ISO 17025 REQUIREMENTS: HOW TO EVALUATE UNCERTAINTY FOR DIOXIN ANALYSIS IN FOOD AND FEED FROM VALIDATION DATA?

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Introduction

Accreditation now based on ISO 17025 requires for laboratories to estimate measurement uncertainty. It is widely recognised that uncertainty estimation is now considered as a part of validation data. As a consequence of this, analytical chemists have to demonstrate the quality of their measurements by associating the evaluation of uncertainty with their results.

Eurachem[1] published a guide to evaluate uncertainty in analytical chemistry. More recently, VAM[2] established a protocol for uncertainty evaluation from validation data. The process to evaluate uncertainty is based on 4 stages:

- First step is to specify the measurand
- Second step is to identify uncertainty sources
- Third step is to quantify uncertainty components
- Fourth step is to calculate combined or total uncertainty

There are several ways of quantifying uncertainty components but mainly two different approaches are frequently used. One is to evaluate uncertainty by quantification of each individual sources and the other one is to estimate uncertainty from validation experiments. We will focus on the second method.

Materials and Methods

Uncertainty for dioxin analysis in food was estimated using in-house validation studies. Precision, trueness and possible other uncertainty contributions was investigated through the whole analytical process.

The overall precision was estimated by performing replicates analysis over an extended time period (more than one year) on a reference material RM 533 spray dried milk powder and internal quality controls (QC) such as beef fat and serum. We also included estimates of reproducibility from a collaborative study done on an animal feed raw material[3]. Factors such as operators, time, calibrations (mass spectrometers, balances, syringes), solvent batches, consummables, temperature and instruments maintenance are therefore included in the global precision term, providing the uncertainty of the whole analytical process. Trueness was studied by carrying out replicates analysis on a certified reference material BCR 607 spray dried milk powder. The others potential uncertainty (purity, homogeneity,…) were evaluated.

Results and discussion

- The first step of the process is to define the measurand. Dioxins results are expressed on TEQ basis. The individual concentration of the congener i is calculated by :

$$C_i = \frac{Area\,C_{12,i} * C_{std\,i} * \text{Volume}_{std}}{Area\,C_{13,i} * RRF_i * m_{sample}}$$

$$C_{PCDD/F} = \sum C_i * TEF_i$$

Where:
Area C_{12}: Peak integration for native congener i
Area C_{13}: Peak integration for standard C_{13} congener i
$C_{std\,i}$: concentration of the standard congener i (pg μL^{-1})
V_{std}: spiked volume (µL)
RRF_i: Relative Response Factor of the congener i (calculated by calibration)
M_{sample} : Sample weight (g)

- The second step is to identify sources of uncertainty :

Identification of all the possible sources of uncertainty is a critical step. The aim is to record a list of sources that is relevant for the analytical method. An alternative and useful method for recording a list of uncertainty sources is the cause and effect analysis. This approach uses a cause and effect diagram as shown in figure 1. The equation parameters form the main branches of the diagram. Additional main branches from the validation study as trueness and precision were added. For each branch, add contributory factors. Remove duplicates terms and simplify as much as possible by grouping sources of uncertainty in a set of experiments.

- The third step is to quantify uncertainty components :

We used data from the validation of the methods to evaluate as much as possible uncertainty sources included in the set of experiments. We checked if the experimental results are covering the whole analytical process, the range of matrices and analytes concentration. The sources that was not covered by the validation studies have to be quantify separately. All the contributions to uncertainty are expressed as standard deviation.

The precision study

Table 1 summarised the results over a period of approximately two years. The precision study was covering a rang of matrices and a range of analyte concentrations.

Table 1. Precision study

Sample	Mean pg-TEQ/g	standard deviation pg-TEQ/g	relative standard deviation	n
QC beef-1	4.24	0.440	0.104	50
QC beef-2	4.80	0.422	0.090	42
RM 533	2.96	0.313	0.106	55
QC serum	0.22	0.026	0.116	23
QC animal feed intercal.	1.97	0.15	0.076	13

Table 1 shows that the precision (SD) is proportional to the TEQ level across the concentration range. The corresponding RSDs look constant at a value of ± 0.1. In this case the RSDs can be pooled using the equation :

$$RSD_{pool} = \sqrt{\frac{(n_1-1)*RSD_1^2+(n_2-1)*RSD_2^2+\ldots}{(n_1-1)+(n_2-1)+\ldots.}} = 0.102 \qquad (1)$$

Trueness study

Trueness is estimated in terms of overall recoveries R_m. R_m is defined as the ratio of the concentration observed to the certified value. We used a certified reference material milk powder BCR 607 to estimate the trueness. We analysed 5 times the same batch of BCR 607 in repeatability conditions. Table 2 gives the results.

