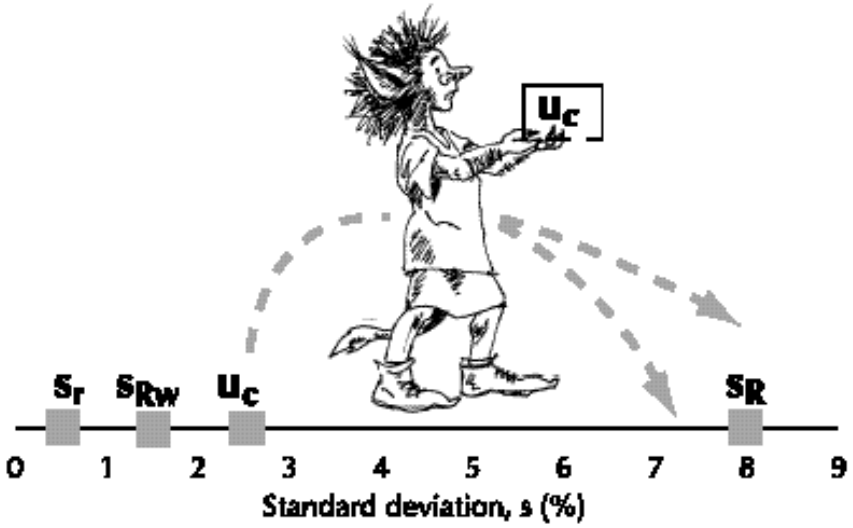
Handbook
for

Calculation of Measurement Uncertainty

in
Environmental Laboratories



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Environmental Laboratories

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Nordtest project 1589-02

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Update for version 4 – Minor revision of text and update of terminology and references. New references include motivation for the treatment of bias, a software for the uncertainty calculations according to the approach presented here as well an ISO standard describing in detail the uncertainty calculations for water analysis.

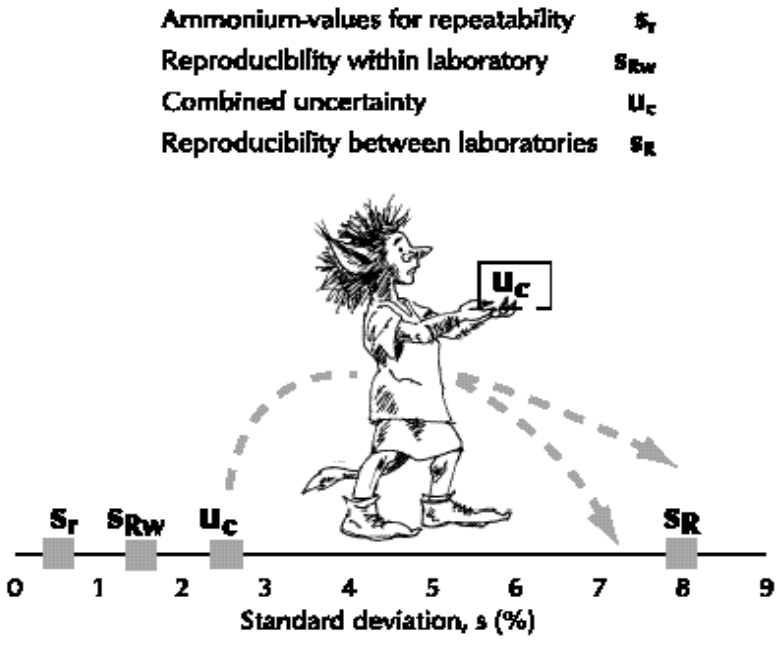
This guide should be cited as: B. Magnusson, T. Näykki, H. Hovind and M. Krysell, *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories* Nordtest tecn report 537 (2013) www.nordtest.info.

Contents:

1 Definitions and abbreviations

s	An estimate of the population standard deviation σ from a limited number (n) of observations (x_i). The unit could be absolute i.e. in concentration units or relative in % (CV%).
$\bar{x}$	Mean value.
$u(x)$	Individual standard uncertainty component (GUM, /1/).
u_c	Combined standard uncertainty (GUM, /1/).
U, $k=2$	Expanded uncertainty with a coverage factor k of 2 which provides an interval with a level of confidence of approximately 95 %.
r	Repeatability limit – performance measure for a test method or a defined procedure. It indicates the maximum difference between two values which can be expected with 95 % probability when the test results are obtained under **repeatability conditions**. **Repeatability conditions:** Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. Repeatability (precision under repeatability conditions) is also sometimes called "within run precision" (ISO 3534-1, /6/).
s_r	Repeatability standard deviation of a measurement (can be estimated from a series of duplicate analyses).
R	Reproducibility limit – performance measure for a test method or procedure. It indicates the maximum difference between two values which can be expected with 95 % probability when the test results are obtained under **reproducibility conditions**. **Reproducibility conditions:** Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment. Reproducibility (precision under reproducibility conditions) is also sometimes called "between laboratory precision" (ISO 3534-1, /6/).
s_R	Reproducibility standard deviation of a measurement. s_R can be estimated from method validation studies with many participating laboratories or from other interlaboratory comparisons, e.g. proficiency testing (PT) schemes. Note: $R = 2.8 \cdot s_R$
R_w	Within-laboratory reproducibility = intermediate measure between r and R, where *operator* and/or *equipment* and/or *time* and/or *calibration* can be varied, but in the same laboratory. An alternative name is intermediate precision

s_{Rw}	Within-laboratory reproducibility standard deviation (can be estimated from standard deviation of a control sample over a certain period of time, preferably one year).
$u(R_w)$	Uncertainty component for within-laboratory reproducibility. The component is equal to s_{Rw} or when control samples are not similar to test samples $\sqrt{s_{Rw}^2 + s_{difference}^2}$ where $s_{difference}$ is the increased standard deviation due to differences between test samples and control sampleo
CRM	Certified Reference Material.
Certified value	Value assigned to a CRM, quantified through a certification process, with documented traceability and uncertainty.
Nominal value	Nominal value is the assigned value, e.g. in an interlaboratory comparison where it is the organiser's best representation of the "true value".
u(Cref)	Uncertainty component from the certified or nominal value
Bias	Difference between mean measured value from a large series of test results and an accepted reference value (a certified or nominal value). The measure of trueness is normally expressed in term of bias. Bias for a measurement, e.g. for a laboratory or for an analytical method.
u(bias)	Uncertainty component for bias. The *u*(bias), is always included in the measurement uncertainty calculations.
RMS_{bias}	$\sqrt{\frac{\sum (bias_i)^2}{n}}$ see further ref /17/.
Interlaboratory comparison	General term for a collaborative study normally aimed at documenting either method performance, laboratory performance (proficiency testing) or at characterising properties of a material.



2 Introduction

2.1 Scope and field of application

This handbook is written for environmental testing laboratories in the Nordic countries, in order to give support to the implementation of the concept of measurement uncertainty for their routine measurements. The aim is to provide a practical, understandable and common way of measurement uncertainty calculations mainly based on already existing quality control and validation data, according to an EA guideline /12/, a Eurolab Technical Report /3/ and the standard ISO 21748 /8/. Nordtest has supported this project economically in order to promote and enhance harmonisation between laboratories on the Nordic market.

Practical examples, taken directly from the everyday world of environmental laboratories, are presented and explained. However, the approach is very general and should be applicable to most testing laboratories in the chemical field.

The handbook covers the steps in the analytical chain, from the arrival of the sample in the laboratory until the data has been reported. It is, hence, important to notice that other uncertainty sources, which can constitute a significant part of the total measurement uncertainty are not included here, e.g. sampling, sample transportation.

The recommendations in this document are primarily for guidance. An ISO standard for water analysis has implemented this approach to estimate uncertainty/18/. It is recognised that while the recommendations presented do form a valid approach to the evaluation of measurement uncertainty for many purposes, other suitable approaches may also be adopted – see references in Section 9. Especially the EURACHEM/CITAC-Guide /2/ is useful in cases where sufficient previous data is not available, and therefore the modelling approach may be used.

Basic knowledge in the use of quality control and statistics is required. In order to make it possible for the reader to follow the calculations, some raw data is given in appendices. Appendix 10 is an example of a printout from a software available /16/ thanks to work at SYKE, for the uncertainty calculations according to this handbook, www.environment.fi/syke/envical,

2.2 Comment to customers

Previously, laboratories usually reported uncertainty as the standard deviation calculated from data for an internal control sample. The measurement uncertainty also taking into account method and laboratory bias and using a coverage factor of 2, can give uncertainty values which may be a factor of 2 to 5 times higher than previously (Figure 1). However, this does not reflect a change in the performance of the laboratory, just a much better estimation of the real variation between laboratories.

In Figure 1, the ammonium results from two laboratories are in good agreement – the difference is about 5 %. You can see this if you look to the right where measurement uncertainty is calculated correctly, but not if you look to the left, where the uncertainty is calculated directly from a control sample and presented as the standard deviation (± 1*s*).

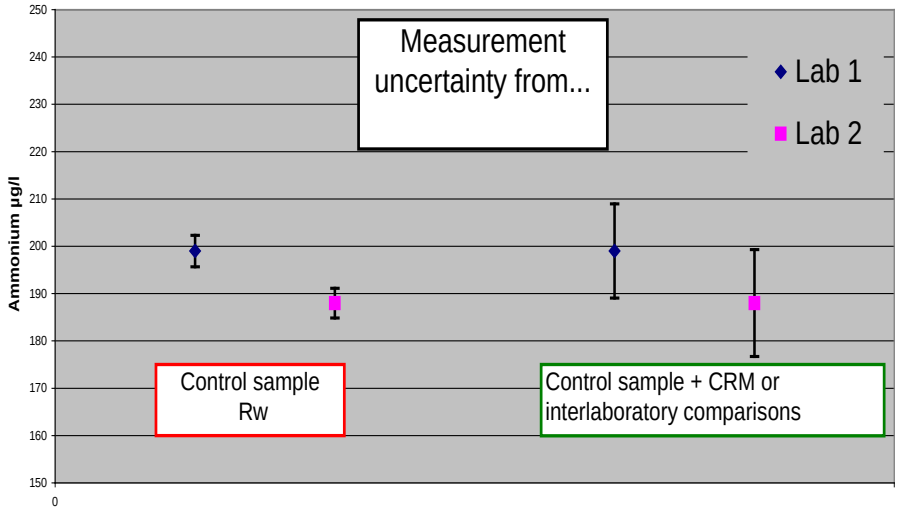


Figure 1. Comparing ammonium results from two laboratories, Lab 1 = 199 µg/L and Lab 2 = 188 µg/L. To the left the error bars are calculated from results on control samples (± 1s) and to the right the error bars are expanded measurement uncertainties (*U*, *k*=2).

