CODEX ALIMENTARIUS COMMISSION

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CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
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PROPOSAL TO AMEND THE *GUIDELINES ON MEASURMENT UNCERTAINTY* (CAC/GL 54-2004)

Report of the EWG

Background

1. At the 37th session of CCMAS, the Committee agreed to establish an eWG, taking as a basis the document contained in CRD 26, to:

Identify areas for improvement and amendments of *Guidelines on Measurement Uncertainty* (CAC/GL 54-2004).

Recommend procedures if necessary for determining uncertainty of measurement results including sub-sampling, sample processing and analysis into CAC/GL 54-2004.

Avoid any kind of overlapping with the *Guidelines on Estimation of Uncertainty of Results* (CAC/GL 59-2006).

2. The eWG consisted of more than 70 members of different working areas representing much analytical experience. The list of participants is attached as Appendix II.

3. There have been some reservations about the premature presentation of a revised draft GL54 before having identified the areas for improvement. However, during the 33th session of CCMAS in 2010 much time had been spent discussing amendments and it might be more effective and less time consuming to provide a revised draft serving as basis of possible improvements and taking into consideration, the discussion of 2010[1].

4. In order to keep the GL 54 as simple as possible, as discussed in the last session of CCMAS:

- The Explanatory Notes have been relieved from redundancies and are now integrated into the main text.
- Based on the document contained in CRD 26[2] of the last session, a new chapter with recommended procedures for determining uncertainty of measurement results has been introduced.
- The examples have been revised for strict accordance with the cited standards and international guidelines.
- The Table of the anticipated measurement uncertainties is now harmonized with the Codex Procedure Manual, section II chapter 1.3.

5. Apart from that, all the aspects of general importance of measurement uncertainty (hereafter referred to as MU) of the original GL54 are maintained in the now proposed Draft (Appendix I), but it was necessary to clarify why MU is important in its influence on sampling plans - that means on the procedure of lot assessment - and its role in conformity assessment of a particular analytical test sample.

6. Therefore, in this draft revised GL54, the influence of MU on sampling plans and the corresponding decisions of lot compliance is explained and it contains a link to the concerning ISO standards on sampling.

EWG discussion

7. There have been 12 feedbacks from the eWG with suggestions for improvement and amendment and only one feedback rejecting substantial change. The latter argued, that the draft GL including procedures for estimation of MU is too comprehensive and should be readily understood by those who

[1] ALINORM 10/33/23
[2] CRD 26 can be found here.

discuss and agree the Codex specifications and need to know what its effect on sample acceptance will be.

8. The identified areas for improvement and amendments of CAC/GL 54-2004 and the corresponding recommendations and passages of the submitted draft revised GL 54 are:

- The main body of the original (current) GL54 consists of 3 sections, introduction, terminology and recommendations, with explanatory notes for further explanation in an annex. Avoiding redundancies, in the draft document, the structure is different from that of the original.
- Consideration should be given to which structure is more user-friendly. There exists reservation against the implementation of the practical procedures for determining MU (section 4).
  - It might be discussed whether GL 54 should also be for practical use, as done in the *General Guidelines on Sampling* (CAC/GL 50 – 2004) for sampling plans and CAC/GL 59 for MU in pesticide analysis, providing more than general aspects or not. The alternative would be to split up the theoretical and the practical part into a guideline and a corresponding information document.
- Some members of the eWG expressed their demand for practical exemplary calculations of MU in particular situations.
  - That would take a large extend of the text and would contradict the intention to keep the GL 54 as simple as possible. Generally it should be discussed, whether it is really necessary to demonstrate calculations, if we suspect, that the concerned laboratories do have much experience on application of formulas.
- The reasons for estimation of MU are to be clarified.
  - As stated in the first and in the seventh paragraph of introduction in GL54, the legal reason is given by the requirements of the ISO 17025 and the practical reason is given by the assessment of the test sample with a reasonable level of confidence.
- It was pointed out, that in the situations ii and iii of Figure 1, the suggested procedure for use of MU in sample assessment can allow acceptance of samples whose true values lie above the maximum level.
  - In that case, taking into account the ‘null hypothesis’ of compliance, the probability of non-compliance is less than the required 95% (before last paragraph of chapter 1).
- It was recommended to point out the pros and cons of the particular procedures for estimation of MU.
  - The choice of the procedure is the business of the particular laboratory. It depends on the personnel and financial resources as well as on the available time. As stated in paragraph 1 of section 4, there is no "hierarchy".
- Should MU be estimated for each combination of test method and matrix?
  - The consideration of different matrices is discussed in section 4.1.2
- The difference between conformity assessment and acceptance sampling and the influence of MU on sampling plans are to be clarified.
  - This was done in section 1, paragraphs 2-4, giving reference to the corresponding ISO standards.
- The different types of analytical methods are to be harmonised with the Codex Procedural Manual
  - If applicable, the corresponding Codex types were added in parenthesis.
- There are reservations against the anticipated values of measurement uncertainty estimates based on the Horwitz/Thompson equation (section 6).
  - The accordance with the Codex Procedural Manual was pointed out.
- The consideration of Microbiological Methods was requested.
  - According to the discussion in 37th session of CCMAS (Microbiological Methods are outside the mandate of CCMAS) the estimation of MU for Microbiological Methods was not included.

