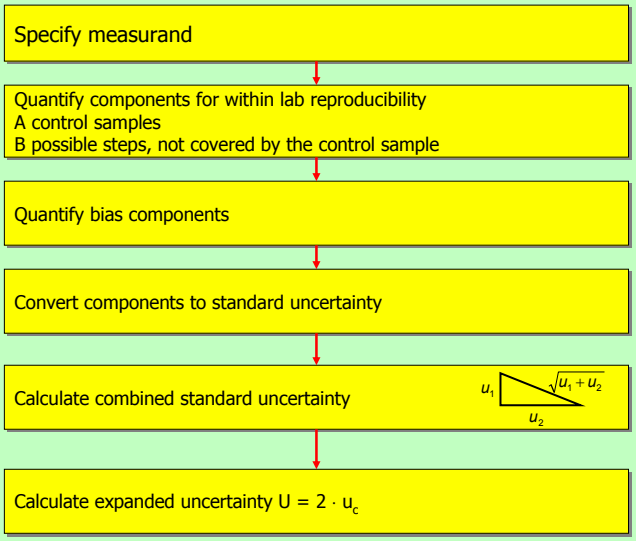
Flowchart for Nordtest Method (a)



Reproducibility within the Laboratory R_w

Control Sample Covering the Whole Analytical Process

- If
  - The control sample covers the whole analytical process and
  - Has a matrix similar to the samples,
- The within-laboratory reproducibility at that concentration level can simply be estimated from the analyses of the control sample
- If the analyses performed cover a wide range of concentration levels, also control samples of other concentration levels should be used.

		value	rel. Uncertainty	Comments
Reproducibility within the lab R_w				
Control sample 1 x = 20.01 µg/l	s_{Rw}	standard deviation 0.5 µg/l	2.5 %	from 75 measure-ments in 2002
Control sample 2 x = 250.3 µg/l	s_{Rw}	standard deviation 3.7 µg/l	1.5 %	from 50 measure-ments in 2002
Other components		---		

Reproducibility within the Laboratory R_w

Control Samples for Different Matrices and Concentrations

- If
  - A synthetic control solution is used for quality control, and
  - The matrix type of the control sample is **not** similar to the natural samples
- We have to take into consideration uncertainties arising from different matrices
- These can be estimated from the repeatability with different matrices (range control chart)

		value	u(x)	Comments
Reproducibility within the lab R_w				
Low level (2-15 µg/l)	s_{Rw}	0.5 µg/l from the mean control chart 0.37 µg/l from the range control chart	0.6 µg/l	Absolute: $u(x)=(0.5^2 + 0.37^2)^{1/2}$
High level (>15 µg/l)	s_{Rw}	1.5 % from the mean control chart 3.6 % from the range control chart	3.9 %	Relative: $u(x)=(1.5\%^2 + 3.6\%^2)^{1/2}$

Note: The repeatability component is included twice!!

Reproducibility within the Laboratory R_w

Unstable Control Samples

- If
  - The laboratory does not have access to stable control samples (e.g. measurement of dissolved oxygen)
- It is possible only to estimate uncertainty components from repeatability via the range control chart
- The „long-term“ uncertainty component (from batch to batch) has to be estimated e.g. by a qualified guess

		value	u(x)	Comments
Reproducibility within the laboratory R_w				
Duplicate measurements of natural samples	s_r	s = 0.024 mg/l mean: 7.53 mg/l	0.32 %	from 50 measurements
Estimated variation from differences in calibration over time		s = 0.5 %	0.5 %	based on experience
Combined uncertainty for R_w Repeatability + Reproducibility in calibration		$\sqrt{0.32\%^2 + 0.5\%^2} = 0.59\%$		

Method and Laboratory Bias

- Can be estimated from
  - The analysis of certified reference materials
  - The participation in proficiency tests
  - From recovery experiments
- Sources of bias should always be eliminated if possible
- According to GUM a measurement result should always be corrected if the bias is significant and based on reliable data such as a CRM.
- In many cases the bias can vary depending on changes in the matrix. This can be reflected when analysing several matrix CRMs

Method and Laboratory Bias u_{bias} Components of Uncertainty

- The bias (as % difference from the nominal or certified value)
- The uncertainty of the nominal/certified value $u(C_{ref})$ u_{bias} can be estimated by:

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{ref})^2} \quad with \quad RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}}$$