2.3 About measurement uncertainty

What is measurement uncertainty?

- The number after ±. For example, a result reported as 10 ± 1 mg/L is normally interpreted as that the true value is in the interval 9-11 mg/L. If the laboratory estimates the uncertainty correctly, this interpretation is valid.
- All measurements are affected by a certain error. The measurement uncertainty tells us how large that error **might** be. Therefore, the measurement uncertainty is an important part of the reported result.
- Definition: Measurement uncertainty is ”A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand” /1/.

Who needs measurement uncertainties?

- The customer needs it together with the result to make correct decisions.
- The laboratory to know its own measurement quality and to improve to quality if necessary.

Why should the laboratory report a measurement uncertainty?

- The customers need it to make correct decisions. The uncertainty of the result is important, e.g. when assessing compliance with legal limits. Some regulations state limits for the maximum allowed uncertainty.
- An estimation of the measurement uncertainty is required for laboratories accredited to ISO/IEC 17025 /9/.

How is a measurement uncertainty obtained?

- The basis for the evaluation is a measurement, evaluated using statistical and other information, and where the different uncertainty sources are estimated and combined into a single value.
- "*Basis for the estimation of measurement uncertainty is the existing knowledge (no special scientific research should be required from the laboratories). Existing experimental data should be used (quality control charts, validation, interlaboratory comparisons, CRM etc.)*" /12/.
- Guidelines are given in GUM /1/, further developed in, e.g., EA guidelines /12/, the Eurachem/CITAC guide /2/, in a Eurolab technical report /3/ and in ISO 21748 /8/.

How is the measurement uncertainty reported?

- Measurement uncertainty should normally be expressed as an expanded uncertainty $U = k \cdot u_c$, where k is the coverage factor and u_c the combined standard uncertainty. Usually k is set to 2 which provides an interval with a level of confidence of approximately 95 %.
- It is often useful to state how the measurement uncertainty was obtained.
- Example, where ± 7 is the measurement uncertainty: Ammonium (NH_4-N) = 148 ± 7 µg/L. The measurement uncertainty, 7 µg/L (expanded uncertainty U, with coverage factor k=2, corresponding approximately to 95 % level of confidence) is estimated from control samples and from regular interlaboratory comparisons.

How should measurement uncertainty be used?

- It can be used as in Figure 1, to decide whether there is a difference between results from different laboratories, or results from the same laboratory at different occasions (time trends etc.).
- It is necessary when comparing results to allowable values, e.g. tolerance limits or allowable (legal) concentrations.

3 Calculation of expanded uncertainty, *U*

A common way of presenting the different contributions to the total measurement uncertainty is to use a so-called fish-bone (or cause-and-effect) diagram. We propose a model (Figure 2), where either the within-laboratory reproducibility (u_{Rw}) is combined with estimates of the method and laboratory bias, (error model in Appendix 3) or the reproducibility s_R is used more or less directly, ISO 21748 /8/. The alternative way is to construct a detailed fish-bone diagram and estimate the individual uncertainty contributions. This approach may prove very useful when studying or quantifying individual uncertainty components. It has been shown, however, that in some cases this methodology underestimates the measurement uncertainty /3/, partly because it is hard to include all possible uncertainty contributions in such an approach. By using existing and experimentally determined quality control (QC) and method validation data, the probability of including all uncertainty contributions is increased.

Measurement uncertainty model – fish-bone diagram

covering the analytical process from sample arrival to report

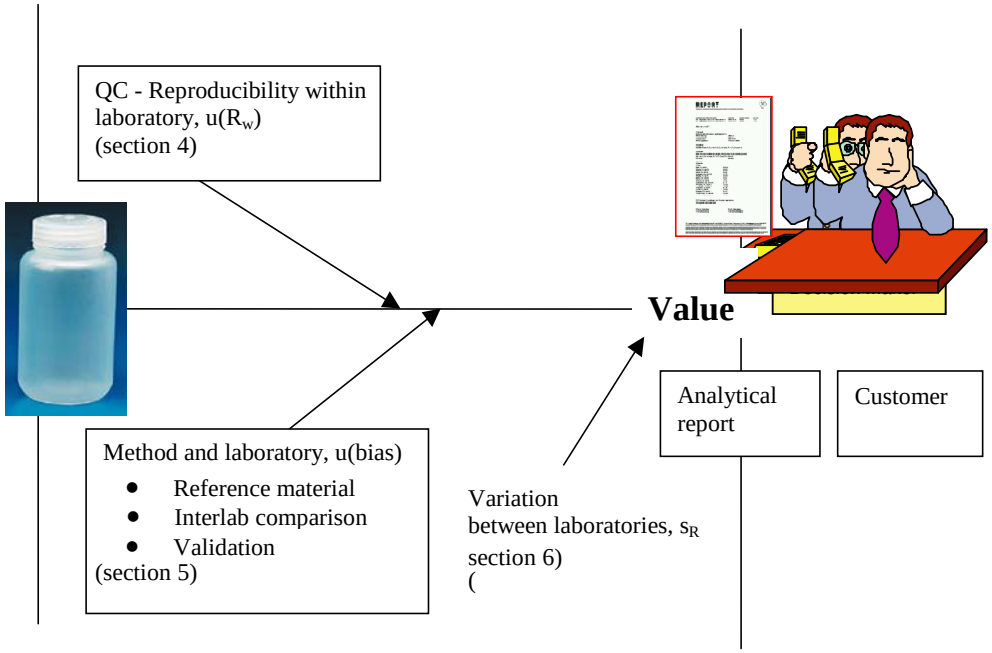


Figure 2. Measurement uncertainty model (fish-bone diagram), where the within-laboratory reproducibility is combined with estimates of the method and laboratory bias. Alternatively, according to ISO 21748 /8/, the combined standard uncertainty u_c can be directly estimated from the reproducibility between laboratories (s_R). This approach is treated in section 6.

3.1 Customer needs

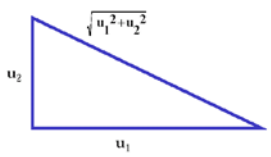
Before estimating the measurement uncertainty, it is recommended to find out what the needs of the customers are. After that, the main aim of the actual uncertainty evaluation process will be to find out if the laboratory can fulfil the customer demands with the analytical method in question. However, customers are not used to specifying demands, so in many cases the demands have to be set in dialogue with the customer. In cases where no demands have been established, a guiding principle could be that the expanded uncertainty, *U*, should be approximately equal to, or less than, 2 times the reproducibility, s_R.

3.2 Flow scheme for uncertainty calculations

The flow scheme presented in this section forms the basis for the method outlined in this handbook. The flow scheme, involving six defined steps, should be followed in all cases. The example with NH_4-N in water shows the way forward for calculating the measurement uncertainty using the flow scheme. Explanations of the steps and their components will follow in the succeeding chapters. For each step, there may be one or several options for finding the desired information.

Background for the NH_4-N example – automatic photometric method: The laboratory has participated in six interlaboratory comparisons recently. All results have been somewhat higher than the nominal value. The laboratory therefore concludes that there may be a small positive bias. On average, the bias has been +2.2 %. This observed bias is considered small by the laboratory and is not corrected for in their analytical results, but exists, and is thus another uncertainty component. The raw data for this example is found in Appendix 4.

For this method, the main sources of uncertainty are contamination and variation in sample handling, both causing random uncertainty components. These uncertainty sources will be included in the calculations below. An output from these calculations below using the MU kit software /16/ is given in Appendix 10.

Step	Action	Example – Ammonium NH_4-N
1	***Specify measurand and analyte***	Concentration of ammonium-nitrogen (NH_4-N) measured in water according to EN/ISO 11732 /11/. The required uncertainty (U, k=2) is 10 %
2	***Quantify R_w component*** ***A control sample*** ***B possible steps not covered by the control sample***	A: Control limits are set to ± 3.34 % (95 % confidence limit) B: The control sample includes all analytical steps in the laboratory
3	***Quantify bias component***	From interlaboratory comparisons over the last 3 years the bias results were 2.4, 2.7, 1.9, 1.4, 1.8 and 2.9. The root mean square (RMS) of the bias is 2.26 %. The uncertainty of the nominal values is $u(Cref)$ = 1.52 %. (see Appendix 4 for explanations)
4	***Convert component to a standard uncertainty u(x)***	Confidence intervals and similar distributions can be converted to standard uncertainty /1, 2, 14/. $u(R_w)$ = 3.34/2 = 1.67 % $u(bias) = \sqrt{RMS_{bias}{}^2 + u(Cref)^2}$ $= \sqrt{2.26^2 + 1.52^2} = 2.73\,\%$
5	***Calculate combined standard uncertainty, u_c*** 	Independent standard uncertainties expressed in the same unit can be summed by taking the square root of the sum of the squares $u_c = \sqrt{u(R_w)^2 + u(bias)^2} = \sqrt{1.67^2 + 2.73^2} = 3.20\,\%$
6	***Calculate expanded uncertainty,*** $U = 2 \cdot u_c$	The reason for calculating the expanded uncertainty is to reach a high enough confidence (app. 95 %) in that the "true value" lies within the interval given by the measurement result ± the uncertainty. $U = 2 \cdot 3.20 = 6.40 \approx 6$ %.

The measurement uncertainty for NH_4-N will thus be reported as 6 % at this concentration level.

3.3 Summary table for uncertainty calculations

The results of the calculations done in the flow scheme will then be summarised in a summary table.

Ammonium in water by EN/ISO 11732

Measurement uncertainty *U*, *k*=2 (approx. 95 % level of confidence) is estimated to 6 % at a level of 200 µg/L The customer demand is 10 %. The calculations are based on control chart limits and interlaboratory comparisons.

		Value	***Relative u(x)***	***Comments***
Within-laboratory reproducibility , R_w				
Control sample $\overline{X}$ = 200 µg/L	s_{Rw}	Control limits is set to ± 3.34 %	1.67 %	
Other components		--		
Method and laboratory bias				
Reference material	bias	--		
Interlaboratory comparison	bias	RMS_{bias}= 2.26 % $u(Cref)$ = 1.52 %	2.73 %	$u(bias) = \sqrt{RMS_{bias}^2 + u(Cref)^2}$
Recovery test	bias	--		
Reproducibility between laboratories				
Interlaboratory comparison	s_R	--	8.8 %	Data - see Section 6.2
Standard method	s_R	--	8 %	

The combined standard uncertainty u_c is calculated from the control sample limits and bias estimation from interlaboratory comparisons. The s_R from interlaboratory comparisons can also be used (see 6.2) if a higher uncertainty estimation is acceptable.

