ANALYSIS OF THE MEASUREMENT UNCERTAINTY IN THE DETERMINATION OF PCDD/Fs AND PCBs IN SOIL: PRACTICAL VS THEORETICAL APPROACH

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Introduction

Dioxins and furans (PCCD/Fs) and polychlorinated biphenyls (PCBs) are usually monitored in foodstuffs, feed and environmental samples, such as soil. Thus, several incidents involving soil contamination have been reported; e.g., in 2001, the first Dutch case of contamination of free-range eggs was discovered, being soil the most likely source; other cases were later detected in Dutch and German farms[1]. Besides, the occurrence of PCCD/Fs and PCBs in clay has been also demonstrated[2]. Consequently, a method for the determination of PCDD/Fs and PCBs in different types of soil has been validated, and measurement uncertainty was assessed. Method validation and measurement uncertainty calculation will provide the required confidence in the results. However, in literature, the calculation of the measurement uncertainty is not commonly carried out[3-7], and consequently, efforts and new data on uncertainty assessments are very valuable.

In this study, uncertainty assessment was determined applying two different approaches: (i) a practical approach based on the use of experimental data from a thorough method validation; (ii) a theoretical approach based on the identification of the uncertainty sources. The protocol applied herein was based on the method described in the document Eurachem "Quantifying Uncertainty in Analytical Measurement, 2nd Edition"[8], this guide is subsequently based on the guide published by ISO in 1993, "Guide to the Expression of Uncertainty in Measurement"[9]. In this case, uncertainty means "the variation in the results due to variables that can reasonably contribute to the result". The uncertainty can be determined as the standard deviation or a multiple thereof, or as a confidence interval. The estimation of the uncertainty is a process that is completed in four steps: (1) determination of what exactly is being measured; (2) identification of all the sources that can contribute to the uncertainty of the result; (3) quantification of the uncertainty of the individual sources which contribute to the total uncertainty; (4) calculation of the combined uncertainty due to the individual contributions.

Materials and methods

As aforementioned, measurement uncertainty was accomplished using two approaches. The first approach was based on the use of experimental data from the performance parameters determined in the validation according to internal RIKILT SOPs, which were based on NEN 7777[10]. Thus, measurement uncertainty was calculated using the standard deviation of the within-laboratory reproducibility (S_{RL}) and expanded measurement uncertainty was determined using a coverage factor (k) of 2. Some authors indicate that the use of the inter-laboratory reproducibility standard deviation may not cover some significant sources[7].

In this approach, the study was focused on the determination of PCDD/Fs and PCBs in agricultural soil; to this aim, four types of soil were included in the validation: soil with high percentage of organic matter, sandy soil, clay soil and sandy/clay soil. The validation levels for PCDD/Fs + non-*ortho*-PCBs (DIOXNOP; Note: acronym from "dioxins" and "non-*ortho*-PCBs") and non-dioxin-like-PCBs + mono-*ortho*-PCBs (MOPIP; Note: acronym from "mono-*ortho*-PCBs", and "indicator-PCBs", now named as non-dioxin-like-PCBs) is shown in Table 1.

Table 1. Validation levels used in the uncertainty assessement

	Analyte	**Spiking level in pg/g[a]**	**Spiking level in pg-TEQ/g**
PCDD/Fs and Non-*ortho*-PCBs (DIOXNOP)	Sum PCDD/Fs-TEQ	10	31.61
	Sum Non-*ortho*-PCBs-TEQ	10	1.30
Mono-*ortho*-PCBs and Non-DL-PCBs (MOPIP)	Sum Mono-*ortho*-PCBs-TEQ	1000	0.24
	Sum Non-DL-PCBs	1000	-
	Sum DL-PCBs-TEQ	-	1.54
	Sum PCDD/Fs + DL-PCBs-TEQ	-	33.15

[a] Concentrations for the individual congeners, dry weight.

The extraction method is based on the solid-liquid extraction by pressurized-liquid extraction (also known as accelerated solvent extraction, ASE™), clean-up, purification and fractionation by column chromatography using an automated system (Powerprep™) and further determination by GC-HRMS. A detailed description of the method can be found in the European Standard EN 16215[11].