- There were several technical and editorial recommendations and corrections.
  - Most of them were taken into account.

Finally, in order to achieve a target-oriented further procedure, the concrete action based on the submitted draft revised GL 54 is recommended.

Recommendation

Based on the summary provided above and the proposed revised draft (Appendix I), the Committee, is invited to consider whether work should be initiated on the revision of the *Guidelines on Measurement Uncertainty* GL54.

Appendix I

Proposed draft revised Guidelines on Measurement Uncertainty (CAC/GL 54-2004)

(For information)

1. Introduction:

One of the requirements of the ISO/IEC 17025:2005 (1) Standard that Codex has adopted by reference is that testing laboratories should have and should apply procedures for estimating uncertainty of measurement. Information on measurement uncertainty is needed in test reports when it is relevant to the validity or application of the test results, when a customer's instruction so requires, or when the uncertainty affects compliance to a specification limit. The Codex Alimentarius Commission has developed *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Foods* CAC/GL 27-1997 that require laboratories involved in the import/export of foods to comply with general criteria in ISO/IEC 17025.

There should be no confusion between the activities of conformity assessment and acceptance sampling. Measurement uncertainty does have a role in metrology, where the 'null hypothesis' of compliant must necessarily be assumed. But measurement uncertainty only pertains to the uncertainty of results for a single laboratory sample as such. It does not cover the uncertainty involved with sampling from a lot of product.

Hence also for quantitative estimations on test samples, in case of inspection by variables and inspection by attributes (if they depend on the results of quantitative estimations) the acceptance of a lot is based on the criteria of the corresponding sampling plans.

However, if the measurement uncertainty is not negligible or dominant compared to the sampling uncertainty (which is to be proven just by estimation of the measurement uncertainty), for inspection by attributes (depending on quantitative estimations), it does have influence on the decision whether or not test samples meet the specification i.e. on the acceptance/rejection number (ISO 2859-1/-2 (2,3)). For inspection by variables it does have influence on the sampling size (ISO 3951-2, Annex P (4) or ISO 10725 Annex B (5)).

Measurement uncertainty can be regarded as the variability around the reported result of a test sample within which the "true" value of the test sample may be expected to lie with a reasonable probability.

Thus, as stated in the Guidelines, most quantitative analytical results are reported in the form of "a ± U" where "a" is the estimate of the value of the measurand and "U" is the expanded uncertainty at 95% level of confidence.

It is important that measurement uncertainty be considered when deciding whether or not a test sample meets the specification.

The significance of this can be illustrated by an example, shown in the diagram (Fig.1), which shows the simplest case when decisions are made based on a single test sample.

The example shown here is one where the test result is compared against the specification consisting of a maximum level. It illustrates how the concept of measurement uncertainty could be taken into account when interpreting analytical results on a tested sample.