Analyte	***Combined standard uncertainty u_c***	***Expanded uncertainty U, k=2***
Ammonium	$\sqrt{1.67^2 + 2.73^2} = 3.20$ %	$3.18 \cdot 2 = 6.4 \approx 6$ %

4 Within-laboratory reproducibility - $u(R_w)$

In this section the three most common ways of estimating the within-laboratory reproducibility component, $u(R_w)$, for the measurement uncertainty calculation are explained:

1. Stable control samples covering the whole analytical process. Normally one sample at the lower and one sample at the upper part of the measurement range.
2. Control samples not covering the whole analytical process. Uncertainties estimated from control samples and from duplicate analyses of real samples with varying concentration levels.
3. Unstable control samples.

It is of utmost importance that the estimation must cover all steps in the analytical chain and all types of matrices – worst-case scenario. The control sample data should be run in exactly the same way as the samples e.g. if the mean of duplicate samples is used for ordinary samples, then the mean of duplicate control samples should be used for the calculations.

It is likewise important to cover long-term variations of some systematic uncertainty components **within** the laboratory, e.g. caused by different stock solutions, new batches of critical reagents, recalibrations of equipment, etc. In order to have a representative basis for the uncertainty calculations and to reflect any such variation the number of results should ideally be more than 50 and cover a time period of approximately one year, but the need differs from method to method.[1]

4.1 Customer demands

Some laboratories choose to use the customer demand when setting the limits in their control charts. The actual performance of the method is not interesting, as long as it meets the customer demands on expanded uncertainty. If, for example, the customer asks for data with an (expanded, k=2) measurement uncertainty of 10 %, then, from our experience, a good starting point is to set the control limits ± 5 %. The $u(R_w)$ used in the calculations will then be 2.5 %.[2] This is just a proposal and the measurement uncertainty calculations will show if these control limits are appropriate.

[1] When a method is developed and validated in-house, the initial uncertainty estimate is often based on a much smaller number of measurements

[2] Treating the control limits according to GUM /1/ as type B estimate with 95 % confidence limit

4.2 Control sample covering the whole analytical process

When a stable control sample is covering 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 samples. If the analyses performed cover a wide range of concentration levels, also control samples of other concentration levels should be used. Example: For NH_4-N two control sample levels (20 µg/L and 250 µg/L) were used during year 2002. The results for the manual analysis method are presented in the table below.

		Value	***Relative uncertainty*** $u(x)/\overline{x}$	***Comments***
Within-laboratory reproducibility , u(R_w)				
Control sample 1 $\overline{X}$ = 20.01 µg/L	s_{Rw}	Standard deviation 0.5 µg/L	2.5 %	From measurements in 2002, n = 75
Control sample 2 $\overline{X}$ = 250.3 µg/L	s_{Rw}	Standard deviation 3.7 µg/L	1.5 %	From measurements in 2002, n = 50
Other components		--		

4.3 Control sample for different matrices and concentration levels

When 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. Example: To estimate the repeatability in different matrices, duplicate analysis of ammonium is performed, and the s_r is estimated from the corresponding r%-chart /13/, giving the repeatability of analysing natural samples having a normal matrix variation at different concentration levels.

The data set consists of 73 duplicate analyses in the range of 2 µg/L – 16 000 µg/L. Most of the results were below 200 µg/L. The data is divided into two parts:

< 15 µg/L and > 15 µg/L

The s_r can be estimated from R%-charts constructed for both concentration ranges. The data is given in Appendix 5. The standard deviation is estimated from the range of duplicates (see Appendix 8): $s = range / 1.128$.

		Value	*Relative uncertainty* $u(x)/\bar{x}$	*Comments*
Within-laboratory reproducibility , u(R_w)				
Variation from duplicate analysis 2-15 µg/L: > 15 µg/L:	s_R		5.7 % 3.6 %	n = 43 ($\bar{x}$ = 6.50 µg/L) n = 30 ($\bar{x}$ = 816 µg/L)

At very low levels it is often better to use an absolute uncertainty rather than a relative, as relative numbers tend to become large. In this example the absolute uncertainty contribution (repeatability conditions) *u(r)* becomes 0.37 µg/L for the natural sample (mean concentration 7 µg/L) and 0.5 µg/L for the control sample in Section 4.2 (mean concentration 20 µg/L).

As the estimate from duplicate analysis gives the repeatability (s_r) only, it should be combined with the control sample results from Section 4.2 to give a better estimate of s_{Rw}. This way, the repeatability component will be included two times, but it is normally small in comparison to the between-days variation.

		Value	*Uncertainty*	*Comments*
Within-laboratory reproducibility , R_w				
Low level (2-15 µg/L)	s_{Rw}	0.5 µg/L from control sample and 0.37 µg/L from duplicates	0.6 µg/L	Absolute $u(x) = \sqrt{0.5^2 + 0.37^2}$
High level (> 15 µg/L)	s_{Rw}	1.5% from control sample and 3.6% from duplicates	3.9 %	Relative $u(x)/\bar{x} = \sqrt{1.5^2 + 3.6^2}$

It can be noticed that the sample matrix as well as the low level (< 10 µg/L) have some effect on the variation of the results. The quantification limit of the measurement was 2 µg/L and the relative standard deviation usually becomes higher near that limit (cf. Figures 4 and 5 in Section 7.4).

4.4 Unstable control samples

If the laboratory does not have access to stable control samples, the repeatability can be estimated using analysis of natural duplicate samples. The results from the duplicate sample analysis can either be treated in an R-chart, where the difference between the first and second analysis is plotted directly, or as an R %-chart, where the absolute difference between the sample pair is calculated in % of the average

value of the sample pair. The latter approach is particularly useful when the concentration varies a lot from time to time.

In this example, duplicate samples for oxygen have been analysed on 50 occasions. The raw data is given in Appendix 6. The concentration variation is limited, so an R-chart approach is chosen. The difference between the first and the second analysis is calculated and plotted in a chart, see Figure 3. In this case, the second result is always subtracted from the first when constructing the R-chart, as it is important to look for systematic differences between the first and the second sample. The standard deviation for the results can be estimated from the average range of the duplicate samples (see Appendix 8), and in this case becomes 0.024. The control limits at ±2s thus lies at ±0.048. The average value of the first determination is 7.53, and the s_r thus equals 100·0.024/7.53 = 0.32 %.

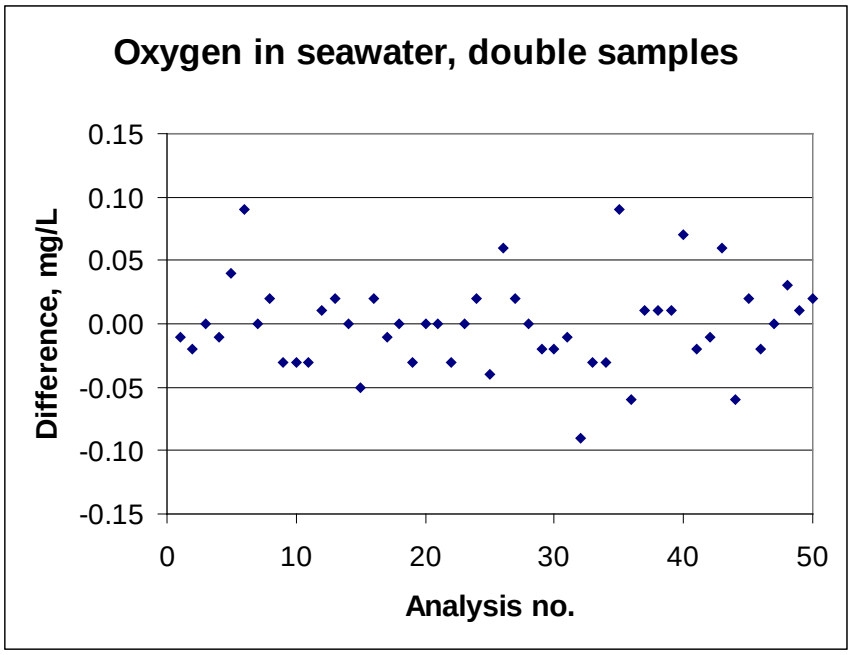


Figure 3. The difference between duplicate measurements of oxygen concentration plotted in an R-chart.

However, this only gives the within-day variation (repeatability, s_r) for sampling and measurement, and there will also be a "long-term" uncertainty component from the variation in the calibration (here the thiosulphate used for titrating or the calibration of the oxygen probe, depending on method). For this particular analysis, the uncertainty component from the long-term variation in calibration is hard to measure, as no stable reference material or CRM is available for dissolved oxygen. One method would be to calibrate the same thiosulphate solution several times during a few days, and use the variation between the results. Here we choose to

estimate that component by a qualified guess, but laboratories are encouraged to also try the experimental approach.

The total within-laboratory reproducibility for dissolved oxygen thus becomes:

		Value	***Relative uncertainty u(x)/$\overline{X}$***	***Comments***
Within-laboratory reproducibility , u(R_w)				
Duplicate measurements of natural samples, difference used in r-chart	s_R	s = 0.024 mg/L $\overline{X}$ = 7.53 mg/L	0.32 %	Measurements in 2000-2002, n= 50
Estimated variation from differences in calibration over time		s = 0.5 %	0.5 %	Estimate, based on experience
Combined standard uncertainty for R_w				
Repeatability + within-laboratory reproducibility in calibration		$\sqrt{0.32^2 + 0.5^2} = 0.59$ %		

5 Method and laboratory bias – *u*(bias)

In this chapter the most common ways of estimating the bias components of the uncertainty will be outlined. These are the use of CRMs, participation in proficiency testing (PT) schemes and recovery experiments. Sources of bias should always be eliminated if possible. According to GUM /1/ a measurement result should always be corrected if the bias is significant and based on reliable data such as a CRM. However, even if the average bias is zero, it has to be estimated and treated as an uncertainty component. In many cases the bias can vary depending on changes in matrix. This can be reflected when analysing several matrix CRMs, e.g. the bias could be both positive and negative. Examples are given and explained for the proposed calculations.