The procedure used in the second approach (theoretical) was based on the descriptions indicated by Eurachem[8]. As this second approach can be more subject to variations depending on the sources considered, it is important to define the uncertainty sources included in the calculations, as shown in the Ishikawa diagram (Figure 1). In the theoretical approach, the well-known formulas considered for the determination of the dioxin content (equation [1] and [2]) were the starting point to specify all the sources of uncertainty.

$$C_{diox} = \frac{A_n}{A_i} \times RRF_i \times C_i \times V_i \qquad \text{Equation [1]}$$

Where:
- C_{diox} : content of a dioxin congener (ng)
- A_n : peak area of the native dioxin congener
- A_i : peak area of the labeled dioxin congener (internal standard)
- RRF_i : relative response factor of the native dioxin congener relative to the labelled
- C_i : concentration of the added internal standard solution (ng/ml)
- V_i : volume of the added internal standard solution (ml)

$$RRF_i = \frac{A_i}{A_n} \times \frac{C_n}{C_i} \qquad \text{Equation [2]}$$

Where:
- Ai : Peak area of the labelled dioxin congener (external standard)
- An : Peak area of the native dioxin congener (external standard)
- Cn : Concentration of the native dioxin congener in the external standard (ng/mL)
- Ci : Concentration of the labelled dioxin congener in the external standard (ng/mL)

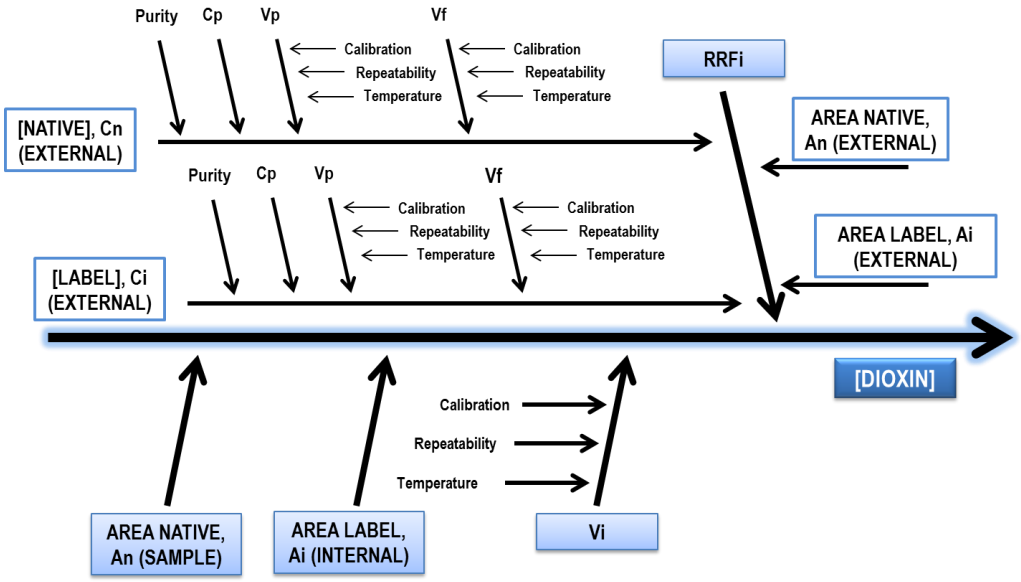

Figure 1. Ishikawa diagram applied in this study

The calculation of the uncertainty was therefore started from the calculation of the uncertainties of the primary, stock and other standard solutions used in the method. Afterwards, the uncertainty of the relative response factor was determined and finally, the uncertainty of the concentration of each congener in TEQ was set. Bearing in

mind the formula to calculate the dioxin content, C_{diox}, the calculation of the uncertainty was accomplished considering each source as follows (Equation [3]):

$$\frac{u\,(C_{Component})}{C_{Component}} = \sqrt{\left(\frac{u\,(A_n)}{A_n}\right)^2 + \left(\frac{u\,(A_{i(spiked)})}{A_{i(spiked)}}\right)^2 + \left(\frac{u\,(RRF_i)}{RRF_i}\right)^2 + \left(\frac{u\,(C_{i(spiked)})}{C_{i(spiked)}}\right)^2 + \left(\frac{u\,(V_{i(spiked)})}{V_{i(spiked)}}\right)^2} \qquad \text{Equation [3]}$$

Results and discussion

The uncertainty values obtained when applying the experimental approach are shown in Table 2. The results are expressed as TEQ-base, as usual for result reports, and divided in the representative groups. Overall, the expanded uncertainty was always lower that 22%, with the lowest value for mono-ortho-PCBs (10.2%) and the highest for non-dioxin-like-PCBs (21.1%).

Table 2. Summary of the results obtained for RSD_r, RSD_{RL}, EMO and proposed EMO[a] using the experimental approach.