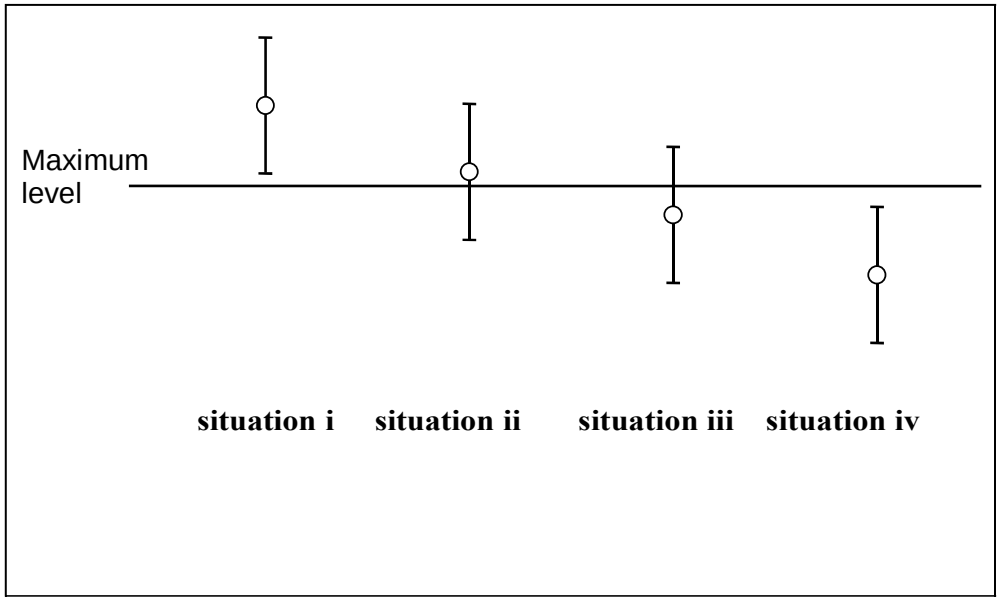

Fig.1: Comparing test results with a Maximum Level taking into account the expanded measurement uncertainty

Situation i

The analytical result minus the expanded measurement uncertainty exceeds the maximum level. The result indicates that the measured analyte in the test sample is above the specification.

Situation ii

The analytical result exceeds the maximum level by less than the expanded measurement uncertainty.

Situation iii

The analytical result is less than the maximum level by less than the expanded measurement uncertainty.

Situation iv

The analytical result is less than the maximum level by more than the expanded measurement uncertainty.

Obviously, in the situations ii and iii, the suggested procedure for use of measurement uncertainty in sample assessment can allow acceptance of samples whose true values lie above the maximum level. But taking into account the 'null hypothesis' of compliance, the probability of non-compliance is less than the required 95%.

The implications of the situations i to iii in case of testing MRL compliance are extensively discussed in the *Guidelines on estimation of uncertainty of results* (CAC/GL 59-2006).

2. Definition:

The international definition for Measurement Uncertainty is:

"Non-negative Parameter characterising the dispersion of the quantity values being attributed to a measurand**"** (6).

NOTES:

108. The parameter may be, for example, a standard deviation (or a given multiple of it), or the half-width of an interval having a stated level of confidence.

109. Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of results of a series of measurements and can be characterised by standard deviations. The other components, which can also be characterised by standard deviations, are evaluated from assumed probability density functions based on experience or other information.

110. It is understood that all components of uncertainty, including those arising from

systematic effects (bias), such as components associated with corrections and reference standards, contribute to the dispersion.

3. General Recommendations:

1. A testing laboratory should have and should apply a procedure to estimate the measurement uncertainty (ISO/IEC 17025).
2. The measurement uncertainty of an analytical result may be estimated by a number of procedures, notably those described by JCGM (GUM) (7) and EURACHEM (8). These documents recommend procedures based on a component-by-component approach, method validation data, internal quality control data and proficiency test data. In many cases the overall uncertainty may be determined by an inter-laboratory (collaborative) study by a number of laboratories and a number of matrices by the IUPAC/ISO/AOAC INTERNATIONAL (9), by the ISO 5725 Protocols (10-13) and/or by the corresponding guide of ISO 21748 (14).
3. The measurement uncertainty and its level of confidence must, on request, be made available to the user (customer) of the results (ISO/IEC 17025, Paragraph 5.4.6).

4. Recommended Procedures for Estimating Measurement Uncertainty:

There are many procedures available for estimating the measurement uncertainty of a result. The Codex guidelines do not recommend any particular approach, but it is important that whatever approach is used, the procedure is scientifically credible. No one approach may be said to be better than any other provided the procedure used is appropriate and credible - i.e. there is no “hierarchy” of the procedures.

In general, procedures are based on a component-by-component (“bottom-up”) approach or on a “top-down” approach using data from collaborative trials, proficiency studies, validation studies or intra-laboratory quality control samples, or a combination of such data.