For every estimation of the uncertainty from the method and laboratory bias, two components have to be estimated to obtain *u(bias)*:

1) the root mean square (RMS) of the bias values /17/ (as % difference from the nominal or certified value for each CRM)

2) the uncertainty of the nominal/certified value, *u(Cref)* or for recovery experiments the uncertainty in the amount added, *u(Crecovery).*

The uncertainty of the bias, *u(bias)* can be estimated by

$$u(bias) = \sqrt{RMS_{bias}{}^2 + u(Cref)^2} \text{ where } RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n_{CRM}}}$$

and if only one CRM is used also the standard deviation obtained in the *n* measurement of the CRM, s_{bias} have to be included and *u(bias)* can the be estimated /14, 15/ by

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

5.1 Certified reference materials

Regular analysis of a CRM can be used to estimate and check the bias. The reference material should be analysed in at least five different analytical series (e.g. on five different days) before the values are used.

In this example the certified value is 11.5 ± 0.5. The stated uncertainty is expressed as a 95 % confidence interval. The average analytical result is 11.9 with a standard deviation of 2.21 %.

Uncertainty component from the uncertainty of the certified value	
Step	**Step**
Convert the confidence interval to *u(Cref)*	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(Cref)*	100·(0.26/11.5) = 2.21 %

3 ***Quantify method and laboratory bias***

$\text{bias} = 100 \cdot (11.9 - 11.5)/11.5 = 3.48\,\%$

s_{bias} = 2.2 % (n = 12)

u(Cref) = 2.21 %

4 ***Convert component to a standard uncertainty u(x)***

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

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

If **several CRMs** are used, we will get different values for the bias. The uncertainty of the bias estimation will be calculated in the following way (see also section 5.2).

3 ***Quantify method and laboratory bias***

bias CRM1 is 3.48 %, s=2,2 (n=12), *u(Cref)*=2.21 %
bias CRM2 is -0.9 % s=2,0 (n=7)), *u(Cref)*=1.8 %
bias CRM3 is 2.5 %, s= 2,8 (n=10), *u(Cref)*=1.8 %
For the bias the RMS_{bias} = 2.50
mean *u(Cref)*=1,9 %

4 ***Convert component to a standard uncertainty u(x)***

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

$$\sqrt{2.50^2 + 1.9^2} = 3.1\%$$

5.2 Proficiency testing data

Results from proficiency testing (PT) schemes can also be used to estimate the bias. In order to have a reasonably clear picture of the bias from results, a laboratory should participate at least 6 times within a reasonable time interval.

Since bias can be positive or negative, it is important to state it with its sign, e.g. – 1.5 %. Even if the measurements appear to give a positive bias on certain occasions and negative on others, all bias values can be used to estimate the uncertainty component, RMS_{bias} /17/.

The way forward is very similar to that for reference materials. However, the estimation of the bias from PT has more uncertainty to it, and thus usually becomes a bit higher than if CRMs are used. This is partly due to the fact that the certified value of a CRM normally is better defined than a nominal or assigned value in an interlaboratory comparison exercise. In some cases the calculated uncertainty *u(Cref)* from an interlaboratory comparison becomes too high and is not valid for estimating the *u(bias)*.

Uncertainty component from the uncertainty of the nominal value	
Step	**Example**
Find the between laboratory standard deviations, s_R, for the exercises.	The s_R has been on average 9 % in the 6 exercises.
Calculate *u(Cref)*	Mean number of participants = 12. $u(Cref) = \frac{s_R}{\sqrt{n_{Lab}}} = \frac{9\ \%}{\sqrt{12}} = 2.6\ \%$

The bias has been 2 %, 7 %, -2 %, 3 %, 6 % and 5 %, in the six PT rounds where the laboratory has participated.

3 ***Quantify method and laboratory bias***

RMS_{bias} = 4.6 %,
u(Cref)= 2.6 %

4 ***Convert component to a standard uncertainty u(x)***

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

5.3 Recovery

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.

In an experiment the recoveries for an added spike were 95 %, 98 %, 97 %, 96 %, 99 % and 96 % for 6 **different** sample matrices. The average is 96.8 %. The spike of 0.5 mL was added with a micropipette.

Uncertainty component from the definition of 100 % recovery, u(Crecovery)	
Step	**Example**
Uncertainty of the 100 % recovery. Main components are concentration, *u(conc)* of standard and volume added *u(vol)*	*u(conc)* - *C*ertificate ± 1.2 % (95 % conf. limit) gives = 0.6 % *u(vol)* - This value can normally be found in the manufacturer's specifications, or better use the limits specified in your laboratory. Max bias 1 % (rectangular interval) and repeatability max 0.5 % $u(vol) = \sqrt{\left(\frac{1}{\sqrt{3}}\right)^2 + 0.5^2} = 0.76\,\%$
Calculate *u(Crecovery)*	$\sqrt{u(conc)^2 + u(vol)^2} = \sqrt{0.6^2 + 0.76^2} = 1.0\,\%$

3 ***Quantify method and laboratory bias***

RMS_{bias} = 3.44 %
u(Crecovery)=1.0 %

4 ***Convert component to a standard uncertainty u (x)***

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

6 Reproducibility – s_R

If there is no need for a detailed evaluation of the uncertainty, it can be possible to directly use the s_R as an approximation of u_c /8/. In such case the expanded uncertainty is: $U = 2 \cdot s_R$. 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.

6.1 Use of data given in a standard method

In order to use a figure taken directly from the standard method, the laboratory must prove that they are able to perform in accordance with the standard method /8/, i.e. demonstrating control of bias and verification of the repeatability, s_r. Reproducibility data can either be given as a standard deviation s_R or as *reproducibility limit R).* To calculate a standard deviation from a limit you simply divide by 2.8. E.g. $s_R = R/2.8$.

The example below is taken from ISO 15586 *Water Quality — Determination of trace elements by atomic absorption spectrometry with graphite furnace*. The matrix is wastewater. The combined uncertainty u_c for wastewater is taken from the s_R from interlaboratory comparison exercises quoted in the ISO method.

Table 1. ISO 15586 - Results from the interlaboratory comparison – Cd in water with graphite furnace AAS. The wastewater was digested by the participants.

Cd		**n_{Lab}**	**Outliers**	**Nominal value** µg/L	**Mean** µg/L	**Recovery,** %	**s_r** %	**s_R** %
Synthetic	Lower	33	1	0.3	0.303	101	3.5	17.0
Synthetic	Higher	34	2	2.7	2.81	104	1.9	10.7
Fresh water	Lower	31	2		0.572		2.9	14.9
Fresh water	Higher	31	3		3.07		2.1	10.4
Waste water		27	2		1.00		3.1	27.5

Analyte	**Combined standard uncertainty u_c**	**Expanded uncertainty(k=2) $U = 2 \cdot u_c$**
Cd	27.5 %	55 %

6.2 Use of proficiency testing data

Proficiency Testing (PT) schemes are valuable tools in uncertainty evaluation. The variation between the participants is normally given directly in reports from the exercises. When all participants use the same method, the standard deviation observed is an estimate of the methods reproducibility s_R.

The PT data may well be used by a laboratory (having performed satisfactorily in the scheme) as the standard uncertainty of the analysed parameter, provided that the comparison covers all relevant uncertainty components and steps (see section 5.4.6.3 in /9/). For example, a standard deviation in a round of a PT scheme, s_R, can be directly used as a combined standard uncertainty, u_c.

Table 2. Summary results (mean values) from ten rounds of six PT schemes that Lab A has participated in. The level is approximately the same in each round. The reproducibility standard deviation is given in absolute units, s_R and in relative units s_R %.

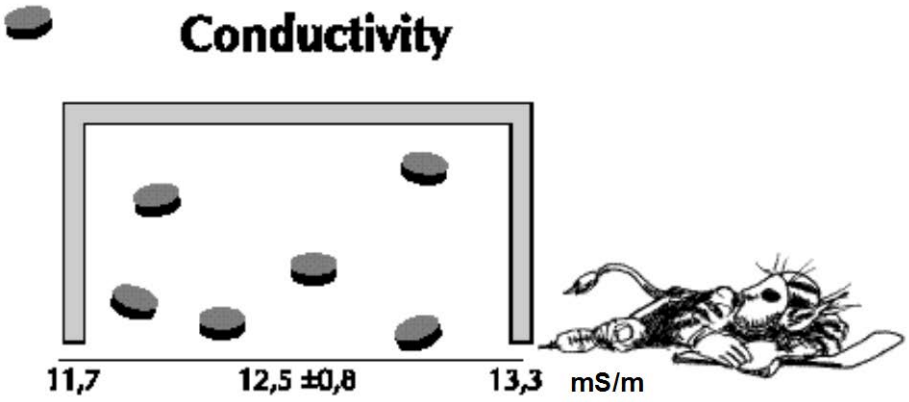
Parameter	**Mean nominal value**	**Lab A % mean deviation**	***Mean*** s_R *(abs)*	***Mean*** s_R *%*	**Mean No. labs**	**Mean No. excluded results**
pH	7.64	-0.037	0.101		90	5
Conductivity, mS/m	12.5	-2.8	0.40	3.2	86	6
Alkalinity, mmol/L	0.673	2.3	0.026	3.9	60	3
Turbidity, FNU	1.4	-9.1	0.1	14.2	44	3
NH_4-N, µg/L	146	2.2	12.0	8.8	34	5
NO_3-N, µg/L	432	-1.6	16.3	3.7	39	6

In Table 2 we find that for conductivity, for instance, the mean value for the results from 10 rounds of the PT scheme is 12.5 mS/m (125 µS/cm). The mean reproducibility relative standard deviation is 0.40 mS/m (3.2 %), which is an average (or pooled) standard deviation between the laboratories in the different rounds and this value can be taken as an estimate of the combined standard uncertainty i.e:

u_c *(conductivity)* = s_R = 0.40 mS/m, thus U = 2·0.40 mS/m = 0.80 mS/m or ≈ 6 %.

If we take the ammonium results, we have a mean nominal value of 146 µg/L, and we find that the reproducibility, s_R, is 8.8 %. Thus U = 2·8.8 % = 17.6 % = 18 % at this concentration level.

Comment: In Section 3 the expanded uncertainty for ammonium is 6 % using an automated method in one highly qualified laboratory.



7 Examples

In this chapter, practical examples on how measurement uncertainty can be calculated using the method of this handbook are given.