- And if only one CRM is used

$$u_{bias} = \sqrt{(bias)^2 + \left(\frac{s_{bias}}{\sqrt{n}}\right)^2 + u(C_{ref})^2}$$

Method and Laboratory Bias u_{bias}
Use of <u>One</u> Certified Reference Material

- The reference material should be analysed in at least 5 different analytical series
- Example: Certified value: 11.5 ± 0.5 (95% confidence interval)

Uncertainty component from the uncertainty of the certified value	
Convert the confidence interval	The confidence interval is ± 0.5. Divide this by 1.96 to convert it to standard uncertainty: 0.5/1.96=0.26
Convert to relative uncertainty $u(C_{ref})$	100·(0.26/11.5)=2.21%

Method and Laboratory Bias u_{bias}
Use of <u>One</u> Certified Reference Material

- Quantify the bias
  - The CRM was analysed 12 times. The mean is 11.9 with a standard deviation of 2.2%
  - This results in:
    bias = 100 · (11.9 – 11.5) / 11.5 = 3.48% and
    s_{bias} = 2.2% with n = 12
  - Therefore the standard uncertainty is:

$$u_{bias} = \sqrt{(bias)^2 + \left(\frac{s_{bias}}{\sqrt{n}}\right)^2 + u(C_{ref})^2} =$$

$$\sqrt{(3.48\%)^2 + \left(\frac{2.2\%}{\sqrt{12}}\right)^2 + 2.21\%^2} = 4.2\%$$

Method and Laboratory Bias u_{bias} Use of Several Certified Reference Materials

- Quantification of the bias
  - Bias CRM1 is 3.48%, s=2.2% (n=12), $u(C_{ref})$=2.21%
  - Bias CRM2 is –0.9%, s=2.0% (n=7), $u(C_{ref})$=1.8%
  - Bias CRM3 is 2.4%, s=2.8% (n=10), $u(C_{ref})$=1.8%
  - RMS_{bias} then is:

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} = \sqrt{\frac{3.48\%^2 + (-0.9\%)^2 + 2.4\%^2}{3}} = 2.5\%$$

  - And the mean uncertainty of the certified value $u(C_{ref})$: 1.9%
  - This results in the total standard uncertainty of the bias:

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{ref})^2} = \sqrt{2.5\%^2 + 1.9\%^2} = 3.1\%$$

Method and Laboratory Bias u_{bias} Use of PT Results

- In order to have a reasonably clear picture of the bias from interlaboratory comparison results, a laboratory should participate at least 6 times within a reasonable time interval

Uncertainty component from the uncertainty of the nominal value	
Between laboratory standard deviations s_R	s_R has been on average 9% in the 6 exercises
Convert to relative uncertainty $u(C_{ref})$	Mean number of participants= 12 $u(C_{ref}) = \frac{s_R}{\sqrt{n}} = \frac{9\%}{\sqrt{12}} = 2.6\%$

Method and Laboratory Bias u_{bias} Use of PT Results

- Quantification of the bias
  - In the 6 participations the differences between the lab results and the assigned value biases have been: 2%, 7%, -2%, 3%, 6% and 5%
  - Therefore RMS_{bias} is:

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} = \sqrt{\frac{2\%^2 + 7\%^2 + (-2\%)^2 + 3\%^2 + 6\%^2 + 5\%^2}{6}} = 4.6\%$$

  - And the total standard uncertainty of the bias:

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{ref})^2} = \sqrt{4.6\%^2 + 2.6\%^2} = 5.3\%$$

Method and Laboratory Bias u_{bias} From Recovery Tests

- Recovery tests, for example the recovery of a standard addition to a sample in the validation process, can be used to estimate the systematic error. In this way, validation data can provide a valuable input to the estimation of the uncertainty.
- Example: In an experiment the recoveries for an added spike were 95 %, 98 %, 97 %, 96 %, 99 % and 96 % for 6 different sample matrices. The spike of 0.5 ml was added with a micropipette.

uncertainty component from spiking	
Uncertainty of the concentration of the spike u(conc)	from the certificate: 95% confidence intervall = ± 1.2 % u(conc) = 0.6 %
Uncertainty of the added volume u(vol)	from the manufacturer of the micro pipette: max. bias: 1% (rectangular interval), repeatability: max. 0.5% (standard dev.) $u(vol) = \sqrt{\left(\frac{1\%}{\sqrt{3}}\right)^2 + 0.5\%^2} = 0.76\%$
Uncertainty of the spike u($c_{recovery}$)	$\sqrt{u(conc)^2 + u(vol)^2} = \sqrt{0.6\%^2 + 0.76\%^2} = 1.0\%$