The following procedures for Estimation of Measurement uncertainty should be regarded as practical examples, which are applicable in many day-to-day situations. In order to achieve acceptance by both trading partners, the concepts are strictly based on internationally recommended guidelines and standards (JCGM 100:2008: Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM), the EURACHEM / CITAC Guide CG 4: Quantifying Uncertainty in Analytical Measurement and ISO Protocols).

The development of the examples cannot be exhaustive and in special situations, other rational procedures might be applied by agreement. Furthermore they do not apply situations, where legal specifications or other internationally accepted guidelines define special rules for the estimation of the measurement uncertainty (e.g. the empirical Horwitz equation). In particular, for pesticide residues, the procedures do not infringe on provisions in the *Guidelines on estimation of uncertainty of results* (CAC/GL 59-2006).

In order to consider as many analytical situations as possible, the procedures are developed for different classes of analytical methods (standard or in-house methods). Multi-factor experimental designs, analysed by ANOVA, and Propagation of distributions using a Monte Carlo method are not included in this document but reference to literature is provided (15-18).

Measurement uncertainty, which is a parameter of the test result, is based on precision data of the method, taking into account the steps of analysis that may include sub-sampling, sample processing and instrumental analysis. The uncertainty components are combined according to the error propagation rules. Basically, N uncertainty standard deviations $s_{1...N}$ (or relative standard deviations i.e. coefficients of variation $cv_{1...N}$) of the evaluation (statistical analysis of series of experimental observations on one or more components of the analytical process) and of the evaluation (usually based on a pool of comparatively reliable information) can be combined to the total standard uncertainty u (or relative total standard uncertainty u_{rel}) (GUM 5.1.2, 5.1.5, 5.1.6) :

$$u = \sqrt{s_1^2 + s_2^2 + \ldots + s_N^2} \qquad \text{or} \qquad u_{rel} = \sqrt{cv_1^2 + cv_2^2 + \ldots + cv_N^2} \quad *)$$

*) The formulas refer to measurands given by the sum and/or the difference of parameters (left) or given by the product and/or the quotient of parameters (right). Since in practice, most of the analytical measurands are given by formulas with products and/or quotients of parameters, in the following text the second formula will be used. For simplicity, the parameters are regarded as non-correlated.

This has the practical advantage that particular precision data from Single-Laboratory method validation or from inter-laboratory method validation (after proving fitness for purpose of the particular test laboratory by verification of that precision data) can be used in combination.

The following procedures are ordered according to the particular class of the analytical method:

4.1. Standard Methods

4.1.1 Defining Methods

4.1.2 Rational Methods (Reference Methods)

4.2. Single Laboratory validated Methods (Alternative Approved Methods)

4.2.1 Established Methods

4.2.1.1 Combination of repeatability precision of all single steps of analysis

4.2.1.2 Precision estimated by series of analysis

4.2.1.2.1 ISO 5752-2 and 5752-3 Approach

4.2.1.2.2 Duplicate Approach

4.2.2. Ad-hoc Methods

4.1. Standard Methods

For Standard methods, the uncertainty is established utilising appropriate validation including precision data. Generally, these data are based on extensive inter-laboratory method validation, mostly performed according to the IUPAC/ISO/AOAC International Harmonized Guideline, ISO 5725-6 or the AOAC International Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis (19). A basic assumption underlying ISO 5725-1 is that, for a standard measurement method, repeatability will be, at least approximately, the same for all laboratories applying the standard procedure, so that it is permissible to establish one common average repeatability standard deviation s_r which will be applicable to any laboratory. However, any laboratory should, by carrying out a series of measurements under repeatability conditions, verify that the average repeatability standard deviation is applicable under given conditions (ISO 5725-6). The reproducibility standard deviation s_R of the standard method is obtained by combining s_r with the between-laboratory standard deviation s_L (ISO 5725-2).

4.1.1 Defining Methods

Defining methods achieve comparability between laboratories measuring the same material with no intent to obtain an absolute measure of the true amount of analyte present. Corrections for method bias or matrix effect are ignored by convention. For an defining method, for which collaborative trial data are available, at least the repeatability should be evaluated in the particular laboratory and proven to be comparable to that s_r predicted by the collaborative trial and documented in the method i.e. the repeatability standard deviation should be less or equal s_r (EURACHEM Example A6). A priori, no bias contribution must be considered and it is therefore appropriate to use the relative reproducibility standard deviation (i.e. the coefficient of variation) CV_R values from the collaborative trial or method publication as relative standard uncertainty u_{rel} within the tested range of analyte levels (EURACHEM 7.6.3).