7.1 Ammonium in water

The measurement of ammonium-nitrogen in water has already been treated in section 3.2 and section 6.2 . The results are summarised in Table 3.

Table 3. Measurement uncertainty for NH_4-N concentrations in water – comparison of different calculations

Basis for calculation	Relative expanded uncertainty, U_{rel} (k=2)	Comment
Control sample + PT	6 %	Uncertainty for one good laboratory – level 200 µg/L.
PT	18 %	Uncertainty in general among laboratories – level 150 µg/L

7.2 Biological oxygen demand in wastewater

Biological oxygen demand, BOD, is a standard parameter in the monitoring of wastewater. This example shows how data from internal quality control can be used together with CRM results or PT data to calculate the within-laboratory reproducibility and bias components of the measurement uncertainty. The results are summarised in Table 4.

Table 4. Measurement uncertainty of BOD in water - comparison of different calculations

Basis for calculation	Relative expanded uncertainty, U_{rel} (k=2)	Comment
Control sample + CRM	10 %	
Control sample + PT	10 %	n = 3 for PT, unreliable estimate
PT	16 %	Uncertainty in general among laboratories

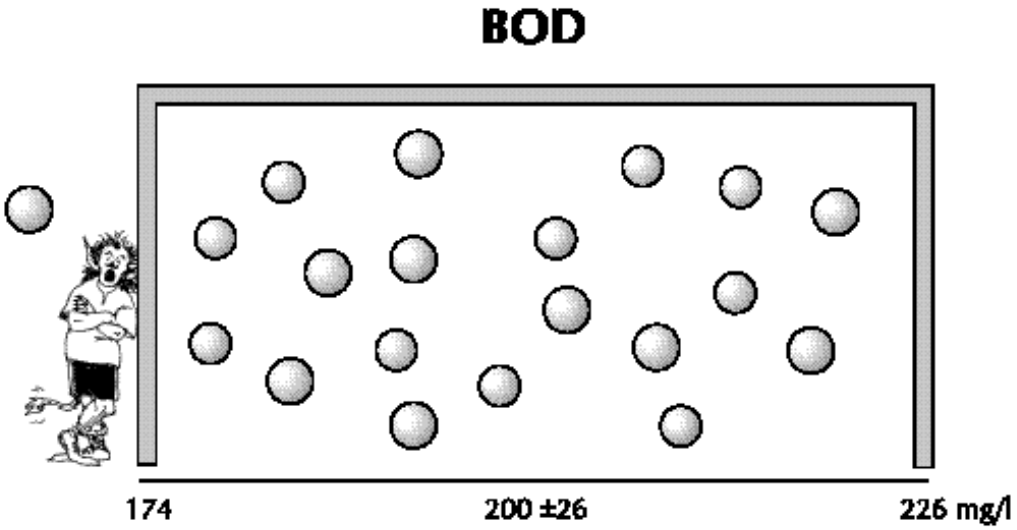
For BOD at high concentrations, using the dilution analytical method, the major uncertainty sources are the actual oxygen measurement and variation in the quality of the seeding solution. These uncertainty contributions will be included in the calculations.

The raw data from the internal quality control, used for the calculations, is shown in Appendix 7.

The laboratory has only participated in three rounds of the PT scheme during the last 2 years (Table 5). At least six would be needed, so here we estimate the bias in two different ways – with CRM and from the PT data.

Table 5. BOD results from PT.

Exercise	Nominal value	Laboratory result	Bias	s_R	Number of labs
	mg/L	mg/L	%	%	
1	154	161	+ 4.5	7.2	23
2	219	210	- 4.1	6.6	25
3	176	180	+2.3	9.8	19
$\overline{X}$			+0.9	7.87[3]	22.3
RMS_{bias}			3.76	-	-



[3] If s_R or the number of participants vary substantially from exercise to exercise, then a pooled standard deviation will be more correct to use. In this case, where the variation in s_R is limited, we simply calculate the mean s_R (the corresponding pooled standard deviation becomes 7.82, an insignificant difference).

Example A: BOD with Internal quality control and CRM data

Step	*Action*	*Example: BOD in wastewater*
1	Specify measurand and analyte	BOD in wastewater, measured with EN1899-1 (method with dilution, seeding and ATU). The required expanded uncertainty (k=2) is 20 %.
2	Quantify $u(R_w)$ A control sample B possible steps not covered by the control sample	A: The control sample, which is a CRM, gives an s = 2.6 % at a level of 206 mg/L O_2. s = 2.6 % is also when setting the control chart limits. B: The analysis of the control sample includes all analytical steps after sampling
3	Quantify method and laboratory bias	The CRM is certified to 206 ±5 mg/L O_2. The average result of the control chart is 214.8. Thus, there is a bias of 8.8 mg/L = 4.3 %. The s_{bias} is 2.6 % (n=19) The $u(Cref)$ is 5 mg/L / 1.96 = 1.2 %
4	Convert component to a standard uncertainty $u(x)$	$u(R_w)$ = 2.6 % $u(bias) = \sqrt{bias^2 + \frac{s_{bias}}{\sqrt{n}}^2 + u(Cref)^2}$ $= \sqrt{4.3^2 + \left(\frac{2.6}{\sqrt{19}}\right)^2 + 1.2^2} = 4.5\ \%$
5	Calculate combined standard uncertainty, u_c	$u_c = \sqrt{2.6^2 + 4.5^2} = 5.2\ \%$
6	Calculate expanded uncertainty, $U = 2 \cdot u_c$	$U = 2 \cdot 5.2 = 10.4 \approx 10\ \%$

Example B: BOD with Internal quality control + interlaboratory comparison results

Step	*Action*	*Example: BOD in wastewater*
1	Specify measurand and analyte	BOD in wastewater, measured with EN1899-1 (method with dilution, seeding and ATU). The required expanded uncertainty (k=2) is 20 %.
2	Quantify $u(R_w)$) A control sample B possible steps not covered by the control sample	A: The control sample, which is a CRM, gives an s of 2.6 % at a level of 206 mg/L O_2. s = 2.6 % is also used as s when setting the control chart limits. B: The analysis of the control sample includes all analytical steps after sampling.
3	Quantify method and laboratory bias Data from Table 5	RMS_{bias} = 3.76 % $u(Cref) = \frac{s_R}{\sqrt{n_{Lab}}} = \frac{7.9}{\sqrt{22.3}} = 1.67\,\%$
4	Convert component to a standard uncertainty $u(x)$	$u(R_w)$ = 2.6 % $u(bias) = \sqrt{RMS_{bias}{}^2 + u(Cref)^2} =$ $\sqrt{3.76^2 + 1.67^2} = 4.11\%$
5	Calculate combined standard uncertainty, u_c	$u_c = \sqrt{2.6^2 + 4.11^2} = 4.86$ %
6	Calculate expanded uncertainty, $U = 2 \cdot u_c$	$U = 2 \cdot 4.86 = 9.7 \approx 10\,\%$

7.3 Polychlorinated biphenyls (PCBs) in sediment

In this example, the $u(R_w)$ is estimated from a quality control sample and the u(bias) is estimated from two different sources: in the first example the use of a CRM and in the second example participation in PT scheme. In the summary table both ways of calculating the *u(bias)* will be compared.

For this analysis, the sample-work up is a major uncertainty source (both for random and systematic errors), and it is thus crucial that this step is included in the calculations. The number of PT results is too low to get a good estimate.

Example C: PCB with Internal quality control and a CRM

Step	Action	Example: PCBs in sediment
1	Specify measurand and analyte	Sum of 7 PCBs (mass fraction) in sediment by extraction and GC-MS (SIM). The required expanded uncertainty (k=2) is 20 %.
2	Quantify $u(R_w)$ A control sample B possible steps not covered by the control sample	A: The control sample, which is a CRM, gives an s_{Rw} = 8 % at a level of 150 µg/kg dry matter. s_{Rw} = 8 % is also used when setting the control chart limits. B: The analysis of the control sample includes all steps except for drying the sample to determine the dry weight. The uncertainty contribution from that step is considered small and is not accounted for.
3	Quantify method and laboratory bias	The CRM is certified to 152 ± 14 µg/kg. The average result of the control chart is 144. Thus, there is a bias = -5.3 %. The s_{bias} = 8 % (n=22) *u(Cref)* 14 µg/kg/1.96, which is 4.7 % relative.
4	Convert component to a standard uncertainty *u(x)*	$u(R_w)$ = 8 % $u(bias) = \sqrt{bias^2 + \frac{s_{bias}^2}{\sqrt{n}} + u(Cref)^2}$ $= \sqrt{5.3^2 + \left(\frac{8}{\sqrt{22}}\right)^2 + 4.7^2} = 7.29$
5	Calculate combined standard uncertainty, u_c	$u_c = \sqrt{8^2 + 7.29^2}$ = 10.8 %
6	Calculate expanded uncertainty, $U = 2 \cdot u_c$	$U = 2 \cdot 10.8 = 21.6 \approx 22$ %

Example D: PCB with internal quality control and PT data

Step	Action	Example: PCB in sediment
1	Specify measurand and analyte	Sum of 7 PCB:s (mass fraction) in sediment by extraction and GC-MS(SIM). The required expanded uncertainty is 20 %.
2	Quantify $u(R_w)$ A control sample B possible steps not covered by the control sample	A: The control sample, which is a stable in-house material, gives s_{Rw} = 8 % at a level of 150 µg/kg dry matter. s_{Rw} = 8 % is also used as s when setting the control chart limits. B: The analysis of the control sample includes all steps except for drying the sample to determine the dry weight. The uncertainty contribution from that step is considered small and is not accounted for.
3	Quantify method and laboratory bias	Participation in 3 PT rounds with concentration levels similar to the internal quality control. The "bias"[4] in the 3 exercises has been –2 %, -12 % and –5 %. RMS_{bias} = 7.6 %. The s_R in the three exercises has been 12 %, 10 % and 11 %, on average s_R = 11 % (n_{Lab}=14). $u(Cref) = \frac{11}{\sqrt{14}} = 2.9\ \%$
4	Convert component to a standard uncertainty $u(x)$	The $u(R_w)$ is 8 % $u(bias) = \sqrt{RMS_{bias}^{\ 2} + u(Cref)^2} =$ $\sqrt{7.6^2 + 2.9^2} = 8.1\%$
5	Calculate combined standard uncertainty, u_c	$u_c = \sqrt{8^2 + 8.1^2} = 11.4$

[4] Ideally this should be an average measured several times to be a bias. Since only measured once it is only a difference.

