Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry

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Table of Contents

1. Scope and Field of application

This document on measurement uncertainty was developed within the network of the European Union Reference Laboratory (EURL) for Dioxins and PCBs in Feed and Food and the respective National Reference Laboratories (NRLs) of member states. Detailed guidance is given on the evaluation of measurement uncertainty in the quantitative analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs), to assist laboratories performing official feed and food control within the European Union, especially National Reference Laboratories (NRLs) and Official Laboratories (OFLs). It provides useful key elements contributing to further harmonization of compliance assessment and outlines practical aspects related to measurement uncertainty estimation.

A new concept placing special emphasis on the inclusion of current method performance data is presented. The concept covers the full analytical process from sample receipt at the laboratory through sample storage, preparation and analysis, to data processing and reporting. In particular, it focuses on the role of analytical variability generally known as "measurement uncertainty" (MU) in the interpretation of analytical results for assessment of their compliance with a specification. Effects from sampling [EURACHEM/CITAC 2007, /18/] and transport also contributing to MU are acknowledged but not treated within the scope of this document.

Two selected approaches for measurement uncertainty estimation are proposed for the determination of PCDD/Fs and PCBs in food and feed by gas chromatography-mass spectrometry (GC-MS) using internal standard stable isotope labelled analogues. An empirical, or “top-down”, approach combines contributions from intermediate (intra-laboratory) precision and trueness (expressed as bias) to estimate measurement uncertainty, both for individual congeners and for sum parameters. The working group recommends the use of the empirical approach as described in this document as the main option for MU estimation, because it is designed and developed to cover the whole analytical process and also includes the opportunity to reassess or update MU on a regular basis.

However, an alternative methodology based on a semi-empirical approach following the EURACHEM/CITAC guide [EURACHEM/CITAC 2012, /12/] is also presented. It has been designed for laboratories new to this type of analysis that have generated data from initial validation studies. In this case the semi-empirical approach may be a good starting point, however the authors recommend implementing the empirical or top-down approach once enough data have been gathered.

Figure 1 provides a flow chart for the estimation of measurement uncertainty applying the different approaches described in this document.

Practical examples based on laboratory data help connect theory with the application, thus making the theoretical basis of the approach more accessible to the analyst.

NOTE 1: This guidance document supports implementation and practical realisation of the requirements given in the ISO/IEC 17025 standard [ISO/IEC 17025:2005-08, /14/] and in the relevant EU regulations on analytical criteria [COM 2014, /27/; COM 2009, /28/]. The concepts and recommendations given form an integral part of state-of-the-art analytical performance and quality control.

NOTE 2: The scope of the approaches presented in this guidance document can be extended to include the analysis of other contaminants that use isotope dilution techniques.

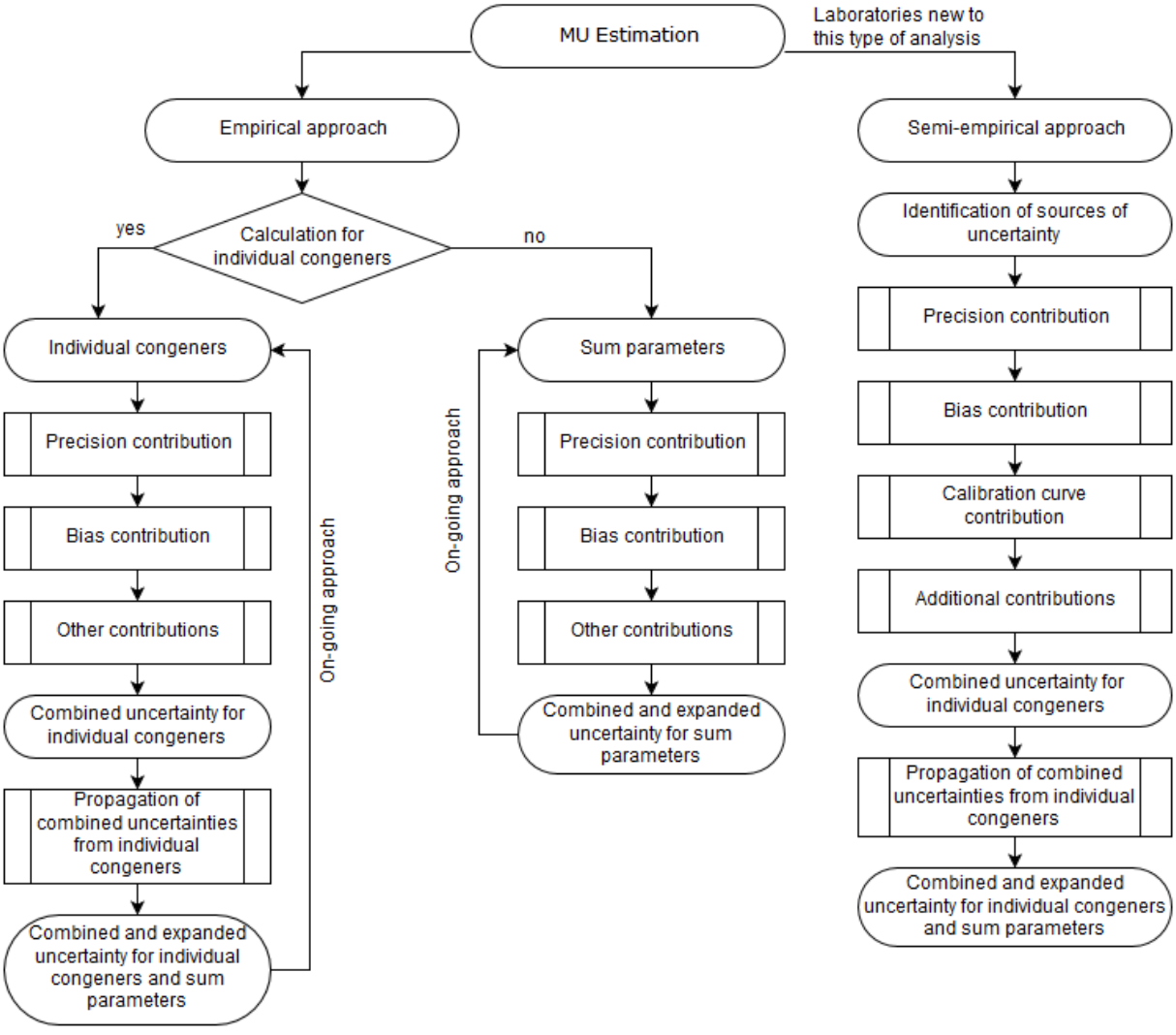


Figure 1: Flow chart for estimation of measurement uncertainty using an on-going empirical (top-down) and a semi-empirical approach.

2. Acronyms

Acronym	Definition
BIPM	International Bureau of Weights and Measures
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
DIN	German Institute for Standardization (Deutsches Institut fuer Normung)
EPA	Environmental Protection Agency
EU-RL	European Union Reference Laboratory
EURACHEM	Network of analytical chemistry organisations in Europe
GUM	Guide to the Estimation of Uncertainty in Measurement
IEC	International Electrotechnical Commission
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
ISO	International Standardisation Organisation
IUPAC	International Union of Pure and Applied Chemistry
IUPAP	International Union of Pure and Applied Physics
NIST	National Institute of Standards and Technology
NRL	National Reference Laboratory
NT	Nordtest (Nordic Innovation)
OIML	International Organization of Legal Metrology
OFL	Official Laboratory
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PCBs	Polychlorinated biphenyls
DL-PCBs	Dioxin-like polychlorinated biphenyls
NDL-PCBs	Non dioxin-like polychlorinated biphenyls
SEMATEC	Semiconductor Manufacturing Technology
TEF	Toxic equivalency factor
TEQ	Toxic equivalency
WHO	World Health Organisation

3. Introduction

Measurement uncertainty is a subject that is both complex and continually evolving. Scientists generally take great care to identify the types and sources of measurement error to reduce its impact on results to acceptable levels, and to characterise the extent of residual measurement uncertainty within a set of data.

The concepts proposed in this guidance document consider the uncertainty associated with the analytical procedure only. The uncertainties related to, for example, sampling, homogeneity or stability of the sample also contribute to the total uncertainty but these aspects are discussed elsewhere [EURACHEM/CITAC 2007, /18/]. An additional and significant element of uncertainty arising from the use of toxic equivalence factors (WHO-TEFs) to derive WHO-PCDD/F- and WHO-PCB-TEQ is similarly beyond the scope of this document.

An analytical result cannot be properly interpreted without knowledge about its uncertainty. Estimation of measurement uncertainty is not only a requirement of ISO/IEC 17025 [ISO/IEC 17025:2005-08, /14/] for testing laboratories. In the feed and food sector, legislation setting maximum levels addresses how analytical results shall be expressed and interpreted. All reported analytical results actually take the form ‘x ± U’, where x is the analytical result (the best estimate of the true value) and U the expanded measurement uncertainty, at a specified level of confidence (e.g. 95%). Two times U is the range within which the unknown true value of the real sample analysed is assumed to fall, with a high probability (depending on the coverage factor k selected). The value of U is the uncertainty generally reported by analysts.

Three general strategies for MU estimation are considered:

- Empirical or top-down approach based on performance data of the whole method taking into account trueness and precision contributions
- Theoretical or bottom-up approach based on a mathematical model of the measurement process, estimating individual contributions of the relevant sources of uncertainty
- Semi-empirical approach based on a combination of the theoretical and empirical approach

They are based on the following steps: specifying the measurand, identifying the uncertainty sources, quantifying uncertainty components, and finally combining all individual contributions to calculate the combined uncertainty [EURACHEM/CITAC 2012, /12/].

In the first sections of this guide, the concept and importance of measurement uncertainty are introduced, along with a glossary of symbols and definitions. Details are then given of how to estimate uncertainties in real measurement situations by the empirical (top-down) and semi-empirical approaches. The main steps involved in calculating the uncertainty for a measurement are outlined, with examples found in the Annex. Finally, a list of publications

for further reading is included to direct the reader's attention towards the next steps in understanding and calculating measurement uncertainties.

NOTE: Although in this guidance document, U is calculated from individual contributory terms expressed in relative units to more easily accommodate these terms, e.g. various concentrations, concentration ranges or various similar matrices, U is expressed in absolute units when associated with an analytical result for reporting and compliance assessment.

4. Compliance Assessment

4.1 General

The generally accepted procedure for compliance assessment [EURACHEM/CITAC 2007, /26/; EC 2004, /34/] is to report samples as containing 'not less than (x-U)' in situations where the statutory limit is a maximum permissible concentration. Here any enforcement action is only taken when the analyst is sure that the specification limit is exceeded. The interpretation of results is depicted on Figure 2. In practice, if we are considering a maximum value in legislation, the analyst will determine the analytical level and estimate the measurement uncertainty at that level, subtract the uncertainty from the reported concentration (x-U) and use that value to assess compliance. If that value is larger than the legislation limit the sample is considered to be non-compliant (for details concerning PCDD/Fs and PCBs, see chapter 4.2.1). Thus, according to the accepted procedure, only the result in situation 4 is non-compliant beyond reasonable doubt (Figure 2).

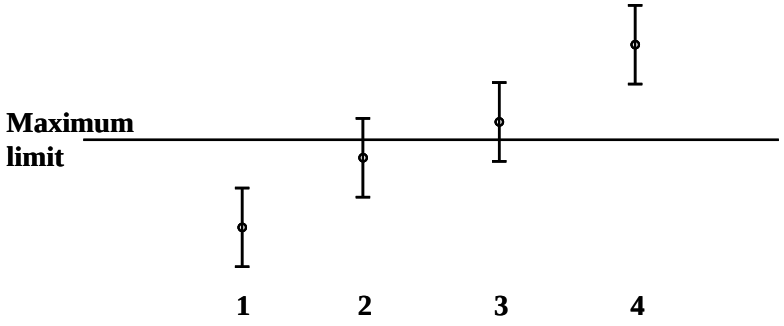


Figure 2: Interpretation of results for compliance assessment; dots represent analytical results, bars indicate uncertainty intervals of 2U. Four situations are illustrated:

1. The analytical result, either with the expanded measurement uncertainty (U) added or subtracted, is below the maximum limit (ML): The sample is compliant.
2. The analytical result plus U exceeds the ML, however with U subtracted it is below the ML: The sample is compliant.
3. The analytical result is above the ML, but non-compliance is not determined beyond reasonable doubt since the result minus U is below the ML with a certainty of 95%: The sample is compliant.
4. The result, even with the subtraction of U, is above the maximum limit: The sample is deemed non-compliant beyond reasonable doubt.

4.2 Compliance Assessment in PCDD/F and PCB Analysis

4.2.1 Legal Requirements for official control

By definition and in principle, measurement uncertainty (MU) is associated with a measurand (e.g. a congener concentration derived from a signal value). In the specific case of PCDD/Fs and related dioxin-like compounds, the concept of Toxic Equivalents (TEQs) was introduced by toxicologists with the objective of obtaining an estimate of the summed PCDD/F and PCB toxicity of a sample irrespective of its congener pattern. *Stricto sensu*, this TEQ value is not a measurand but a sum of individual congener concentrations each multiplied by its assigned weighting factor, the TEF value [Van den Berg M et al. 2006, /19/]. Within EU legislation, maximum levels are expressed in TEQs, therefore, MU values must be assessed for these TEQ sum parameters for decision making and compliance assessment.

For compliance assessment, analytical results of a sample expressed as WHO-PCDD/F-TEQ, WHO-PCB-TEQ or WHO-PCDD/F-PCB-TEQ are compared with maximum levels and/or action levels/thresholds given in TEQ units, taking into account measurement uncertainty [COM 2006, /29/; COM 2013, /30/; DIRECTIVE 2002, /31/].

Legislation [COM 2009, /28/; COM 2014, /27/] further requires:

"The *lot is accepted*, if the result of a single analysis […] performed by a confirmatory method does not exceed the respective maximum level of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs as laid down in Regulation (EC) No 1881/2006 taking into account the measurement uncertainty."

"The lot is *non-compliant* with the maximum level as laid down in Regulation (EC) No 1881/2006, if the upperbound analytical result obtained with a confirmatory method and confirmed by duplicate analysis, exceeds the maximum level beyond reasonable doubt taking into account the measurement uncertainty. The *mean of the two determinations*, taking into account the measurement uncertainty is used for verification of *[non-]compliance*. The duplicate analysis is necessary if the result of the first determination applying confirmatory methods with the use of ^{13}C-labelled internal standard for the relevant analytes is *not compliant*."

"The measurement uncertainty may be taken into account […]:

- by calculating the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %. A lot or sublot is *non-compliant* if the measured value minus U is above the established permitted level. In case of a separate determination of PCDD/Fs and dioxin-like-PCBs the sum of the estimated expanded uncertainty of the separate analytical results of PCDD/Fs and dioxin-like PCBs has to be used for the estimated expanded uncertainty of the sum of PCDD/Fs and dioxin-like PCBs."

Estimated expanded uncertainty for the sum of PCDD/Fs and dioxin-like PCBs

According to legislation [COM 2009, /28/; COM 2014, /27/], the expanded uncertainty U for the *sum* of PCDD/Fs and DL-PCBs may be assessed by summing up both absolute U values estimated for WHO-PCDD/F-TEQ results, and for WHO-PCB-TEQ results, respectively.

Measurement uncertainty of the mean calculated from results of two separate analyses

In this document, measurement uncertainty is estimated for a result from single analyses. According to legislation [COM 2009, /28/; COM 2014, /27/], however, a mean result from duplicate analysis is required for verification of non-compliance.

If the results from two separate determinations differ by no more than the intermediate precision limit ($R_w = 2.8 \cdot s_{Rw}$), the individual measurement uncertainties are propagated according to the following formula:

$$u_{c,mean} = \frac{\sqrt{u_{c1}^2 + u_{c2}^2}}{2} \qquad \textbf{Eq. 1}$$

$u_{c,mean}$ = combined standard uncertainty of the mean from results of two separate analyses

u_{c1}, u_{c2} = individual combined standard uncertainties of results 1 and 2, with $u_{c1} \approx u_{c2}$

NOTE: Equation 1 shows that the combined standard uncertainty of the mean of two separate results are approximately by $\sqrt{2}/2$ smaller than the uncertainty of each individual result.

4.2.2 Expression of Results and Compliance Assessment

Reporting of results as TEQ for PCDD/Fs and DL-PCBs and the sum of NDL-PCBs for compliance assessment shall include the analytical result x and its associated expanded uncertainty U, including the applied coverage factor for calculation of U. The results are reported as x ± U, calculated using a coverage factor of 2 (level of confidence of ca. 95%).

In addition, according to EU regulations on analytical criteria [COM 2009, /28/; COM 2014, /27/], the results for PCDD/Fs and PCBs shall be expressed in the same units and with at least the same number of significant figures as the maximum levels.

For rounding of results and significant digits, refer to chapter 9.6.

4.3 Estimation of Target Measurement Uncertainty from Legal Requirements

In case of PCDD/F and PCB analysis in feed and food, Commission Regulations (EU) No 589/2014 (food) [COM 2014, /27/] and (EC) No 152/2009 (feed) [COM 2009, /28/] require that confirmatory methods used within official control should not exceed the following performance criteria for sum-parameters in the range of the maximum level:

- Trueness, expressed as bias, must fall within the range ± 20% for TEQ results, or ± 30% for the sum of PCBs 28, 52, 101, 138, 153 and 180.
- Relative intermediate precision must be less than 15% for TEQ results, or ≤ 20% for the sum of PCBs 28, 52, 101, 138, 153 and 180.

Further, each laboratory must evaluate the measurement uncertainty associated with the analytical results that it produces. As maximum acceptable values for measurement uncertainties are not defined in the above mentioned regulations, practical considerations suggest definition of a target measurement uncertainty as an additional method performance parameter [EURACHEM/CITAC 2015, /36/].

The maximum tolerable standard uncertainty $u_{c,max}$ can be calculated by combining the uncertainty components of the required precision and trueness values specified in the regulations mentioned above:

$$u_{c,max} = \sqrt{u_{Rw,max}^2 + u_{bias,max}^2} \qquad \textbf{Eq. 2}$$

$$u_{bias,max} = \left(\frac{bias_{max}}{\sqrt{3}}\right) \qquad \textbf{Eq. 3}$$

where $u_{Rw,max}$ is the maximum tolerable intermediate precision expressed as s_{Rw}, and $bias_{max}$ is the maximum tolerable bias with its corresponding uncertainty component $u_{bias,max}$. The uncertainty component $u_{bias,max}$ is calculated from a rectangular distribution which is the half-width of the full interval (± bias) divided by the square-root of 3 [EURACHEM/CITAC 2015, /36/].

NOTE: The selection of a rectangular distribution reflects the acceptable bias distribution within the range of ± 20% for TEQ results, or ± 30% for the sum of PCBs 28, 52, 101, 138, 153 and 180

With a coverage factor of 2, the maximum tolerable expanded measurement uncertainty U_{max} becomes:

$$U_{max} = 2 \cdot u_{c,max} \qquad \textbf{Eq. 4}$$

Table 1 shows that in PCDD/F and PCB analysis, the expanded measurement uncertainty shall not exceed ± 38% for TEQ results, and ± 53% for the sum of PCBs 28, 52, 101, 138, 153 and 180.

In principle, the estimated expanded measurement uncertainty should not exceed the expanded target measurement uncertainty [EURACHEM/CITAC 2015, /36/].

Table 1: Requirements according to Commission Regulations (EU) No 589/2014 (food), (EC) No 152/2009 (feed) and discussed amendments for NDL-PCBs, and resulting combined target standard uncertainty and expanded uncertainty.

Parameter	**Trueness** $bias_{max}$ **(%)**	**Precision** s_{Rw} **(%)**	**Target standard uncertainty** u_{max} **(%)**	**Target expanded uncertainty** U_{max} **(%)**
PCDD/Fs and DL-PCBs	20	15	18.9	38
NDL-PCBs by isotope dilution*	20	15	18.9	38
NDL-PCBs**	30	20	26.5	53

* when all six ^{13}C-labelled analogues are used as internal standards

** other techniques

5. Grouping of Matrices

In principle, each matrix in the scope of validation requires individual MU assessment within the working range. If this is not possible for some matrices, e.g. due to the limited availability of suitable CRMs or proficiency tests, then these may be grouped with similar matrices (for which identical or similar analytical procedures provide equivalent performance) in order to estimate the relative MU.

The assessment of MU for an analytical procedure covering different matrices or identical/similar procedures providing equivalent performance should be based on a range of representative matrices and concentration ranges. It may be possible to use a single matrix that covers all the sample types specified in a particular group if there is evidence to suggest that the uncertainties are comparable. However, different sample matrices and/or analyte concentration ranges can behave differently in some cases and would therefore require separate uncertainty estimates. E.g. the precision may not be proportional to the analyte level over the entire concentration range as expected and/or the magnitude of the precision may vary from matrix to matrix at comparable concentrations [Barwick, Ellison 2000, /9/].

A possible grouping of matrices for PCDD/F and PCB analysis according to the applied methods is given in Annex A.1.

NOTE: Grouping of matrices is recommended for both empirical (top-down) and semi-empirical approaches developed in this document.

6. The Empirical or Top-Down Approach

The "top-down" or empirical approach is based on the performance of the full method, acknowledging trueness and precision contributions to MU.

International bodies recognise historic data from validation processes, interlaboratory studies, and from the use of RMs and/or CRMs, as a valid basis for estimation of MU in analytical work. However, such an estimated MU does not necessarily reflect the current uncertainty associated with daily routine results.

This guide therefore proposes a top-down approach that integrates relevant historical data and more recent data from internal and external quality controls. Moreover, daily (or batch) performance indicators such as the actual limits of quantification, matrix and procedural blank effects, should be included in order to provide a realistic and current estimate of MU associated with the results being reported (see chapter 6.5).

Basic principles are adopted from Nordtest's Report TR 537 "Handbook for calculation of measurement uncertainty in environmental laboratories" [Nordtest 2012, /3/]. Therein, a procedure is suggested that uses routine quality control data acquired from RMs and/or CRMs, results from interlaboratory and/or PT studies, and validation data for a realistic estimate of MU. Nordtest's keynote is to make use of results and data which are already available, without adding to the laboratory's workload. From these data, the contribution affecting precision and the overall contribution to method and laboratory bias are determined. This concept was more recently adopted by ISO 11352:2012-07, or DIN ISO 11352:2013-03, "Water quality – Estimation of measurement uncertainty based on validation and quality control data" [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/].

According to Barwick and Ellison [Barwick, Ellison 2000, /9/], two sets of experiments can be carried out, a precision study and a trueness study, which will provide the information required to estimate the combined uncertainty of the method. They should be planned in a way that as many sources of uncertainty as possible are covered.

Within the scope of this guidance document, the experimental design consists of a long-term precision study for looking at intermediate precision by using RMs or CRMs as QC matrix samples. These QC samples should be representative of the matrix and the levels of interest.

