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MEASUREMENT UNCERTAINTY ESTIMATION FOR LABORATORIES PERFORMING PCDD/F AND PCB ANALYSIS BY ISOTOPE DILUTION MASS SPECTROMETRY

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Introduction

A European SANTE (European Commission, Directorate-General for Health and Food safety) guidance document on Measurement Uncertainty (MU) for laboratories performing PCDD/F and PCB analysis using isotope dilution mass spectrometry has been prepared 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). As a measure to further harmonise compliance assessment, this document provides practical guidance through two approaches that will allow laboratories to estimate measurement uncertainty on an ongoing basis. It reflects more than three years of collaboration between expert laboratories in the field of dioxin and PCB analysis. Final conclusions are presented here.

A new approach 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 - in this case maximum residue limits. Effects from sampling [1], transport and the representation of measured concentrations as toxic equivalents that together contribute 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 (EA), it combines contributions from intermediate (intra-laboratory) precision and trueness (expressed as bias) to estimate measurement uncertainty [2,3], both for individual congeners and for sum parameters. A semi-empirical approach (S-EA) following the EURACHEM/CITAC guide [4] is also presented. This last approach has been designed for laboratories new to this type of analysis, and relies mostly on data that was generated from initial validation studies together with elements of ongoing QA/QC implementation. For inexperienced laboratories the semi-empirical approach may be a good starting point, but the authors recommend implementing the EA approach once enough external data on bias control have been gathered. Figure 1 provides a flow chart for the estimation of measurement uncertainty applying the two approaches described in the document. The EA proposes calculation of MU by two different means.

One option is to calculate MU associated with each congener and to propagate the MU to the TEQ by combining the standard uncertainty for individual congeners. The second option is to calculate MU for the sum of congeners directly from the TEQ. Regarding the S-EA, MU is calculated and associated with each congener. Combined uncertainty for individual congeners in TEQ follows the same rule as the one proposed for the empirical approach, i.e. by combining through the root sum of squares (RSS).

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

RESULTS and DISCUSSION

Measurement uncertainty and compliance assessment for official food control

A major aim of this guidance document is to harmonize approaches for estimating MU in the context of using expanded MU for compliance assessment with maximum food and feed EU legislation. This arose from the many discrepancies that were (and are still) observed between dioxin NRLs, official or private laboratories, from unrealistically low to unexpectedly large MU estimates, which can lead to controversial decisions when a food lot has to be released on the EU market. However, the guidance document has also been designed to respond to a growing demand by laboratories to expand the use of isotope dilution-mass spectrometry based technique for other contaminants or trace analysis in food, feed or environmental samples.

EA and S-EA approaches requires data from precision studies

The underlying questions when discussing how to use data from precision are related to the selection of matrices, the number of replicates and the concentration range to cover. All these issues are discussed in the document. In principle, each matrix in the scope of validation requires individual MU assessment within the working range. However, due to the limited availability of suitable relevant RMs and internal QC samples, matrices for which an identical or similar analytical procedure provides equivalent performance, can be grouped for estimation of the expanded MU (NOTE the same issue is addressed when discussing trueness studies with limited availability of CRMs and Proficiency testing). A possible grouping of matrices for PCDD/F and PCB analysis according to the applied methods is given in the guidance document. Within the working range, as a minimum, precision studies have to be carried out at half the maximum limit (ML), at the ML and twice the ML, with 6 replicates at each of these levels. The possibility of estimating a single precision contribution value by using a pooled relative intermediate standard deviation $s_{Rw,pool,rel}$ of a range of matrices is also discussed in the document.

EA and trueness studies

The isotope dilution technique is applied to quantify individually the target analytes. In theory, the losses of native analytes during the analytical process should be reflected by equivalent losses of the stable isotope-labelled compounds introduced at the early stage of the analytical method. However if the remaining bias is beyond the acceptable trueness range specified in the EU legislation, these sources of bias should be identified and eliminated.