Step	Action	Example: PCB in sediment
6	Calculate expanded uncertainty, $U = 2 \cdot u_c$	$U = 2 \cdot 11.4 = 22.8 \approx 23\,\%$

Summary table for PCB measurement uncertainty calculations

PCBs in sediment by extraction and GC-MS (SIM)

The relative measurement uncertainty U (k=2) is estimated to 20 % for the sum of 7 PCB:s in sediments at a level of 150 µg/kg dry weight. The customer's demand is 20 %. The calculations are based on internal quality control using a stable sample, CRM and the participation in a small number of PT exercises.

		Value	u(x)	Comments
Within-laboratory reproducibility , R_w				
Control sample $\overline{X}$ = 160 µg/kg dry weight	$u(R_w)$	12.8 µg/kg dry weight	8 %	
Other components		too small to be considered		
Method and laboratory bias				
Reference material		Bias: 5.3 % s_{bias} = 8 ; n = 22 $u(Cref)$ = 4,7 %	$u(bias)$ = 7.29	$u(bias) = \sqrt{bias^2 + \frac{s_{bias}^2}{\sqrt{n}} + u(Cref)^2}$
PT results n = 3		RMS_{bias} = 7.6 $u(Cref)$ = 2.9 %	$u(bias)$ = 8.1	$u(bias) = \sqrt{RMS_{bias}^2 + u(Cref)^2}$

The combined standard uncertainty, u_c, is calculated from internal quality control and the maximum bias observed from the PT results.

Measurand	Combined standard uncertainty u_c	Expanded uncertainty U, k=2
Mass fraction of PCBs	$u_c = \sqrt{8^2 + 8.1^2} = 11.4$	$U = 2 \cdot u_c = 2 \cdot 11.4 = 22.8 \approx 23\,\%$

Conclusion: In this case the calculation of the *u(bias)* gives similar results regardless of whether CRM or PT results are used. Sometimes PT will give considerably higher values, and it might in such cases be important to evaluate which estimate to use.

7.4 Variation of uncertainty with level

The measurement uncertainty will normally vary with the level measured, e.g. concentration, both in absolute and relative terms. If the concentration range of the reported data is large, it is thus often necessary to take this into account. For lead (Pb) in water, a recovery experiment was carried out a number of times to investigate within-laboratory reproducibility over the measurable range – the major component of the measurement uncertainty at low levels. The following results were obtained:

Table 6. Within-laboratory reproducibility and recovery for Pb determined with ICP-MS at different concentration levels.

Added concentration, µg/L	Recovery Pb, %	*s*, %
0.01	109.7	53.8
0.1	125.2	12.1
0.4	91.8	5
1	98.4	3.0
10	98	1.7
10	100.5	1.3
100	105.5	1.4

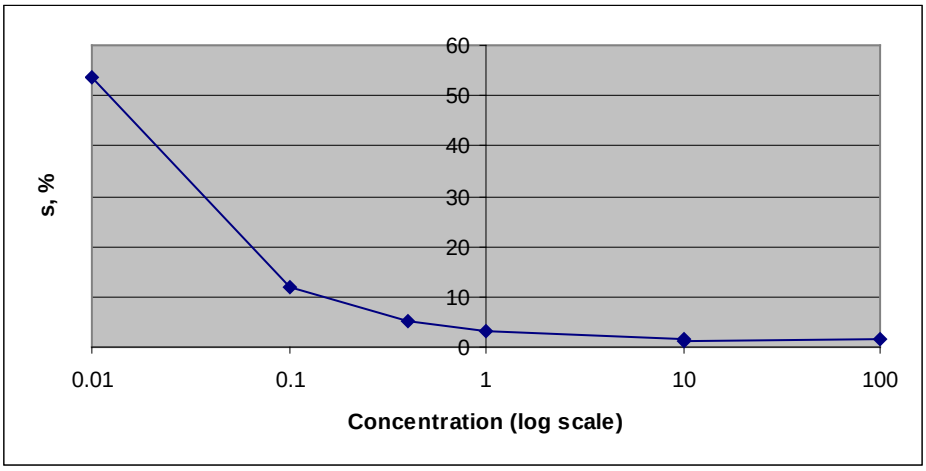


Figure 4. Within-laboratory reproducibility for Pb over the concentration range.

It is clear from the results that the measurement uncertainty, here represented by relative *s*, is strongly concentration dependent. Two approaches are recommended for using these data:

(1) To divide the measurable range into several parts, and use a fixed relative measurement uncertainty or absolute uncertainty – see Table 7.

Table 7. Within-laboratory reproducibility for Pb divided into three concentration ranges

Within-laboratory reproducibility Pb		
Range (µg/L)	$s_{Rw}(rel)$	s_{Rw} *(abs)*
0.01-0.09	50 %	0.01 (µg/L)
0.1 - 10	10 %	
> 10	2 %	

In the second column *s* is relative and given in %. In the third column an absolute value is given in the lower range close to the detection limit.

To use an equation that describes how the measurement uncertainty varies with concentration

Plotting *s* % against 1/concentration gives a straight line, and a relatively simple equation. (see Figure 5).

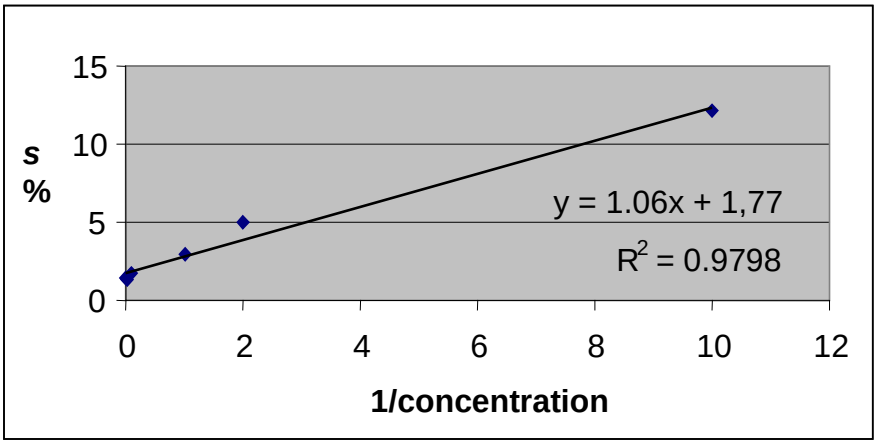


Figure 5: The relationship between within-laboratory reproducibility and the inverted concentration for Pb in the range 0.1 – 100 µg/L.

The straight-line equation above tells us that the within-laboratory reproducibility equals 1.06 multiplied with 1/concentration plus 1.77. For example, at a concentration of 2 µg/L the within-laboratory reproducibility becomes 1.06·1/2 + 1.77 = 2.3 %. When reporting to customers, the laboratory can choose between quoting the formula or calculating the measurement uncertainty for each value, using the formula. For further reading, see Appendix E5 in /2/.

8 Reporting uncertainty

This is an example on how an analytical report could look like, when measurement uncertainty has been calculated and is reported together with the results. The company and accreditation body logotypes are omitted, and the report does not contain all information normally required for an accredited laboratory. It is recommended to use either relative or absolute values for the benefit of the customer.

Analytical Report

Sample identification: P1 – P4
Samples received: 14 December 2002
Analysis period: 14 –16 December 2002

Results

NH_4-N (µg/L):

Sample	Result	*U, k=2* *	Method
P1	103	6 %	23B
P2	122	6 %	23B
P3	12	10 %	23B
P4	14	10 %	23B

TOC (mg/L)

Sample	Result	*U, k=2* *	Method
P1	40	4.0	12-3
P2	35	3.5	12-3
P3	10	1.0	12-3
P4	9	0.9	12-3

Signed: Dr Analyst

The laboratory should also prepare a note explaining how the measurement uncertainty has been calculated for the different parameters. Normally, such an explanatory note should be communicated to regular customers and other customers who ask for information. An example is given below:

Note on measurement uncertainty from Dr Analyst's laboratory

Measurement uncertainty:
U = expanded measurement uncertainty, estimated from control sample results, proficiency testing data and the analyses of certified reference materials. It is calculated by multiplying the combined standard uncertainty u_c with a coverage factor (*k*) equal to 2. A result ± *U* corresponds to an intervall with a level of confidence of approximately 95 %.

NH_4-N: *U* is estimated to 6 % above 100 µg/L and 6 µg/L below 100 µg/L.

TOC: *U* is estimated to 10 % over the whole concentration range.

References:

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9 References

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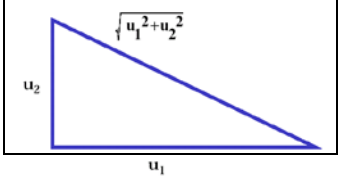
18. ISO 11352:2012 Water quality – Estimation of measurement uncertainty based on validation and quality control data
19. ISO 13528:2005 Statistical methods for use in proficiency testing by interlaboratory comparisons

10 Appendices

Appendix 1: Empty flow scheme for calculations

Before starting: Always identify the main uncertainty sources, to make sure that they are included in the calculations.

Step	*Action*	*Measurand*
1	*Specify measurand and analyte*	(measurand) in (matrix) by (method) The customer demand on expanded uncertainty is ± _ %.
2	*Quantify $u(R_w)$* *A control sample* *B possible steps not covered by the control sample*	A: B:
3	*Quantify method and laboratory bias*	
4	*Convert component to a standard uncertainty u(x)*	
5	*Calculate combined standard uncertainty, u_c* 	
6	*Calculate expanded uncertainty, $U = 2 \cdot u_c$*	

Appendix 2: Empty summary table

(measurand) in (matrix) by (method)
Measurement uncertainty *U* (*k*=2) is estimated to ± _ % (relative) for (measurand) in (matrix) at a level of _ (unit). The customer demand is ± _ %. The calculations are based on (control samples/control limits/CRM/interlaboratory comparison/other).

		Value	***Relative u(x)***	***Comments***
Within-laboratory reproducibility , R_w				
Control sample $\overline{X}$ = (conc) (unit)	s_{Rw}			
Other components				
Method and laboratory bias				
Reference material	bias			
Interlaboratory comparison	bias			
Recovery test	bias			
Reproducibility between laboratories				
Interlaboratory comparison	s_R			
Standard method	s_R			

The combined standard uncertainty, u_c, is calculated from __ and bias from __.

