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CLOSING QUALITY CONTROL GAPS IN ENVIRONMENTAL MONITORING: ERM-CE100, A NOVEL FISH REFERENCE MATERIAL CERTIFIED FOR THE PRIORITY SUBSTANCES HEXACHLOROBENZENE AND HEXACHLOROBUTADIENE

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Introduction

The EU Water Framework Directive (WFD) [1] aims at maintaining and improving the quality of European waters. Among the specific measures for pollution control, the establishment of Environmental Quality Standards (EQS) for Priority Substances (PS) plays a critical role. In the context of the mandatory monitoring of the water status by the EU Member States (MS), the use of biota is encouraged as a fit-for-purpose and cost-effective alternative matrix to water. As a matter of fact, Directive 2013/39/EU [2] introduced new EQS for a number of PS for which biota becomes the "default" monitoring matrix. The quality and comparability of the results generated by the analytical laboratories officially appointed by the MS are ensured by fixing minimum performance criteria for the applied methods as laid down in another EU Directive [3]. Furthermore, this Directive requires the monitoring laboratories to demonstrate their competence through the use of Reference Materials (RMs) that are representative of collected samples and that contain appropriate levels of the PS in relation to the respective EQS. This contribution describes the development and production of ERM-CE100, a fish paste matrix RM certified for hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD), two PS with biota EQS set to 10 and 55 µg/kg wet weight, respectively. ERM-CE100 was produced by the Institute for Reference Materials and Measurements of the Joint Research Centre (JRC-IRMM) following an alternative approach aiming at simulating as close as possible the routinely analysed fish samples and is provided as a "wet" material in contrast to the commonly available freeze-dried powder Certified Reference Materials (CRMs).

Materials and methods

The starting material was naturally contaminated wild Wels catfish (Silurus glanis) originating from the Ebro river, Spain. The fish was filleted, divided in head and tail parts and delivered frozen to JRC-IRMM (Geel, Belgium).

An in-house validated analytical method [4] was used for the sample screening during processing and in the subsequent homogeneity and stability studies. In brief, the fish samples were extracted by accelerated solvent extraction (ASE) and the extract cleaned-up on a Florisil and Na_2SO_4 packed column. After solvent evaporation, HCB and HCBD were quantified in the extract by gas chromatography-isotope dilution mass spectrometry (GC-IDMS), using ^{13}C-HCB and ^{13}C-HCBD as internal standards.

Results and discussion

Processing of the material

After screening all frozen heads and tails for HCD and HCBD, the filleted fish was manually cut, cryogenically milled, homogenised and stored at - 20 °C. With the support of simultaneous in-house screening of the analytes' levels, heads and tails of specific specimens were selected and mixed in a multi-step scheme (see Figure 1) to reach the target levels of HCB and HCBD in the CRM. The final batch was pre-cooked, further mixed and the obtained fish paste was filled into glass jars, which were subsequently sterilised in an autoclave at 121 °C. Over 1000 CRM units containing approximately 40 g of fish paste were produced and thereafter stored at 4 °C.

Homogeneity and stability studies

A key requirement for any RM is the equivalence between the produced units. For the homogeneity study, two independent samples of 2 g each from twelve CRM units were analysed. Following the application of statistical checks to ensure the consistency of the dataset, the results were subjected to analysis of variance (ANOVA). The resulting between-unit variation was taken as homogeneity uncertainty