Collaborative trials provide homogenised mostly stabilised material and hence do not cover physical preparation steps (e.g. grinding, drying) material. The uncertainty contributions of that analytical part should be additionally taken into consideration (EURACHEM 7.6.1), provided that the contribution is significant (i.e. >1/3 CV_R (EURACHEM 7.2.2)).

In the case of significant laboratory sample inhomogeneity, the uncertainty contribution of subsampling should be considered. The significance might be assessed by using a homogeneity check like ISO 13528 (20), Annex B by comparing the relative between-subsamples standard deviation cv_s with the relative standard deviation for proficiency assessment CV_σ (σ is used for the estimation of the z-scores) of the standard method. The laboratory sample may be considered to be adequately homogeneous if, $cv_s \leq 0.3\ CV_\sigma$.

The between-subsamples standard deviation s_s might be estimated by the procedure given in ISO 13528, Annex B1 and using the formula given in Annex B3. That duplicate test also gives information on the uncertainty contribution of the physical preparation procedure:

Select a number *g* of the subsamples from the laboratory sample at random, where *g* ≥10.

- Prepare two test portions from each subsample using techniques appropriate to the test material to minimize between-test-portion differences.
- Taking the 2*g* test portions in a random order, obtain a measurement result on each, completing the whole series of measurements under repeatability conditions.
- Calculate the general average $\bar{\bar{x}}$

$$\bar{\bar{x}} = \frac{\sum_{t=1}^{g} \bar{x}_t}{g} \qquad \text{with} \qquad \bar{x}_t = \frac{x_{t,1} + x_{t,2}}{2}$$

- Calculate the standard deviation s_x of sample averages

$$s_x = \sqrt{\frac{\sum_{t=1}^{g} (\bar{x}_t - \bar{\bar{x}})^2}{g-1}}$$

- Calculate the within-subsamples standard deviation s_w which is a measure of the physical preparation uncertainty

$$s_w = \sqrt{\frac{\sum_{t=1}^{g} w_t^2}{2g}} \qquad \text{with} \qquad w_t = \left| x_{t,1} - x_{t,2} \right|$$

- Calculate the between-subsamples standard deviation s_s with the factor ½ on s_w due to the mean of duplicate analyses being used

$$s_S = \sqrt{s_x^2 - \frac{s_W^2}{2}}$$

- and the relative standard deviation of sample inhomogeneity

$$cv_S = \frac{s_S}{\bar{x}}$$

In case that the sample inhomogeneity is significant ($cv_s > 0.3\ CV_\sigma$), the relative standard measurement uncertainty u_{rel} is given by the combination:

$$u_{rel} = \sqrt{cv_R^2 + cv_S^2}$$

Taking into account the uncertainty contribution of sample preparation (the standard deviation is divided by √2 to correct from a standard deviation for pairwise differences to the standard uncertainty for single values),

$$cv_P = \frac{1}{\sqrt{2}} \frac{s_w}{\bar{x}}$$

the relative standard measurement uncertainty u_{rel} is given by the combination:

$$u_{rel} = \sqrt{cv_R^2 + cv_S^2 + cv_P^2}$$

Notice: In formulas for calculating the analytical result, the influence of subsampling differences due to inhomogeneity and preparation variability can be implemented as factors, which are dispersed around 1 (EURACHEM A4.3).

4.1.2 Rational Methods (Reference Methods)

For rational standard methods, trueness is an issue, which should be considered in the estimation of measurement uncertainty. The current procedure applies to the situation where no bias is to be taken into account. But this assumption should be proven by appropriate recovery experiments. For many rational standard methods, certified reference materials are supplied. As an alternative, samples can be spiked with a known level of the analyte (with preference of matrices, which do not contain the analyte), bearing in mind the different behaviour of the spiked substance and the native counterpart.