Measurand	***Combined standard uncertainty u_c***	***Expanded uncertainty U, k=2***
		$2 \cdot u_c$ =

Appendix 3: Error model used in this handbook

This model is a simplification of the model presented in the ISO guide /8/:

$$y = m + (\delta + B) + e$$

y measurement result of a sample

m expected value for y

δ method bias

B laboratory bias – the uncertainty for these are combined to *u(bias)*

e random error at within-laboratory reproducibility conditions, R_w

Uncertainty estimation in section 3 to 5

$$u(y)^2 = u_{Rw}{}^2 + u(bias)^2$$

$u_{Rw}{}^2$	The estimated variance of e under within-laboratory reproducibility conditions – intermediate precision. In the ISO guide the repeatability, s_r is used as an estimate of e.
$u(bias)^2$	The estimated variance of method bias and laboratory bias.

Uncertainty estimation in section 6

The combined standard uncertainty *u(y)* or u_c can also be estimated by from reproducibility data.

$u(y)^2 = s_L{}^2 + s_r{}^2 = s_R{}^2$ - equation A6 ref. /8/

where $s_R{}^2$ is the estimated variance under reproducibility conditions and where $s_L{}^2$ is either the estimated variance of B if one method is used by all laboratories or an estimated variance of B and δ if several different methods have been used in the collaborative study and $s_r{}^2$ is the estimated variance of e.

Comment
For samples that are more inhomogeneous and have big variations in matrix the estimation of the measurement uncertainty of the method can become too low. However we recommend the use of repeatability limit for duplicate analyses $r = 2.8 \cdot s_r$ in order to control sample inhomogeneity.

Appendix 4: Uncertainty of bias for NH_4-N in section 3.2

Results for a laboratory from proficiency testing data of NH_4-N in water.

Exercise	Nominal value x_{ref}	Laboratory result x_i	Bias[5] (difference)	s_R	Number of labs
	µg/L	µg/L	%	%	
1999 1	81	83	2.5	10	31
2	73	75	2.7	7	36
2000 1	264	269	1.9	8	32
2	210	213	1.4	10	35
2001 1	110	112	1.8	7	36
2	140	144	2.9	11	34
$\overline{X}$			**+ 2.20**	8.8	34
RMS_{bias}			**2.26**	-	-

$$RMS \text{ of the bias } = \sqrt{\frac{\sum bias_i^{\,2}}{n}} = \sqrt{\frac{2.4^2 + 2.7^2 + ...2.9^2}{n}} = 2.26 \text{ \% (rel)}$$

$$\boldsymbol{u(Cref)} = \frac{s_R}{\sqrt{n_{Lab}}} = \frac{8.8}{\sqrt{34}} = 1.5 \textbf{ \% (rel)}$$

A *t*-test shows that the bias (+2.20 %) is not significant taking into account the standard uncertainty of nominal value of 1.5 %. However, in order not to complicate the calculations when the bias is small, *t*-test are normally not performed.

The mean value of s_R is used. If differences in number of laboratories and s_R are very big pooled standard deviations should be used. In this case the pooled standard deviation is 8.9 % for s_R which is the same as the mean value of 8.8 %.

If the nominal value is a median or a robust mean PT value is used in each round can be calculated:

$$u(\text{Cref}) = 1{,}25 \cdot \frac{s_R}{\sqrt{n_{Lab}}}$$

(ISO 13528 /19)

[5] Ideally this should be an average measured several times to be a bias. Since only measured once it is only a difference.

Appendix 5: Raw data for NH_4-N in section 4.3

The estimation of the standard deviation from the range is explained in Appendix 8

concentration < 15 µg/L

$$\overline{X} = \frac{x_{i1} + x_{i2}}{2} \qquad d = x_{i1} - x_{i2} \qquad 100 \cdot \frac{|d|}{\overline{X}} = r\%$$

Sample	X1	X2			
1	7.46	7.25	7.355	0.210	2.855
2	9.01	9.17	9.090	-0.160	1.760
3	3.6	3.1	3.350	0.500	14.925
4	6.48	6.48	6.480	0.000	0.000
5	14.49	14.12	14.305	0.370	2.587
6	10.84	9.89	10.365	0.950	9.165
7	4.61	5	4.805	-0.390	8.117
8	2.6	2.42	2.510	0.180	7.171
9	2.8	2.62	2.710	0.180	6.642
10	5.84	6.19	6.015	-0.350	5.819
11	2.12	2.5	2.310	-0.380	16.450
12	2.3	2.11	2.205	0.190	8.617
13	2.52	2.89	2.705	-0.370	13.678
14	3.71	3.71	3.710	0.000	0.000
15	7.43	7.43	7.430	0.000	0.000
16	8.83	8.51	8.670	0.320	3.691
17	9.12	8.79	8.955	0.330	3.685
18	8.24	7.9	8.070	0.340	4.213
19	2.62	2.78	2.700	-0.160	5.926
20	3.33	3.33	3.330	0.000	0.000
21	2.69	2.69	2.690	0.000	0.000
22	12.09	12.09	12.090	0.000	0.000
23	4.24	4.24	4.240	0.000	0.000
24	10.49	10.64	10.565	-0.150	1.420
25	3.68	3.52	3.600	0.160	4.444
26	9.37	9.37	9.370	0.000	0.000
27	2.22	2.06	2.140	0.160	7.477
28	6.1	6.1	6.100	0.000	0.000
29	2.96	2.86	2.910	0.100	3.436
30	14.02	13.7	13.860	0.320	2.309
31	4.24	3.62	3.930	0.620	15.776
32	5.1	4.61	4.855	0.490	10.093
33	2.78	2.62	2.700	0.160	5.926
34	8.52	6.81	7.665	1.710	22.309
35	12.82	14.05	13.435	-1.230	9.155
36	3.17	2.4	2.785	0.770	27.648
37	11.28	11.43	11.355	-0.150	1.321
38	14.31	13.82	14.065	0.490	3.484
39	4.01	4.48	4.245	-0.470	11.072
40	3.27	3.58	3.425	-0.310	9.051
41	9.98	10.29	10.135	-0.310	3.059
42	12.56	13.66	13.110	-1.100	8.391
43	3.35	2.88	3.115	0.470	15.088
		Mean:	**6.499**		**6.4363** = mean range (%)

s(r) % = range(mean)/1.128 = 5.71 %

concentration > 15 µg/L

$\overline{X} = \frac{x_{i1} + x_{i2}}{2}$ $d = x_{i1} - x_{i2}$ $100 \cdot \frac{|d|}{\overline{X}} = r\%$

Sample	X1	X2				
1	*37.62*	*36.85*	37.235	0.770	2.068	
2	*16.18*	*16.56*	16.370	-0.380	2.321	
3	*28.82*	*28.65*	28.735	0.170	0.592	
4	*4490*	*4413*	4451.500	77.000	1.730	
5	*135.7*	*124.7*	130.200	11.000	8.449	
6	*62.56*	*62.25*	62.405	0.310	0.497	
7	*158.9*	*159.2*	159.050	-0.300	0.189	
8	*16540*	*16080*	16310.000	460.000	2.820	
9	*31.26*	*30.12*	30.690	1.140	3.715	
10	*58.49*	*60.11*	59.300	-1.620	2.732	
11	*740.5*	*796.2*	768.350	-55.700	7.249	
12	*130.3*	*126.9*	128.600	3.400	2.644	
13	*29.35*	*29.19*	29.270	0.160	0.547	
14	*1372*	*1388*	1380.000	-16.000	1.159	
15	*36.55*	*44.74*	40.645	-8.190	20.150	
16	*22.57*	*23.37*	22.970	-0.800	3.483	
17	*34.75*	*33.15*	33.950	1.600	4.713	
18	*92.93*	*94.01*	93.470	-1.080	1.155	
19	*40.6*	*42.23*	41.415	-1.630	3.936	
20	*80.36*	*86.36*	83.360	-6.000	7.198	
21	*15.76*	*18.54*	17.150	-2.780	16.210	
22	*78.22*	*73.76*	75.990	4.460	5.869	
23	*48.89*	*50.91*	49.900	-2.020	4.048	
24	*17.65*	*16.72*	17.185	0.930	5.412	
25	*36.56*	*35.3*	35.930	1.260	3.507	
26	*51.89*	*52.2*	52.045	-0.310	0.596	
27	*197.5*	*206.5*	202.000	-9.000	4.455	
28	*70.32*	*69.22*	69.770	1.100	1.577	
29	*29.99*	*30.62*	30.305	-0.630	2.079	
30	*31.9*	*32.36*	32.130	-0.460	1.432	
		Mean:	**816.331**		**4.0843**	= mean range (%)

s(r) % = range(mean)/1.128 = 3.62 %

Appendix 6: Raw data for oxygen in Section 4.4

Data plotted in Figure 3. “Range” equals the absolute value of the difference between Result 1 and Result 2.

Res. 1 mg/L	Res. 2 mg/L	Range mg/L
8.90	8.91	0.01
8.99	9.01	0.02
8.90	8.90	0.00
9.11	9.12	0.01
8.68	8.64	0.04
8.60	8.51	0.09
8.81	8.81	0.00
8.02	8.00	0.02
7.05	7.08	0.03
6.98	7.01	0.03
7.13	7.16	0.03
6.79	6.78	0.01
6.55	6.53	0.02
4.68	4.68	0.00
5.28	5.33	0.05
7.42	7.40	0.02
7.62	7.63	0.01
5.88	5.88	0.00
6.03	6.06	0.03
6.33	6.33	0.00
5.90	5.90	0.00
6.24	6.27	0.03
6.02	6.02	0.00
9.13	9.11	0.02
9.10	9.14	0.04
8.50	8.44	0.06
8.73	8.71	0.02
8.09	8.09	0.00
7.56	7.58	0.02
6.30	6.32	0.02
6.43	6.44	0.01
7.25	7.34	0.09
7.28	7.31	0.03
8.00	8.03	0.03
8.38	8.29	0.09
9.23	9.29	0.06
9.09	9.08	0.01
9.37	9.36	0.01
9.38	9.37	0.01
9.32	9.25	0.07
8.47	8.49	0.02
8.27	8.28	0.01
8.37	8.31	0.06
8.09	8.15	0.06
8.05	8.03	0.02
7.38	7.40	0.02
7.49	7.49	0.00
4.52	4.49	0.03
4.45	4.44	0.01
4.29	4.27	0.02
	mean range:	0.026
	mean range/1.128:	0.024

Appendix 7: Raw data for BOD in section 7.2

Results in mg/L O_2 consumption. The certified value and expanded uncertainty of the CRM is 206 ± 5 mg/L. As the average of two results is always reported for ordinary samples, the s is also calculated from the average of each sample pair in the internal quality control.