A trueness study, by means of relevant matrix CRMs, fortified RMs (blank or low contaminated), interlaboratory studies or PTs, provides estimation of the uncertainty component of the bias.

NOTE 1: Contributions of precision, and of a bias, respectively, to the combined standard uncertainty from which the expanded uncertainty is calculated can be based on individual congeners or expressed for the sum-TEQ parameter. For example, a comparison of MU

values calculated for individual congeners following the Eurachem Guide [EURACHEM/CITAC 2012, /12/] together with MUs estimated for total PCDD/F- and PCB-WHO-TEQs has been published elsewhere [Fernandes et al. 2012, /13/].

NOTE 2: In some QC samples, not all of the congeners may have a value assigned to them, e.g. due to very low concentrations. Assigned values missing for certain individual congeners may then be supplemented by results from analysis of fortification experiments involving fortified (blank matrix) samples.

6.1 Precision Studies

The uncertainty component for random variations u_{Rw} should be estimated under conditions that are also valid during routine analysis. Therefore, intermediate conditions (between batches) should apply rather than repeatability or reproducibility conditions. The same conditions apply for QC charts. Therefore, the guide proposes *long-term precision studies* to evaluate this parameter under intermediate precision (R_W) conditions.

Suitable control samples must be carefully selected. Ideally, they should be representative of the samples being analysed, in terms of both the physical and chemical *composition* of the matrix and the *concentration* of the analyte.

NOTE 1: It is acknowledged that the use of representative QC samples may not always be possible. In practice, a laboratory may have only few suitable matrices available.

The QC sample (e.g. one per series of samples) should be treated in exactly the same way, covering the whole analytical procedure. It can be a fortified matrix sample (with undetectable or low levels of contamination) or an appropriate reference material, if available. QC samples must be characterized and show sufficient homogeneity and long-term stability.

CRMs can also be used, but may prove quite expensive for this purpose, unless the uncertainty contribution of the bias is estimated simultaneously from the same CRM.

The intermediate precision contribution to uncertainty u_{Rw} may be calculated as

$$u_{Rw} = s_{Rw} \qquad \textbf{Eq. 5}$$

$$u_{Rw,rel} = s_{Rw,rel} \qquad \textbf{Eq. 6}$$

s_{Rw} = intermediate precision standard deviation
$s_{Rw,rel}$ = relative intermediate precision standard deviation

Examples for intermediate precision studies for PCDD/Fs and DL-PCBs in selected matrices are given in Annex B.1.

NOTE 2: The intermediate precision standard deviation may also be calculated using data from the results of duplicate analysis performed for similar sample types and using the same method (e.g. 10 duplicate analyses under intermediate precision conditions of samples in the range of the level of interest) [IUPAC 1997, /41/].

If the same method is used for various matrices defined within a matrix group (Annex A.1), and it covers a suitable range of analyte concentrations, it may be possible to estimate a single precision contribution value by using a pooled relative intermediate standard deviation $s_{Rw,pool,rel}$ of the included matrices. In this case, $s_{Rw,rel}$ (equation 7) should be constant to the

analyte level over the entire working range. An estimation of $s_{Rw,pool,rel}$, is obtained from equation 8:

$$s_{Rw,rel} = \frac{\sqrt{\frac{\sum_i (x_i - \bar{x})^2}{m-1}}}{\bar{x}} \qquad \textbf{Eq. 7}$$

$$s_{Rw,pool,rel} = \sqrt{\left(\frac{(m_1-1)\cdot s^2_{Rw,rel1} + (m_2-1)\cdot s^2_{Rw,rel2} + \ldots}{(m_1-1)+(m_2-1)+\ldots}\right)} \qquad \textbf{Eq. 8}$$

m_i = number of measurements of QC sample *i*
$s_{Rw,rel\ i}$ = relative intermediate standard deviation, from *m* measurements of QC sample *i*

Examples for evaluation of the intermediate precision of PCDD/Fs and DL-PCBs in matrices intended to be pooled are given in Annex B.2.

NOTE 3: $s_{Rw,pool,rel}$ estimates which cover a wide range of matrices and levels may then lead to an underestimation in the combined uncertainty for some matrices and to an overestimation for others. Pooling the precision estimates, however, should not lead to a significant over or underestimate of the combined uncertainty for a particular matrix [Barwick, Ellison 2000, /9/].

NOTE 4: Deciding whether or not there is a "significant" difference between the standard deviations obtained for each sample is ultimately up to the analyst. Statistical tests can be used, but their relevance depends very much on the number of results available for each sample. If 10 or more replicates have been made for each sample, the standard deviations can be compared using F-tests assuming a Gaussian distribution of the data [Barwick, Ellison 2000, /9/].

6.2 Trueness or Bias Studies

One of the most important steps in the validation of an analytical procedure is the assessment of trueness and/or bias. Measurements are liable to two components of bias, referred to as method and laboratory bias. The *method bias* arises from systematic errors inherent in the method, whichever laboratory uses it. The *method bias* can generally only be assessed by collaborative studies that give rise to an interlaboratory mean. The *laboratory bias* arises from additional systematic errors associated with the laboratory and its interpretation or application of the method. A single laboratory can only estimate the total bias.

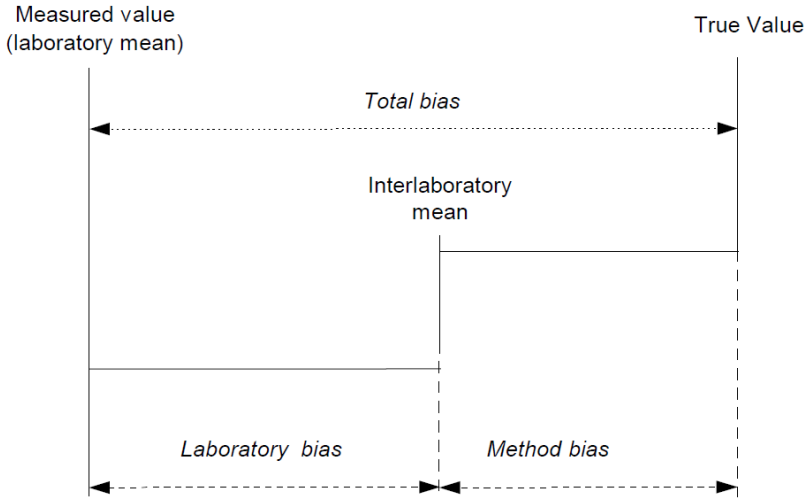


Figure 3: Interpretation of the bias [EURACHEM 2014, /21/]. Laboratory and method biases are shown here acting in the same direction. In reality, this is not always the case, and may also vary for different congeners or homologue groups.

The isotope dilution technique is applied to quantify concentrations of target analytes. Losses of these analytes during sample processing, and interferences during measurement should be reflected in the stable isotope-labelled compound, thus compensating for the bias to a considerable extent. If the remaining bias is outside the acceptable trueness range, according to the relevant European legislation, sources should be identified and eliminated.

NOTE: In all equations given in this guidance document for calculation of the bias contribution to measurement uncertainty, it is assumed that the bias is within the accepted trueness range.

The uncertainty component of the bias u_{bias} can be estimated from:

- Analyses of certified reference materials (CRMs)
- Results from participation in interlaboratory studies

- Fortification experiments using fortified blank sample or samples with low levels of contamination

and consists of several sub-components:

- the bias
- the uncertainty of the determination of the bias
- the uncertainty of the certified/assigned value or the fortifying concentration.

If CRMs are not available, participation in interlaboratory studies, e.g. proficiency tests (PTs) is a good alternative. In cases where interlaboratory studies are not available for the required matrix type and/or concentration range, fortification experiments can be carried out by fortifying suitable blank samples, or samples with low levels of contamination, at the respective levels of interest.

The estimate of the combined (relative) bias is for

- CRMs: the difference between laboratory's results x_i from analyses of $n = 1, 2 \ldots i$ CRMs and the respective certified values x_{cert} (divided by x_{cert}),
- PT results: the difference of the laboratory's results x_i from analyses of $n = 1, 2 \ldots i$ PT samples and their assigned values $x_{a,i}$ (divided by $x_{a,i}$),
- fortified samples: the difference between the mean $\bar{x}_i$ from analyses of $n = 1, 2 \ldots i$ fortified samples and the fortifying concentration x_{fort} (divided by x_{fort}).

$$bias_{CRM} = (x_i - x_{cert}) \text{ or } bias_{CRM,rel} = \frac{(x_i - x_{cert})}{x_{cert}} \qquad \textbf{Eq. 9}$$

$$bias_{PT} = (x_i - x_{a,i}) \text{ or } bias_{PT,rel} = \frac{(x_i - x_{a,i})}{x_{a,i}} \qquad \textbf{Eq. 10}$$

$$bias_{fort} = (\bar{x}_i - x_{fort}) \text{ or } bias_{fort,rel} = \frac{(\bar{x}_i - x_{fort})}{x_{fort}} \qquad \textbf{Eq. 11}$$

It should be noted that $bias_{CRM}$ and $bias_{fort}$ can be based on multiple analyses, while $bias_{PT}$ is calculated individually for each PT sample (to be consecutively converted to an RMS value).

6.2.1 Estimating u_{bias} using a Representative Matrix CRM

Regular analyses of CRM samples which are representative of the samples to be analysed as regards matrix type, concentration and physico-chemical properties, can be used to estimate the trueness.

According to the Nordtest Report 537 [Nordtest 2012, /3/], adopted by DIN ISO 11352:2013-03, or ISO 11352:2012-07 [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/], the bias contribution u_{bias} to MU for a **single CRM** analysed m times may be calculated as:

$$u_{bias,CRM,rel} = \sqrt{(bias_{CRM,rel})^2 + \left(\frac{s_{bias,rel}}{\sqrt{m}}\right)^2 + u_{CRM,rel}^2} \qquad \textbf{Eq. 12}$$

A single CRM should be analysed at least six times (m ≥ 6). The mean value(s), $\bar{x}_i$ of these analyses can be used for the estimation of u_{bias} as shown in the example given in Annex C.1.1.

If **several CRMs** are used and analysed <u>once,</u> which may be preferable to cover a range of concentrations and/or matrix properties, different values will be obtained for the bias and s_{bias} does not need to be included. u_{bias} may then be estimated by

$$u_{bias,CRM,rel} = \sqrt{RMS_{bias,CRM}^2 + u_{CRM,rel}^2} \qquad \textbf{Eq. 13}$$

$$RMS_{bias,CRM} = \sqrt{\frac{\sum(bias_{CRM,rel,i})^2}{n}} \qquad \textbf{Eq. 14}$$

$$u_{CRM,rel} = mean(u_{CRM,rel,i}) \qquad \textbf{Eq. 15}$$

n = number of different CRMs analysed *(n = 1, 2, … i)*

An example is given in Annex C.1.2.

6.2.2 Estimating u_{bias} using Results from Interlaboratory Studies

In principle, results from Interlaboratory Studies are used in the same way as results from several certified reference materials, to estimate u_{bias}. According to Nordtest [Nordtest 2012, /3/], the uncertainty associated with the bias is calculated as:

$$u_{bias,PT,rel} = \sqrt{RMS_{bias,PT}^2 + u_{Cref,rel}^2} \qquad \textbf{Eq. 16}$$

$$RMS_{bias,PT} = \sqrt{\frac{\sum(bias_{PT,rel,i})^2}{n}} \qquad \textbf{Eq. 17}$$

$$u_{Cref,rel} = mean(u_{Cref,rel,i}) \qquad \textbf{Eq. 18}$$

n = number of samples *(n = 1, 2, … i)* from interlaboratory studies, or from PTs

An example for the estimation of u_{bias} using results of proficiency tests is given in Annex C.2.

If $bias_{PT,i}$ is calculated from results of interlaboratory studies or PTs, in which a variety of analytical methods may have been applied by participants, the uncertainty $u_{Cref,i}$ of the assigned value $x_{a,i}$ could sometimes be relatively large. In these cases, the contribution of $bias_{PT,i}$ should not be included in the estimation of $u_{bias,PT}$.

As described in Annex C.4, it is reasonable that $u_{Cref,rel,i}$ should not exceed 30% of $bias_{PT,rel,i}$ for the interlaboratory study i:

$$\left|\frac{u_{Cref,rel,i}}{bias_{PT,rel,i}}\right| \leq 0.3 \qquad \textbf{Eq. 19}$$

However, when a laboratory performs very well in a PT (i.e. reported value very close to assigned value, and thus providing very small $bias_{PT,rel,i}$), it might be possible that the criteria in equation 19 cannot be met while $u_{Cref,rel,i}$ is more than acceptable. In this case, the use of $\sigma_{p,rel}$ is recommended rather than $bias_{PT,rel,i}$ in equation 19 provided that $u_{Cref,rel,i} \leq 0.3 \cdot \sigma_{p,rel}$.

$\sigma_{p,rel}$: fitness-for-purpose-based "standard deviation for proficiency assessment" expressed as relative standard deviation

6.2.3 Estimating u_{bias} from fortification experiments

Fortification experiments are frequently performed during validation or verification of analytical procedures. A pre-analysed sample with low or undetectable contamination levels is fortified with the analytes of interest and measured before and after fortification. From the difference of the results and the fortified amount the bias can be calculated. If the results are not biased, the average bias should be around 0%.

The uncertainty u_{fort} of the fortified amount of an analyte may be calculated from the uncertainty of the concentration of the standard solution u_{conc} and from the uncertainty of the added volume u_{vol}:

$$u_{fort} = \sqrt{u_{conc}^2 + u_{vol}^2} \qquad \textbf{Eq. 20}$$

To calculate the uncertainty contribution of the bias $u_{bias,fort}$ the relative biases from the fortification experiments have to be included.

$$RMS_{bias,fort} = \sqrt{\frac{\sum(bias_{fort,rel,i})^2}{n}} \qquad \textbf{Eq. 21}$$

$$u_{bias,fort,rel} = \sqrt{RMS_{bias,fort}^2 + u_{fort}^2} \qquad \textbf{Eq. 22}$$

n = number of different fortified samples analysed *(n = 1, 2, … i)*

A full example on the derivation of the uncertainty components of the concentration and the volume of a standard solution used for fortification of a sample, followed by calculation of the bias contribution to MU, is given in Annex C.3.

6.3 Combined and Expanded Uncertainties

The **combined standard uncertainty** u_c is calculated from the combination of the uncertainty component describing the random variations u_{Rw} with the uncertainty component describing the method and laboratory bias u_{bias}:

$$u_c = \sqrt{u_{Rw}^2 + u_{bias}^2} \qquad \textbf{\textit{Eq. 23}}$$

u_c describes the estimated uncertainty of the measurement result at a level of confidence of the standard deviation (approx. 68 %). What is often required is a measure of uncertainty that defines an interval about the measurement result within which the value of the measurand can be confidently assumed to lie. The measure of uncertainty intended to meet this requirement is termed **expanded uncertainty** *U*. To convert u_c to a higher level of confidence it is multiplied with a coverage factor *k*.

$$U = k \cdot u_c \qquad \textbf{\textit{Eq. 24}}$$

The choice of *k* determines the level of confidence. Usually, a coverage factor $k = 2$ is used, corresponding to a level of confidence of about 95%.

6.4 Moving Time Window Scheme

If u_{Rw} is to be estimated from internal QC, representative RM, or from fortified samples, at least 10 independent analyses of the sample (e.g. each in one series of samples) should have been performed within an adequate time interval, before the values are used. Once laboratories have collected for example, 20 results [IUPAC 1995, /35/], a *moving time window* can be implemented as the computation period. With each new result, the oldest is removed thus always keeping 20 values in the time window. The frequency of updating U and the number of QC data included in the calculation are left to the responsibility of the analyst.

NOTE: The moving time window of 20 results is given as an indicative value and shall be adapted according to the sample throughput of the laboratory.

If u_{bias} is to be estimated from either relevant PTs, representative matrix CRM or from fortified (low contaminated) samples, at least 6 independent samples (whatever their origin) should have been analysed within an adequate time interval, before the values are used. Especially for beginner laboratories, it may be helpful to use a mix of the before mentioned 3 types of samples to achieve the minimum required number of six samples. Once laboratories have collected the required minimum of 6 results [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/], it is proposed to implement a *moving time window* as computation period (indicative value of up to 3 years). With each new result, the oldest is removed keeping always six values within the time window as illustrated in figure 4:

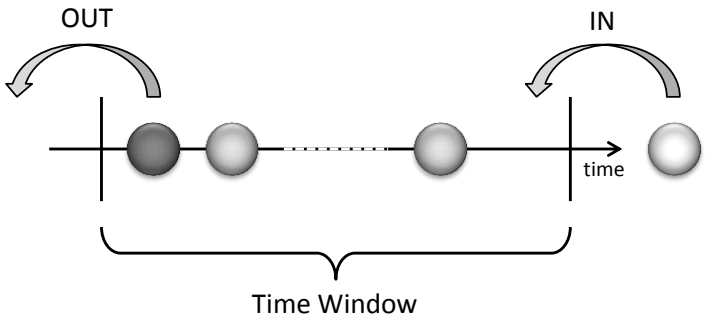


Figure 4: *Moving time window* as computation period. With each new result, the oldest is removed keeping always the required number of values within the time window.

For exclusion of potential outliers please refer to chapter 9.3.

6.5 Contributions from Current Performance

Precision and bias studies provide a valid "snapshot" of current laboratory performance and their contributions to MU. However, the uncertainty estimate might be even more realistic and meaningful if contributions arising from daily performance (reflected by e.g. procedural blanks, LOQs) are integrated into the calculation of the combined uncertainty, while using the presented top-down approach. Unpredictable "special incidents" might occur in routine analysis and should also be accounted for, such as: extraction issues, low recoveries, insufficient clean-up, injections of "dirty" sample extracts, poor resolution during chromatographic separation and GC-MS sensitivity problems.

6.5.1 Contributions to MU from LOQs and procedural blanks

Contributions of current performance to MU may be calculated by combining u_{Rw} and u_{bias} with the actual limit of quantification (LOQ) of the respective congener, determined using the procedural blank of the relevant sample batch. The combined uncertainty $u_{c,i(LOQ)}$ of a congener *i* is then calculated as:

$$u_{c,i(LOQ)} = \frac{\sqrt{(u_{Rw,i}^2 + u_{bias,i}^2) \cdot x_i^2 + LOQ_i^2}}{x_i} \qquad \textbf{\textit{Eq. 25}}$$

$u_{c,i(LOQ)}$: Combined uncertainty including contributions of LOQ and procedural blank of congener *i*, expressed in relative units or %

$u_{Rw,i}$ and $u_{bias,i}$: for congener *i*, both expressed here as %

x_i: Concentration of congener *i* expressed in pg/g or ng/kg (or ng/g as appropriate)

LOQ_i: Limit of quantification (LOQ) of congener *i* expressed in pg/g or ng/kg (or ng/g as appropriate)

The expanded uncertainty $U_{i(LOQ)}$ is then calculated according to equation 24:

$$U_{i(LOQ)} = k \cdot u_{c,i(LOQ)} \qquad \textbf{\textit{Eq. 26}}$$

$U_{i(LOQ)}$: Expanded uncertainty of a congener *i* including contributions of LOQ and procedural blank

Congener-based LOQs are calculated according to the approaches described for PCDD/F and PCB analysis using isotope dilution mass spectrometry in the "Guidance Document on the

Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (EURL Guidance Document 2016, /40/). One approach is based on the evaluation of the signal-to-noise ratios measured using the ion chromatograms of the individual congeners in a particular sample. The second approach is based on a calibration model proposed for low levels of noise.

In addition, procedural blanks are analysed with every batch of test samples providing information on method performance, such as effects/interferences from the test method. It is recommended that procedural blanks are monitored in QC charts and checked for acceptance of a batch of samples by comparing the measured blank with these charts. If acceptance criteria are met, calculated LOQs are applied. In case these criteria are not met, the analyst must check, if the batch of samples has to be repeated or re-analysed.

Alternatively, if calculated LOQs or measured analyte contents of procedural blanks are higher than analyte contents in respective test samples of the same batch, the values estimated/measured in the procedural blanks should be applied as LOQs for these test samples (taking into account sample intake). If the estimated/measured values of procedural blanks are lower than the values of test samples, the values of the test samples are used.

An example for the estimation contributions of LOQs and procedural blanks to MU is given in Annex D.

7. The Theoretical or Bottom-Up Approach

The theoretical or "bottom-up" approach presupposes a mathematical model of the measurement process, estimating individual contributions of all relevant sources of uncertainty and combining them.

When a "bottom-up approach" is used, the Guide to the Expression of Uncertainty in Measurement (GUM) [GUM 2008, /1/] provides valuable insight for the laboratory. The main principles of the GUM are that:

- uncertainty evaluation is comprehensive, accounting for all relevant sources of measurement error,
- uncertainties arising from random and systematic effects are treated alike, i.e. are expressed and combined as variances of associated probability distributions,
- statistical evaluation of measurements (Type A) and alternative techniques, based on other data / information (Type B), are recognised and utilised as equally valid tools,
- uncertainties of final results are expressed as standard deviations (standard uncertainty) or by multiples of standard deviations (expanded uncertainty) using a specified numerical or coverage factor.

In PCDD/F and PCB analysis, additional requirements apply:

- in principle, the bottom-up approach applies to each PCDD/F and PCB congener, individually, meaning that the combined uncertainty must be assessed for each congener separately
- next, the combined uncertainty (in TEQ) is calculated from individual congener uncertainties (see Annex E)

NOTE: However, when it comes to evaluating the uncertainty of the results in quantitative analysis – especially in conjunction with isotope dilution based analyses – the GUM is often criticised as being less than ideal. This may be due to the fact that the GUM approach includes a tedious and error-prone series of calculations, while it almost exclusively presents a single approach for uncertainty evaluation.

The GUM approach includes identification and quantification of the relevant sources of uncertainty followed by combination of the individual uncertainty estimates. The combination is done by means of the 'error propagation theory', which consists of a first order Taylor series:

$$u(x(y_1,...,y_n)) = \sqrt{\sum_{i=1}^{n}\left(\frac{\partial x}{\partial y_i}u(y_i)\right)^2}\ ,$$

where x is the measurement result which depends on parameters y_i, each y_i being a certain uncertainty source; $u(y_i)$ is the standard uncertainty related to this uncertainty source and $\partial x/\partial y_i$ the partial derivative of x with respect to y_i. Note that this equation relates to independent variables (covariance term omitted).

The GUM method was adapted for quantitative chemical measurement in the Eurachem Guide [EURACHEM/CITAC 2012 /12/]. For the theoretical or bottom-up approach, the Eurachem Guide suggests the identification and recording of a list of sources of uncertainty relevant to the analytical method. It seems useful to structure this process, both to ensure comprehensive coverage and to avoid over-counting. In practice, it might be helpful to construct a cause and effect diagram (Ishikawa diagram). This is a tool that consists of a hierarchical structure of causes which culminate in a single effect. The effect in the context of measurement uncertainty is the result obtained.