In a first step, from *n* recovery experiments on certified reference material or homogenized spiked material (e.g. homogenized samples are split and one portion spiked) with the reference concentration x_{ref} , the found concentrations of the analyte x_i , and the bias b_i , the average laboratory bias $\bar{b}$ is estimated

$$\bar{b} = \frac{1}{n}\sum_{i=1}^{n} b_i \qquad \text{with} \qquad b_i = x_i - x_{ref}$$

and compared with the standard uncertainty *u* at the reference concentration (by multiplying u_{rel} with the concentration of the analyte) combined with the certified uncertainty of the reference material or the experimental uncertainty of spiked material estimated by homogeneity tests u_{ref} (see 4.1.1). Laboratory bias can be neglected if

$$|\bar{b}| \leq 2\sqrt{\left(\frac{u^2}{n}\right) + u_{ref}^2}$$

Otherwise, the bias is significant (EURACHEM 7.16) and the analytical result might be corrected for the bias, making due allowance for the uncertainty of the correction. In that case, the standard deviation s_B of the average bias is given by

$$s_B = \frac{1}{\sqrt{n}}\sqrt{\frac{\sum_{i=1}^{n}(b_i-\bar{b})^2}{n-1}}$$

In case that the matrix might have an impact on the bias, the recovery experiments should be applied on samples from different matrices and the uncertainty contribution of that particular matrix, which corresponds to the sample should be used.

Notice: It should be avoided to take the effect of bias (this is not the uncertainty of bias) into account by enlarging the “uncertainty” assigned to the result instead of correcting for bias. Evaluating the uncertainty of a measurement result should not be confused with assigning a safety limit to some quantity (Guide to the expression of uncertainty in measurement (GUM), 6.3.1).

4.2. Single-laboratory Validated Methods (Alternative Approved Methods)

Contrary to standard methods, for Single-laboratory validated methods no published standard precision data are available. Therefore, they are subjects of extensive validation procedures. Despite of ad-hoc situations, the validation provides precision data. Nevertheless, in case that the Single-laboratory validated method is a modification of a corresponding standard method, the estimation of precision should focus on the uncertainty contributions of that modification. The uncertainty contributions should be compared to the relative reproducibility standard deviation (i.e. coefficient of variation) CV_R values from the collaborative trial or standard method publication. If the uncertainty contribution of modifications is negligible, it is appropriate to use CV_R as relative standard uncertainty u_{rel} and to proceed according to Procedures 4.1.

There are two general approaches to estimate the precision:

- The combination of the repeatability precision of all single steps of analysis (e.g. weighing, drying, extracting, diluting and analytical measurement) with the involved calibrations and other uncertainty sources (e.g. purity of reference standards, experience of test personnel)
- Precision estimated by series of analysis as far as possible over an extended time period allowing natural variation of all impact factors.

In practice, a combination of these types is usually necessary and convenient.

4.2.1 Established Single-laboratory validated Methods (in-house Methods)

4.2.1.1 Combination of the repeatability precision of all single steps of analysis

The uncertainty components associated with *N* potential sources of uncertainty are identified, quantified as standard deviations u_i, multiplied with sensitivity coefficients c_i, and combined (GUM 5.1.3):

$$u = \sqrt{\sum_{i=1}^{N} c_i \cdot u_i^2}$$

In the case that the different components are not statistically independent, corresponding correlation factors are to be introduced.

The sources are for example:

- Standard substances (certified uncertainty/purity)
- Physical/chemical variability (extraction, derivatisation, stoichiometry)
- Application of measuring devices for preparation of the test samples (balances, pipettes, thermometers etc.)
- Application of analytical instruments (stability, calibration, contamination etc.)
- Different experience of test personnel

The procedure begins with the critical reflection of the formula of the measurand i.e. the relationship between the result and the input values. All parameters are to be checked for their uncertainty relevance.

Therefore, for example, the uncertainty of the sample preparation is separated into the uncertainties of the individual steps of weighing, homogenizing, drying, extracting, diluting etc., which are to be combined.

The uncertainty of weighing itself, for example, is estimated from the separate contributions of calibration and traceability (including certified uncertainty of the weights) and the uncertainty of the reading (analogue/digital-display).

Obviously, the subject of this type of estimation is too complex to be sufficiently described in the current paper. Therefore, for further information, reference is made to the JCGM 100:2008: Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM) and the EURACHEM / CITAC Guide CG 4: Quantifying Uncertainty in Analytical Measurement.