Date	Res. 1	Res. 2	Average
12-09-00	218.90	214.77	216.84
01-03-01	206.46	220.83	213.65
13-03-01	221.18	210.18	215.68
02-04-01	215.00	206.50	210.75
14-08-01	194.96	218.03	206.50
05-09-01	218.65	216.55	217.60
19-09-01	223.86	212.19	218.03
16-10-01	215.58	213.01	214.30
07-11-01	196.26	214.93	205.60
28-11-01	210.89	206.89	208.89
11-12-01	228.40	222.73	225.57
13-12-01	206.73	229.03	217.88
15-01-02	207.00	208.47	207.74
22-01-02	224.49	213.66	219.08
30-01-02	201.09	214.07	207.58
11-02-02	218.83	223.13	220.98
06-03-02	216.69	218.22	217.46
18-09-02	206.36	227.96	217.16
02-10-02	215.21	226.18	220.70
		Average:	214.84
		s:	5.58
		s%:	2.60

Appendix 8: Estimation of standard deviation from range

Number of samples (*n*)	Factor , d_2	**Estimation of standard deviation from range (max-min), /1/ and /13, page 11/.**
2	1.128	
3	1.693	
4	2.059	
5	2.326	The standard deviation, s can be estimated from $s = \frac{range}{d_2}$
6	2.534	
7	2.704	
8	2.847	
9	2.970	
10	3.078	
		where d_2 is dependent on number of samples (*n*) to calculate the range
For comparison		
Rectangular interval	3.464	(Example, see Appendix 5 and 6)
95 % conf. limit.	3.92	

Note: pooling of individual standard deviations is an alternative way to estimate the standard deviation.

Appendix 9: Detailed template for evaluation of measurement uncertainty

Measurement Uncertainty

from

Quality Control and Validation Data

Name of analytical procedure:	
Measurand and analyte:	
Measurement ranges	Uncertainty in? • Concentration (absolute) • Per cent (relative)
Measurement range 1	
Measurement range 2	
Measurement range 3	
Short description of the analytical procedure	
Corresponding standard procedure/method	
Customer demand on uncertainty?	

Reproducibility within laboratory R_w – ($_w$=within lab)

Control sample:	**Low**	**Middle**	**High**
Composition of control sample			
Mean value			
Standard deviation, *s*			
Number of determinations, *n*			
Number of months			
Nominal value			

Preliminary estimate of s_{Rw} from the warning limits of the control chart

Warning limits ±				
$s_{Rw}^{prel} = \frac{warning\ limits}{2} =$	Conc. (abs)			
	% (relative)			

List of differences in the procedure/method or property of control samples compared with test samples and if possible also an indication of size. From size an estimate of standard uncertainty, *u* can be made.

	Difference	Size	u
1			
2			
3			
4			

Differences could e.g. be sample amount or matrix, instability, temperature, inhomogeneity, impurities that affect the analytical result. Inhomogeneity of test samples can be assessed by running duplicates. If there are important differences increased within-laboratory reproducibility can be calculated below.

Estimation of an increased s_{Rw}

Control sample			

$s_{Rw} = \sqrt{(s_{Rw}^{prel})^2 + s_{differences}^2} =$			

Bias – method and laboratory bias from CRM

Bias is a systematic error or mean difference from a nominal value.
Use one page for each matrix or concentration level where appropriate.
(Here you have a choice to do the calculations in concentration (absolute) or relative in %.
Please fill in unit used for each column)

Concentration Range:

One CRM. Uncertainty in the nominal value u(Cref) = *U*(Cref)/2.

CRM	**Own laboratory results**		**Cert. value**	***U*(Cref)**	**u(Cref)**	***n***	**bias = Laboratory – CRM**	***Relative bias = (Laboratory-CRM)/CRM *100***
	Mean	**S_{bias}**						

If there is only **one** CRM there is only one bias value but several measurements and the following equation apply:

$$u(bias) = \sqrt{(bias)^2 + \left(\frac{s_{CRM}}{\sqrt{n}}\right)^2 + u(Cref)^2}$$

Where *n* = number of measurement on the CRM and s_{bias} is the obtained standard deviation from measurements on the CRM.

Several CRM – uncertainty in nominal value is u(Cref) = *U*(Cref)/2

CRM	**Own laboratory results**		**Cert. value**	***U*(Cref)**	**u(Cref)**	**bias = Laboratory – CRM**	***Relative bias = (Laboratory-CRM)/CRM *100***
	Mean	**S_{CRM}**					
					RMS_{bias}		

n_{CRM} = number of CRM samples

Root Mean Square is $RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n_{CRM}}} =$

Mean value of $u(Cref) =$

Estimate from several CRM - $u(bias) = \sqrt{RMS_{bias}^{\ 2} + u(Cref)^2} =$

Bias – method and laboratory bias from proficiency testing data

Bias is calculated as the mean difference from a nominal value. In this case it is a only difference therefore marked "bias" in table heading. Use one page for each matrix or concentration level where appropriate. Here you have a difficult choice: To do the calculations in concentration (absolute) or relative in %. Please fill in unit used for each column)

Concentration range:

Proficiency Testing (PT)

Data from the last ten rounds of a PT scheme (minimum six!) see Appendix 4. .

Year	**Sample ¤**	**Laboratory value**	**PT value**	**"bias" = Laboratory –PT**	***Relative bias = (Laboratory-PT)/PT *100***		n_{Lab}	s_R
			RMS_{bias}			Mean		

Number of PT, n_{PT} =

Root Mean Square, $RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n_{PT}}}$ =

Uncertainty in nominal PT **mean**[6] value u(Cref) = $\frac{s_R}{\sqrt{n_{Lab}}}$ =…..

where n_{Lab}= mean value of participating laboratories and s_R is the mean (or pooled) standard deviation of the different PT rounds

Calculation of *u*(bias)

See chapter 5 in Nordtest handbook.

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

[6] If a median PT value is used $u(\text{Cref}) = 1{,}25 \cdot \frac{s_R}{\sqrt{n_{Lab}}}$ according to ISO 13528 /19/..

Calculation of expanded measurement uncertainty

$$U = 2 \cdot u_c = 2 \cdot \sqrt{s_{Rw}{}^2 + (u(bias))^2}$$

where u_c is the combined standard uncertainty, covering approximately 68 % of the values (similar to 1 standard deviation).

Low range – Measurement uncertainty:

Bias from	s_{Rw}	*u(bias)*	u_c	$U = 2*u_c$
CRM				
PT				

Middle range – Measurement uncertainty:

Bias from	s_{Rw}	*u(bias)*	u_c	$U = 2*u_c$
CRM				
PT				

High – Measurement uncertainty:

Bias from	s_{Rw}	*u(bias)*	u_c	$U = 2*u_c$
CRM				
PT				

List over the main contributions to measurement uncertainty and if possible also an indication of size in concentration (e.g. mg/l) or in % (relative).

	Source	Size
1		
2		
3		
4		
5		
6		

Appendix 10: Example of a report from measurement uncertainty calculations using the software MUkit

Step	Action	Determination of ammonium in water	2012-11-22
1	Specify Measurand	Analyte measured: Ammonium Concentration range: 50 - 500 µg/l Matrix: Water Analysis method: EN/ISO 11732	
2	Quantify within-laboratory reproducibility $u(R_w)$ Control sample that covers all the steps in the analytical process	**Control samples:** Matrix: Water Period of measurements: 2001-01-01 - 2002-01-01 Number of control samples: 135 Average concentration: 100 µg/l Standard deviation, s_{Rw}: 1,67 % $u(R_w) = s_{Rw}$ = 1,67 %	

Step	Action	Determination of ammonium in water	2012-11-22

3 — Quantify method and laboratory bias, $u(bias)$

Method and laboratory bias from interlaboratory comparisons: (PT)

Interlaboratory comparison count, N: 6

I	1	2	3	4	5	6
Assigned concentration, $c_{ref\,i}$	81 µg/l	73 µg/l	264 µg/l	210 µg/l	110 µg/l	140 µg/l
Measured concentration, c_i	83 µg/l	75 µg/l	269 µg/l	213 µg/l	112 µg/l	144 µg/l
$bias_i = \frac{c_i - c_{ref\,i}}{c_{ref\,i}} \cdot 10$	2,47 %	2,74 %	1,89 %	1,43 %	1,82 %	2,86 %
Between laboratories standard deviation, $s_{R\,i}$	10,0 %	7,00 %	8,00 %	10,00 %	7,00 %	11,00 %
Consensus value robust mean or median, $s_{R(fixed)i} = 1{,}25$	No	No	No	No	No	No
Fixed standard deviation, $s_{R(fixed)i}$	10,0 %	7,00 %	8,0 %	10,0 %	7,0 %	11,0 %
Number of participating laboratories, n_i	31	36	32	35	36	34
$u(c_{ref\,i}) = \frac{s_{R(fixed)i}}{\sqrt{n_i}}$	1,80 %	1,17 %	1,41 %	1,69 %	1,17 %	1,89 %
Analyte measured	Ammonium					
Matrix	water	water	water	water	water	water
Date	1999-03-01	1999-09-01	2000-03-03	2000-10-04	2001-04-04	2001-10-11

		<table><tr><td>Arranger</td><td>NIVA</td><td>NIVA</td><td>NIVA</td><td>NIVA</td><td>NIVA</td><td>NIVA</td></tr><tr><td>Additional information</td><td></td><td></td><td></td><td></td><td></td><td></td></tr></table> $u(c_{ref}) = \frac{\sum_{i=1}^{N} u\,(c_{ref\,i})}{N}$ = 1,52 % $RMS_{bias} = \sqrt{\frac{\sum_{i=1}^{N} bias_i{}^2}{N}}$ = 2,26 % $u(bias) = \sqrt{RMS_{bias}{}^2 + u(c_{ref})^2}$ = 2,73 %
4	Convert component to a standard uncertainty	$u(R_w)$ = 1,67 % $u(bias)$ = 2,73 %
5	Calculate combined standard uncertainty u_c	$u_c = \sqrt{u(Rw)^2 + u(bias)^2}$ = 3,20 %
6	Calculate expanded uncertainty U	$U = 2 \cdot u_c$ = 6,4 %