In addition to the bottom-up approach, the Eurachem Guide describes also the possibility of estimating measurement uncertainty based on method performance data, also in combination with contributions of individual sources (see chapter 8).

Practical Recommendations

1. It goes without saying that calculations should be updated on a regular basis as individual parameter values may change over time, or uncertainties may be refined with increasing experience of the analyst. Changes to either parameters or uncertainties will then be reflected both in the overall result, and in the combined standard uncertainty.
2. The mathematical model should be revised when the observed data demonstrate that the model is incomplete.

Conclusions of the working group

A full bottom-up approach is not recommended for PCDD/Fs and PCBs mainly due to the complexity of the whole analytical process and the difficulty in quantifying separately, all the sources of uncertainties [Horwitz 2003, /37/].

8. The Semi-empirical Approach

8.1 Introduction

The proposed methodology here is based on the approach taken in the ISO "Guide to the Expression of Uncertainty in Measurement" applied to analytical chemistry by EURACHEM/CITAC [GUM 2008, /1/; EURACHEM/CITAC 2012, /12/]).

The semi-empirical approach derives from a combination of the top-down and the bottom-up procedures, providing an uncertainty estimation based on the results obtained from validation studies, expressed in terms of precision and bias, and additional uncertainty sources not covered by validation data, such as calibration factors and reference standards.

This model may seem quite laborious but provides a clear understanding of the analytical steps which contribute significantly to the uncertainty budget and which therefore may be identified as critical points to keep under control and thus reduce the measurement uncertainty. In fact, the largest contributions to the combined uncertainty can be identified during a preliminary study and a reliable estimate of uncertainty can be made by considering only the main sources.

The uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of series of measurements and can be characterized by experimental standard deviations (type A evaluation). The other components, which also can be characterized by standard deviations, are evaluated from assumed probability distributions based on experience or other information (type B evaluation). It is important not to "double-count" uncertainty components. If a component of uncertainty arising from a particular effect is obtained from a type B evaluation, it should be included as an independent component of uncertainty in the calculation of the combined standard uncertainty of the measurement result only to the extent that the effect does not contribute to the observed variability of the observations. The first step is to define the measurement procedure identifying each source of uncertainty.

The next stage of the process is the planning of experiments, which will provide the information required to obtain an estimate of the combined uncertainty. In practice, method validation studies produce data on overall performance and on individual factors which influence the estimation of uncertainty associated with the results based on precision and trueness data.

The starting point is the analysis of a series of observations obtained under within-laboratory reproducibility conditions.

Among the potential sources of uncertainty, it should be decided if a given parameter is sufficiently covered by a given set of data or planned experiments. The parameters which are

not accounted for become the subject of further study, either through planned experiments or by locating appropriate standing data, such as calibration certificates or manufacturing specifications. The resulting contributions, obtained from a mixture of validation studies, standing data and any additional studies on single effects can be then combined according to ISO guidelines.

In the case of PCDD/F and PCB analysis the analyte concentration is calculated according to the equation 27:

$$C_i = \frac{A_{12C,i} \cdot C_{13C,i} \cdot V_{13C,i}}{A_{13C,i} \cdot RRF_i \cdot m_{sample}} \qquad \textbf{\textit{Eq. 27}}$$

where:

C_i: concentration of the congener *i* (pg/g)

$A_{12C,i}$: peak area of native congener *i*

$A_{13C,i}$: peak area of labelled congener *i*

$C_{13C,i}$: concentration of the labelled congener *i* (pg/µL)

$V_{13C,i}$: spiked volume of the labelled congener *i* (µL)

RRF_i: relative response factor of congener *i*

m_{sample}: weight of sample aliquot (g)

The uncertainties associated with these parameters contribute to the overall uncertainty in the final result.

8.2 Estimation of MU using the Semi-empirical Approach

The following sources of uncertainty can be identified (see flow chart, figure 5):

1) intermediate precision from validation study
2) bias from validation study
3) calibration curve
4) volume
5) standard concentration
6) sample aliquot weight

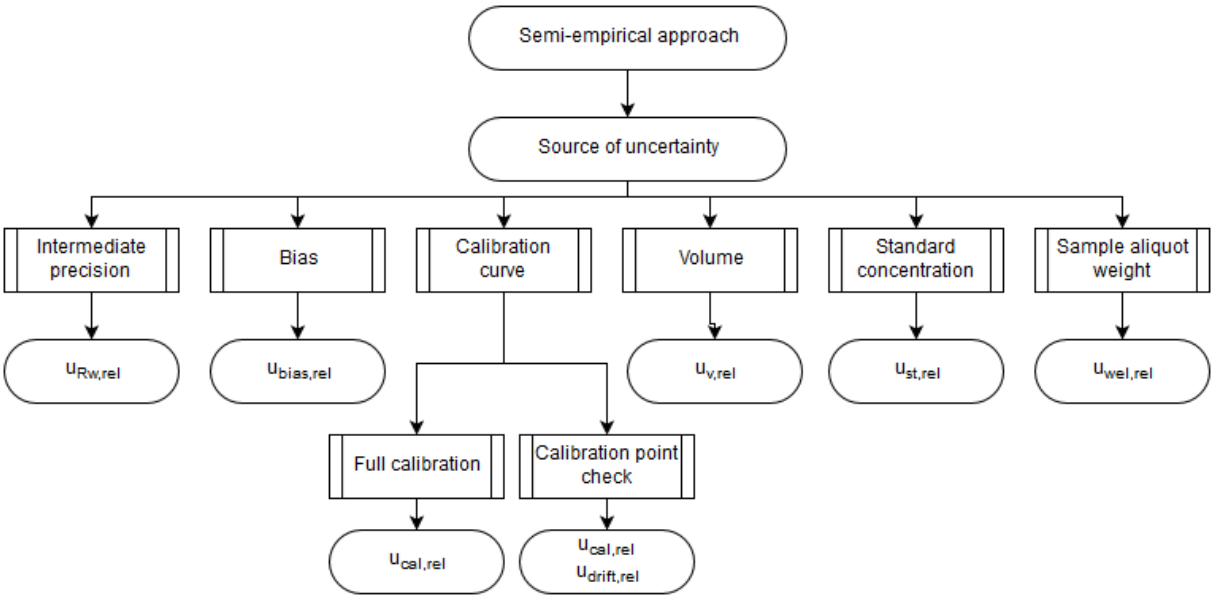


Figure 5: Flow chart for estimation of measurement uncertainty using a semi-empirical approach.

The different contributions to the combined uncertainty can be usefully represented by a diagram showing the magnitude of single components. Only parameters with uncertainties greater than one third of the magnitude of the largest contribution to the uncertainty budget need to be considered as significant sources of uncertainty for the method. Indeed, the basic principle of uncertainty propagation is underlining the influence of the quantities with the highest values. Generally, type B parameters have a minor influence over the uncertainty budget, and their relative contribution may be neglected if this condition is satisfied.

NOTE: Even though isotope dilution analysis should largely compensate for the bias, its uncertainty component can be estimated from recovery experiments using fortified samples with low or undetectable contamination levels, performed during validation of the analytical procedure. In this way, the uncertainty associated with losses of analytes during the extraction and clean-up steps are also considered.

8.3 Precision Contribution

8.3.1 Intermediate precision uncertainty

The precision study is a useful tool to estimate the random error. Because an estimate of intermediate precision is available from the validation study for the procedure as a whole, there is no need to consider all the precision contributions individually. They are therefore grouped into one contribution.

The uncertainty associated with the intermediate precision (u_{Rw}) is calculated as the standard deviation of *n* test results in the precision study during method validation.

The relative intermediate precision standard uncertainty ($u_{Rw,\ rel}$) is calculated as the ratio between the standard deviation and the mean of analytical results of *n* samples analysed in the precision study.

$$u_{Rw,rel} = \frac{s_{Rw}}{\bar{x}} \qquad \textbf{\textit{Eq. 28}}$$

s_{Rw}: intermediate precision standard deviation

$\bar{x}$: mean of analytical results

8.4 Bias Contribution

8.4.1 Bias uncertainty

The uncertainty component associated with bias (u_{bias}) can be estimated from the same experiments performed in the precision study. To calculate $u_{bias,rel}$ the relative biases from the fortification experiments have to be included using the mean bias value for each of *n* fortification levels:

$$RMS_{bias,mean} = \sqrt{\frac{\sum(bias_{fort,rel,i})^2}{n}} \qquad \textbf{\textit{Eq. 29}}$$

The uncertainty u_{fort} of the fortified amount of analyte should be also taken into account and may be calculated as already described in paragraph 6.2.3 (equation 20).

Finally, the uncertainty contribution $u_{bias,rel}$ is calculated combining $RMS_{bias,\ mean}$ and u_{fort}

$$u_{bias,rel} = \sqrt{(RMS^2_{bias,mean} + u^2_{fort})} \qquad \textbf{\textit{Eq. 30}}$$

8.5 Calibration Curve Uncertainty

With reference to calibration, two different approaches can be adopted by the laboratory depending on whether the calibration curve is prepared for each analytical batch or, as an alternative, periodically.

8.5.1 Full calibration (Option 1)

If a full calibration is performed for each analytical batch, the standard deviation of the mean Relative Response Factor (RRF) of a congener represents the uncertainty contribution related to calibration. The RRF value is usually calculated as the mean value obtained from the analysis of appropriately prepared standard solutions that contain known amounts of the analyte and the internal standard.

At least five calibration levels should be used to construct the average RRF model. If the relative standard deviation (RSD) of variation in the factors is ≤ 20%, the linear model is generally representative over the range of calibration standards [US EPA 2014, /25/].

The calibration curve linearity uncertainty component (u_{cal}) relies on the variation of relative response factors (RRFs) among the points of the calibration curve. This uncertainty component is calculated as the standard deviation of RRF_i divided by the square root of the number (n) of calibration points.

$$u_{cal,rel} = \frac{s_{RRF,rel}}{\sqrt{n}} \qquad \textbf{Eq.31}$$

NOTE: A worst-case scenario is to consider the maximum acceptable variation of RRF_i established by reference methods (e.g. 20% coefficient of variation according to EN 16215 [EN 16215:2012-07, /39/] and US EPA Method 8000D [US EPA 2014, /25/]), thus calculating the maximum permitted uncertainty associated with the calibration curve. In practice, the actual RRF standard deviation reflects the daily or session-based performance and, for this reason, its use is recommended.

8.5.2 Calibration point check (Option 2)

When the calibration curve is not carried out daily, a calibration verification procedure should be adopted. This procedure represents an instrumental bias check using an independently prepared reference solution. A term representing the uncertainty due to this drift also needs to be included in the uncertainty budget. The calibration curve drift standard uncertainty (u_d) can be calculated using the actual value measured for each congener when the calibration verification procedure is carried out [Barwick et al. 1999, /39/]. The maximum permitted deviation is 20% [US EPA 2014, /25/]. In practice, 15% or lower should be achievable. If there is no evidence of lower probability towards the extremes of the drift values range, this can be treated as a rectangular distribution:

$$u_{d,rel} = \frac{d_{RRF,rel}}{\sqrt{3}} \qquad \textbf{Eq. 32}$$

If the relative response factor differs by more than the acceptance limit from the mean relative response factor at calibration, the calibration curve needs to be re-run.

The uncertainty component $u_{d,rel}$ is combined with the $u_{cal,rel}$ related to the mean RRF obtained from the last full calibration (equation 31).

NOTE: Alternatively, the RRF value of the calibration point checks can be used for calculations. In this case, the uncertainty contribution for this approach needs to be included.

8.6 Additional Contributions

8.6.1 Volume uncertainty

The volume uncertainty (u_v) is related to the glassware (e.g. volumetric flasks, cylinders, pipettes, syringes) and micropipettes used for the preparation and addition of standard solutions.

The volume standard uncertainty could be taken from the calibration certificate of glassware, syringes and micropipettes or considering a maximum deviation accepted by the laboratory and assuming a rectangular distribution.

If limits of ± a are given without a confidence level and there is reason to expect that extreme values are likely, it is normally appropriate to assume a rectangular distribution, with a standard deviation of:

$$u_v = \frac{a}{\sqrt{3}} \qquad \textbf{\textit{Eq. 33}}$$

The volume relative standard uncertainty ($u_{v,\mathrm{rel}}$) is obtained dividing u_v by the volume amount.

Then all contributions are combined to give the standard uncertainty of the volume.

NOTE: In cases when extreme values are unlikely on the basis of prior laboratory experience, it is appropriate to assume a triangular distribution, with a standard deviation of:

$$u_v = \frac{a}{\sqrt{6}} \qquad \textbf{\textit{Eq. 34}}$$

8.6.2 Standard solution concentration uncertainty

The standard solution concentration uncertainty (u_{st}) is related to the concentration of the labelled compound fortification (internal standard) mixture and the unlabelled calibration standards.

The uncertainty of standard solution concentration can be obtained from the supplier.

Concentration relative standard uncertainty ($u_{st,rel}$) could be taken from the supplier's certificate of analysis. If the uncertainty provided by the supplier is the expanded uncertainty (calculated with a coverage factor) then the standard uncertainty is calculated dividing the expanded uncertainty by the coverage factor.

NOTE: The uncertainty associated with the concentration of the labelled compounds does not have to be taken into account if the same standard solution is used to fortify the samples and to prepare the calibration standard solutions.

8.6.3 Sample aliquot weighing uncertainty

Two contributions arise from sample weighing: a random error due to the sample weighing and a systematic error associated with the calibration of the balance. The first component has been already included in the component of uncertainty obtained from the precision study. The second component does not vary at all during the precision study. For example, during the precision study the same balance was used to weigh out all the samples and the same calibration value was related to all of the samples weighed. Although the precision associated with this operation is included in the overall precision estimate, the effect of the accuracy of the balance has not been included in the uncertainty budget so far [Barwick, Ellison 2000, /9/].

The weight uncertainty (u_w) is derived from the calibration certificate and in the absence of other information, a rectangular distribution is assumed:

$$u_w = \frac{a}{\sqrt{3}} \qquad \textbf{\textit{Eq. 35}}$$

The relative weight standard uncertainty ($u_{w,rel}$) is calculated dividing u_w by the amount of sample. This contribution has to be counted twice, once for the tare and once for the gross weight, because each weighing is an independent observation and the linearity effects are not correlated. The two contributions have to be combined to give the standard uncertainty of the weight.

8.7 Combined and Expanded Uncertainty

The **combined standard uncertainty** u_c is calculated from the combination of the relative uncertainty components describing the random variations (u_{Rw}), the bias contribution (u_{bias}), the calibration curve uncertainty components (u_{cal}, u_d) and the type B contributions (u_v, u_{st}, u_w).

In case of full calibration (Option 1) performed for each analytical batch, the following equation is used:

$$u_{c,rel} = \sqrt{u_{Rw,rel}^2 + u_{bias,rel}^2 + u_{cal,rel}^2 + u_{v,rel}^2 + u_{st,rel}^2 + u_{w,rel}^2} \qquad \textbf{\textit{Eq. 36}}$$

When the calibration point check procedure (Option 2) is adopted, the equation includes the additional term for calibration curve drift:

$$u_{c,rel} = \sqrt{u_{Rw,rel}^2 + u_{bias,rel}^2 + u_{cal,rel}^2 + u_{d,rel}^2 + u_{v,rel}^2 + u_{st,rel}^2 + u_{w,rel}^2} \qquad \textbf{\textit{Eq. 37}}$$

As already described in the paragraph 6.3 for the top-down approach, the **expanded uncertainty** U is calculated by multiplying the combined standard uncertainty by the coverage factor k (equation 24).

A full example on how to calculate the uncertainty following the semi-empirical approach is given in Annex H.

9. Practical Implementation

9.1 Combined Uncertainty in TEQ from Individual Congeners

In this section, a strategy is presented as to how a TEQ-based MU value can be derived and propagated from individual congeners' uncertainties.

A congener-based combined uncertainty u_c is calculated as described in chapter 6.3. The TEQ-based MU value calculated from individual congener uncertainties is dependent on the congener level profile because the combined standard uncertainty associated with each congener u_c is dependent on the level of concentration in most cases (see precision studies in annex B). When u_c is calculated individually for each congener, different rules of propagation can be applied to calculate the combined TEQ-based u_c.

In annex F, four possible approaches are treated to compare the TEQ-based standard uncertainty u_c obtained directly from empirical TEQ-data collected within the EU-RL/NRL network, and the TEQ-based standard uncertainty calculated from each congener u_c, using RSS, SUM, average and median approaches as propagation rules of uncertainty. None of the approaches gave a perfect fit between empirical TEQ-based u_c values and those recalculated from the congener-based u_c data. However, the RSS approach provided the best agreement of empirical with calculated data and is therefore recommended by the authors.

The combined uncertainty (RSS approach) expressed in TEQ may be calculated as:

$$u_c(TEQ = TEF_1 \cdot u_{c1} + \cdots + TEF_{29} \cdot u_{c29}) = \sqrt{\sum_{i=1}^{29}(TEF_i \cdot u_{ci})^2} \qquad \textbf{\textit{Eq. 38}}$$

9.2 Laboratories new to Isotope Dilution Analysis

For laboratories new to the field and lacking historical QC data, preliminary MU values may be estimated from precision and trueness data acquired during initial method validation. As a start in routine analysis, the use of available relevant PT samples is recommended. If such materials are not available, fortification studies should be performed until sufficient data have been gathered.

For estimating the contribution of the precision to the measurement uncertainty, representative quality control samples or test samples from proficiency tests can be analysed under intermediate precision conditions (at least 10 complete analyses of a representative sample in different batches reflecting routine conditions). Further evaluation of the precision study is given in chapter 6.1.

The bias contribution can be estimated by analysing representative certified reference materials, reference materials, and test materials from relevant and valid PT studies or fortified samples. Therefore at least 6 samples are analysed. The evaluation of the data is performed according to chapter 6.2.

Another option is to use the semi-empirical approach as a starting point (see chapter 8). Once sufficient data from quality control programmes has been acquired by the laboratory, then measurement uncertainty according to the top-down approach can be calculated and compared to the value obtained using the semi-empirical approach.

9.3 Exclusion of Data

Values which are substantially biased compared to other results should attract our attention. They may be outliers and often are a product of systematic error in analysis. However, such data can also describe some unusual but real events in the laboratory environment. Exclusion of a potential outlier thus demands a very careful and reasoned approach. It is necessary to decide, whether and which potential outlier will be removed from the data, since they could highly bias the final results of MU evaluation.

9.4 Factors affecting a Timeline-based Evaluation of MU

Following the scheme proposed above, measurement uncertainty is updated on a regular basis. Several factors in the time scale, however, might impact MU, to varying degrees. We identify these factors as being of major and of minor influence.

Major factors

- Method changes (important modifications)
- Standard solutions (newly obtained, freshly prepared, or too long in use)
- New instruments obtained: from sector to sector instrument replacement to a lesser extent, but changing from HRMS to MS/MS, or major repairs, might have a significant impact

Minor factors

- New staff (i.e. performing the analysis for the first time, even if correctly trained)
- New kinds of matrices (e.g. feed or baby food)
- Recoveries of internal standards
- Linearity of the instrument, RRF values
- LOQs
- Other factors

As an intrinsic part of an on-going statistical process, such factors should be identified, if necessary. Their causes should carefully be evaluated, and affected results may eventually be eliminated.

The described top-down approach based on ISO 11352:2012-07, or DIN ISO 11352:2013-03 [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/], is used for estimating the measurement uncertainty from a set of results of control samples gathered under "in-control" conditions using a specific analytical method. The resulting estimated measurement uncertainty can be applied to all results of samples analysed under the same intermediate precision conditions, independent e.g. of sample matrix and staff, as long as a quality control programme is successfully performed.

In practice, a re-evaluation of the estimation of the measurement uncertainty is necessary if any part of the whole analytical procedure undergoes major changes (see major factors, as above), or after a certain period of time (e.g. one year).

9.5 MU Estimation when ad hoc amendments to methods are used (for Matrices not covered by the Matrix Groups)

Ad-hoc methods are methods established to carry out analysis of certain matrices within short notice. Such methods are typically based on established methods within the laboratory, but parts of the established method are modified substantially which do not generally justify or allow, due to the limited time, full validation studies [EURACHEM/CITAC 2012, /12/]. This refers to matrices requiring specific methods of analysis, which are analysed less frequently, or even only very rarely, according to official control plans, or only in cases of emergency.

Since limited resources will be available to establish the relevant uncertainty contributions, it is necessary to rely largely on the known performance of established methods or parts of methods for uncertainty estimation.

As a minimum, it is essential that an indication of bias and precision be available for the ad-hoc method. A minimum *precision* experiment consists of 6 full analyses of the sample in question. The precision should be compared with that for the related methods; the standard deviation for the ad-hoc method should be comparable.

The *bias* will ideally be measured against a CRM, but will in practice more commonly be assessed from fortification experiments using the standard addition technique. As a minimum, duplicate analysis of the sample in question, or the fortified sample is recommended. The bias from resulting application of the ad-hoc method should be comparable to that observed from related methods. Alternatively, the semi-empirical approach may also be used in this context.

NOTE: If it can be shown, that the ad-hoc method meets all criteria established in respective EU regulations, the target measurement uncertainty calculated according to chapter 4.3 may be applied as the worst case scenario.

9.6 Rounding of Results and Significant Digits

Rounding refers to the replacement of the result by the nearest multiple of the rounding interval. This procedure always implies an additional error, the rounding error (round-off error) or rounding bias being the difference between the approximation of a number (by rounding) and its exact value. Too few significant digits cause information to be lost and unnecessarily increase the rounding error, while too many significant digits reflect an accuracy that analytical methods may not be capable of providing. The relative error (%-error) caused by rounding may be considerable, depending on the number of significant digits the result is rounded to.

Rounding of analytical result, and of the measurement uncertainty, shall be done only after all calculations have been completed. The numerical values of the result and its uncertainty should then not be given with an excessive number of digits. According to the Eurachem Guide [EURACHEM/CITAC 2012, /12/] it is seldom necessary to give more than two significant digits for expanded uncertainty U or standard uncertainty u. The corresponding results should then be rounded to be consistent with the uncertainty [EURACHEM/CITAC 2007, /26/].

10. Inter-laboratory Studies

10.1 Information from PT Providers for participating Laboratories

In their reports to participating laboratories, PT providers are requested to include the following data:

- the accreditation status of the provider with respect to performing PTs or inter-laboratory studies, e.g. according to the requirements of ISO/IEC 17043 [ISO/IEC 17043:2010-02, /5/]
- the uncertainty of the assigned value, with level of confidence
- the PT target standard deviation values that were used for the evaluated parameters

It is strongly encouraged that providers of inter-laboratory studies and PTs assess the uncertainties associated with the assigned values according to ISO 13528 [ISO 13528:2015-08, /11/]. Results from these PTs used by laboratories for performance and MU assessment may otherwise lead to inadequate interpretation and results.