contribution (u_{bb}, Table 1) to the uncertainty of the certified values and estimated as 2.0 % and 1.3 % for HCB and HCBD, respectively.
The stability studies, necessary to establish the conditions for storage (long-term stability) as well as transport of the CRM to the customers (short-term stability), were carried out following an "isochronous design" [5]. Samples were stored for certain periods of time at selected temperatures, afterwards moved to reference conditions (usually lower temperature) where degradation is assumed to be negligible and finally analysed simultaneously. The outcome of the 4-week short-term stability studies shows that ERM-CE100 must be transported below 18 °C. The long-term behaviour of the CRM was studied during two years and the relative uncertainty contribution of stability (u_{lts}, Table 1) for storage at 4 °C was estimated as 0.9 % and 2.6 % for HCB and HCBD, respectively.
Characterisation and value assignment
The RM characterisation (determination of the property values) was carried out by an interlaboratory comparison of eleven laboratories of demonstrated competence. Each laboratory reported results for three sub-samples of two CRM units and measurements on two quality control (QC) samples (one being a fish CRM). A variety of extraction (e.g. ASE, Soxhlet, liquid-liquid extraction) and clean-up methods (e.g. Florisil / silica gel columns, gel permeation chromatography) with different quantification techniques [GC-electron capture detection (ECD), GC-MS, GC-high resolution MS, GC-MS/MS] were applied, ensuring the absence of method bias. The evaluation of the QC samples' results was used for judging the validity of the submitted datasets. The mean of the means of the accepted datasets was assigned as certified value for HCB and HCBD while its standard error was taken as the uncertainty contribution of the characterisation (u_{char}, Table 1). The final uncertainty of the certified value was calculated by quadratic combination of contributions related to characterisation (u_{char}), potential between-unit inhomogeneity (u_{bb}) and potential degradation during long-term storage (u_{lts}), Table 1.

Conclusion
ERM-CE100 was produced as a "wet" biota matrix CRM by following a novel approach in support of the implementation of the WFD. It is the first biota CRM ever available for HCBD. The material was not freeze-dried in order to resemble the fresh fish samples as close as possible but also in consideration of the fact that EQS are expressed as mass fraction relative to wet weight. The range of analytical methods applied during the characterisation of ERM-CE100 confirms its commutability to routine biota samples. The natural levels in the starting material dictated the final certified values of HCB and HCBD, especially for the latter very well matching the EQS. The CRM will be used to assess the performance of the analytical methods employed in the mandatory monitoring of water bodies under the WFD.

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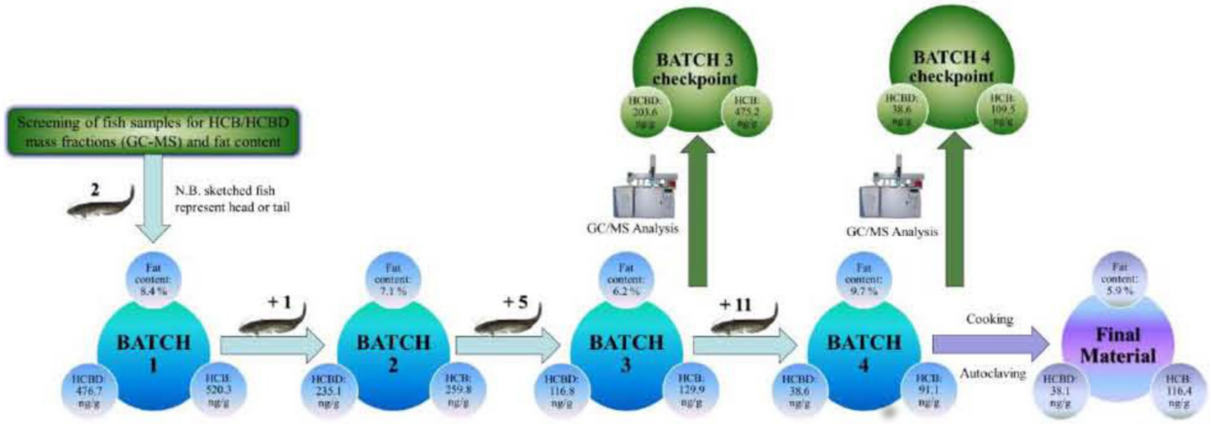

Figure 1. Processing scheme of ERM-CE100 (blue: theoretical mass fractions of HCB/HCBD, green: actual mass fractions measured, purple: indicative fat content and mass fractions of HCB/HCBD in the produced CRM; mass fractions are expressed on wet weight basis)

Table 1: Certified values and their uncertainties for ERM-CE100

Analyte	Certified value[1)] [µg/kg]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%] $U_{CRM,rel} = k \cdot \sqrt{u^2_{char,rel} + u^2_{bb,rel} + u^2_{lts,rel}}$	U_{CRM}[2)] [µg/kg]
HCB	**120**	2.4	2.0	0.9	6.5	**8**
HCBD	**36**	4.0	1.3	2.6	9.9	**4**

[1)] The certified values and their uncertainties are expressed as mass fractions relative to wet weight
[2)] Expanded (k=2) and rounded uncertainty