Monte Carlo Simulation
- SIST-IS ISO/TS 21748: Uncertainty estimation based on standard deviations – **repeatability** and **reproducibility** of the measurement results (validation data, QA/QC data, inter-lab comparisons)

The process: ***ISO GUM approach***

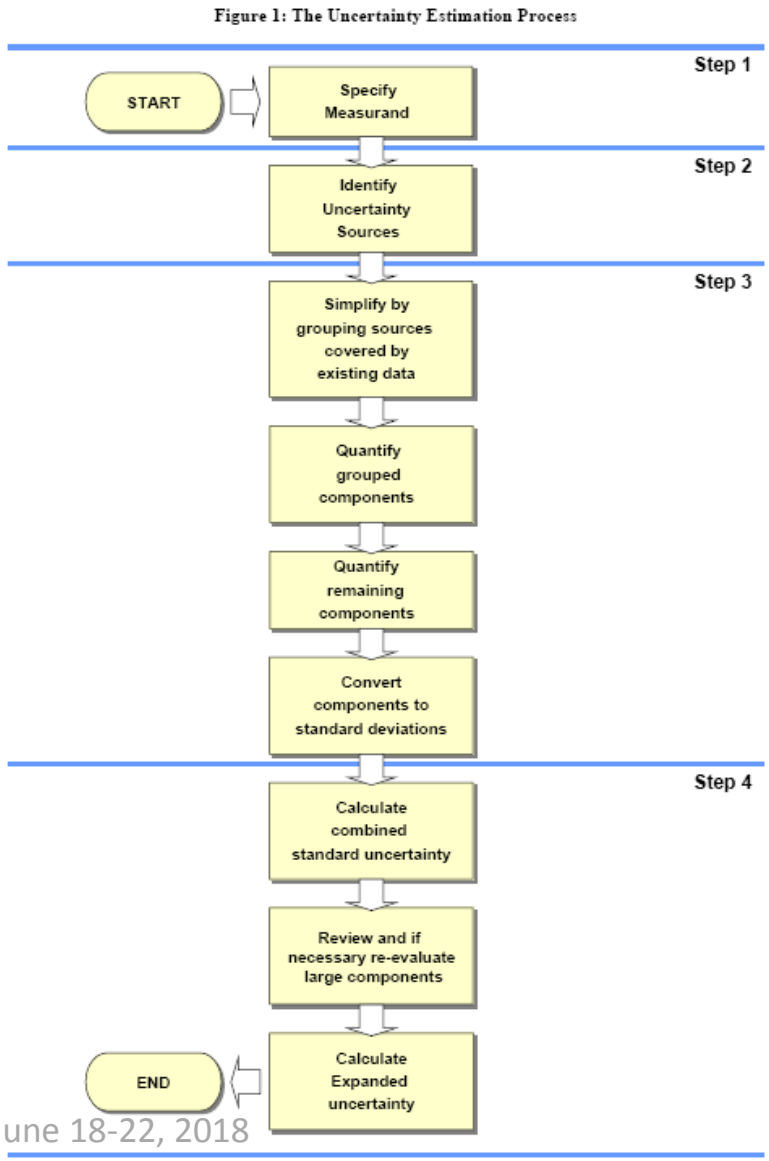
Step 1: Specify Measurand

Step 2: Identify Uncertainty Sources

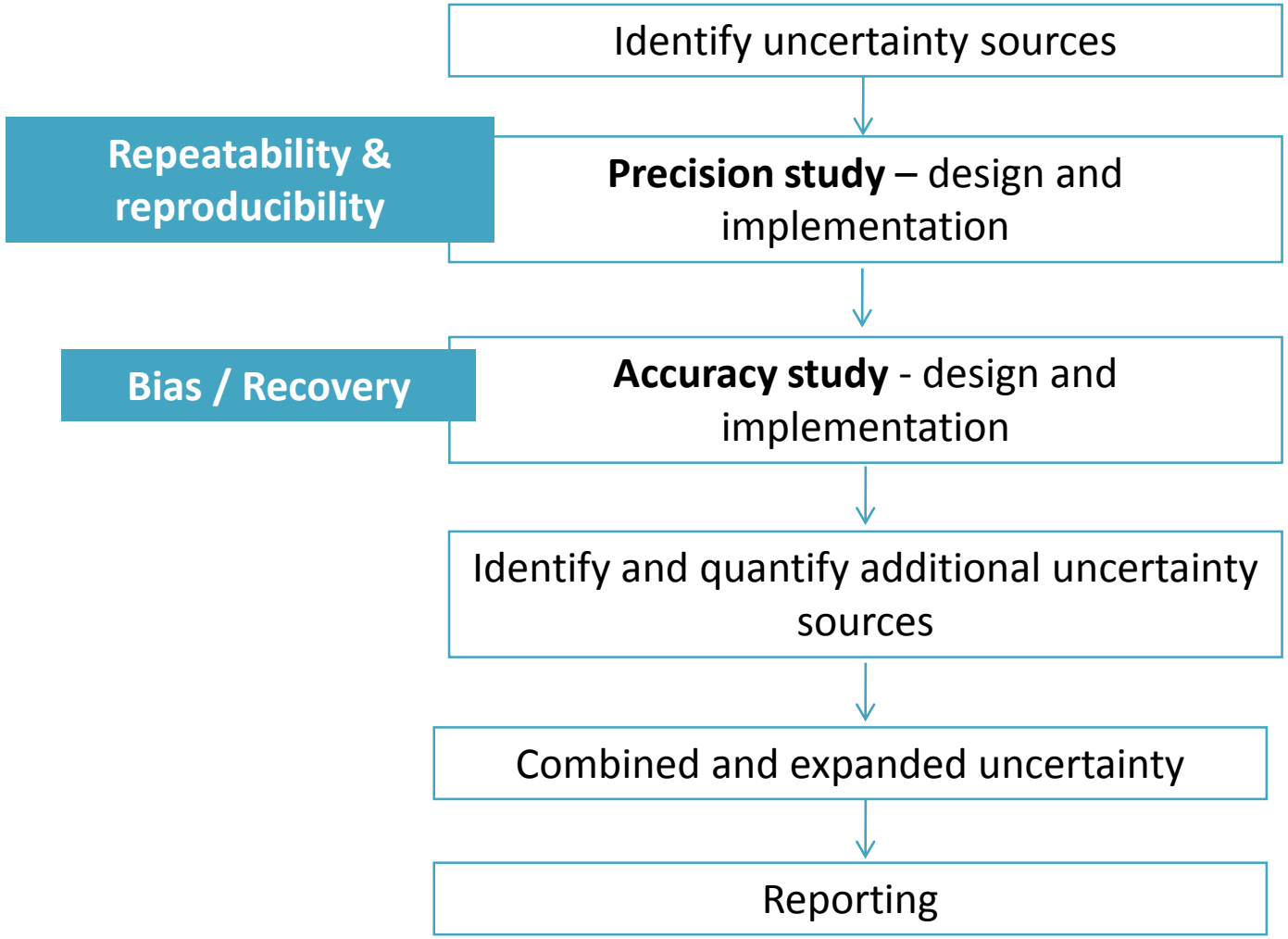
Step 3: Quantify Uncertainty Components

Step 4: Calculate Combined Uncertainty

Source: EURACHEM guide



Alternative approach: Estimating uncertainty using validation data



Alternative approach: Estimating uncertainty using validation data

Design of validation is of crucial importance!

It has to include:

- total method range;
- regular laboratory practice;
- representative range of matrices;
- representative range of analyte concentration

Reproducibility, u(rep)

takes into account long-term variation of results within one lab

Includes sample preparation

Ideally:

- The same sample
- Routine sample OR sample similar to test samples – matrix, concentration, homogeneity
- Different days (preferably over 1 year)
- Different persons
- Different reagent batches
- u(rep) calculated separately for different matrices and different concentration levels!