Table 2. Trueness study

	Certified values			Measured values		
Sample	Concentration	Uncertainty 95%	standard Uncertainty	Mean	standard deviation	n
	pg-TEQ/g	pg-TEQ/g	pg-TEQ/g	pg-TEQ/g	pg-TEQ/g	
BCR 607	2.43	0.084	0.043	2.50	0.103	5

The uncertainty associated to R_m, $u(R_m)$, is calculated using :

$$u(\bar{R}_m) = \bar{R}_m \sqrt{\left(\frac{s_{obs}^2}{n * \bar{C}_{obs}^2}\right) + \left(\frac{u(C_{CRM})}{C_{CRM}}\right)^2}$$

R_m= 1.028 and the corresponding uncertainty $u(R_m)$ = 0.0263. $u(R_m)/R_m$= 0.0255

Evaluation of other sources of uncertainty

1. The purity of C_{13} labelled standard is 98% ± 2% per congener. Assuming a rectangular distribution,

$$u_{(congénère)} = \frac{0.02}{\sqrt{3}} = 0.0115 \qquad u_{(PCDD/F)} = \sqrt{\sum_{i=1}^{17} u_i^2} = 0.0476$$

2. Homogeneity, published data for BCR 607 estimate homogeneity at $u_{(homogeneity)}$= 0.024

The combined relative uncertainty

$$\frac{u(c_{pcddf})}{c_{pccdf}} = \sqrt{(0.102)^2 + (0.0255)^2 + (0.0426)^2 + (0.024)^2} = 0.118$$

Expanded uncertainty

$$\boldsymbol{U} = k * u = 2 * 0.118 = 0.236$$

References

1. Quantifying uncertainty in analytical chemistry, EURACHEM/CITAC guide 2000.
2. Barwick V.J., Ellison S.L.R. Development and harmonisation of measurement uncertainty principles. Part (d): Protocol for uncertainty evaluation from validation data, VAM Project 3.2.1.
3. G. Eppe , E. De Pauw, O*rganohalogen compounds*, 2002, submitted.

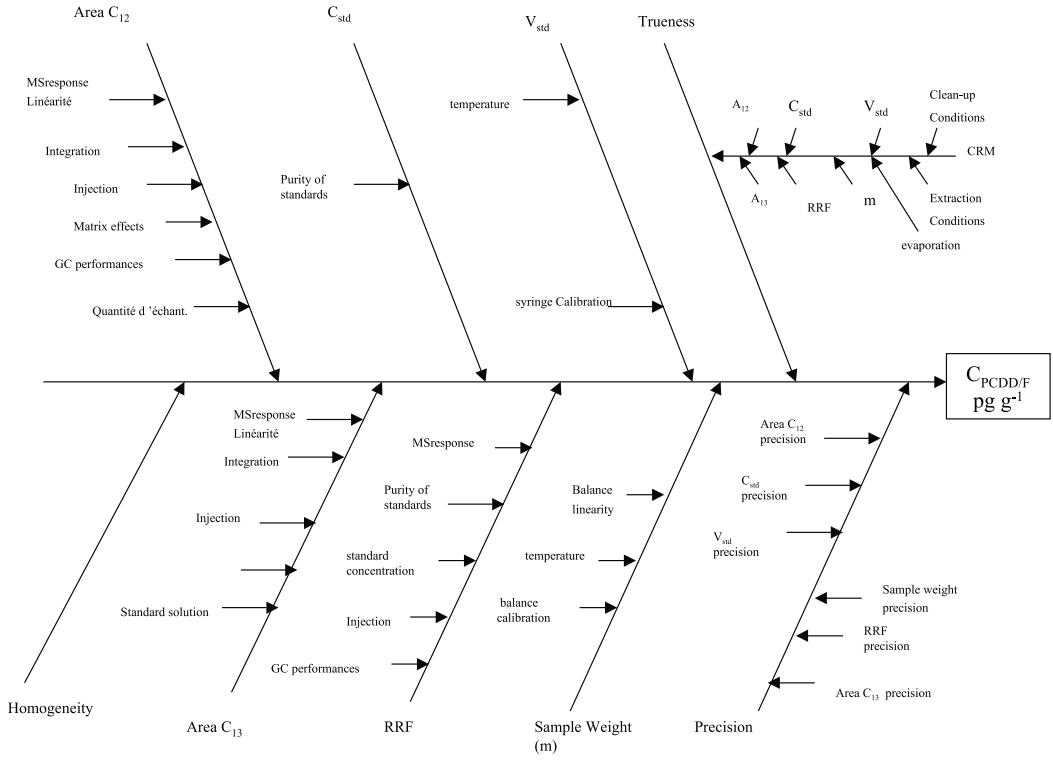

Figure 1. Cause-and –effect diagram

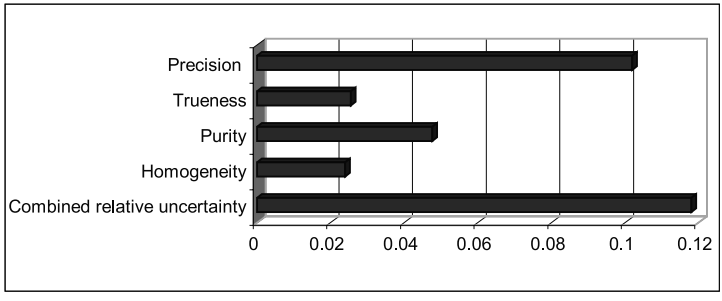

Figure 2. Contribution to the measurement uncertainty for dioxins analysis in food and feed.