Laboratories are additionally encouraged to participate in particular, in those inter-laboratory schemes which are accredited according to EN ISO/IEC 17043 [ISO/IEC 17043:2010-02, /5/] and which provide the above data.

10.2 Evaluation of Participant's Performance and reported MU: z - and zeta-Scores

How realistic is an uncertainty estimate? This question can be answered by examining the results from PTs. Within PT schemes, participating laboratories' performance is usually assessed by conversion of participants' results (x_i) into z-scores, enabling the participant to immediately appreciate their significance:

$$z = \frac{x - x_a}{\sigma_p} \qquad \textit{Eq. 39}$$

σ_p is the fitness-for-purpose-based "standard deviation for proficiency assessment"; the term $(x - x_a)$ represents the individual laboratory's error in measurement.

Most participants will operate with a biased mean, and with a run-to-run standard deviation differing from σ_p. Laboratories performing in accordance with the PT scheme's requirements will usually receive z-scores in the range ± 2.

Interpretation of z-scores:

$\lvert$ z-score $\rvert \leq 2$	satisfactory performance
$2 < \lvert$ z-score $\rvert < 3$	questionable performance (warning signal)
$\lvert$ z-score $\rvert \geq 3$	unsatisfactory performance (action signal)

A laboratory's PT results can also be used to check the validity of the reported measurement uncertainty. Zeta (ζ)-scores can be evaluated as follows [ISO 13528:2015-08, /11/]:

$$\zeta = \frac{x - x_a}{\sqrt{u_c^2 + u_{Cref}^2}} \qquad \textit{Eq. 40}$$

u_c is the combined uncertainty in x.

According to ISO 13528:2015-08 the calculation of zeta-scores according to the above mentioned equation may strictly be used only if the assigned value is not calculated using the reported results by the participants. In other cases the assigned value is correlated with the results reported by the participants. The zeta-score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation of its reported result from the assigned value. Interpretation is similar to that of z-scores: absolute values over 3 should be regarded as cause for further investigation. The reason might be underestimation of the combined uncertainty, but might also be due to gross error causing the deviation ($x - x_a$) to be large. The latter condition would usually be expected to result in a high z-score. If a z-score is high, due to a large deviation ($x - x_a$), but at the same time, a small zeta-score is observed, then u_{Cref} might be too large. Both examples show that it is important to consider z- and zeta-scores together.

Examples for the comparison of z-scores and zeta-scores and conclusion for applied measurement uncertainty are given in Annex G.

11. Symbols, Terms and Definitions

Symbol, Term	Definition
a	*Semi-range* of an interval ± a
Aliquot	A known amount of a homogeneous material, assumed to be taken with negligible sampling error. [IUPAC 1997, /20/]
Assigned value (x_a)	An estimate of the value of the measurand that is used for the purpose of calculating scores. [IUPAC 2014, /15/] Value attributed to a particular property of a proficiency test item. [ISO/IEC 17043:2010-02, /5/; ISO 13528:2015-08, /11/] ***NOTE:*** Within the scope of this document, an assigned value may also be an estimate of the value of the measurand assigned to a well characterized QC sample from the laboratory's own analyses.
Bias	The difference between the calculated mean of the measurement results and an accepted reference value. [ISO 5725-1:1993, /17/]
$bias_{max}$	*Maximum tolerable bias*
$bias_{rel}$	*Relative bias:* The ratio of the absolute bias and the accepted reference value, expressed e.g. as a percentage.
Blank value	A reading or result originating from the matrix, reagents and any residual bias in the measurement device or process, which contributes to the value obtained for the quantity in the analytical procedure. [IUPAC 1997, /20/]
Consensus value	Value derived from a collection of results in an inter-laboratory comparison [ISO 13528:2015-08, /11/]
(Quality) Control	Material used for the purpose of internal quality control and subjected to the same or part of the same procedures as that used for test materials

Symbol, Term	Definition
material	[IUPAC 1995, /35/]
CRM	*Certified Reference Material:* Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated expanded uncertainty, and a statement of metrological traceability. [ISO Guide 30:2008, /6/; ISO Guide 35:2006 /7/; ISO 13528:2015-08, /11/]
$d_{RRF,rel}$	Relative deviation of relative response factor (RRF) of calibration check from RRF value of applied calibration
Duplicate analysis	Separate analysis of the analytes of interest using a second representative aliquot of the same homogenized sample.
Inter-laboratory comparison	Organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions. [ISO/IEC 17043:2010-02, /5/; ISO 13528:2015-08, /11/]
Isotope Dilution	A technique for mass spectrometric quantitation of an analyte of interest in which a stable isotope-labelled compound is used as both a surrogate and an internal standard for a non-labelled compound. The stable isotope-labelled compound is added to the sample that then undergoes preparation and analysis. Losses of the analyte during preparation and interferences during analysis should be mirrored in the isotope-labelled compound, and thus should not have an adverse effect on quantitation. [EPA 2015, /22/] A technique of mass spectrometry based analysis in which each analyte of interest is quantified using a stable isotope-labelled internal (extraction) standard. For the scope of this document – the analysis of polychlorinated dioxins, furans and biphenyls – such standards should be fully carbon-13 labelled compounds. In this context each standard should be the exact analogue of its corresponding native analyte, i.e. having the same structure (isomer) and differing only in the substitution of all native ^{12}C atoms with ^{13}C. E.g. the target analyte $^{12}C_{12}$-2,3,7,8-TCDD would be quantified by reference to the labelled standard $^{13}C_{12}$-2,3,7,8-TCDD. To minimise measurement uncertainty, isotope dilution should be used for all analytes that contribute to

Symbol, Term	Definition
	a sample's toxic equivalent concentration (TEQ). The labelled standards are added to the sample prior to preparation and analysis, therefore any losses affecting an analyte during sample preparation, and certain interferences during analysis, should similarly apply to its standard such that the resultant concentration is implicitly corrected.
k	*Coverage factor:* Numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty. *k* is typically in the range 2 to 3. [GUM 2008, /1/] ***NOTE:*** Preferably, a coverage factor of $k = 2$ shall be selected according to EU legislation.
Matrix group	Matrices for which an identical or similar analytical procedure provides equivalent performance.
Matrix spike (Spiked Sample or Fortified Sample)	A sample prepared by adding a known mass of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency. [EPA 2015, /22/] ***NOTE:*** In the field of PCDD/Fs and PCBs, generally no real blank samples are available; therefore, samples with low levels of contamination are used for fortification.
Method performance study	An inter-laboratory study in which all laboratories follow the same written protocol and use the same test method to measure a quantity in sets of identical test items [test samples, materials]. The reported results are used to estimate the performance characteristics of the method. Usually these characteristics are within-laboratory and among-laboratories precision, and when necessary and possible, other pertinent characteristics such as systematic error, recovery, internal quality control parameters, sensitivity, limit of determination, and applicability. [IUPAC 1995, /23/]
MU	*Measurement uncertainty* is a metrological term, which is defined as follows: a parameter associated with the result of a measurement that characterizes the dispersion of the value that could reasonably be attributed to the

Symbol, Term	Definition
	measurand. [GUM 2008, /1/] ***NOTE:*** The wording "measurement uncertainty" does not imply the chosen level of confidence.
Precision	Closeness of agreement between independent test/measurement results obtained under stipulated conditions. [ISO 3534-2:2006, /24/]
Procedural blank	The simplest form of a blank […] where the analytical procedure is executed in all respects apart from the addition of the test portion. This kind of blank, in fact, tests more than the purity of the reagents. For example it is capable of detecting contamination of the analytical system originating from any source, e.g., glassware and the atmosphere […]. [IUPAC 1998, /32/] In this context *procedural blank* means the complete analytical procedure applied without the test portion or using an equivalent amount of suitable solvent in place of the test portion.
Proficiency Testing (PT)	Evaluation of participant performance against pre-established criteria by means of inter-laboratory comparisons. [ISO/IEC 17043:2010-02, /5/]
r	*Repeatability* (precision 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 3534-2:2006, /24/]
R	*Reproducibility* (precision 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 lab precision".

Symbol, Term	Definition
	[ISO 3534-2:2006, /24/]
R	*Recovery:* Proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is extracted and presented for measurement. [IUPAC 1998, /32/]
$\bar{R}$	*Average recovery* obtained from multiple analysis of a fortified sample using the same method.
Reference quantity value	*Quantity value* used as a basis for comparison with values of quantities of the same kind. … A reference quantity value with associated measurement uncertainty is usually provided with reference to, e.g. a certified reference material. [ISO/IEC Guide 99-12:2007, /8/]
RM	*Reference Material:* Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. [ISO Guide 30:2008, /6/; ISO Guide 35:2006 /7/; ISO 13528:2015-08, /11/] ***NOTE:*** Within the scope of this document, a reference material may be a QC sample sufficiently characterized using the laboratory's own analyses.
RMS	*Square Root of Mean Squares*
RMS_{bias}	*Square Root of Mean Squares* of individual bias contributions: $\sqrt{\frac{\sum(bias_i)^2}{n}}$ [[ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/]
RSS	*Root of Sum of Squares*
R_w	*Intermediate Precision (*precision under intermediate conditions, also expressed as within-laboratory precision) *Intermediate precision conditions:* Conditions where test results or measurement results are obtained with the same method, on identical test/measurement items in the same test or measurement facility, under some different operating condition. There are four elements to the operating

Symbol, Term	Definition
	condition: time, calibration, operator and equipment. [ISO 3534-2:2006, /24/]
s	*Sample standard deviation:* An estimate of the population standard deviation σ from a sample of n results [EURACHEM/CITAC 2012, /12/]
s_{bias}	*Standard deviation of the bias*, expressed e.g. as a percentage
$S_{bias,rel}$	*Relative standard deviation of the bias*, expressed e.g. as a percentage.
Fortified QC sample	A sample matrix, free from the analytes of interest, fortified with verified known amounts of analytes or a material containing known and verified amounts of analytes from the same source as the calibration standards. It is generally used to establish intra-laboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system. based on [EPA 2015, /22/]
s_r	*Repeatability standard deviation:* Standard deviation of test results or measurement results obtained under *repeatability* conditions. [ISO 3534-2:2006, /24/] s_r is a measure of the repeatability r and can be estimated from simple replication studies.
s_R	*Reproducibility standard deviation:* Standard deviation of test results or measurement results obtained under *reproducibility* conditions. [ISO 3534-2:2006, /24/] s_R is a measure of the reproducibility R and can be estimated from validation studies with many participating laboratories or from proficiency testing data.
$s_{r,rel}$	*Relative repeatability standard deviation*, expressed e.g. as a percentage.
$s_{R,rel}$	*Relative reproducibility standard deviation*, expressed e.g. as a percentage.
s_{rel}	*Relative standard deviation*: An estimate of the standard deviation of a population from a (statistical) sample of n results divided by the mean of that

Symbol, Term	Definition
	sample. Often known as coefficient of variation (CV). Also frequently stated as a percentage. [EURACHEM/CITAC 2012, /12/]
$s_{RRF,rel}$	Relative standard deviation of relative response factors (RRF) of calibration
s_{Rw}	*Intermediate precision standard deviation:* Standard deviation of test results or measurement results obtained under *intermediate precision* conditions. [ISO 3534-2:2006, /24/] s_{Rw} is a measure of the intermediate precision R_w and can be estimated from the standard deviation of a control sample over a certain period of time.
$s_{Rw,pool}$	*Pooled intermediate precision standard deviation:* Standard deviation of test results or measurement results obtained from *various grouped sample matrices* under otherwise *intermediate precision* conditions. $s_{Rw,pool}$ is a measure of the intermediate precision in the special case of using pooled matrices, and can be estimated from the standard deviation of *various grouped sample matrices* over a certain period of time.
$s_{Rw,pool,rel}$	*Pooled relative intermediate precision standard deviation*, expressed e.g. as a percentage.
$s_{Rw,rel}$	*Relative intermediate precision standard deviation*, expressed e.g. as a percentage.
s_{source}	*Standard deviation* associated with an uncertainty source
$s_{\bar{x}}$	*Standard deviation of the mean:* The standard deviation of the mean $\bar{x}$ of n values taken from a population is given by $$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$ The terms "standard error" and "standard error of the mean" have also been used to describe the same quantity. [EURACHEM/CITAC 2012, /12/]
σ	*Population standard deviation* [EURACHEM/CITAC 2012, /12/]

Symbol, Term	Definition
σ_p	*Fitness-for-purpose-based "standard deviation* for proficiency assessment". [ISO 13528:2015-08, /11/]
$\sigma_{p,rel}$	Fitness-for-purpose-based "standard deviation for proficiency assessment", expressed e.g. as a percentage. [ISO 13528:2015-08, /11/]
Target measure-ment uncertainty	Measurement uncertainty specified as an upper limit and decided on the basis of the intended use of measurement results [ISO/IEC Guide 99-12:2007, /8/]
True value	Value which characterizes a quantity or quantitative characteristic perfectly defined in the conditions which exist when that quantity or quantitative characteristic is considered. ***NOTE*:** The true value of a quantity or quantitative characteristic is a theoretical concept and, in general, cannot be known exactly. [ISO 3534-2:2006, /24/; EURACHEM/CITAC 2012, /12/]
Trueness	Closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value. [ISO/IEC Guide 99-12:2007, /8/] ***NOTE:*** Trueness is generally expressed as the overall bias.
U	*Expanded [combined] uncertainty:* Quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand. ***NOTE 1*:** The fraction may be viewed as the coverage probability or level of confidence of the interval. [GUM 2008, /1/; EURACHEM 2012, /12/] ***NOTE 2:*** The expanded uncertainty provides an interval within which the value of the measurand is believed to lie with a higher level of confidence EURACHEM/CITAC 2012, /12/]. Preferably, a level of confidence of the interval of 95 % shall be chosen. ***NOTE 3:*** In this document, for compliance assessment, U is expressed in absolute units.

Symbol, Term	Definition
$u_{(xi)}$	*Individual standard uncertainty component:* Uncertainty of the result x of a measurement i expressed as a standard deviation. [GUM 2008, /1/] ***NOTE:*** For calculation of u according to the provisions given in this document, individual contributory terms $u_{(xi)}$ are expressed in relative units, to simplify the calculations and, on the practical level, facilitate e.g. the accommodation of various concentrations.
u_{bias}	*Uncertainty component for the bias*. [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/]
$u_{bias,max}$	*Uncertainty component* corresponding to the *maximum tolerable bias.*
u_c	*Combined standard uncertainty:* Standard uncertainty of the result of a measurement when that result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances or co-variances of these other quantities weighted according to how the measurement result varies with changes in these quantities. [GUM 2008, /1/]
$u_{c,max}$	*Maximum tolerable combined standard uncertainty.*
u_{conc}	*Uncertainty component for the concentration.*
u_{Cref}	*Uncertainty component of the assigned value* of a RM, or in a PT. [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/] ***NOTE:*** If the uncertainty of the assigned value is too large in comparison with the standard deviation for proficiency assessment, there is a risk that some laboratories will receive a questionable or unsatisfactory performance (zeta-score) because of inaccuracy in the determination of the assigned value, not due to any cause within those laboratories.
u_{CRM}	*Uncertainty component of the certified value* of a CRM. [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/] *Uncertainty of a Certified Value:* An estimate attached to a certified value of a quantity which characterizes the range of values within which the "true

Symbol, Term	Definition
	value" is asserted to lie with a stated level of confidence. [ISO Guide 30:2008, /6/]
U_{max}	*Maximum tolerable expanded measurement uncertainty.*
u_{mean}	*Standard uncertainty* associated with the arithmetic mean calculated from the results of 2 independent analyses.
Upper bound approach	*'Upper-bound'* means the concept which requires using the limit of quantification for the contribution of each non-quantified congener. [COM 2014, /27/; COM 2009, /28/]
u_r	*Uncertainty component for the repeatability.*
u_{Rw}	*Uncertainty component for within-laboratory reproducibility*. [ISO 11352:2012-07, or DIN ISO 11352:2013-03, /4/]
$u_{Rw,gr}$	*Uncertainty component for intermediate precision* (within-laboratory reproducibility) in the special case when matrices are grouped.
$u_{Rw,max}$	*Maximum tolerable intermediate precision* expressed as s_{Rw}.
u_{fort}	*Fortification procedure uncertainty*. Uncertainty component of the amount of analyte a blank or low contaminated sample is fortified with.
u_{vol}	*Uncertainty component for the volume.*
$\bar{x}$	*Mean value:* Arithmetic mean value of a sample of n results. [EURACHEM/CITAC 2012, /12/] *Note: Laboratory mean value* means the arithmetic mean value of a sample of n results analysed in an individual laboratory.
x_{cert}	*Certified (property) value:* The certified (property) value is attributed to a quantity representing a property of the CRM. [ISO Guide 35:2006, /7/]

Symbol, Term	Definition
x_{fort}	*Fortification concentration:* Amount of analyte used for fortification of a blank or low contaminated sample.
zeta-score	$\zeta = \frac{x - x_a}{\sqrt{u_c^2 + u_{Cref}^2}}$ *Zeta-score:* Standardized measure of performance, calculated using the participant result, assigned value and the combined standard uncertainties for the result and the assigned value. [ISO 13528:2015-08, /11/]
z-score	$z = \frac{x - x_a}{\sigma_p}$ *z-score:* Standardized measure of performance, calculated using the participant result, assigned value and the standard deviation for proficiency assessment. [ISO 13528:2015-08, /11/]

12. References

1. BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML: *Guide to the Expression of Uncertainty in Measurement (GUM).* International Organization for Standardization, Geneva. ISBN 92-67-10188-9, (1st ed. 1993), corrected and reprinted 1995. Reissued as ISO Guide 98-3 (2008). Available at http://www.bipm.org as JCGM 100:2008.
2. ISO 3534-1:2006, *Statistics - vocabulary and symbols - Part 1: Probability and General Statistical Terms*, International Organization for Standardization, Geneva.
3. Magnusson B, Näykki T, Hovind H, Krysell M. Nordtest Technical Report 537: *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*. (NT TR 537 - ed. 3.1, 2012)
4. DIN ISO 11352:2013-03, or ISO 11352:2012-07, *Water quality – Estimation of Measurement Uncertainty based on Validation and Quality Control Data*. International Organization for Standardization, Geneva.
5. ISO/IEC 17043:2010-02, *Conformity assessment – General requirements for proficiency testing.* International Organization for Standardization, Geneva.
6. ISO Guide 30:2008, *Terms and definitions used in connection with reference materials*. International Organization for Standardization, Geneva.
7. ISO Guide 35:2006, *Reference materials – General guidance for the assignment of property values.* International Organization for Standardization, Geneva.
8. ISO/IEC Guide 99-12:2007, *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms* (VIM).
9. Barwick VJ and Ellison SLR. *Uncertainty Principles*, Part (d): *Protocol for uncertainty evaluation from validation data.* January 2000. LGC/VAM/1998/088
10. Maroto A, Boqué R, Riu J and Rius FX. *Should non-significant bias be included in the uncertainty budget?* Accred Qual Assur 7 (2002) 90–94
11. ISO 13528:2015-08, *Statistical methods for use in proficiency testing by interlaboratory comparisons.* International Organization for Standardization, Geneva.
12. Ellison SLR and Williams A (eds). EURACHEM/CITAC guide: *Quantifying Uncertainty in Analytical Measurement*, (3rd ed. 2012). ISBN 978-0-948926-30-3. Available at www.eurachem.org and at http://www.citac.cc.
13. Fernandes A, Rose M, Smith F and Holland J. 2012. Organic Environmental Contaminants in the 2012 Total Diet Study samples. Food Standards Agency Report. Available at http://www.food.gov.uk/sites/default/files/research-report-total-diet-study.pdf

14. ISO/IEC 17025:2005-08, *General requirements for the competence of testing and calibration laboratories.* International Organization for Standardization, Geneva.

15. Thompson M, Ellison SLR and Wood R. *The International Harmonised Protocol for the proficiency testing of chemical analytical laboratories*. IUPAC, Pure and Applied Chemistry 78, 145–196 (2006). Last update: 2014-02-24; version: 2.3.3.

16. EURACHEM Guide – *Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories*, (2nd ed. 2011). Available at www.eurachem.org.

17. ISO 5725-1:1993, *Accuracy (trueness and precision) of measurement methods and results.* Part 1: *General principles and definitions.* International Organization for Standardization, Geneva.

18. Ramsey MH and Ellison SLR (eds.) EURACHEM/EUROLAB/CITAC/Nordtest/ AMC Guide: *Measurement uncertainty arising from sampling: A guide to methods and approaches,* (1st ed. 2007). ISBN 978-0-948926-26-6. Available at www.eurachem.org.

19. Van den Berg M et al., The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. *Toxicological Sciences* 93 (2) (2006) 223–241

20. IUPAC. *Compendium of Chemical Terminology*, 2nd ed. (The "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8. doi:10.1351/goldbook

21. Magnusson B and Örnemark U (eds.) EURACHEM Guide: *The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics,* (2nd ed. 2014). ISBN 978-91-87461-59-0. Available at www.eurachem.org.

22. United States Environmental Protection Agency (EPA). *Vocabulary Catalog*, *Forum on Environmental Measurements (FEM) Glossary* (August 2015). Available at http://www.epa.gov/fem/.

23. Horwitz W, Protocol for the design, conduct and interpretation of method performance studies. *Pure Appl. Chem.* 67 (1995) 331-343. Available at www.iupac.org.

24. ISO 3534-2:2006, *Statistics - vocabulary and symbols - Part 2: Applied statistics,* International Organization for Standardization, Geneva.

25. US EPA 2014, Method 8000D, Determinative Chromatographic Separation. Revision 4

26. EURACHEM/CITAC Guide: *Use of uncertainty information in compliance assessment*, (1st ed. 2007). Available at www.eurachem.org.