Even if the bias is acceptable according to the EU legislation criteria, it nevertheless contributes to the total uncertainty associated with a result. The document proposes 3 means of assessing the uncertainty component of the bias (ubias) : analyses of certified reference materials (CRMs), results from participation in interlaboratory studies (Proficiency Testing), recovery experiments using spiked blank samples or samples with very low contamination levels. The objective is to cover as much as possible the scope of matrices by using one or a combination of the three options.

S-EA and trueness studies

Initially, the uncertainty component associated with bias (ubias) can be estimated from the same experiments performed in the precision study at different levels of concentration. Then, the contribution from ubias is estimated through calibration procedure. Two options are available for the estimation, depending on whether the calibration curve is prepared for each analytical batch or, as an alternative, periodically by checking one calibration point, both options are described in the guidance document.

Combined expanded uncertainty for EA and S-EA approaches

The combined standard uncertainty uc for the EA is calculated from the combination of the uncertainty component describing the random variations uRw from precision with the uncertainty component

describing the method and laboratory bias ubias. The uc for the S-EA is also a combination of uRw and ubias and additional minor contributions arising from volume, weight or standard purity.

On-going approach for EA

Because MU is associated with a result reported, it must reflect as best as possible the performance of the laboratory on the day those data are produced. One of the major outcomes of this document is the strategy developed to tackle this challenge. The core-working group (CWG) placed great emphasis on the integration of QA/QC data generated daily (or on a regular basis) to allow frequent reassessment of MU. The CWG proposed the use of a moving data time window scheme where a limited number of QC, PT, RM or CRM data from a relevant time window are integrated in order to reassess uRw and ubias regularly. This allows exclusion of historical QA/QC data that may no longer reflect the performance of the methodology (Figure 2). The number of QC data, frequency of collection and other recommendations are given in the document.

Figure 2: Moving time window as computation period

In addition, MU might be even more realistic and meaningful if contributions arising from daily performance (reflected by e.g. procedure blanks, LOQs) are integrated into the calculation of the combined uncertainty, while using the presented empirical approach. Unpredictable "unexpected 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, sensitivity problems. These issues are already partially treated in the first version of the released Guidance document.

Practical implementations and recommendations

One the features of this guidance document is that draws on the extensive practical experiences of isotope dilution techniques of the core working group members and explains in detail, with practical examples, how to estimate MU even for unusual circumstances. For instance, the guidance document explains how to calculate MU of the mean calculated from results of two separate analyses for verification of non-compliance according to EU legislation; it gives recommendations on how to exclude data; on how to round off results and the associated MU; it provides recommendations for laboratories new to isotope dilution technique. Additionally, the document also provides a means of assessing whether the obtained MU estimates are realistic, by comparing it with performances obtained at PTs or by providing a measure of a target MU value [5]. This is estimated from the upper limits of the criteria for measurement that are laid down in the EU performance criteria document for sampling and measurement of dioxins and PCBs [6]. The estimated expanded MU calculated by laboratories using either EA or S-EA should not exceed this target expanded MU, at the maximum limit. Finally, the document also provides guidance on propagating MU of the individual congeners to the total TEQ value for the sample.