4.2.1.2 Precision estimated by series of analysis

According to ISO 5725-3, precision estimated in one laboratory is the so-called intermediate precision measure, which is smaller than the reproducibility standard deviation based on inter-laboratory method validation and hence more appropriate for the individual laboratory. That intermediate precision condition of measurement includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time, but may include other conditions involving changes like new calibrations, calibrators, operators, and measuring systems.

Therefore, it is recommended to start at the situation, which is similar to the participation on collaborative trials (homogenised and dried material of a particular matrix) and to implement the additional components.

To this end, the Single-Laboratory estimation of precision should take into account all parts of the analysis, which basically would be involved in case of participation on a corresponding inter-laboratory validation of a standard method. That comprises at least the extraction/derivatisation/digestion procedures (recovery variation) and the complete measurement process including calibration and traceability.

A typical test sample containing an appropriate amount of analyte (e.g. homogenised and dried or processed to assure stability of the matrix and analyte(s)) might be analysed several times over a period of time, using different analysts and equipment where possible (e.g. the results of measurements on quality control samples) thus verifying Single-Laboratory reproducibility conditions (EURACHEM 7.7.2) or intermediate precision conditions.

The relative intermediate standard deviation cv_{int} estimated by use of the following procedures, like corresponding collaborative trials, does not cover effects of sample preparation, sample inhomogeneity and subsampling. In order to take into account these uncertainty components, they should be combined with cv_{int} as described in Procedures 4.1.

For the identification and uncertainty estimation of bias, the approaches described in the Procedure 4.1.2 have to be applied.

In case that the uncertainty might depend on analyte levels, the precision experiments should be carried out at different levels in any case, according to ISO/IEC 17025, including the level, which is relevant for compliance assessment. The significance of influence might be checked by the F-test or the Cochran test for homogeneity of the variances from different experiments on different levels of the analyte.

Finally, the uncertainty of the calibration standards (which obviously might be much higher than the certified uncertainty of reference material) or of the reference materials (negligible in most cases) should be considered.

4.2.1.2.1 ISO 5725-2 and ISO 5725-3 Approach

An appropriate norm-consistent approach might be the as-far-as-possible-application of the procedure given in ISO 5725-2 where the reproducibility standard deviation s_R of an inter-laboratory method validation is obtained by combining the mean repeatability standard deviation s_r of all laboratories with the between-laboratory standard deviation s_L.

A typical test sample (homogenised and dried) is analysed over a period of time on *n* different days by different analysts (with a new extraction/digestion, recalibration). Each of the days, a number of *k* replicates of the particular extract/digest are measured with the results $x_{j=1...k}$ under repeatability conditions (measurement within a short time, the same instrument and calibration used by the same operator) and the following parameters are calculated:

- Each day *i* : From the *k* replicate results $x_{j=1...k}$ the mean value $\bar{x}_i$ and the repeatability standard deviation $s_{r\,i}$ are estimated:

$$\bar{x}_i = \frac{1}{k}\sum_{j=1}^{k} x_j$$

$$s_{r\,i} = \sqrt{\frac{\sum_{j=1}^{k}\left(x_j - \bar{x}_i\right)^2}{k-1}}$$

- From the repeatability standard deviations of the different days $s_{r\,i=1...n}$, the mean repeatability standard deviation $s_{r\,mean}$ is calculated:

$$s_{r\,mean} = \sqrt{\frac{\sum_{i=1}^{n} s_{r\,i}^2}{n}}$$

- The "between-days" standard deviation s_d of the mean values $\bar{x}_{i=1...n}$ of the different days is calculated:

$$s_d = \sqrt{\frac{\sum_{i=1}^{n}(\bar{x}_i - \bar{\bar{x}})^2}{n-1}}$$

with the total mean value $\bar{\bar{x}} = \frac{1}{n}\sum_{i=1}^{n}\bar{x}_i$

- According to ISO 5725-3, the intermediate standard deviation is given by :

$$s_{int} = \sqrt{s_{r\,mean}^2 + s_d^2}$$

Finally, the relative intermediate standard deviation is given by:

$$cv_{int} = \frac{s_{int}}{\bar{\bar{x}}}$$

4.2.1.2.2. Duplicate Approach

As an alternative to the above-mentioned ISO 5725-2 and ISO 5725-3 approach, the overall run-to-run variation can be performed with a number *n* of duplicate tests (homogenised samples each divided into two test samples, each of the test samples subjected to complete extraction/digestion and determination procedure including recalibration)(EURACHEM 7.7.2 and A4.4).