Recovery, u(rec)

u(rec) calculated from

analysis of the same samples with a reference procedure

analysis of certified reference materials (CRMs)

Inter-laboratory comparison measurements

spiking experiments

Separately for different sample matrices and different concentration levels

Alternative approach: Estimating uncertainty using inter-lab comparison data

Inter-laboratory comparison data can be used in measurement uncertainty estimation in cases:

- requirement for measurement uncertainty is low,
- laboratory results are sufficient,
- types of samples in the inter-lab comparisons are similar to the routine samples,
- laboratory participated in inter-lab comparisons at least 6 times

Measurement of total Hg in biological samples

- Thermal combustion, amalgamation and detection by AAS

- Acid digestion of samples followed by cold vapour AAS or AFS

Standard operating procedures

DETERMINATION OF TOTAL MERCURY IN CORD BLOOD BY COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY (CV AAS). Majda Pavlin and Milena Horvat. Jožef Stefan Institute. Ljubljana, Slovenia

Analysis of mercury in human scalp hair by Thermal Decomposition-Gold Amalgamation-Atomic Absorption Spectroscopy. Susana Pérez Argudo, Argelia Castaño Calvo. National Centre for Environmental Health, Instituto de Salud Carlos III. Madrid, Spain.

EXAMPLE 1

ISO GUM approach

Total Hg in hair by acid digestion and CVAAS detection

Sample digestion flask

HNO_3-$HClO_4$ (1+1), 2 ml
H_2SO_4, 5 ml

Urine samples, 2 ml

Add dropwise while swirling slowly.
Heat at 200-230°C for 30 min.

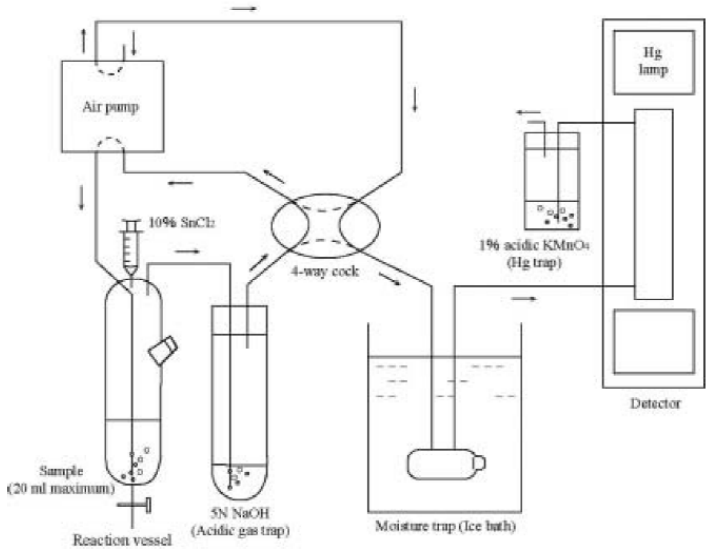
Digested samples

Cool.
Top up to 50 ml with distilled water.

Test solution, a fixed volume (usually 5 ml)