Analyte	RSD_r (%)	RSD_{RL} (%)	Measured expanded uncertainty EMO (%)	Proposed expanded uncertainty EMO (%)
PCDD/Fs and Non-*ortho*-PCBs (DIOXNOP)				
Sum PCDD/Fs-TEQ	2.5	5.3	10.6	15
Sum Non-ortho-PCBs-TEQ	10.7	9.8	19.6	25
Mono-*ortho*-PCBs and Non-DL-PCBs (MOPIP)				
Sum Mono-ortho-PCBs-TEQ	3.9	5.1	10.2	15
Sum Non-DL-PCBs	15.9	10.5	21.1	25
Sum DL-PCBs-TEQ	10.4	9.3	18.6	25
Sum PCDD/Fs + DL-PCBs-TEQ	1.5	4.9	9.9	15

[a] TEQ-based

Abbreviations: RSD_r: relative standard deviation of repeatability; RSD_{RL}: within-laboratory reproducibility; EMO: the relative expanded measurement uncertainty

In the second approach (Table 3), the relative standard uncertainty ($u(C_{diox})/C_{diox}$), and the combined standard uncertainty ($u(C_{diox})$ in pg) were calculated considering the aforementioned spiking levels (a content of 10 pg/g and 1000 pg/g for DIOXNOP and MOPIP, respectively) and the use of two standards at low (DIOXNOP: 0.005 pg/µL; MOPIP: 0.10 pg/µL) and at high concentration (DIOXNOP: 0.050 pg/µL; MOPIP: 1.00 pg/µL).

Table 3. Summary of the results obtained for RSD_r, RSD_{RL}, EMO and proposed EMO using the theoretical approach.

					Validation	
	C_{SUM}-TEQ (pg-TEQ)	u (C_{SUM}-TEQ) (pg-TEQ)	U (C_{SUM}-TEQ) (pg-TEQ)[a]	%U	Calculated (%)	Proposed (%)
PCDD/Fs-TEQ	31.61	1.95	3.91	12.4	10.6	15
Non-ortho-PCBs	1.30	0.13	0.27	20.6	19.6	25
Mono-ortho-PCBs	0.24	0.02	0.04	16.0	10.4	15
Non-DL-PCBs	6000	550.78	1101.55	18.4	21.1	25
DL-PCBs	1.54	0.14	0.27	17.5	18.6	25
PCDD/Fs + DL-PCBs	33.15	1.45	2.89	8.7	9.9	15

The following step was the application of the corresponding TEF value to determine the uncertainty of the TEQ value, as shown in Equation [4].

$$u(C_{SUM-TEQ}) = \sqrt{(TEF_1 \cdot u(C_1))^2 + (TEF_2 \cdot u(C_2))^2 + \cdots + (TEF_n \cdot u(C_n))^2} \qquad \text{Equation [4]}$$

However, in order to perform the comparison with the values obtained for the uncertainty according to the first approach, the uncertainty was calculated for the representative groups of congeners. A summary of the results is shown in Table 3.

Considering the obtained results, several questions can be asked:

- The values of the expanded uncertainty calculated in the theoretical approach developed herein are comparable to the results obtained in the experimental approach.
- The highest difference is observed for mono-*ortho*-PCBs, with a value of expanded U around 6% higher. Nevertheless, the contribution of this group to the total TEQ is very low.
- There is no clear opinion which approach is the most correct; some authors described the approach commented in this report as a mere "theoretical approach" not linked to the "real approach", which is based on experimental data (precision, recoveries, etc.). However, it is important to notice, that this is not totally accurate because the "theoretical approach" makes use of the information of the standards, formulas which are in fact used for the determination of the dioxin content and other "real" data (pipette information, flask information, peak area, etc.). Furthermore, the "experimental approach" does not consider any of the sources of uncertainty in the preparation of the standards solutions, which are major contributors to the overall uncertainty.
- In any case, the proposed values for the expanded uncertainty are quite realistic considering both approaches; perhaps the value for mono-*ortho*-PCBs could be increased another 5% from 15 to 20% in the practical approach.
- Concentration of standards: volume (native and labelled compounds) and concentration (native compounds) of primary solution are apparently major contributors to the uncertainty of the concentration.
- Content of dioxin: Apparently, the main contributor to the uncertainty is the RRF_i value and the peak area of the native compound (for the examples evaluated). The concentration of the labelled internal standard seems also a relevant source of uncertainty for MOPIP compounds.

The results obtained in the present study can be compared somehow to the results reported in other studies even though this comparison is limited due to the differences in the matrix and procedure employed in the calculations, but it can be justified because of the scarce available information in this topic. The uncertainty values reported were as follows: (i) PCDD/Fs in fish: 9.3-28.8% (referred to pg/g and per congener, k=2)[3], (ii) PCDD/Fs in beef fat: 17.0-19.4% (referred to pg-TEQ/g, k=2)[4] (iii) PCDD/Fs and PCBs in fly ashes: 13% (total I-TEQ) and 31% (TEQ), respectively[5]; (iv) PCBs in dust: 22.4-23.3% (relative uncertainty) [6]

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