27. COMMISSION REGULATION (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012 (OJ L 164, 03.06.2014, p. 18)
28. COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.02.2009, p. 1)
29. COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (OJ L 364, 20.12.2006, p. 5)
30. COMMISSION RECOMMENDATION of 3 December 2013 on the reduction of the presence of dioxins, furans and PCBs in feed and food (2013/711/EU) (OJ L 323, 04.12.2013, p. 37)
31. DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed (OJ L 140, 30.05.2002, p. 10)
32. IUPAC. *Compendium of Analytical Nomenclature (definitive rules 1997)* – The Orange Book, 3rd Edition, Compiled by J. Inczedy, T. Lengyel, and A.M. Ure. Chapter 18.6.1 Terms concerned with internal quality control procedures. Blackwell Scientific Publications, Oxford (1998).
33. IUPAC. *Harmonised Guidelines for the use of Recovery Information in Analytical Measurement* (Technical Report). *Pure Appl. Chem.* 71 (2), 337-348 (1999)
34. EC Guidance document: Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU Food and Feed legislation (2004).
    Available at http://ec.europa.eu/food/safety/docs/cs_contaminants_sampling_analysis-report_2004_en.pdf
35. IUPAC. *Harmonized guidelines for internal quality control in analytical chemistry* (Technical Report) *Pure Appl. Chem.* 67 (4), 649-666 (1995)
36. EURACHEM/CITAC Guide: *Setting and Using Target Uncertainty in Chemical Measurement*. R. Bettencourt da Silva, A. Williams (Eds) (1st ed. 2015). Available at www.eurachem.org
37. Horwitz W, The Certainty of Uncertainty. *J AOAC Int.* 86, 109-111 (2003).
38. EN 16215:2012-07, Animal feeding stuffs. Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

39. Barwick V.J., Ellison S.L.R, Fairman B. Estimation of uncertainties in ICP-MS analysis: a practical methodology. Analytica Chimica Acta 394, 281-291 (1999)

40. EU Reference Laboratories Guidance Document: Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food, EURL for Polycyclic Aromatic Hydrocarbons, EURL for Dioxins and PCBs in Feed and Food, EURL for Heavy Metals in Feed and Food and EURL for Mycotoxins (2016). Available at: http://www.eurl-dioxin-freiburg.eu/doc/LOD_LOQ_guidance document _food contaminants_2016.pdf

41. IUPAC. *Compendium of Analytical Nomenclature (definitive rules 1997)* – The Orange Book, 3rd Edition, Compiled by J. Inczedy, T. Lengyel, and A.M. Ure. Chapter 2.3 Quantities related to replicate measurements. Blackwell Scientific Publications, Oxford (1998).

Annex A – General

A.1 Grouping of Matrices

A possible grouping of matrices for PCDD/F and PCB analysis is given in Table A.1-1.

Table A.1-1: Guidance for grouping of matrices according to physico-chemical properties and applied (extraction) methods

Group 1
Food
Subgroup 1.1 Fat-containing food
Meat and meat products: Bovine, sheep, poultry, pigs
Subgroup 1.2 Milk
Milk and dairy products
Subgroup 1.3 Egg
Eggs and egg products
Subgroup 1.4 Fats/oils
Marine oils
Animal fat
Vegetable oils and fats
Subgroup 1.5 Liver of terrestrial animals
Liver of terrestrial animals: Bovine, sheep, poultry, pigs
Subgroup 1.6 Muscle meat of fish and fish liver
Muscle meat of fish, fishery products, crustaceans
Liver of fish
Subgroup 1.7[*] Infant food
Foods for infants and young children
Subgroup 1.8 Non-fat containing food
Cereals
Fruits and vegetables
Clays as food supplement

[*] specific working range level due to very low maximum limits

Group 2
Feed
Subgroup 2.1 Feed matrices (other than fats/oils)
Feed materials of plant origin
Feed materials of animal origin
Other land animal products including milk and milk products and eggs and egg products
Fish, other aquatic animals, and products derived thereof with the exception of fish oil, hydrolysed fish protein containing more than 20 % fat and crustacean meal
Hydrolysed fish protein containing more than 20 % fat**
Feed materials of mineral origin
Feed additives
Binders and anti-caking agents
Compounds of trace elements
Compound feed
Premixtures
Subgroup 2.2 Fats/oils
Animal fat, including milk fat and egg fat
Fish oil**
Vegetable oils and their by-products

** significantly higher levels of interest

Assessing MU at 0.5x, 1x and 2x the maximum limit is consistent with current EU legislation. For groups of matrices, however, the working range will then expand and cover concentrations from 0.5x the lowest of the maximum limits assigned to the selected matrices up to 2x the highest of those maximum limits, assuming a constant relative combined uncertainty.

Annex B – Intermediate Precision Studies

B.1 Evaluation of intermediate precision contribution to MU

As an example, several matrix quality controls naturally contaminated or fortified at different levels can be used for this study. Table B.1-1 shows the mean value and their corresponding intermediate precision for 85 replicates of mixed animal fat from the EU-RL.

Table B.1-1: Intermediate precision study for PCDD/Fs and DL-PCBs in mixed animal fat QC samples (n = 85)

Mixed animal fat	**Mean concentration** $\bar{x}$	**Intermediate Precision** s_{Rw}	**Rel. Intermediate Precision** $s_{Rw,rel}$
Congeners	pg/g fat	pg/g fat	%
2,3,7,8-TCDD	0.20	0.04	20
1,2,3,7,8-PeCDD	0.23	0.05	22
1,2,3,4,7,8-HxCDD	0.10	0.04	40
1,2,3,6,7,8-HxCDD	0.28	0.05	18
1,2,3,7,8,9-HxCDD	0.09	0.04	44
1,2,3,4,6,7,8-HpCDD	0.62	0.14	23
1,2,3,4,6,7,8,9-OCDD	2.27	0.42	19
2,3,7,8-TCDF	0.84	0.09	11
1,2,3,7,8-PeCDF	0.10	0.03	30
2,3,4,7,8-PeCDF	0.61	0.07	11
1,2,3,4,7,8-HxCDF	0.24	0.05	21
1,2,3,6,7,8-HxCDF	0.18	0.02	11
2,3,4,6,7,8-HxCDF	0.22	0.04	18
1,2,3,7,8,9-HxCDF	0.01	0.01	100
1,2,3,4,6,7,8-HpCDF	0.25	0.04	16
1,2,3,4,7,8,9-HpCDF	0.02	0.02	100
1,2,3,4,6,7,8,9-OCDF	0.14	0.07	50
PCB 105	388	29	7.5
PCB 114	24.1	4.8	20
PCB 118	1480	110	7.4

Mixed animal fat	**Mean concentration** $\bar{x}$	**Intermediate Precision** s_{Rw}	**Rel. Intermediate Precision** $s_{Rw,rel}$
Congeners	pg/g fat	pg/g fat	%
PCB 123	15.1	3.8	25
PCB 156	281	22	7.8
PCB 157	50.5	19	38
PCB 167	176	17	10
PCB 189	33.3	6.3	19
PCB 77	21.6	1.5	6.9
PCB 81	1.53	0.30	20
PCB 126	14.1	1.1	7.8
PCB 169	1.92	0.30	15

Mixed animal fat	**Mean concentration** $\bar{x}$	**Intermediate Precision** s_{Rw}	**Rel. Intermediate Precision** $s_{Rw,rel}$
Sum parameters	pg WHO-TEQ/g fat	pg WHO-TEQ/g fat	%
SUM PCDD/Fs	0.82	0.08	9.8
SUM DL-PCBs	1.54	0.11	7.1
SUM PCDD/Fs + DL-PCBs	2.37	0.15	6.3

According to equation 5, the contribution of the intermediate precision to MU for the sum parameters is given by

$$u_{Rw\,(SUM\ PCDD/Fs)} = s_{Rw\,(SUM\ PCDD/Fs)} = 0.08\ pg\ WHO-TEQ/g\ fat$$

$$u_{Rw\,(SUM\ DL-PCBs)} = s_{Rw\,(SUM\ PDL-PCBs)} = 0.11\ pg\ WHO-TEQ/g\ fat$$

$$u_{Rw\,(SUM\ PCDD/Fs+DL-PCBs)} = s_{Rw\,(SUM\ PCDD/Fs+DL-PCBs)} = 0.15\ pg\ WHO-TEQ/g\ fat$$

Or according to equation 6, the contribution of the relative intermediate precision to MU for the sum parameters is given by

$$u_{Rw,rel\ (SUM\ PCDD/Fs)} = s_{Rw,rel\ (SUM\ PCDD/Fs)} = 9.8\ \%$$

$$u_{Rw,rel\ (SUM\ DL-PCBs)} = s_{Rw,rel\ (SUM\ PDL-PCBs)} = 7.1\ \%$$

$$u_{Rw,rel\ (SUM\ PCDD/Fs+DL-PCBs)} = s_{Rw,rel\ (SUM\ PCDD/Fs+DL-PCBs)} = 6.3\ \%$$

B.2 Evaluation of intermediate precision from various matrices

Table B.2-1 summarizes the results from intermediated precision studies for various matrices and concentration levels of PCDD/Fs and DL-PCBs in TEQ.

NOTE: Only sum in TEQ are presented here, but the same exercise can be done by congener.

Table B.2-1: Intermediate precision study for PCDD/Fs and DL-PCBs in various biological QC samples grouped according to annex A

Sum Parameter	**QC matrix**	**Mean concentration** $\overline{x}$	**Intermediate Precision** s_{Rw}	**Rel. Intermediate Precision** $s_{Rw,rel}$	***n***
	Subgroup 1.1	pg WHO-TEQ/g	pg WHO-TEQ/g	%	
SUM PCDD/Fs	Pork	0.69	0.05	7.2	17
	Lard	1.26	0.06	4.8	13
	Bovine	2.93	0.16	5.5	24
	Sheep	4.36	0.22	5.0	12
SUM DL-PCBs	Pork	2.50	0.11	4.4	17
	Lard	1.15	0.06	5.2	13
	Bovine	1.55	0.08	5.2	24
	Sheep	3.78	0.21	5.6	12

Sum-Parameter	**QC matrix**	**Mean concentration** $\overline{x}$	**Intermediate Precision** s_{Rw}	**Rel. Intermediate Precision** $s_{Rw,rel}$	***n***
	Subgroup 1.4	pg WHO-TEQ/g	pg WHO-TEQ/g	%	
SUM PCDD/Fs	Fish oil 1	1.88	0.15	8.0	21
	Olive oil	3.42	0.15	4.4	29
	Fish oil 2	2.26	0.08	3.5	10
	Mixed fat	0.82	0.08	9.8	85

Sum-Parameter	QC matrix	Mean concentration $\bar{x}$	Intermediate Precision s_{Rw}	Rel. Intermediate Precision $s_{Rw,rel}$	*n*
	Subgroup 1.4	pg WHO-TEQ/g	pg WHO-TEQ/g	%	
SUM DL-PCBs	Fish oil 1	10.06	0.27	2.7	21
	Olive oil	4.13	0.14	3.4	29
	Fish oil 2	9.08	0.27	3.0	10
	Mixed fat	1.54	0.11	7.1	85

When plotting the concentrations of PCDD/Fs, and of DL-PCBs, respectively, shown in Table B.2-1 against their corresponding s_{Rw} values for subgroup 1.1 (see figures B.2-1 and B.2-2), resulting regression lines exhibit slopes close to 0.049 (PCDD/Fs) and 0.056 (DL-PCBs). The (absolute) intermediate precision is seen to be proportional to the analyte level across the selected concentration range for subgroup 1.1.

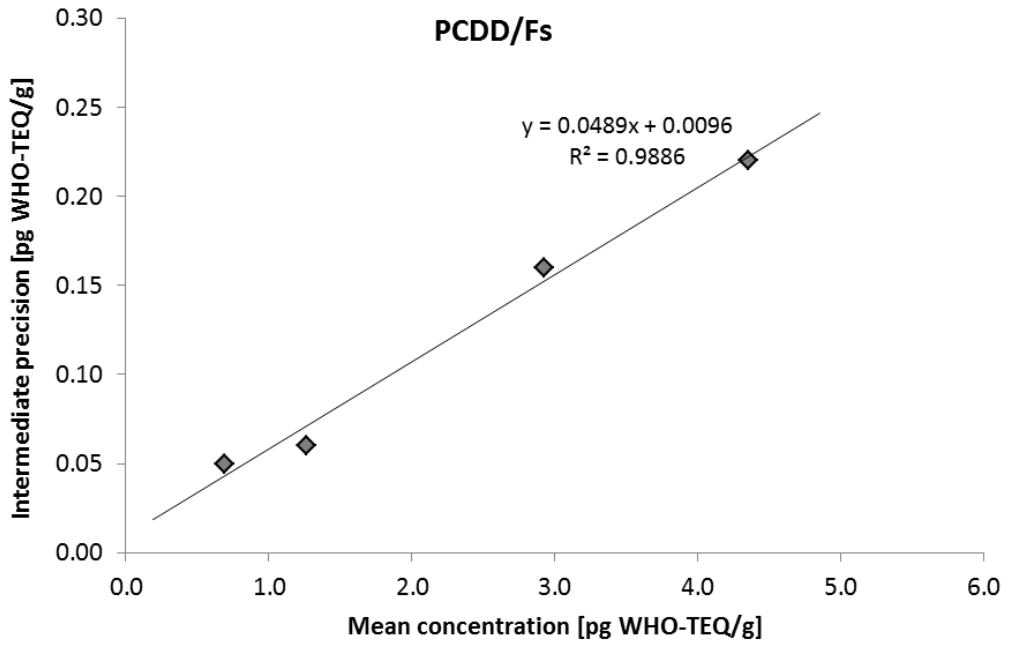


Figure B.2-1: Intermediate precision for PCDD/Fs plotted vs. their corresponding concentrations (in TEQ), for subgroup matrices 1.1.

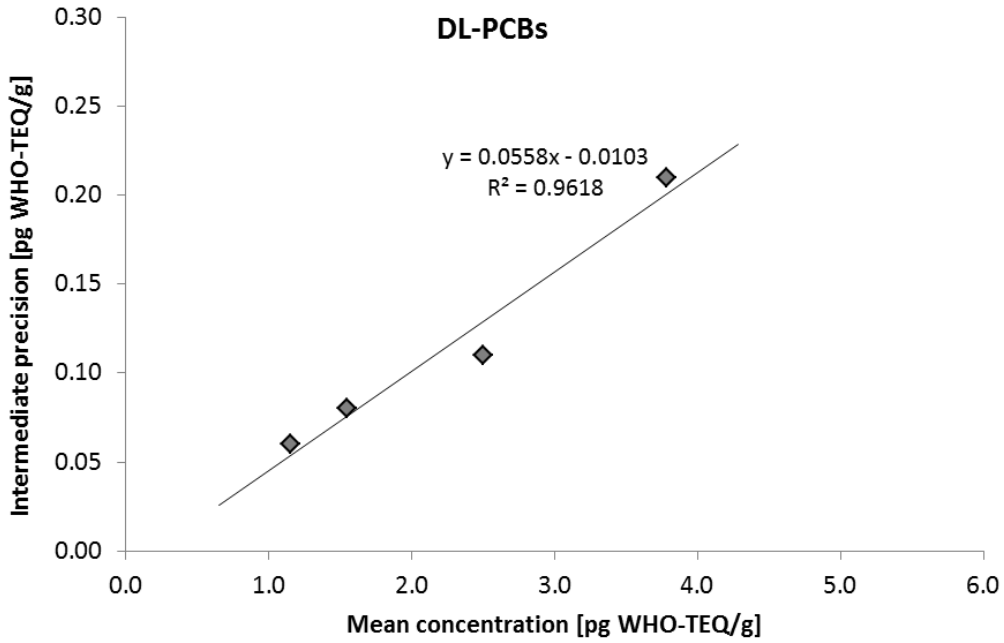


Figure B.2-2: Intermediate precision for DL-PCBs, plotted vs. their corresponding concentrations (in TEQ), for subgroup matrices 1.1.

In such cases, the relative $s_{Rw,rel}$ is rather constant through the concentration range for subgroup 1.1 as shown in figures B.2-3 and B-2-4.

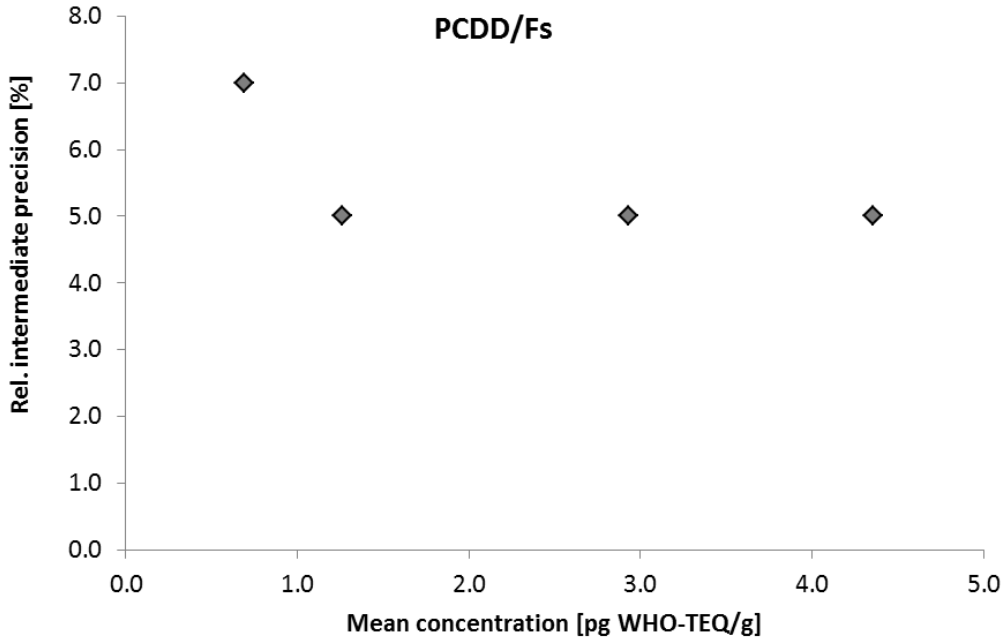


Figure B.2-3: Relative intermediate precision (%) for PCDD/Fs plotted vs. their corresponding concentrations (in TEQ), for subgroup matrices 1.1.

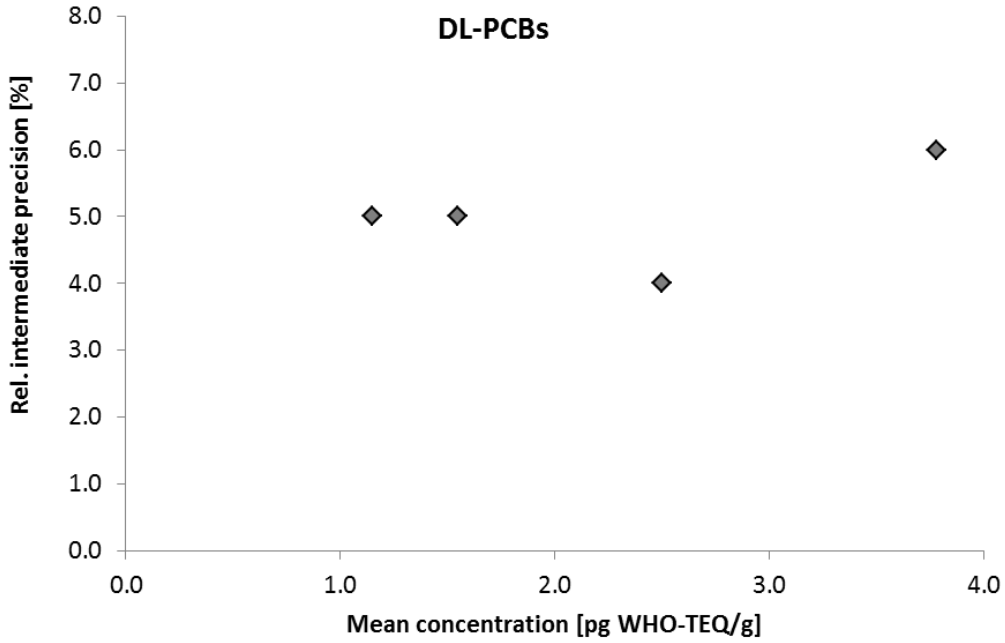


Figure B.2-4: Relative intermediate precision (%) for DL-PCBs, plotted vs. their corresponding concentrations (in TEQ), for subgroup matrices 1.1.

In such case, it may be possible to estimate a single precision contribution value by using a pooled relative intermediate standard deviation $s_{Rw,pool,rel}$ of the included matrices as given by equation 8 for the sum of PCDD/Fs:

$$s_{Rw,pool,rel} = \sqrt{\frac{(17-1)\cdot 7.2^2+(13-1)\cdot 4.8^2+(24-1)\cdot 5.5^2+(12-1)\cdot 5.0^2}{(17-1)+(13-1)+(24-1)+(12-1)}} = 5.8\ \%$$

and for the sum of DL-PCBs:

$$s_{Rw,pool,rel} = \sqrt{\frac{(17-1)\cdot 4.4^2+(13-1)\cdot 5.2^2+(24-1)\cdot 5.2^2+(12-1)\cdot 5.6^2}{(17-1)+(13-1)+(24-1)+(12-1)}} = 5.1\ \%$$

Annex C – Trueness (Bias) Studies

C.1 Evaluation of the bias contribution to MU from CRM

C.1.1 Bias contribution from single CRM

As an example, a well-characterized fish tissue CRM is used to assess the trueness (or bias) contribution to MU in the analysis of PCDD/Fs and DL-PCBs by GC/HRMS using isotope dilution. Six replicate analyses (i.e. 6 replicates) were performed. Table C.1.1-1 provides the results obtained for the 29 individual congeners and for their sum–TEQ parameters as well.