10% $SnCl_2$ solution, 1 ml

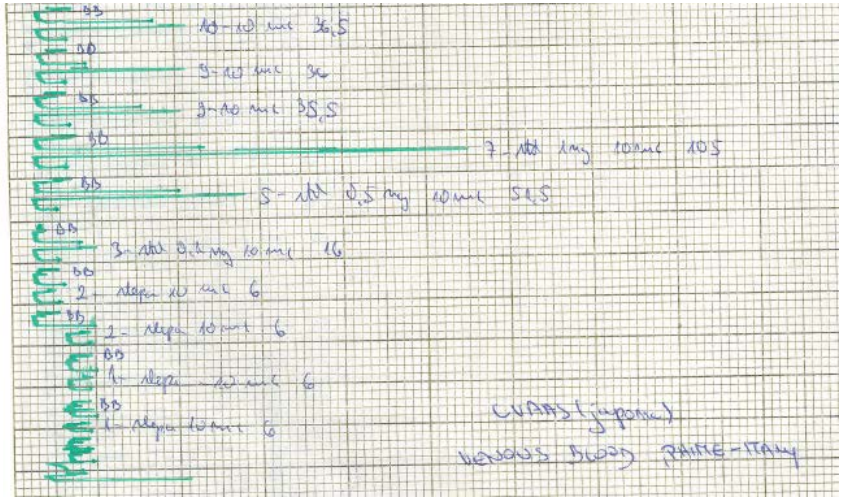
CVAAS



Step 1: Specify Measurand

Quantitative expression relating the value of the measurand to the parameters on which it depends

Mass fraction of Hg in hair (unit *ng/g*)



$$c_{sample} = \frac{h_{sample} - h_{blk}}{h_{STD} - h_{blk}} \cdot \frac{V_{tot}}{V_{analysed} \cdot m_{sample}} \cdot c_{STD} \cdot V_{STD}$$

h_{blk} – blank signal (height of absorbance peak in mm)
h_{sample} – sample signal (mm)
h_{STD} – standard solution signal (mm)
V_{tot} – volume of a sample (ml)

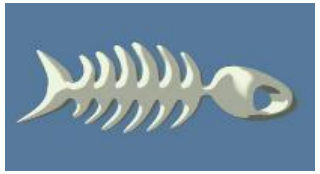
$V_{analysed}$ – volume of a sample aliquot analysed (ml)
m_{sample} – sample mass (g)
c_{STD} – concentration of standard solution (ng/ml)
V_{STD} – volume of standard solution (ml)

Step 2: Identify Uncertainty Sources

- List the possible sources of uncertainty. This will include sources that contribute to the uncertainty on the parameters in the relationship specified in Step 1, but may include other sources and must include sources arising from chemical assumptions.

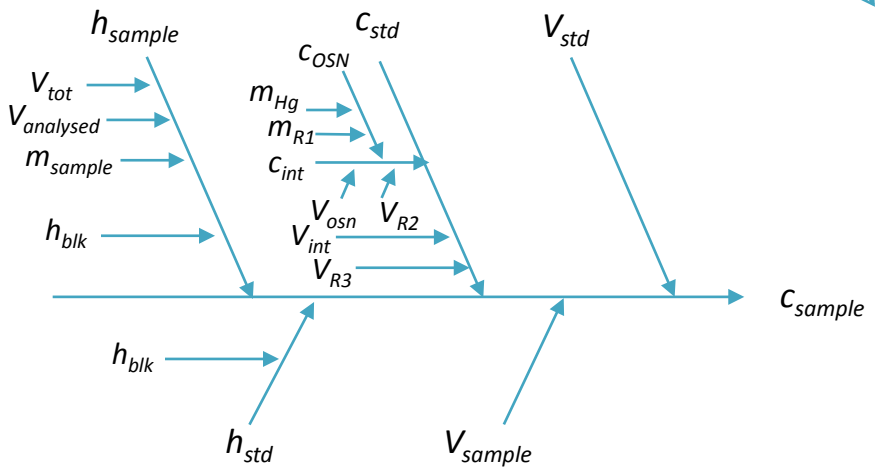
- start with the **basic expression** used to calculate the measurand from intermediate values

- **The cause and effect diagram**



Step 2: Identify Uncertainty Sources

$$c_{sample} = \frac{h_{sample} - h_{blk}}{h_{STD} - h_{blk}} \cdot \frac{V_{tot}}{V_{analysed} \cdot m_{sample}} \cdot c_{STD} \cdot V_{STD}$$



$$c_{STD} = c_{INT} \cdot \frac{V_{INT}}{V_{R3}}$$

$$c_{INT} = c_{OSN} \cdot \frac{V_{OSN}}{V_{R2}}$$

$$c_{OSN} = \frac{m_{Hg}}{m_{R1}} \cdot 1000$$

Step 3: Quantify Uncertainty Components

Estimate standard measurement uncertainties of the identified sources:

$u(h_{sample})$, $u(m_{sample})$, $u(c_{STD})$, $u(V_{STD})$,…

We use

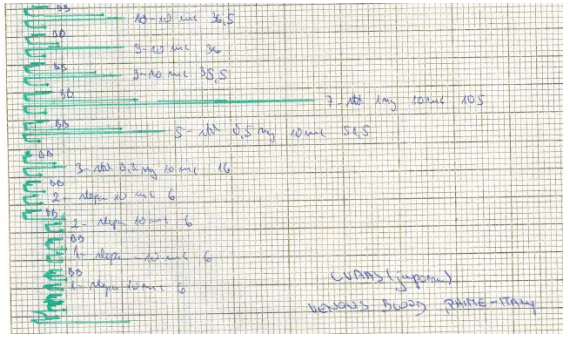
Experimental variation from **validation studies**

Standing data obtained in the **QA/QC laboratory system**

data based on **experience or other information** (e.g. literature, producer‘s data, calibration certificates data,...)

Step 3: Quantify Uncertainty Components

Input parameter	Value	Standard uncertainty	Relative standard uncertainty [%]
h_s	30.0	0.5 mm	1.6
m_s	20 mg	0.06 mg	0.29
$V_{tot\ (vol.\ flask)}$	50 mL	0.12 mL	0.24
$V_{analysed}$	5 mL	0.0095 mL	0.2
c_{STD}	10 ng/mL	0.014 ng/mL	0.14
V_{STD}	0.1000 mL	0.00094 mL	0.94



certificate of the producer

±0.0095
(± SD of 12 readings)

±0.00094
(± SD of 10 readings)

Step 3: Quantify Uncertainty Components

Input parameter	Value	Standard uncertainty	Relative standard uncertainty [%]
h_s	30.0	0.5 mm	1.6
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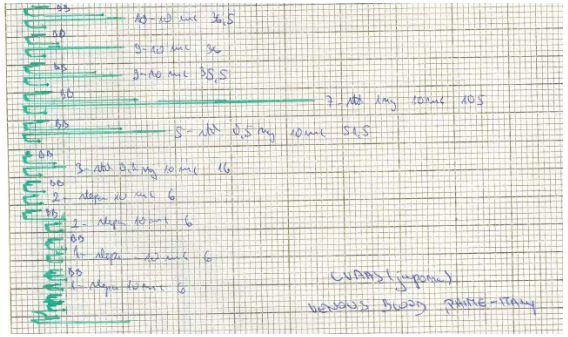


Step 3: Quantify Uncertainty Components

Repeatability & reproducibility

Sample	Result D1 (ng/g)	Result D2 (ng/g)	Mean value (D1+D2/2)	Difference (D1-D2)	Relative difference (D1-D2/mean)
Hair Hg-1	254	254	254	0	0.00
Hair Hg-2	165	165	165	0	0.00
Hair Hg-3	145	156	151	-10	-0.07
Hair Hg-4	303	291	297	12	0.04
Hair Hg-5	94	94	94	0	0.00
Hair Hg-6	621	602	611	19	0.03
Hair Hg-7	298	274	286	24	0.08
Hair Hg-8	531	520	525	11	0.02
Hair Hg-9	666	641	654	25	0.04
Hair Hg-10	298	273	286	25	0.09
Hair Hg-11	325	337	331	-12	-0.04
Hair Hg-12	1534	1516	1525	18	0.01
Hair Hg-13	467	508	487	-41	-0.08
Hair Hg-14	273	262	267	11	0.04
Hair Hg-15	111	122	117	-11	-0.10
Hair Hg-16	574	553	564	20	0.04
Hair Hg-17	400	428	414	-29	-0.07

$$u(rep) = \frac{s_d}{\sqrt{n}} = 0.039$$

Step 3: Quantify Uncertainty Components

Recovery

Measurements of reference material IAEA-086

Reference material (CRM):	Human hair
Name RM:	IAEA-086
Element:	T-Hg
Recommended value:	573
Uncertainty of the value (95% CI):	39
Unit:	ng/g