Method and Laboratory Bias u_{bias} From Recovery Tests

- Quantification of the bias:
- RMS_{bias}:

$$RMS_{bias} = \sqrt{\frac{5\%^2 + 2\%^2 + 3\%^2 + 4\%^2 + 1\%^2 + 4\%^2}{6}} = 3.44\%$$

- Therefore the total standard uncertainty of the bias is:

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{recovery})^2} = \sqrt{3.44\%^2 + 1.0\%^2} = 3.6\%$$

Combination of the Uncertainties
(Reproducibility within the Laboratory and Bias)

- Reproducibility (R_w) (from control samples and other estimations)
- Bias u_{bias} (from CRM, PT or recovery tests)
- Combination:

$$u_c = \sqrt{u(R_w)^2 + u_{bias}{}^2}$$

Calculation of the Expanded Uncertainty

- For the conversion to an approx. 95% confidence level

$$U = 2 \cdot u_c$$

Method b) - Direct Use of Reproducibility Standard Deviations

- If the demand on uncertainty is low
- $u_c = s_R$
- The expanded uncertainty becomes $U = 2 \cdot s_R$
- This may be an overestimate depending on the quality of the laboratory – worst-case scenario
- It may also be an underestimate due to sample inhomogeneity or matrix variations

Reproducibility Standard Deviation from a Standard

- The laboratory must first prove that they are able to perform in accordance with the standard method
  - No “unusual” bias
  - Verification of the repeatability
- The expanded uncertainty then is:

$$U = 2 \cdot s_R$$

Reproducibility standard deviation from a standard Example – Mercury according to EN 1483

Tabelle 2: Verfahrenskenndaten

reproducibility variation coefficient

	Alle Laboratorien										
	Probe	l	n	NAP %	Wahrer Wert µg/l	$\bar{\bar{x}}$ µg/l	σ_R µg/l	VC_R %	σ_r µg/l	VC_r %	Wieder-findungs-rate %
drinking water	A	21	62	9	0,819	0,831	0,2500	30,1	0,1310	15,8	101,5
surface water	B	20	59	13	1,474	1,459	0,3918	26,9	0,1855	12,7	99,0
waste water	C	21	68	0	5,732	5,799	1,3745	23,7	0,5746	9,9	101,2

- Expanded uncertainty for drinking water:
  $U = 2 \cdot VC_R \approx 60\ \%$

Reproducibility Standard Deviation from a PT

- The laboratory must have been successfully participating in the PT
- If the comparison covers all relevant uncertainty components and steps (matrix?)
- The expanded uncertainty then also is:

$$U = 2 \cdot s_R$$

Reproducibility Standard Deviation from a PT Example – Mercury in a Univ. Stuttgart PT

Niveau	Vorgabe [µg/l]	rob. Standardab	Standardab	Ausschlussgren	Ausschlussgren	Ausschlussgren	Ausschlussgrenze unten [%]	Anzahl Werte	außerhalb unten	außerhalb oben	außerhalb [%]
		chung [µg/l]	hung [%]	ben [µg/l]	nten [µg/l]	ben [%]					
1	0,584	0,133	22,86	0,889	0,341	52,25	-41,60	37	3	1	10,8
2	1,248	0,225	18,09	1,748	0,830	40,07	-33,46	39	3	1	10,3
3	1,982	0,3502	17,67	2,756	1,333	39,06	-32,75	39	1	0	2,6
4	3,238	0,4726	14,60	4,263	2,352	31,65	-27,36	41	2	2	9,8
5	3,822	0,4550	11,90	4,793	2,960	25,40	-22,55	38	0	1	2,6
6	4,355	0,7704	17,69	6,057	2,927	39,10	-32,78	40	1	0	2,5
7	5,421	0,7712	14,23	7,090	3,973	30,78	-26,71	41	1	1	4,9
8	6,360	0,7361	11,57	7,928	4,963	24,65	-21,96	38	5	1	15,8
9	6,553	0,9177	14,00	8,536	4,829	30,25	-26,31	39	2	0	5,1
10	7,361	0,9965	13,54	9,508	5,486	29,16	-25,48	40	1	3	10,0
11	8,063	1,0672	13,24	10,357	6,051	28,46	-24,94	38	5	2	18,4
12	9,359	0,9854	10,53	11,444	7,481	22,29	-20,06	40	2	2	10,0
							Summe	470	26	14	8,5