In Table C.1.1-1, $s_{bias,rel}$ is calculated from the six replicates, $bias_{CRM,rel}$ is calculated from Equation 9 and $u_{bias,CRM,rel}$ according to Equation 12

Table C.1.1-1: Trueness (bias) study for PCDD/Fs and DL-PCBs in a fish tissue CRM

Fish tissue	$\bar{x}$ (m=6)	$s_{bias,rel}$ (m=6)	**Certified value***	$bias_{CRM,rel}$	u_{CRM} 95% CI**	$u_{bias,CRM,rel}$
Congeners	pg/g		pg/g		pg/g	
2,3,7,8-TCDD	0.097	0.069	0.102	-0.049	0.02	0.113
1,2,3,7,8-PeCDD	0.414	0.076	0.435	-0.048	0.05	0.081
1,2,3,4,7,8-HxCDD	0.118	0.066	0.136	-0.132	0.03	0.174
1,2,3,6,7,8-HxCDD	0.496	0.080	0.5	-0.008	0.05	0.060
1,2,3,7,8,9-HxCDD	0.225	0.086	0.255	-0.118	0.058	0.167
1,2,3,4,6,7,8-HpCDD	2.12	0.119	2.39	-0.112	0.82	0.210
OCDD	2.3	0.088	2.6	-0.115	0.91	0.213
2,3,7,8-TCDF	3.15	0.112	2.52	0.250	0.7	0.290
1,2,3,7,8-PeCDF	0.45	0.099	0.415	0.084	0.086	0.140
2,3,4,7,8-PeCDF	2.05	0.084	1.84	0.114	0.28	0.141
1,2,3,4,7,8-HxCDF	0.106	0.136	0.118	-0.102	0.026	0.160
1,2,3,6,7,8-HxCDF	0.126	0.102	0.118	0.068	0.022	0.123
1,2,3,7,8,9-HxCDF	0.022	0.180	(<0.05)			
2,3,4,6,7,8-HxCDF	0.17	0.112	0.179	-0.050	0.026	0.099

Fish tissue	$\bar{x}$ (m=6)	$s_{bias,rel}$ (m=6)	**Certified value***	$bias_{CRM,rel}$	u_{CRM} 95% CI**	$u_{bias,CRM,rel}$
1,2,3,4,6,7,8-HpCDF	0.088	0.110	0.091	-0.033	0.024	0.143
1,2,3,4,7,8,9-HpCDF			(<0.03)			
OCDF	0.016	0.100	(<0.1)			
WHO-PCDD/F-TEQ	2.02	0.079	1.89	0.069	0.17	0.088

Fish tissue	$\bar{x}$ (m=6)	$s_{bias,rel}$ (m=6)	**Certified value***	$bias_{CRM,rel}$	u_{CRM} 95% CI**	$u_{bias,CRM,rel}$
Congeners	pg/g		pg/g		pg/g	
PCB 77	39.2	0.082	35.7	0.098	4.6	0.122
PCB 81	0.909	0.216	1.39	-0.346	0.26	0.369
PCB 126	14.5	0.047	13.3	0.090	2.6	0.134
PCB 169	3.52	0.049	3.89	-0.095	1.1	0.172
PCB 105	832	0.053	771	0.079	92	0.101
PCB 114	76	0.155	33.7	1.255	11	1.267
PCB 118	2392	0.049	2445	-0.022	208	0.052
PCB 123	26.7	0.064	22.7	0.176	12	0.319
PCB 156	244	0.068	238	0.025	26	0.066
PCB 157	76.2	0.079	70.6	0.079	7.6	0.101
PCB 167	147.9	0.097	136.7	0.082	15	0.106
PCB 189	26.2	0.075	25.1	0.044	2.6	0.074
WHO-PCB-TEQ	2.01	0.05	1.87	0.075	0.26	0.104
WHO-PCDD/F-PCB-TEQ	4.03	0.06	3.76	0.072	0.43	0.095

* Congener values in brackets are not certified

** CRM certificates always provide u_{CRM} as an interval at a level of confidence and not a standard deviation. At 95% of confidence, u_{CRM} has to be divided by a factor 2.

C.1.2 Bias contribution from multiple CRMs

As an example, three well-characterized CRMs (fish tissue, pork tissue and animal fat, group 1, annex A) are used to estimate the trueness or bias contribution to MU in the analysis of PCDD/Fs and DL-PCBs by GC/HRMS using isotope dilution. A single analysis of each CRM is performed. Tables C.1.2-1, C.1.2-2 and C.1.2-3 provide the measured values and the certified values with associated uncertainties for each congener in each of the three CRMs.

NOTE: This example is limited to the use of three CRMs. However it is recommended that at least six CRMs should be analysed.

Table C.1.2-1: PCDD/Fs and DL-PCBs measured in a fish tissue CRM

Fish tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	
2,3,7,8-TCDD	0.095	0.102	0.020	0.098
1,2,3,7,8-PeCDD	0.428	0.435	0.050	0.057
1,2,3,4,7,8-HxCDD	0.141	0.136	0.030	0.110
1,2,3,6,7,8-HxCDD	0.460	0.500	0.050	0.050
1,2,3,7,8,9-HxCDD	0.275	0.255	0.058	0.114
1,2,3,4,6,7,8-HpCDD	2.20	2.39	0.82	0.172
OCDD	2.80	2.60	0.91	0.175
2,3,7,8-TCDF	2.96	2.52	0.70	0.139
1,2,3,7,8-PeCDF	0.398	0.415	0.086	0.104
2,3,4,7,8-PeCDF	2.01	1.84	0.28	0.076
1,2,3,4,7,8-HxCDF	0.129	0.118	0.026	0.110
1,2,3,6,7,8-HxCDF	0.134	0.118	0.022	0.093
1,2,3,7,8,9-HxCDF	0.024	(<0.05)		
2,3,4,6,7,8-HxCDF	0.180	0.179	0.026	0.073
1,2,3,4,6,7,8-HpCDF	0.105	0.091	0.024	0.132
1,2,3,4,7,8,9-HpCDF		(<0.03)		

Fish tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
OCDF	0.021	(<0.1)		
WHO-PCDD/F-TEQ	2.00	1.89	0.17	0.045

Fish tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	pg/g
PCB 77	33.7	35.7	4.6	0.064
PCB 81	1.25	1.39	0.26	0.094
PCB 126	14.1	13.3	2.6	0.098
PCB 169	3.75	3.89	1.1	0.141
PCB 105	884	771	92	0.060
PCB 114	58.0	33.7	11	0.163
PCB 118	2614	2445	208	0.043
PCB 123	24.5	22.7	12	0.264
PCB 156	239	238	26	0.055
PCB 157	69.5	70.6	7.6	0.054
PCB 167	154.7	136.7	15	0.055
PCB 189	25.8	25.1	2.6	0.052
WHO-PCB-TEQ	1.99	1.87	0.26	0.070
WHO-PCDD/F-PCB-TEQ	3.99	3.76	0.43	0.057

Table C.1.2-2: PCDD/Fs and DL-PCBs measured in a pork tissue CRM

Pork tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	pg/g
2,3,7,8-TCDD	0.046	0.059	0.014	0.119
1,2,3,7,8-PeCDD	0.110	0.125	0.016	0.064
1,2,3,4,7,8-HxCDD	0.121	0.148	0.026	0.088
1,2,3,6,7,8-HxCDD	0.126	0.134	0.01	0.037
1,2,3,7,8,9-HxCDD	0.060	0.074	0.016	0.108
1,2,3,4,6,7,8-HpCDD	0.237	0.263	0.092	0.175
OCDD	0.498	0.51	0.099	0.097
2,3,7,8-TCDF	0.011	0.02	0.008	0.200
1,2,3,7,8-PeCDF	0.022	0.025	0.008	0.160
2,3,4,7,8-PeCDF	0.092	0.102	0.018	0.088
1,2,3,4,7,8-HxCDF	0.091	0.111	0.024	0.108
1,2,3,6,7,8-HxCDF	0.086	0.088	0.006	0.034
1,2,3,7,8,9-HxCDF	0.027	0.029	0.012	0.207
2,3,4,6,7,8-HxCDF	0.052	0.063	0.01	0.079
1,2,3,4,6,7,8-HpCDF	0.086	0.10	0.04	0.200
1,2,3,4,7,8,9-HpCDF	0.046	0.044	0.01	0.114
OCDF	0.045	(<0.1)		
WHO-PCDD/F-TEQ	0.264	0.307	0.060	0.098

Pork tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	pg/g
PCB 77	9.44	8.75	1.7	0.097

Pork tissue	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
PCB 81	0.431	0.679	0.128	0.094
PCB 126	0.422	0.51	0.102	0.100
PCB 169	0.671	0.79	0.22	0.139
PCB 105	49.2	41.7	8.8	0.106
PCB 114	10.1	7.5	5.2	0.347
PCB 118	298	292	32	0.055
PCB 123	3.05	2.84	1.78	0.313
PCB 156	73.9	73.9	11	0.074
PCB 157	7.97	7.57	1.22	0.081
PCB 167	49.5	46.6	5.2	0.056
PCB 189	6.62	6.16	1.06	0.086
WHO-PCB-TEQ	0.132	0.139	0.023	0.083
WHO-PCDD/F-PCB-TEQ	0.40	0.45	0.08	0.093

Table C.1.2-3: PCDD/Fs and DL-PCBs measured in an animal fat CRM

Animal fat	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	pg/g
2,3,7,8-TCDD	0.085	0.103	0.016	0.078
1,2,3,7,8-PeCDD	0.107	0.125	0.022	0.088
1,2,3,4,7,8-HxCDD	0.076	0.089	0.016	0.090
1,2,3,6,7,8-HxCDD	0.102	0.1083	0.012	0.055
1,2,3,7,8,9-HxCDD	0.084	0.108	0.028	0.130
1,2,3,4,6,7,8-HpCDD	0.127	0.163	0.032	0.098
OCDD	1.6	2.0	2.1	0.525
2,3,7,8-TCDF	0.58	0.482	0.056	0.058
1,2,3,7,8-PeCDF	0.157	0.141	0.026	0.092
2,3,4,7,8-PeCDF	0.217	0.213	0.024	0.056
1,2,3,4,7,8-HxCDF	0.094	0.103	0.02	0.097
1,2,3,6,7,8-HxCDF	0.099	0.098	0.018	0.092
1,2,3,7,8,9-HxCDF	0.087	0.087	0.018	0.103
2,3,4,6,7,8-HxCDF	0.082	0.096	0.018	0.094
1,2,3,4,6,7,8-HpCDF	0.107	0.124	0.032	0.129
1,2,3,4,7,8,9-HpCDF	0.077	0.09	0.022	0.122
OCDF	0.125	0.166	0.042	0.127
WHO-PCDD/F-TEQ	0.43	0.46	0.07	0.076

Animal fat	**Measured value**	**Certified value***	u_{CRM} 95% CI**	$u_{CRM,rel}$
Congeners	pg/g	pg/g	pg/g	pg/g
PCB 77	17.3	14.7	2.2	0.075

Animal fat	Measured value	Certified value*	u_{CRM} 95% CI**	$u_{CRM,rel}$
PCB 81	1.67	1.66	0.2	0.060
PCB 126	4.9	5.04	0.6	0.060
PCB 169	1.42	1.59	0.18	0.057
PCB 105	311	302	30	0.050
PCB 114	21	21.8	2.6	0.060
PCB 118	1089	1077	150	0.070
PCB 123	32	19.6	8.6	0.219
PCB 156	164	164	16.2	0.049
PCB 157	24.6	24.8	5.2	0.105
PCB 167	255	132	18.2	0.069
PCB 189	14.5	15.4	2.8	0.091
WHO-PCB-TEQ	0.74	0.77	0.06	0.039
WHO-PCDD/F-PCB-TEQ	1.18	1.23	0.13	0.053

* Congener values in brackets are not certified

** CRM certificates always provide u_{CRM} as an interval at a level of confidence and not a standard deviation. At 95% of confidence, u_{CRM} has to be divided by a factor 2.

$u_{bias,CRM,rel}$ is then calculated according to equation 13 through the calculation of $RMS_{bias,CRM}$ and $u_{CRM,rel}$ (equations 14 and 15). All values are summarized in Table C.1.2-4.

Table C.1.2-4: Estimation of bias ($u_{bias,CRM,rel}$**)** for individual PCDD/F and DL-PCB congeners and TEQ parameters, using three CRMs - fish tissue, pork tissue and animal fat.

U_{bias} **for several CRMs**	$RMS^2_{bias,CRM}$	$u^2_{CRM,rel}$	$u_{bias,CRM,rel}$
Congeners			
2,3,7,8-TCDD	0.028	0.010	0.194
1,2,3,7,8-PeCDD	0.012	0.005	0.129
1,2,3,4,7,8-HxCDD	0.019	0.009	0.167
1,2,3,6,7,8-HxCDD	0.004	0.002	0.082
1,2,3,7,8,9-HxCDD	0.030	0.014	0.210
1,2,3,4,6,7,8-HpCDD	0.022	0.022	0.209
OCDD	0.015	0.071	0.293
2,3,7,8-TCDF	0.091	0.018	0.330
1,2,3,7,8-PeCDF	0.010	0.014	0.154
2,3,4,7,8-PeCDF	0.006	0.005	0.108
1,2,3,4,7,8-HxCDF	0.016	0.011	0.165
1,2,3,6,7,8-HxCDF	0.006	0.005	0.108
1,2,3,7,8,9-HxCDF	0.002	0.024	0.160
2,3,4,6,7,8-HxCDF	0.017	0.007	0.155
1,2,3,4,6,7,8-HpCDF	0.021	0.024	0.210
1,2,3,4,7,8,9-HpCDF	0.008	0.014	0.147
OCDF	0.020	0.016	0.191
WHO-PCDD/F-TEQ	0.009	0.005	0.120

U_{bias} **for several CRMs**	$RMS^2_{bias,CRM}$	$u^2_{CRM,rel}$	$u_{bias,CRM,rel}$
Congeners			

U_{bias} for several CRMs	$RMS^2_{bias,CRM}$	$u^2_{CRM,rel}$	$u_{bias,CRM,rel}$
PCB 77	0.014	0.006	0.141
PCB 81	0.048	0.007	0.234
PCB 126	0.011	0.007	0.137
PCB 169	0.012	0.013	0.156
PCB 105	0.018	0.005	0.153
PCB 114	0.214	0.036	0.500
PCB 118	0.002	0.003	0.070
PCB 123	0.137	0.071	0.456
PCB 156	0.000	0.004	0.060
PCB 157	0.001	0.006	0.086
PCB 167	0.296	0.004	0.548
PCB 189	0.003	0.006	0.095
WHO-PCB-TEQ	0.003	0.004	0.082
WHO-PCDD/F-PCB-TEQ	0.006	0.005	0.102

C.2 Evaluation of the bias contribution to MU from PT results

C.2.1 Sum parameters

As an example, results for the sum parameter WHO-PCDD/F-PCB-TEQ for six different PT test samples are shown in table C.2.1-1. Due to the limited number of interlaboratory comparisons for the analysis of PCDD/Fs and PCBs in different food samples, matrices of the food subgroups 1.1, 1.2, 1.3 and 1.4 are combined in order to get the required number of six PT results.

NOTE: The same procedure can be used for intermediate sum parameters like WHO-PCDD/F-TEQ and WHO-PCB-TEQ. Similarly, the same reasoning could be applied to six interlaboratory comparisons or PTs for feed samples (group 2, see Annex A).

Table C.2.1-1: Bias contribution of PT results ($x_{a,i}$: assigned value, $u_{Cref,i}$: uncertainty of assigned value, $u_{Cref,rel,i}$: rel. uncertainty of assigned value, x_i: participant's result)

PT matrix	Para-meter	Unit	$x_{a,i}$	$u_{Cref,i}$	$u_{Cref,rel,i}$	x_i	$bias_{PT,i}$	$bias_{PT,rel,i}$	$\lvert u_{Cref,rel,i} / bias_{PT,rel,i} \rvert$
Fish oil	WHO-PCDD/F-PCB-TEQ	pg/g fat	9.76	0.14	0.014	10.8	1.04	0.11	0.13
Pork	WHO-PCDD/F-PCB-TEQ	pg/g fat	1.42	0.025	0.018	1.15	-0.27	-0.19	0.09
Whole egg	WHO-PCDD/F-PCB-TEQ	pg/g fat	7.21	0.15	0.021	7.53	0.32	0.04	0.53
Egg yolk powder	WHO-PCDD/F-PCB-TEQ	pg/g fat	4.26	0.084	0.020	4.81	0.55	0.13	0.15
Milk powder	WHO-PCDD/F-PCB-TEQ	pg/g fat	3.93	0.089	0.023	3.70	-0.23	-0.06	0.38
Beef	WHO-PCDD/F-PCB-TEQ	pg/g fat	2.53	0.048	0.019	2.31	-0.22	-0.09	0.21

Comparison of $u_{Cref,rel,i}$ with $bias_{PT,rel,i}$ for all PT samples:

For the PT matrices, fish oil, pork, egg yolk powder and beef the ratio $\lvert u_{Cref,rel,I} / bias_{PT,rel,i} \rvert$ is below 0.3, for whole egg and milk powder it is above.

Comparison of $u_{Cref,rel,i}$ with $bias_{PT,rel,i}$ for the PT samples whole egg and milk powder:

The comparison with a given fitness-for-purpose-based "standard deviation for proficiency assessment" $\sigma_{p,rel}$ of 10 % shows that for both PT matrices $u_{Cref,rel,i} \leq 0.3 \cdot \sigma_{p,rel}$. Therefore all PT results can be included in the evaluation.

Estimation of $u_{bias,PT}$:

$RMS_{bias,PT}$ for the six PT matrices is calculated according to equation 17:

$$RMS_{bias,PT} = \sqrt{\frac{(0.11)^2+(-0.19)^2+(0.04)^2+(0.13)^2+(-0.06)^2+(-0.09)^2}{6}} = 0.11\ (= 11\%)$$

The average uncertainty of the assigned value u_{Cref} is calculated according to equation 18:

$$u_{Cref} = \frac{0.014+0.018+0.021+0.020+0.023+0.019}{6} = 0.019\ (= 1.9\ \%)$$

The bias contribution $u_{bias,PT}$ of results from six proficiency test matrices is then given by equation 16:

$$u_{bias,PT} = \sqrt{0.11^2 + 0.019^2} = 0.11\ (= 11\%)$$

C.2.2 Individual congeners

The bias contribution to MU for individual congeners is calculated in the same way as for the sum parameters (see C.2.1). As assigned values are not necessarily provided for all relevant congeners in every PT test sample, results of more than six PT test samples may be necessary to get the required number of six PT results for each congener.

C.3 Evaluation of the bias contribution to MU from fortified samples

When performing fortification experiments, e.g. within a bias study, the fortification procedure is associated with an uncertainty u_{fort}. Both the uncertainty of the concentration of the fortification solution u_{conc} and that of the volume u_{vol} contribute to the uncertainty of the fortification procedure u_{fort} (equation 20):

$$u_{fort} = \sqrt{u_{conc}^2 + u_{vol}^2}$$

The uncertainty of the *concentration* of the standard solution u_{conc} is obtained directly from the manufacturer's certificate. In this example, the latter states that the standard solution contains 50.0 ng 2.3.7.8-TCDD/mL with an uncertainty of ± 0.6 ng/mL at a 95% confidence level. From 0.6 ng/mL, corresponding to 1.2%, the standard uncertainty of the concentration is calculated as:

$$u_{conc} = \pm\left(\frac{1.2}{2}\right)\% = \pm 0.61\%$$

The uncertainty of the dosed *volume* u_{vol} is estimated based on the micropipette manufacturer's specifications: the maximum random error (repeatability) is s_r = 0.5% and the maximum systematic error (bias) is 1.0%:

$$u_{vol} = \sqrt{u_{r,vol}^2 + u_{bias,vol}^2}$$

While $s_{r,vol} = u_{r,vol}$ = 0.5%, the maximum systematic error is converted to a standard deviation $s_{bias} = u_{bias}$ by assuming a rectangular distribution:

$$u_{bias,vol} = \pm\left(\frac{1.0}{\sqrt{3}}\right)\% = \pm 0.58\%$$

NOTE: For conversion of the bias contribution of the pipetted volume to uncertainty, a rectangular distribution is assumed according to QUAM:2012.P1, Appendix E1 "Distribution functions" [EURACHEM/CITAC 2012, /12/], and according to examples in the relevant literature [Nordtest 2012, /3/; ISO 11352:2012-07 or DIN ISO 11352:2013-03, /4/; EURACHEM/CITAC 2012, /12/]. However, a triangular distribution may also be assumed in cases when justified from laboratory experience, e.g. when it is known that the distribution is symmetrical and values close to the mean are more likely than near the bounds.

Finally, random and systematic errors are combined:

$$u_{vol} = \sqrt{0.5\%^2 + 0.58\%^2} = 0.77\%$$

and the fortification procedure uncertainty is calculated as:

$$u_{fort} = \sqrt{u_{conc}^2 + u_{vol}^2} = \sqrt{0.61\%^2 + 0.77\%^2} = 0.98\%$$

As seen from table C.3-1, $RMS_{bias,fort}$ is calculated from the bias values from n=6 fortification experiments as

$$RMS_{bias,fort} = \sqrt{\frac{\sum(bias_{fort,rel,i})^2}{n}}$$

and the bias contribution to MU is finally calculated as

$$u_{bias,fort,rel} = \sqrt{RMS_{bias,fort}^2 + u_{fort}^2}$$

Table C.3-1: Fortification procedure uncertainty, exemplary results from n=6 fortification experiments and calculation of the bias contribution to MU (only 4 digits shown, data not rounded)

n	u_{fort}	u_{fort}^2	*recovery*	$bias_{fort}$	$bias_{fort}^2$	RMS_{bias}	RMS_{bias}^2	u_{bias}
1	0.9789	0.9582	97.4857	2.5143	6.3217			
2	0.9789	0.9582	97.8419	2.1581	4.6574			
3	0.9789	0.9582	97.0876	2.9124	8.4821			
4	0.9789	0.9582	97.2644	2.7356	7.4835			
5	0.9789	0.9582	97.1936	2.8064	7.8759			
6	0.9789	0.9582	96.8869	3.1131	9.6914			
mean					7.4187	2.7237	7.4187	**2.8943**

As an example, results for the fortification of a feed sample are summarized in table C.3-2. The feed sample is fortified with a standard solution containing all 17 unlabelled 2,3,7,8-substituted PCDD/F congeners (standard uncertainty of ± 5 %, level of confidence of 95 %). The manufacturer's specifications of the applied micropipette include a maximum random error of 0.4% and a maximum systematic error of 0.6%. The fortified samples was analysed six times.

Table C.3-2: Bias contribution for individual PCDD/F congeners of fortification of a feed sample

Fortified sample	***Conc. in sample***	u_{conc}	u_{vol}	u_{fort}	$RMS_{bias,fort}$	$u_{bias,fort}$
Congeners	ng/kg product (12 % moisture content)					
2,3,7,8-TCDD	0.10	2.5 %	0.72 %	2.6 %	5.7%	6.3%
1,2,3,7,8-PeCDD	0.50	2.5 %	0.72 %	2.6 %	2.9%	3.9%
1,2,3,4,7,8-HxCDD	0.50	2.5 %	0.72 %	2.6 %	15.2%	15.4%
1,2,3,6,7,8-HxCDD	0.50	2.5 %	0.72 %	2.6 %	13.8%	14.0%
1,2,3,7,8,9-HxCDD	0.50	2.5 %	0.72 %	2.6 %	5.2%	5.8%
1,2,3,4,6,7,8-HpCDD	0.50	2.5 %	0.72 %	2.6 %	6.6%	7.1%
OCDD	1.0	2.5 %	0.72 %	2.6 %	4.0%	4.8%
2,3,7,8-TCDF	0.10	2.5 %	0.72 %	2.6 %	9.4%	9.8%
1,2,3,7,8-PeCDF	0.50	2.5 %	0.72 %	2.6 %	5.0%	5.6%
2,3,4,7,8-PeCDF	0.50	2.5 %	0.72 %	2.6 %	8.1%	8.5%
1,2,3,4,7,8-HxCDF	0.50	2.5 %	0.72 %	2.6 %	6.1%	6.6%
1,2,3,6,7,8-HxCDF	0.50	2.5 %	0.72 %	2.6 %	4.9%	5.5%
1,2,3,7,8,9-HxCDF	0.50	2.5 %	0.72 %	2.6 %	4.9%	5.5%
2,3,4,6,7,8-HxCDF	0.50	2.5 %	0.72 %	2.6 %	15.8%	16.0%
1,2,3,4,6,7,8-HpCDF	0.50	2.5 %	0.72 %	2.6 %	5.5%	6.1%
1,2,3,4,7,8,9-HpCDF	0.50	2.5 %	0.72 %	2.6 %	9.0%	9.4%
OCDF	1.0	2.5 %	0.72 %	2.6 %	14.9%	15.1%
WHO-PCDD/F-TEQ	**1.14**					

C.4 Requirements for the uncertainty component of the assigned value in PTs

If the assigned value from a PT exercise is calculated from the consensus mean of the participants, its uncertainty is:

$$u_{Cref,i} = \frac{s_{R,i}}{\sqrt{n_{P,i}}} \qquad \textit{Eq. C.1}$$

$u_{Cref,i}$ = uncertainty of the assigned value calculated for sample i

$s_{R,i}$ = reproducibility standard deviation among laboratories contributing to calculation of the assigned value for sample i

$n_{P,i}$ = number of participating laboratories contributing to calculation of the assigned value for sample i

If the median or a robust estimation method was used to calculate the mean, the uncertainty of the assigned value is [ISO 13528:2015-08, /11/]:

$$u_{Cref,i} = 1.25 \cdot \frac{s_{R,i}}{\sqrt{n_{P,i}}} \qquad \textit{Eq. C.2}$$

If other methods were used to determine the assigned values, the PT provider has to be asked for the respective uncertainty.