Step 3: Quantify Uncertainty Components

Recovery

Measurements of reference material IAEA-086

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{ref}}$$

$$u(\overline{R}_m) = \overline{R}_m \times \sqrt{\left(\frac{s_{obs}^2}{n \cdot \overline{C}_{obs}^2}\right) + \left(\frac{u(C_{ref})}{C_{ref}}\right)^2}$$

Date	Mean value (ng/g)
19.4.	561
23.5.	620
12.6.	575
13.6.	591
17.7.	522
18.7.	512
19.7.	523
19.7.	522
3.9.	606
4.9.	634
17.9.	602
18.10.	562
23.10.	567
24.10.	578
14.11.	509
16.11.	579
21.11.	593
23.11.	578
29.11.	573
30.11.	592
5.12.	634
6.12.	595
18.12.	587

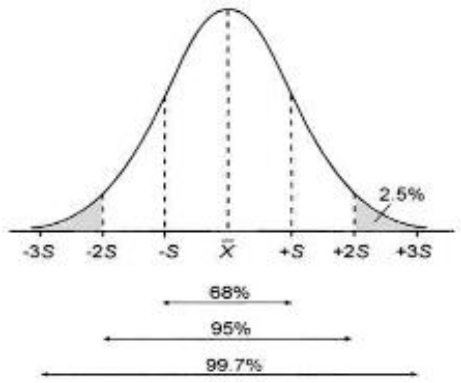
Average C_{obs}	575
s_{obs}	36.4
R_m	1.00
n=	23
C_{ref}=	573
$u(C_{ref})$=	20
u(Rm) =	**0.037**

Ref: Measurement uncertainty: Approaches to the evaluation of uncertainties associated with recovery, V.J.Barwick, S.L.R.Ellison, Analyst, (1999), 124, 981-990.

Step 4: Calculate Combined Uncertainty

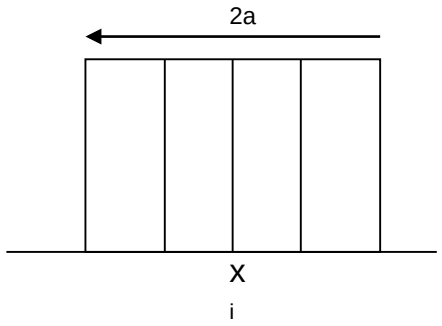
•Before combination, all uncertainty contributions must be expressed as **standard uncertainties**, that is, as **standard deviations**

$$u(x_i) = \frac{s}{1.96}$$



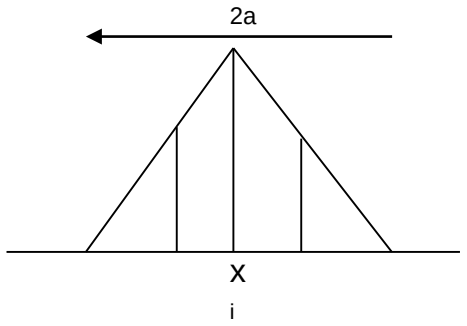
e.g. experimental data

$$u(x_i) = \frac{a}{\sqrt{3}}$$



e.g. scale of the instrument,..

$$u(x_i) = \frac{a}{\sqrt{6}}$$



e.g. volume of volumtric flask, pipets

Step 4: Calculate Combined Uncertainty

Combined uncertainty

$$u_c = \sqrt{u_A^2 + u_B^2} = \sqrt{\sum_i u_X^2}$$

Expanded uncertainty

$$U = k \cdot u_c$$

The choice of the factor *k* is based on the level of confidence desired.

For an approximate level of confidence of 95%, *k* is 2.