reproducibility variation coefficient

- $u_c = s_R \approx 20\%$
- $U \approx 40\%$

Summary of the NORDTEST Approach

- Two different methods to estimate the measurement uncertainty have been introduced:
- Method a)
  - Estimation of the within-lab reproducibility (mainly from control charts)
  - Estimation of the bias (from analyses of CRM, PT results or recovery tests)
  - Combination of both components
- Method b)
  - direct use of the reproducibility standard deviation from standards or PTs as combined standard uncertainty
- As a rule method b) delivers higher measurement uncertainties (conservative estimation)

Expression of Uncertainty

- The statement of uncertainty always has to contain the level of confidence
- If possible also state the estimation method used
- Example:

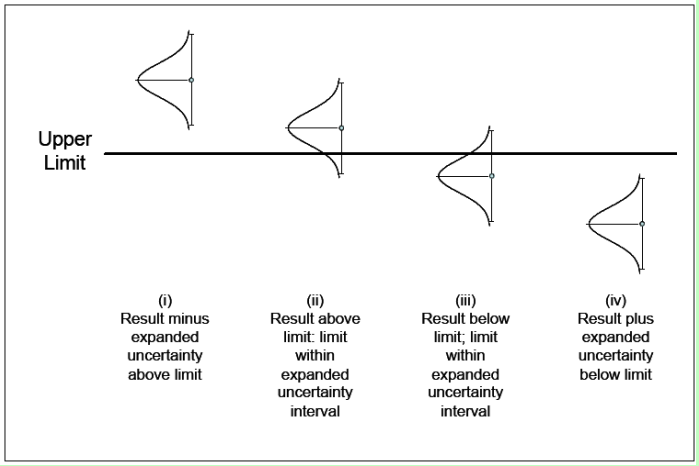
  SO_4^{2-} in waste water (ISO 10304-2): 100 ± 8 mg/l*

  * Measurement uncertainty was derived from results of interlaboratory comparisons. It represents an expanded uncertainty with a coverage factor k=2; this corresponds to a level of confidence of about 95%.

Uncertainties and Limits

- How can we handle uncertainties in the assessment of values with respect to limits
- From: EURACHEM/CITAC Guide “Use of uncertainty information in compliance assessment, 2007

Assessment of Compliance with an Upper Limit



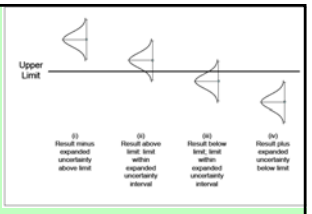
Decision Rules

- The key to the assessment are the „decision rules“
- Based on
  - The measurement result,
  - Its uncertainty,
  - The specification limit
- And taking into account
  - The acceptable level of the probability of making a wrong decision
- These rules give a prescription for the acceptance or rejection of a product

Acceptance Zone – Rejection Zone

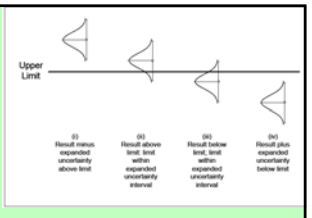
- Based on the decision rules such zones are defined
  - If the measurement lies in the acceptance zone the product is declared compliant
  - If the measurement lies in the rejection zone the product is declared non-compliant

Simple Decision Rules – 1st Example



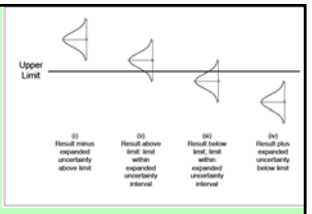
- **„A result implies non-compliance with an upper limit if the measured value exceeds the limit by the expanded uncertainty.“**
  - With this decision rule only case (i) in the figure would imply non-compliance

Simple Decision Rules – 2nd Example



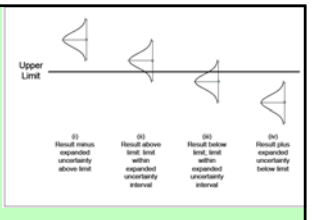
- **„A result implies non-compliance with an upper limit if the measured value exceeds the limit minus the expanded uncertainty.“**
  - With this decision rule only case (iv) in the figure would imply compliance