For each duplicate test *i*, the relative differences $\delta_{i\ rel}$ and the standard deviation of the relative differences $s_{\delta rel}$ are calculated:

$$\delta_{i\,rel} = \frac{\delta_i}{\bar{x}_i}$$

With $\delta_i = x_{i,1} - x_{i,2}$ and $\bar{x}_i = \frac{x_{i,1} + x_{i,2}}{2}$

$$s_{\delta_{rel}} = \sqrt{\frac{\sum_{i=1}^{n}\left(\delta_{i\,rel} - \bar{\delta}_{rel}\right)^2}{n-1}}$$

With $\bar{\delta}_{rel} = \frac{1}{n}\sum_{i=1}^{n}\delta_{i\,rel}$

- Finally, this standard deviation is divided by √2 to correct from a standard deviation for pairwise differences to the standard uncertainty for single values giving the relative intermediate standard uncertainty:

$$cv_{int} = \frac{s_{\delta_{rel}}}{\sqrt{2}}$$

4.2.2 Ad-hoc Methods (Tentative Methods)

In most cases, ad-hoc methods are based on standard or well-established Single laboratory validated methods. They are expanded substantially (e.g. to other analytes or matrices) and will not generally require complete revalidation, but the procedure, which was described in the first paragraph of Procedures 4.2 is highly recommended. Further information on the evaluation of the measurement uncertainty for ad-hoc methods are given in the EURACHEM Guide (EURACHEM 7.10). In order to get an acceptable statistical power, as many replicates as practical of the test (including all relevant parts of method) should be performed. The comparison of the resulting relative standard deviation with the relative standard uncertainty of the basic method gives information about the precision equivalence of the ad-hoc method. Where appropriate, the uncertainty of the basic method should be reported.

5. Reported Measurement Uncertainty

The combined relative standard measurement uncertainty u_{rel}, which was obtained by applying one of the above described procedures, is the basis for the reported expanded measurement uncertainty *U*. It is obtained by multiplying the standard measurement uncertainty by a coverage factor *k*.

For the level of confidence required (normally 95%), for most purposes it is recommended to set *k=2*. In case that the combined uncertainty is based on only few observations (less than about seven i.e. less than six degrees of freedom ν), however, *k* should be set equal to the two-tailed value of Student's t-factor (note that the 95% one-sided confidence limit is equivalent to the 90% two-sided confidence limit) for the so called effective number of degrees of freedom ν_{eff} associated including that ´statistical low power´-contribution. (GUM, Annex G.4.1).

6. Anticipated Values of Measurement Uncertainty Estimates:

Stipulating information on the anticipated values of measurement uncertainty estimates is frequently not supported by analysts. The users of analytical data and the customers of the laboratories producing such data frequently ask for such information regarding the level of uncertainty that may be expected for test results. They have concerns that some laboratories underestimate the size of their uncertainties and so report unrealistically small uncertainties to their customers.

According to the Codex Alimentarius Commission Procedural Manual, for chemical analyses, using the values of s_R from collaborative trials, it would be reasonable to anticipate that the (expanded) uncertainties reported by laboratories would be approximately the following, given by the Horwitz/Thompson equation (21):

	Thompson	*Horwitz equation* ($2C^{-0.1505}$)							
Concentration ratio (C)	$< 10^{-7}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
Concentration unit	< 0.1 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0.1 g/kg	1 g/kg	10 g/kg	100 g/kg	1000 g/kg
$PRSD_R$ (%)	22	22	16	11	8	6	4	3	2
$RSD_R \leq 2 \cdot PRSD_R$ (%)	≤ 44	≤ 44	≤ 32	≤ 22	≤ 16	≤ 12	≤ 8	≤ 6	≤ 4

$PRSD_R$ = predicted value for relative standard deviation of reproducibility.
RSD_R = found value for the relative standard deviation of reproducibility in a collaborative study.

It would be expected that the reported measurement uncertainties by any laboratory would not significantly exceed the value estimated from the s_R at the concentration of carrying out any particular analysis on a regular basis would be expected to obtain uncertainty values less than the values given above.

7. Literature

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