Step 4: Calculate Combined Uncertainty

Combined uncertainty

$$u_c = \sqrt{u_h^2 + u_{Vstd}^2 + u_{rep}^2 + u_{rec}^2} =$$

$$= \sqrt{0.016^2 + 0.0094^2 + 0.039^2 + 0.037^2}$$

Expanded uncertainty

$$U = k \cdot u_c = 2 \cdot 0.054 = 0.11$$

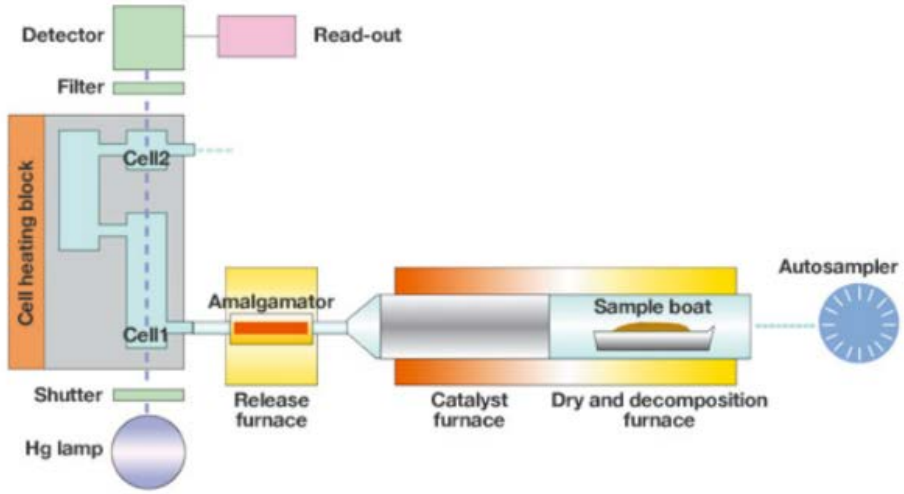
11 %

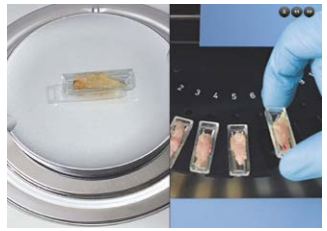
Our result: 413 ± 45 ng Hg/g hair (95 % CI)

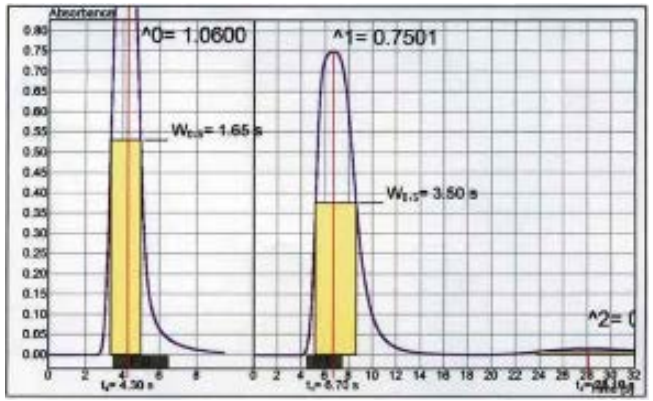
EXAMPLE 2

Alternative approach: uncertainty estimation using validation data

Total Hg in hair, by DMA (Thermal combustion, amalgamation and detection by AAS)







Steps

1. Specify measurand
2. **Quantify repeatability/reproducibility u(rep)**
3. **Quantify recovery u(rec)**
4. Convert components to standard uncertainties u(x)
5. Calculate combined standard uncertainty u_c
6. Calculate expanded uncertainty U

•Remark: Steps 1, 4, 5 and 6: general steps – the same for modeling (i.e. ISO GUM)