Simple Decision Rules – Another Widely Used



- **„A result equal to or above the upper limit implies non-compliance and a result below the limit implies compliance, provided that the uncertainty is below a specified value.“**
  - This is normally used where the uncertainty is so small compared with the limit that the risk of making a wrong decision is acceptable
  - To use such a rule without specifying the maximum permitted value of the uncertainty would mean that the probability of making a wrong decision would not be known
  - With this decision rule, cases (i) and (ii) would imply non-compliance, cases (iii) and (iv) compliance

More Complicated Decision Rules

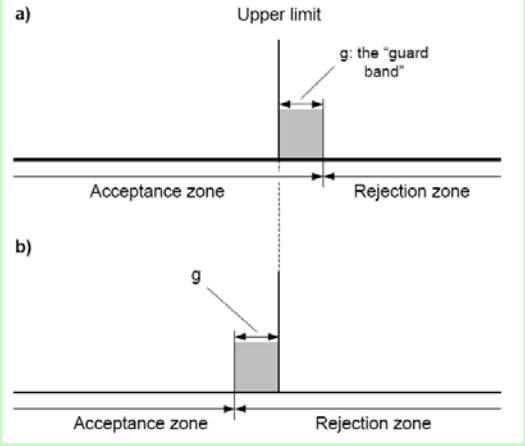


- Decision rules may include, for example, that for cases (ii) and (iii) in the figure, additional measurement(s) should be made, or
- That the manufactured product might be compared with an alternative specification to decide on possible sale at a different price

Basic Requirements for the Decision

- A **specification** giving upper and/or lower permitted limits of the characteristics (measurands) being controlled
- A **decision rule** that describes how the measurement uncertainty will be taken into account with regard to accepting or rejecting a product according to its specification and the result of a measurement
- The limit(s) of the **acceptance or rejection zone** (i.e. the range of results), derived from the decision rule, which leads to acceptance or rejection when the measurement result is within the appropriate zone

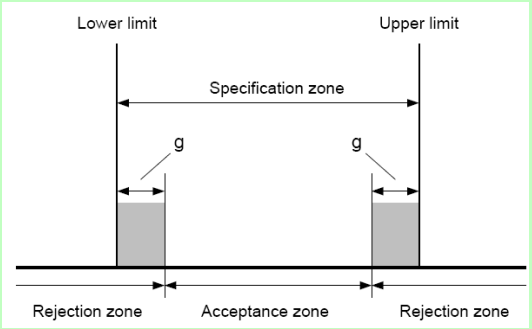
Acceptance and Rejection Zones for an Upper limit



a) high confidence of correct rejection (1st example)

b) high confidence of correct acceptance (2nd example)

Acceptance and Rejection Zones for Simultaneous Upper and Lower Limits



- Acceptance and rejection zones for low risk of false acceptance

Who Defines the Decision Rule?

- The relevant product specification or regulation should ideally contain the decision rules
- Where this is not the case then they should be drawn up as part of the definition of the analytical requirement (i.e. during contract review)
- When reporting on compliance, the decision rules that were used should always be made clear

Guidance Documents

- ISO Guide 98: “Guide to the expression of uncertainty in measurement” (www.bipm.org)
- EURACHEM/CITAC: Quantifying Uncertainty in Analytical Measurement, 2nd Edition (2000) (www.eurachem.org)
- NORDTEST: Handbook for calculation of measurement uncertainty in environmental laboratories. Report TR 537 (www.nordicinnovation.net/nordtest.cfm)
- LGC/VAM: Development and Harmonisation of Measurement Uncertainty Principles Part(d): Protocol for uncertainty evaluation from validation data (www.vam.org.uk)
- Niemelä, S.I.: Uncertainty of quantitative determinations derived by cultivation of microorganisms. MIKES-Publication J4/2003 (www.mikes.fi)
- EA Guidelines on the Expression of Uncertainty in Quantitative Testing EA-4/16 (rev.00) 2003 (www.european-accreditation.org)
- ILAC-G17:2002 Introducing the Concept of Uncertainty of Measurement in Testing in Association with the Application of the Standard ISO/IEC 17025 (www.ilac.org)
- A2LA: Guide for the Estimation of Measurement Uncertainty In Testing, 2002 (www.a2la.net)
- EUROLAB: Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation, 2007 (www.eurolab.org)
- EURACHEM/CITAC Guide “Use of uncertainty information in compliance assessment, 2007 (www.eurachem.org)