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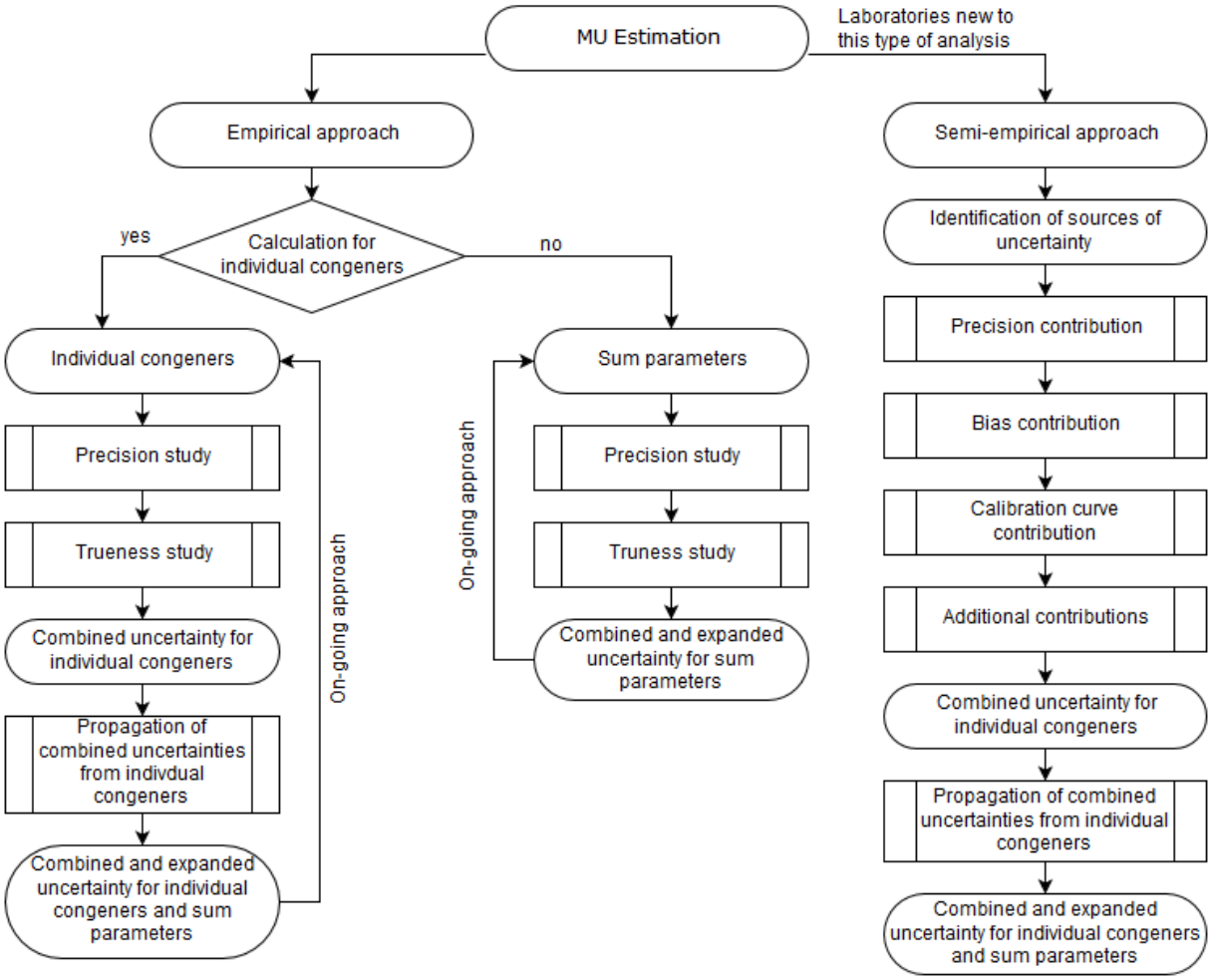

Figure 1: Flow chart for estimation of measurement uncertainty using an on-going empirical (top-down) and a semi-empirical approach.

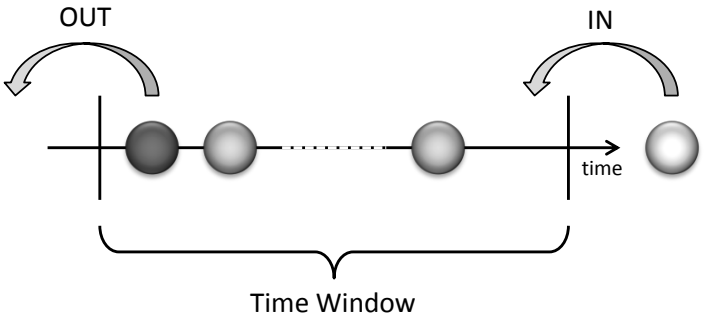

Figure 2: Moving time window as computation period