Step 3: Quantify Uncertainty Components

Repeatability & reproducibility

Sample	Measurement 1 D1	Measurement 2 D2	Mean value (D1+D2)/2	Difference D1-D2	Relative difference D1-D2/mean value
Hair sample 1	241	240	241	2	0.01
Hair sample 2	312	313	312	-1	0.00
Hair sample 3	188	179	183	9	0.05
Hair sample 4	631	637	634	-7	-0.01
Hair sample 5	359	370	365	-11	-0.03
Hair sample 6	235	229	232	6	0.03
Hair sample 7	354	398	376	-44	-0.12
Hair sample 8	616	657	637	-41	-0.06
Hair sample 9	480	484	482	-5	-0.01
Hair sample 10	356	341	348	15	0.04
Hair sample 11	544	546	545	-2	0.00
Hair sample 12	460	415	438	45	0.10
Hair sample 13	299	325	312	-26	-0.08

$$u(rep) = \frac{s_d}{\sqrt{n}} = 0.041$$

Recovery

Based on spiked solution measurements

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{spike}}$$

$$u(\overline{R}_m) = \overline{R}_m \times \sqrt{\left(\frac{s_{obs}^2}{n \cdot \overline{C}_{obs}^2}\right) + \left(\frac{u(C_{spike})}{C_{spike}}\right)^2}$$

Average C_{obs}	101
s_{obs}	1.3
R_m	1.01
n=	23
C_{spike}=	100
$u(C_{spike})$=	1.4
$u(R_m)$ =	**0.014**

Measurement	Measured value (ng/g)
day1	101.2
day1	100.3
day1	101.5
day1	99.4
day1	99.6
day2	100.1
day2	99.8
day2	99.2
day2	102.3
day2	101.4
day3	100.7
day3	103.4
day3	102.1
day3	99.3
day4	101.9
day4	101.4
day4	99.4
day4	99.5
day5	98.6
day5	101.9
day5	101.8
day5	99.3
day5	99.8

Step 4: Calculate Combined Uncertainty (1)

Combined uncertainty

$$u_c = \sqrt{u_{rep}^2 + u_{rec}^2} =$$

$$= \sqrt{0.041^2 + 0.014^2} = 0.043$$

Expanded uncertainty

$$U = k \cdot u_c = 2 \cdot 0.043 = 0.086$$

8.6 %

Our result: 390 ± 34 ng Hg/g hair (95 % CI)

Step 3: Quantify Uncertainty Components (2)

Recovery

Based on reference material data

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{ref}}$$

$$u(\overline{R}_m) = \overline{R}_m \times \sqrt{\left(\frac{s_{obs}^2}{n \cdot \overline{C}_{obs}^2}\right) + \left(\frac{u(C_{ref})}{C_{ref}}\right)^2}$$

Average C_{obs}	531
s_{obs}	20.7
R_m	0.93
n=	18
C_{ref}=	573
$u(C_{ref})$=	19.5
$u(R_m)$ =	**0.032**

Measurement	Measured value (ng/g)
2.7.2014	575
3.7.2014	537
4.7.2014	542
7.7.2014	555
8.7.2014	548
9.7.2014	542
11.7.2014	543
14.7.2014	538
15.7.2014	539
23.7.2014	543
25.7.2014	517
29.7.2014	520
30.7.2014	500
31.7.2014	504
1.8.2014	536
4.8.2014	511
5.8.2014	513
13.8.2014	499

Step 4: Calculate Combined Uncertainty (2)

Combined uncertainty

$$u_c = \sqrt{u_{rep}^2 + u_{rec}^2} =$$

$$= \sqrt{0.041^2 + 0.032^2} = 0.052$$

Expanded uncertainty

$$U = k \cdot u_c = 2 \cdot 0.052 = 0.104$$

10 %

Our result: 390 ± 41 ng Hg/g hair (95 % CI)

Literature

EURACHEM / CITAC Guide CG 4. Quantifying Uncertainty in Analytical Measurement. 2nd Edition.

Measurement uncertainty: Approaches to the evaluation of uncertainties associated with recovery, V.J.Barwick, S.L.R.Ellison, Analyst, (1999), 124, 981-990.

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Speaker’s information

Janja Snoj Tratnik works at the Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia. Her backround is in biology. She has 10 years experience in trace element analysis in biological samples, HBM recruitment, database management and statistical evaluation of the results. In HBM4EU she is part of the Cd chemical group.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 733032.