Estimating the assigned value as the consensus of participants' results

The consensus value of the participant's results is widely used for determination of the assigned value. Results generated by the majority of participants are assumed to be unbiased and their dispersion generally has an easily identifiable mode. To derive a most probable value for the measurand (assigned value) the central tendency of the results, represented, (outliers aside) e.g. by the mode, the median, or a robust mean is evaluated together with its standard error being used as an estimate of its uncertainty. Consensus values are often very close to reliable reference values provided by formulation (addition of a known amount of analyte), expert laboratory consensus, and by reference values from CRMs or from reference laboratories [IUPAC 2006, /15/].

Main disadvantages of participant consensus values are:

- they are dependent on the participants' methods and results, and
- their uncertainty may be too large if the number of laboratories is small.

The lack of independence has two potential effects [IUPAC 2006, /15/]:

- the bias for the population as a whole may not easily be detected, as the assigned value will follow the population;
- if the majority of results are biased, participants whose results are unbiased may unfairly receive extreme z-scores.

Criteria for u_{Cref} associated with the assigned value from PTs

Moreover, the assigned value may, in principle, be defined by the method used. During a PT, a variety of analytical methods may be applied by the participants some of which produce more or less biased results. This might lead to an undesirably wide overall distribution of the results and thus a comparably large uncertainty u_{Cref} of the assigned value x_a. u_{Cref} is therefore compared with the PT-specific fitness-for-purpose-based "standard deviation for proficiency assessment" σ_p. If $u_{Cref} \leq 0.3 \cdot \sigma_p$, then the standard uncertainty u_{Cref} of the assigned value is negligible and does not need to be included in the interpretation of the results of the proficiency test [EURACHEM 2011, /16/].

When used for MU assessment in participating laboratories, the assigned value together with its uncertainty may lead to an unduly high contribution to MU, making it desirable to define a maximum acceptable value for u_{Cref}.

If there is an uncertainty u_{Cref} in the assigned value x_a to be used for uncertainty assessment, the uncertainty component for the bias u_{bias} is calculated as:

$$u_{bias,PT,rel} = \sqrt{RMS_{bias,PT}^2 + u_{Cref,rel}^2} \qquad \textbf{Eq. C.3}$$

$$RMS_{bias,PT} = \sqrt{\frac{\sum(bias_{PT,rel,i})^2}{n}} \qquad \textbf{Eq. C.4}$$

$$u_{Cref,rel} = mean(u_{Cref,rel,i}) \qquad \textbf{Eq. C.5}$$

n = number of samples *(n = 1, 2, ... i)* from interlaboratory studies

If, for example, u_{Cref} does not exceed $0.3 \cdot RMS_{bias}$, the maximum resulting dilation factor for u_{bias} would be as small as 1.04:

$$u_{bias} = \sqrt{(0.3 \cdot RMS_{bias})^2 + RMS_{bias}^2} = \sqrt{1.09 \cdot RMS_{bias}^2} = 1.04 \cdot RMS_{bias} \qquad \textbf{Eq. C.6}$$

Therefore, if the assigned value and its uncertainty are used to assess MU of an individual laboratory, it seems acceptable to require that u_{Cref} shall not exceed 30% of $RMS_{bias,PT}$:

$$\frac{u_{Cref}}{RMS_{bias,PT}} \leq 0.3 \qquad \textbf{\textit{Eq. C.7}}$$

For assessment of the results of a single sample i of an interlaboratory study equation C.7 is adapted as:

$$\left|\frac{u_{Cref,rel,i}}{bias_{PT,rel,i}}\right| \leq 0.3 \qquad \textbf{\textit{Eq. C.8}}$$

$u_{Cref,rel,i}$: uncertainty of the assigned value calculated for sample i

$bias_{PT,rel,i}$: relative basis calculated for sample i

For some analytes $bias_{PT,rel,i}$ may be smaller than $\sigma_{p,rel}$ and therefore in the range of $u_{Cref,rel,i}$. As a consequence the ratio of $u_{Cref,rel,i}$ and $bias_{PT,rel,i}$ may be above 0.3.

In these cases a higher contribution of $u_{Cref,rel,i}$ might also be acceptable provided that $u_{Cref,rel,i} \leq 0.3 \cdot \sigma_{p,rel}$.

$\sigma_{p,rel}$: fitness-for-purpose-based "standard deviation for proficiency assessment" expressed as relative standard deviation

Annex D – Evaluation of contributions from current performance

D.1 Evaluation of contributions to MU from LOQs and procedural blanks

Contributions from LOQs and procedural blanks to MU are calculated by combining the combined standard uncertainty $u_{c,i}$ with the actual limit of quantification (LOQ) of the respective congener in the sample or the procedural blank of the relevant sample batch (equations 25 and 26).

Table D.1-1: Calculation of MU from the combined standard uncertainty $u_{c,i}$ and contributions of LOQ and procedural blank

Beef sample	$u_{c,i}$	**Concentration** x_i	LOQ_i **from sample**	LOQ_i **from procedural blank**	$u_{c,i(LOQ)}$	$U_{i(LOQ)}$
Congener		pg/g fat	pg/g fat	pg/g fat		
2,3,7,8-TCDD	9%	0.11	0.04	0.06	55%	110%
1,2,3,7,8-PeCDD	8%	0.44	0.07	0.06	18%	36%
1,2,3,4,7,8-HxCDD	15%	0.53	0.01	0.03	16%	32%
1,2,3,6,7,8-HxCDD	7%	1.30	0.06	0.05	8%	16%
1,2,3,7,8,9-HxCDD	9%	0.29	0.03	0.04	16%	32%
1,2,3,4,6,7,8-HpCDD	19%	4.81	0.08	0.18	19%	38%
OCDD	19%	5.43	0.25	0.90	25%	50%
2,3,7,8-TCDF	5%	0.41	0.05	0.09	23%	46%
1,2,3,7,8-PeCDF	7%	0.28	0.06	0.04	23%	46%
2,3,4,7,8-PeCDF	8%	1.27	0.12	0.07	12%	24%
1,2,3,4,7,8-HxCDF	7%	0.43	0.06	0.03	16%	32%
1,2,3,6,7,8-HxCDF	6%	0.57	0.05	0.02	11%	22%
1,2,3,7,8,9-HxCDF	16%	0.42	0.02	0.03	18%	36%
2,3,4,6,7,8-HxCDF	5%	< 0.02	0.02	0.03	150%	300%
1,2,3,4,6,7,8-HpCDF	10%	0.65	0.02	0.05	13%	26%

Beef sample	$u_{c,i}$	**Concentration x_i**	LOQ_i **from sample**	LOQ_i **from procedural blank**	$u_{c,i(LOQ)}$	$U_{i(LOQ)}$
1,2,3,4,7,8,9-HpCDF	20%	< 0.05	0.05	0.04	102%	204%
OCDF	23%	0.12	0.08	0.11	95%	190%
WHO-PCDD/F-TEQ					**8 %**	**16 %**

The concentrations of the individual congeners in the beef sample are compared with LOQs individually calculated for this sample (based on signal-to-noise ratio or on calibration) and the LOQs or levels expressed as LOQ from the associated procedural blanks. The respective higher LOQs are used for calculation of the combined uncertainty $u_{c,i(LOQ)}$.

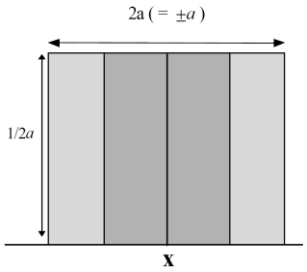
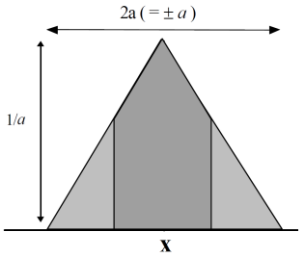
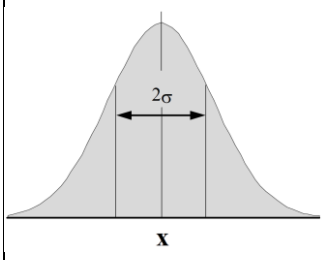
The combined uncertainty for the sum parameter WHO-PCDD/F-TEQ is calculated from the combined uncertainties of the individual congeners according to equation 38.

Annex E – Conversion of Specifications to Standard Uncertainties

E.1 Standard uncertainties from assumed distributions

Sources of uncertainty that influence the measurement process but cannot be assessed by statistical evaluation require alternative strategies. So-called Type B estimates of uncertainty [GUM 2008, /1/] are often based on information given in different forms, e.g. in the form of limits or confidence intervals (Table E.1-1).

Table E.1-1: Calculation of a standard uncertainty from the parameters of the most important distribution functions [EURACHEM/CITAC 2012, /12/].

Probability distribution	**Form**	**Use when:**	***Uncertainty***
Rectangular distribution		– A certificate or specification gives limits without specifying a level of confidence (e.g. 25 mL ± 0.05 mL). – An estimate is made in the form of a maximum range (± a) with no knowledge of the shape of the distribution.	$u = \frac{1}{\sqrt{3}} a$
Triangular distribution		– Values close to x are more likely than near the bounds. – An estimate is made in the form of a maximum range (±a) described by a symmetric distribution.	$u = \frac{1}{\sqrt{6}} a$
Normal distribution		– An estimate is made from repeated observations of a randomly varying process. – An uncertainty is given in the form of a standard deviation s, a relative standard deviation s/x , or a percentage coefficient of variance % CV, without specifying the distribution. – An uncertainty is given in the form of a 95.4 % (or 99.7%) confidence interval x±a without specifying the distribution.	$u = s$ $u = s$ $u = x \cdot s/\bar{x}$ $u = \frac{\%CV}{100} \cdot x$ $u = \frac{a}{2}$ for a at 95.4% $u = \frac{a}{3}$ for a at 99.7%

E.2 Practical examples

Example 1: Standard uncertainty estimation from a calibration certificate

The calibration certificate for an **instrument** states the measurement uncertainty over its range of calibration as ±0.1% at a 95% confidence level. The latter can be assumed to be equivalent to the standard uncertainty *u* being expressed with a coverage factor of k ≈ 2:

$$u = \pm\left(\frac{0.1}{2}\right)\ \% = \pm 0.05\ \%\ \text{ of the reading.}$$

Example 2: Standard uncertainty estimation from the certified value of a CRM

Uncertainties of **CRMs** are usually expressed as expanded uncertainties *U*. It is important to know how U was calculated so that it can be converted back to a standard uncertainty *u*:

$$u = \frac{U}{k}$$

Example 3: Standard uncertainty estimation from a manufacturer's specification

The manufacturing tolerance of a 25 mL Class A glass **pipette** is ±0.03 mL without indication of the distribution or the level of confidence. We may assume that values could occur anywhere within the tolerance range with equal probability (rectangular distribution):

$$u = \pm\left(\frac{0.03}{\sqrt{3}}\right)\ mL = \pm 0.017\ mL$$

If additional information is available leading us to the conclusion that values closer to the centre are more likely than values at its extremes, a triangular distribution may be assumed:

$$u = \pm\left(\frac{0.03}{\sqrt{6}}\right)\ mL = \pm 0.012\ mL$$

Example 4: Standard uncertainty estimation from the purity of a compound

The **purity** of a compound is given by the supplier as 99.9% ±0.1%, without any indication of the distribution or the level of confidence. We may assume that values could occur anywhere within the tolerance range with equal probability, and thus a rectangular distribution:

$$u = \pm\left(\frac{0.1}{\sqrt{3}}\right)\ \% = \pm 0.06\ \%$$

Example 5: Standard uncertainty estimation from the certificate of a standard solution

A certified **standard solution** has a TCDD content of 10 ng/mL with a 95% confidence interval of ±0.2 ng/mL. The standard uncertainty is calculated as

$$u = \pm\left(\frac{0.2}{2}\right)\ ng/mL = \pm 0.1\ ng/mL$$

Table E.2-1: Conversion of manufacturer's specifications to standard uncertainties

	Information	**Standard uncertainty**
Certificate	Interval, with 95% confidence level	value / 2
	Interval / tolerance, with "2s"	value / 2
	Interval / tolerance, with "±a"	value / √3
	Purity, with impurity value	value / √3
Tolerance*	Maximum random error (s_r)	value
	Maximum bias	value / √3
Reading accuracy**	Scale mark	value / √3
	Scale mark, additional information	value / √6
	Interval, with 95% confidence level	value / 2

* e.g. for a microliter pipette

** for an instrument, e.g. volume measuring device, analytical balance, thermometer

Annex F – Combined Uncertainty in TEQ from Individual Congeners

Four different approaches were compared based on authentic quality control data obtained from several of the author's laboratories:

1. The square Root of the Sum of Squares (RSS)

$$u_c(TEQ = TEF_1 \cdot u_{c1} + \cdots + TEF_{29} \cdot u_{c29}) = \sqrt{\sum_{i=1}^{29}(TEF_i \cdot u_{ci})^2} \quad \textbf{Eq. F.1}$$

2. The SUM

$$u_c(TEQ = TEF_1 \cdot u_{c1} + \ldots + TEF_{29} \cdot u_{c29}) = \sum_{i=1}^{29}(TEF_i \cdot u_{ci}) \quad \textbf{Eq. F.2}$$

3. The MEAN of $(TEF_i \cdot u_{ci})_{i=congener}$

4. The MEDIAN of $(TEF_i \cdot u_{ci})_{i=congener}$

Evaluation of the formula for approaches 1 and 2 is given below:

A general equation for uncertainty propagation is given by equation F.3 where a covariance term appears in the second term of the square root [GUM 2008, /1/] reflecting the degree of dependence between the variables (i.e the congener concentration):

$$u_c(y) = \sqrt{\sum_{i=1}^{N} c_i^2 u^2(x_i) + 2\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} c_i c_j u(x_i) u(x_j) r(x_i, x_j)}\ , \quad \textbf{Eq. F.3}$$

with a correlation coefficient:

$$r(x_i, x_j) = \frac{u(x_i, x_j)}{u(x_i)u(x_j)} \quad \text{where } -1 \le r(x_i,x_j) \le 1 \quad \textbf{Eq. F.4}$$

From equation F.3, three cases may, in principle, be distinguished, in which *r* takes the values 0, +1, or -1.

If the variables are considered *independent* (*r = 0*) the combined uncertainty can be calculated as the root of the sum of squares (RSS), applying the following rules:

$$u_c(x_1 \pm x_2) = \sqrt{u^2(x_1) + u^2(x_2)} \quad \textbf{Eq. F.5}$$

$$u_c(x_1 x_2) = (x_1 x_2)\sqrt{\frac{u^2(x_1)}{x_1^2} + \frac{u^2(x_2)}{x_2^2}} \quad \textbf{Eq. F.6}$$

$$u_c\left(\frac{x_1}{x_2}\right) = \left(\frac{x_2}{x_1}\right)\sqrt{\frac{u^2(x_1)}{x_1^2} + \frac{u^2(x_2)}{x_2^2}} \qquad \textbf{\textit{Eq. F.7}}$$

When the variables are assumed to be *highly positively correlated* (*r = +1*), equation F.3 may again be simplified to yield a linear combination of uncertainties (SUM approach).

$$u_c(y) = \sum_{i=1}^{N}[c_i u(x_i)] \qquad \textbf{\textit{Eq. F.8}}$$

Thirteen different matrices corresponding to more than 16 000 individual congener-based results were taken into account. The levels are within the working range including concentrations below and above the maximum limits.

The TEQ-based standard uncertainties u_c, given as empirical standard deviations (in TEQs), are plotted against the TEQ-based standard uncertainties calculated from each congener according to the four approaches mentioned above. The figures below show that in the MEDIAN and MEAN approaches, calculated TEQ-values clearly underestimate the empirical TEQ-values for PCDD/Fs (figure F-1), for DL-PCBs (figure F-2) and for the sum of PCDD/Fs and DL-PCBs (figure F-3).

The SUM approach overestimates the empirical values for the three parameters, while the RSS approach underestimates the experimental standard deviations only slightly for DL-PCBs (figure F-2) and for the sum of PCDD/Fs and DL-PCBs (figure F-3), although it is more pronounced for PCDD/Fs (figure F-1), as can be seen by comparing the different slopes of regression lines.

In the RSS approach the calculated data fit best with empirical data.

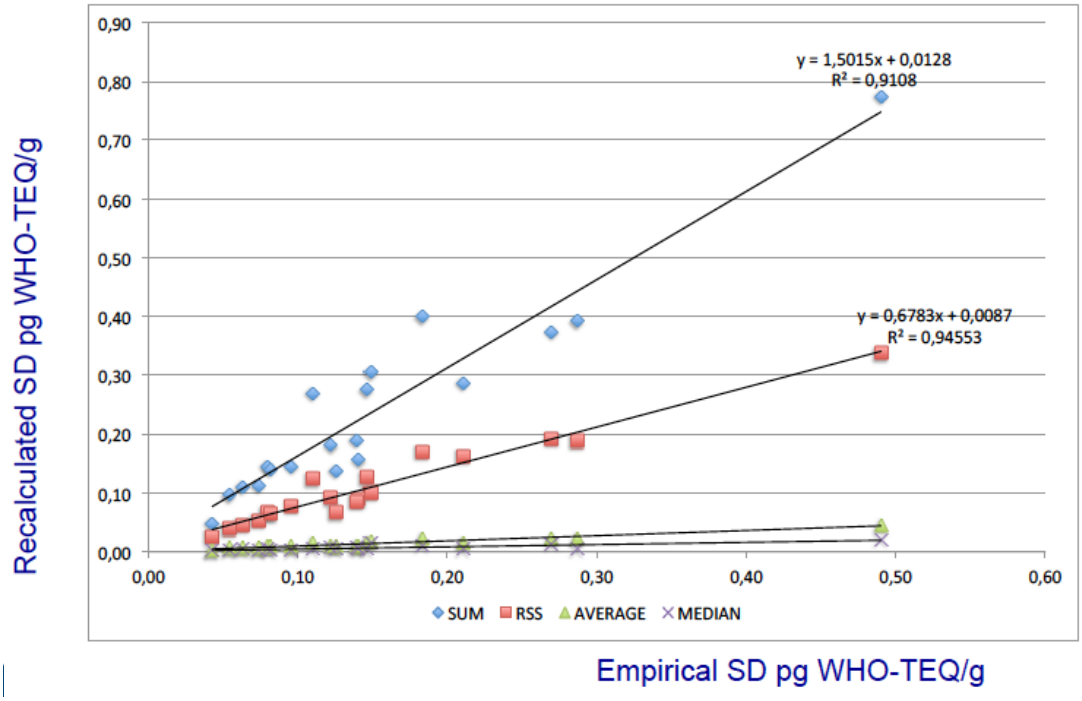


Figure F-1: Empirical SD in TEQ versus recalculated SD in TEQ by four different approaches for PCDD/Fs

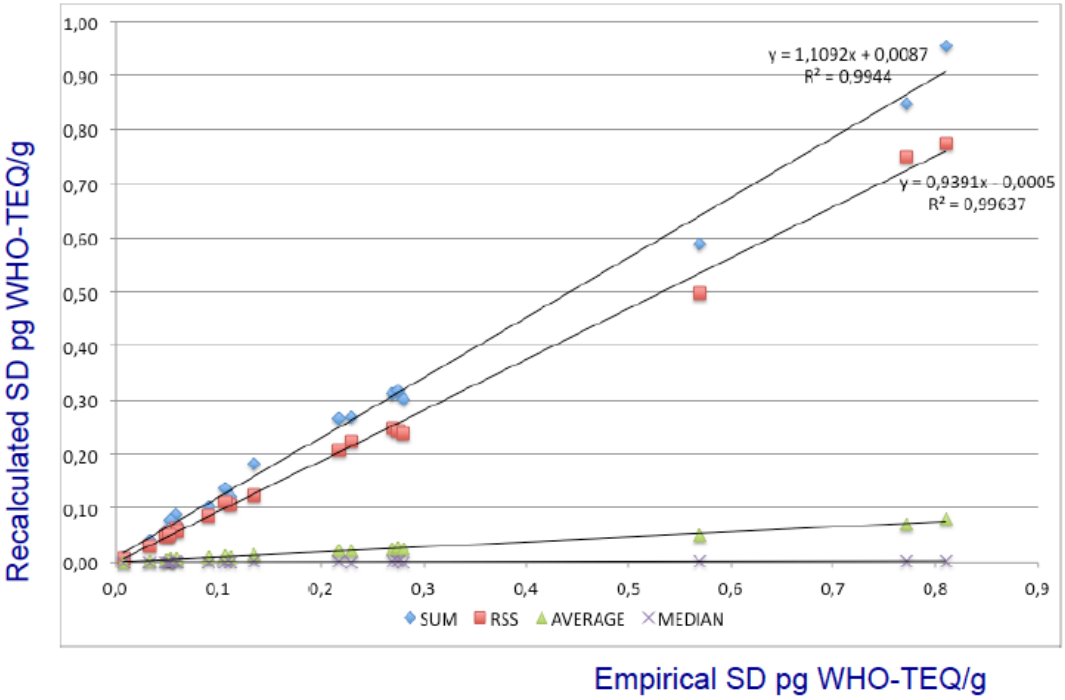


Figure F-2: Empirical SD in TEQ versus recalculated SD in TEQ by four different approaches for DL-PCBs

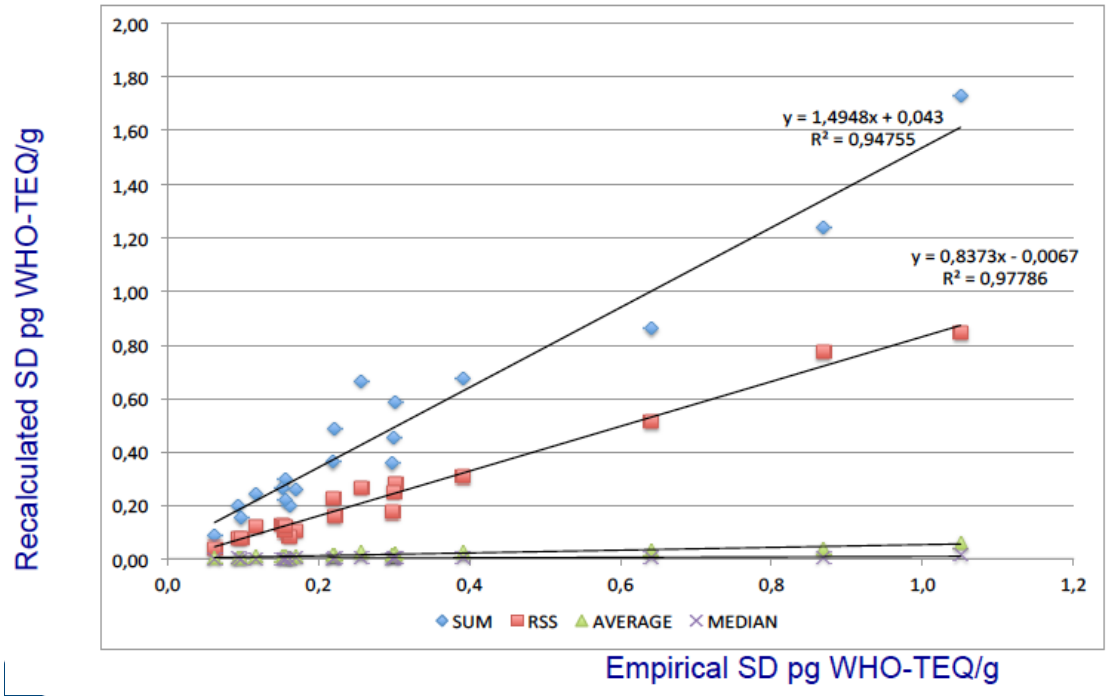


Figure F-3: Empirical SD in TEQ versus recalculated SD in TEQ by four different approaches for the SUM of PCDD/Fs and DL-PCBs

Annex G – Evaluation of Participant's Performance: z- and zeta-Scores

Participants' z-scores and zeta-scores can serve as a tool to check laboratory performance in a proficiency test and the validity of the reported measurement uncertainty. Z-scores and zeta-scores are calculated according to Equation 39 and 40.

Table G-1 shows the assigned value for an analyte, the applied standard deviation for proficiency assessment that is used for calculation of the z-scores and the relative and absolute uncertainty of the assigned value. Table G-2 summarizes results of three participants (A, B, C) for reported values, expanded uncertainty U, the relative and absolute standard uncertainty u and calculated z-scores and zeta-scores.

For participant A, the z-score of -1.0 shows satisfactory performance, whereas the zeta-score of -4.0 – reflecting a possible underestimation of the measurement uncertainty – should be considered as an action signal indicating that further refinement of reported the measurement uncertainty may be required. For participant B, z-scores and zeta-scores are within the acceptable range indicating satisfactory performance and suitable measurement uncertainty representing laboratory performance.

A higher measurement uncertainty as reported by participant C can result in acceptable zeta-scores, but too high z-scores. In this case the high measurement uncertainty possibly reflects the performance of the applied method, but the method does not meet the performance criteria and therefore needs to be adjusted.

In case "action signals" or "warning signals" are obtained for z-scores and/or zeta-scores in successive proficiency tests, further investigation is required.

Table G-1: Assigned value

Assigned value x_a	**Standard deviation for proficiency assessment σ_p**		**Uncertainty of assigned value u_{Cref}**	
pg/g	%	pg/g	%	pg/g
10	20	2.0	3.0	0.30

Table G-2: Participants' results

		Participant A	**Participant B**	**Participant C**
Reported value x	pg/g	8.0	12	16
Expanded uncertainty U (coverage factor k = 2)	%	10	20	40
Standard uncertainty u	%	5.0	10	20
Standard uncertainty u	pg/g	0.40	1.2	3.2
Z-score		-1.0	1.0	3.0
Zeta-score		-4.0	1.6	1.9

Annex H – Measurement Uncertainty: Semi-empirical Approach

H.1 Description of applied method of analysis

The following example is related to the quantification of the uncertainty budget for WHO-PCDD/F-TEQ and WHO-PCB-TEQ based on 17 2,3,7,8-substituted PCDD/F and 12 DL-PCB congeners in milk samples. A general analytical procedure is shown schematically in figure H-1.1.

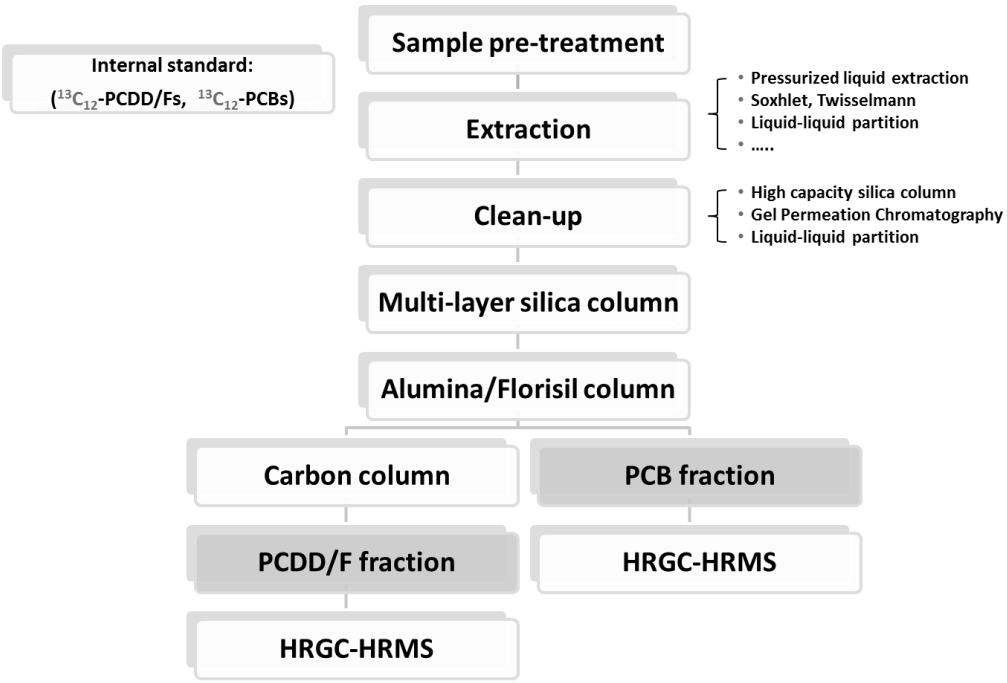


Figure H.1-1: Scheme of an analytical procedure for determination of PCDD/Fs and PCBs in food samples

Sample pre-treatment

Milk sample is homogenized and an aliquot is placed in a separation funnel. A known amount of labelled congeners is added to the sample aliquot before the extraction step.

Extraction

Extraction is performed by a liquid-liquid partitioning process in a separation funnel and the lipid content is determined gravimetrically after evaporation of organic solvents.

Clean-up

The sample clean-up procedure combines two different methods: a direct treatment of the sample extract with sulphuric acid, and then with potassium hydroxide aqueous solution followed by an automated clean-up method using disposable columns (multilayer silica, alumina and carbon). The PCB fraction is collected after elution from the alumina column, while the fraction containing PCDD/Fs is eluted and collected from the carbon column. The two fractions are evaporated to dryness and re-dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labelled congeners).

Instrumental analysis and quantification

PCDD/Fs and PCBs are separated by high resolution gas chromatography (HRGC) on a capillary column and determined by high resolution mass spectrometry (HRMS).

Calibration of the analytical instrument is performed by analysis of five standard solutions containing the target compounds at different concentrations.

The content of each congener in the sample is calculated on a fat basis according to equation 27:

$$C_i = \frac{A_{12C,i} \cdot C_{13C,i} \cdot V_{13C,i}}{A_{13C,i} \cdot RRF_i \cdot m_{sample}}$$

where:

C_i: concentration of the congener *i* (pg/g)

$A_{12C,i}$: peak area of native congener *i*

$A_{13C,i}$: peak area of labelled congener *i*

$C_{13C,i}$: concentration of the labelled congener *i* (pg/µL)

$V_{13C,i}$: spiked volume of the labelled congener *i* (µL)

RRF_i: relative response factor of congener *i*

m_{sample}: weight of sample aliquot (g)

H.2 Estimation of measurement uncertainty

The identification of all relevant uncertainty sources for such a complex analytical procedure is done in accordance with chapter 8, considering precision data, recovery data and other parameters not sufficiently covered by these two estimates.

In this example, all standards are purchased as certified solutions. The same calibration curve is used throughout the intermediate precision study and, in addition, a calibration verification procedure is performed for each analytical batch using an independent calibration verification standard. Finally, commercial "ready to use" standard solutions are used to calculate the Relative Response Factors (RRFs).

As an example, only the congener 1,2,3,7,8-PeCDD is described in detail, and the same procedure is then applied to the rest of PCDD/F and DL-PCB congeners.

H.2.1 Intermediate precision uncertainty

The uncertainty associated with the intermediate precision (u_{Rw}) is calculated as the standard deviation of *n* test results obtained from the precision study during method validation.

A milk sample with a low level of contamination is fortified with a mixture of PCDD/F congeners at three different concentration levels (corresponding to 1.1 – 2.3 – 3.4 pg WHO-TEQ/g fat). Six replicate samples are prepared at each level for a total of eighteen test samples. These samples are analysed under intermediate precision conditions. Analytical results are expressed as concentration and recovery percentage with respect to the fortified amount of native congeners. The mean value and standard deviation for each level are then calculated. Homoscedasticity (homogeneity of variances) is verified over the concentration range of interest.

Therefore, all recovery results are pooled and a single relative standard deviation value is calculated using a pooled relative intermediate standard deviation $s_{Rw,pool,rel}$ according to equations 7 and 8 reported in chapter 6.1.

The relative intermediate precision contribution to uncertainty $u_{Rw,rel}$ may be calculated as $u_{Rw,rel} = s_{Rw,pool,rel}$

If there is no evidence to indicate that the uncertainties at different levels are comparable, a separate uncertainty estimates for each level would be required.

As an example, the results obtained for 1,2,3,7,8-PeCDD are summarized in Table H.2.1-1.

Table H.2.1-1: Intermediate precision data for 1,2,3,7,8-PeCDD from validation study

Fortification level	Fortification concentration pg/g fat	Concentration pg/g	Recovery (%)
1	0.5	0.567	113
		0.466	93.2
		0.402	80.4
		0.537	107
		0.528	106
		0.445	89.0
2	1.0	0.921	92.1
		0.954	95.4
		0.795	79.5
		1.03	103
		1.11	110
		0.811	81.1
3	1.5	1.47	98.1
		1.56	104
		1.58	105
		1.41	94.1
		1.31	87.5
		1.58	105
Relative intermediate precision uncertainty ($u_{Rw,rel}$)		**0.114**	**0.114**

H.2.2 Bias uncertainty

In accordance with chapter 8.4, the contribution of bias related to the analytical method (extraction and clean-up) is quantified using recovery data obtained during the in-house validation study. Starting from recovery data reported in Table H.2.1-1, the mean recovery value for each fortification level is used to calculate $bias_{fort}$. Then, the values of $bias_{fort}$ are summed up to calculate $RMS_{bias,mean}$ according to the following formula:

$$RMS_{bias,mean} = \sqrt{\frac{\sum(bias_{fort,rel,i})^2}{n}} = \sqrt{\frac{(0.0183)^2+(0.0632)^2+(0.0100)^2}{3}} = 0.0384$$

where n is the number of fortification levels.

Since the recovery study has been performed by adding an aliquot of a known solution of the analyte, the uncertainty associated with the fortification solution also has to be calculated, considering the uncertainty related to the analyte concentration (u_{conc}) and the uncertainty related to the added volume (u_{vol}):

$$u_{fort} = \sqrt{u_{conc}^2 + u_{vol}^2} = \sqrt{(0.025)^2 + (0.0077)^2} = 0.0262$$

Calculations details are reported in points H.2.5 and H.2.6 of this annex.

The $u_{bias,rel}$ value is finally obtained:

$$u_{bias,rel} = \sqrt{RMS_{bias,mean}^2 + u_{fort}^2} = \sqrt{(0.0384)^2 + (0.0262)^2} = 0.0465$$

H.2.3 Calibration curve uncertainty

Full calibration (Option 1)

If a full calibration is performed for each analytical batch, the standard deviation of the mean RRF of a congener represents the uncertainty contribution related to calibration (see paragraph 8.5.1).

The amount of 1,2,3,7,8-PeCDD is calculated using a ready to use multi-level calibration curve. For this purpose, five calibration standards (0.1 – 0.5 – 2.0 – 10 - 50 ng/mL) are injected and RRF values, average RRFs and relative standard deviations are calculated.

The calibration curve linearity uncertainty component (u_{cal}) relies on the variation of RRFs among the five points of the calibration curve. This uncertainty component is calculated as the standard deviation of RRFs divided by the square root of the number of calibration points.

In this example, an experimental value of the standard deviation of RRF for 1,2,3,7,8-PeCDD equal to 9.05 % is calculated, the corresponding uncertainty is:

$$u_{cal,rel} = \frac{s_{RRF,rel}}{\sqrt{n}} = \frac{0.0905}{\sqrt{5}} = 0.0405$$

Calibration point check (Option 2)

The calibration curve drift uncertainty component (u_d) has to be taken into account only when a calibration verification procedure is adopted by the laboratory (see paragraph 8.5.2).

For a batch of samples, a calibration standard is periodically analysed to ensure that the instrument response does not drift significantly. According to the in-house method, if a drift above 15% is observed, then a new complete calibration is necessary and the samples are re-analysed. A term representing the uncertainty due to this maximum permitted drift also needs to be included in the budget.

Since there is no evidence of lower probability towards the extremes of the acceptable values range this can be treated as a rectangular distribution and divided by root square of 3 to obtain the standard uncertainty associated with instrument drift.

The example shows the calibration drift uncertainty calculated for 1,2,3,7,8 PeCDD using a maximum acceptable value of 15% and considering a rectangular distribution:

$$u_{d,rel} = \frac{0.15}{\sqrt{3}} = 0.0866$$

H.2.4 Volume uncertainty

The volume uncertainty (u_v) is related to the glassware (e.g. volumetric flasks, cylinders, pipettes) and micropipettes used to prepare standard solutions and for internal standard addition to the sample.

In the following example, a volume of 250 µL of labelled compound solution is diluted to 10 mL to prepare an internal standard solution at a concentration of 5 ppb. A 10 mL volumetric flask and a 250 µL variable volume micropipette are used.

For the volumetric flask, the certificate of the manufacturer gives a tolerance of ± 0.04 mL.

The corresponding uncertainty is calculated assuming a rectangular distribution expecting that all values in the range are equally likely:

$$u_{v1} = \frac{40}{\sqrt{3}} = 23.1$$

The relative standard uncertainty is: $u_{v1,rel} = \frac{23.1}{10000} = 0.00231$

From the micropipette calibration certificate, a maximum value for systematic error of ± 2 µL is deduced. The corresponding uncertainty is calculated assuming a rectangular distribution:

$$u_{v2} = \frac{2}{\sqrt{3}} = 1.15$$

The relative standard uncertainty is:

$$u_{v2,rel} = \frac{1.15}{250} = 0.00460$$

Finally, a 10-100 µL variable volume micropipette is used to add 40 µL of internal standard solution to the sample. From the micropipette calibration certificate, a maximum value for systematic error of ± 0.8 µL is deduced. The corresponding uncertainty is calculated assuming a rectangular distribution:

$$u_{v3} = \frac{0.8}{\sqrt{3}} = 0.462$$

The relative standard uncertainty is:

$$u_{v3,rel} = \frac{0.462}{40} = 0.0116$$

The three contributions are combined to give the standard uncertainty associated with the volume of internal standard added to the sample:

$$u_{v,rel} = \sqrt{(0.00231)^2 + (0.00460)^2 + (0.0116)^2} = 0.0127$$

H.2.5 Standard solution concentration uncertainty

This contribution is associated with the labelled standard solution concentration used in the analytical method.

From the analytical certificate of labelled compounds solution, a standard uncertainty equal to ± 5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is deduced. The standard uncertainty is calculated dividing the expanded uncertainty by the coverage factor:

$$u_{st,rel} = \frac{0.050}{2} = 0.025$$

H.2.6 Sample weighing

The weighing uncertainty (u_w) is calculated assuming a rectangular distribution for the analytical balance. The calibration certificate of the balance quotes ± 0.30 mg for the linearity, the relative uncertainty is obtained dividing u_w by the sample weight (5 g):

$$u_{w1,rel} = \frac{\frac{0.30}{\sqrt{3}}}{5000} = 0.0000346$$

This contribution has to be counted twice, once for the tare and once for the gross weight, because each one is an independent observation and the linearity effects are not correlated. The two contributions are combined to give the standard uncertainty of the weight:

$$u_{w,rel} = \sqrt{(0.0000346)^2 + (0.0000346)^2} = 0.0000490$$

H.3 Combined and expanded standard uncertainty

If the full calibration procedure is adopted (Option 1), the combined standard uncertainty is calculated as follows:

$$u_{c,rel} = \sqrt{u_{Rw,rel}^2 + u_{bias}^2 + u_{cal,rel}^2 + u_{v,rel}^2 + u_{st,rel}^2 + u_{w,rel}^2}$$

The standard uncertainty of each component is expressed as relative standard uncertainty:

$$u_{c,rel} = \sqrt{(0.114)^2 + (0.0384)^2 + (0.0405)^2 + (0.0127)^2 + (0.0250)^2 + (0.0000490)^2} = 0.130$$

The final stage is to multiply the combined standard uncertainty by a coverage factor k=2 (level of confidence 95%) to obtain an expanded uncertainty equal to:

$$U_{rel} = 2 \cdot 0.130 = 0.260$$

If the calibration verification procedure is performed (Option 2), $u_{c,rel}$ is calculated as:

$$u_{c,rel} = \sqrt{u_{Rw,rel}^2 + u_{bias}^2 + u_{cal,rel}^2 + u_{d,rel}^2 + u_{v,rel}^2 + u_{st,rel}^2 + u_{w,rel}^2}$$

The inclusion of the drift contribution increases the overall uncertainty:

$$u_{c,rel} = \sqrt{(0.114)^2 + (0.0384)^2 + (0.0405)^2 + (0.0866)^2 + (0.0127)^2 + (0.0250)^2 + (0.0000490)^2} = 0.156$$

The expanded uncertainty is given by:

$$U_{rel} = 2 \cdot 0.156 = 0.312$$

H.4 Expanded uncertainties for 17 PCDD/F and 12 DL-PCB congeners

As described for 1,2,3,7,8-PeCDD, the expanded standard uncertainty can be calculated for each of the other PCDD/F and DL-PCB congeners.

As an example, the values obtained when using the calibration verification procedure (Option 2) are presented in Table H.4-1. Relative U values are then used to calculate the expanded uncertainties associated with the analytical levels of PCDD/F and DL-PCB congeners in a routine milk sample, as reported in table H.4-2.

Table H.4-1: Relative expanded uncertainty U_{rel} (confidence level 95%) for 17 PCDD/F and 12 DL-PCB congeners in a milk sample.

Congener	Expanded Uncertainty U_{rel} %	Congener	Expanded Uncertainty U_{rel} %
2,3,7,8-TCDD	46.6	PCB 105	25.9
1,2,3,7,8-PeCDD	32.0	PCB 114	24.6
1,2,3,4,7,8-HxCDD	31.1	PCB 118	29.3
1,2,3,6,7,8-HxCDD	33.8	PCB 123	29.5
1,2,3,7,8,9-HxCDD	33.7	PCB 156	23.9
1,2,3,4,6,7,8-HpCDD	36.4	PCB 157	40.8
OCDD	38.4	PCB 167	30.5
2,3,7,8-TCDF	46.8	PCB 189	29.8
1,2,3,7,8-PeCDF	33.7	PCB 77	28.6
2,3,4,7,8-PeCDF	26.8	PCB 81	32.0
1,2,3,4,7,8-HxCDF	31.9	PCB 126	27.7
1,2,3,6,7,8-HxCDF	29.1	PCB 169	27.4
2,3,4,6,7,8-HxCDF	36.1		
1,2,3,7,8,9-HxCDF	31.6		
1,2,3,4,6,7,8-HpCDF	31.3		
1,2,3,4,7,8,9-HpCDF	31.6		
OCDF	36.4		

Table H.4-2: Analytical levels and expanded uncertainty *U* for PCDD/F and DL-PCB congeners in a milk sample.

Congener	Concentration ± *U* pg/g fat	Congener	Concentration ± *U* pg/g fat
2,3,7,8-TCDD	0.99 ± 0.46	PCB 105	142.88 ± 37.07
1,2,3,7,8-PeCDD	2.36 ± 0.75	PCB 114	37.36 ± 9.19
1,2,3,4,7,8-HxCDD	0.71 ± 0.22	PCB 118	394.54 ± 115.46
1,2,3,6,7,8-HxCDD	2.15 ± 0.73	PCB 123	12.42 ± 3.66
1,2,3,7,8,9-HxCDD	0.77 ± 0.26	PCB 156	112.94 ± 27.04
1,2,3,4,6,7,8-HpCDD	0.78 ± 0.28	PCB 157	38.15 ± 15.56
OCDD	3.01 ± 1.16	PCB 167	51.41 ± 15.67
2,3,7,8-TCDF	0.10 ± 0.05	PCB 189	41.54 ± 12.38
1,2,3,7,8-PeCDF	0.04 ± 0.01	PCB 77	2.51 ± 0.72
2,3,4,7,8-PeCDF	4.58 ± 1.23	PCB 81	2.90 ± 0.93
1,2,3,4,7,8-HxCDF	3.29 ± 1.05	PCB 126	18.00 ± 4.99
1,2,3,6,7,8-HxCDF	1.97 ± 0.57	PCB 169	8.32 ± 2.28
2,3,4,6,7,8-HxCDF	0.10 ± 0.04	WHO-PCB-TEQ_{05}	2.08 ± 0.50* (±24.3%)
1,2,3,7,8,9-HxCDF	1.81 ± 0.57		
1,2,3,4,6,7,8-HpCDF	0.57 ± 0.18		
1,2,3,4,7,8,9-HpCDF	0.10 ± 0.03		
OCDF	0.51 ± 0.19		
WHO-PCDD/F-TEQ_{05}	5.83 ± 0.97* (±16.6%)		

* Uncertainty calculated using RSS approach