Guidance document on Extraction Procedures and Confirmation of Results for the analysis of PCDD/Fs and PCBs in feed

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1. Scope and Field of Application

This document on criteria for extraction procedures and confirmation of results for the analysis of PCDD/Fs and PCBs in feed was developed within the network of the European Union Reference Laboratory (EURL) for halogenated POPs in Feed and Food and the respective National Reference Laboratories (NRLs) of EU member states.

The guidance in this document is intended for laboratories involved in the official control of PCDD/Fs and PCBs in feed including laboratories performing control for feed business operators and focuses on criteria for extraction procedures and confirmation of results for determination of PCDD/Fs and PCBs in feed.

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down methods of sampling and analysis for the official control of feed [1] sets analytical criteria and requirements for the determination of the levels of PCDD/Fs and PCBs using confirmatory and screening methods.

The analytical criteria as laid down in this regulation are the basis for the analysis of PCDD/Fs and PCBs in feed. This guidance document gives further recommendations on the implementation of extraction procedures and the confirmation of results in order to achieve the general criteria in laboratories involved in the official control in the EU.

2. Abbreviations

Abbreviation	Definition
EURL	European Union Reference Laboratory
NRL	National Reference Laboratory
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans
POPs	Persistent organic pollutants
CRM	Certified reference material
PT	Proficiency test
GC	Gas chromatography
HRMS	High resolution mass spectrometry

3. Extraction procedures

3.1 Background

Commission Regulation (EC) No 152/2009 [1] does not set specific requirements as regards extraction procedures, techniques or solvents for determination of PCDD/Fs and PCBs in feed. In general, the sample quantity used for the extraction shall be sufficient to fulfil the requirements regarding a sufficiently low working range when checking for compliance with legal limits.

Standard EN 16215:2020 (Animal feeding stuffs: Methods of sampling and analysis - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS) [2] describes aspects regarding the extraction procedure. In particular it states the consecutive use of toluene and toluene/ethanol as extraction solvents.

In 2007 the EURL published a recommendation document [3] with focus on recommendations for extraction procedures for the determination of dioxins in mineral feed, trace elements, premixtures and compound feed. The summarizing conclusions included that for “an unknown premixture or compound feed or a premixture or compound feed with problematic composition, it is recommended to combine two extraction steps, e.g. first with toluene and then with a toluene/polar solvent mixture.”

3.2 Recommendations for extraction of feed

The quantitative extraction of the relevant PCDD/F and PCB congeners from feed matrices is a crucial step in their analysis. As the extraction efficiency in this step cannot be fully checked or covered by the use of isotope-labelled internal standards added before the extraction, the verification of the extraction efficiency of PCDD/F and PCB congeners from a specific feed matrix would require the use of suitable certified reference material(s) (CRMs) or participation in proficiency test(s) (PTs) covering relevant matrices. Depending on the sample matrix and the source of contamination it is very difficult to reflect the distribution of the analytes of interest in the sample matrix or the different compartments of the matrix with the addition of the internal standards. Also binding and adsorption characteristics between native analytes of interest and the internal standards added during analysis might differ. As a consequence, the extraction efficiency of the analytes of interest from the sample matrix and the internal standards might not be fully comparable.

Due to the plethora of different feed matrices a full coverage of these matrices using CRMs or PT participation is not possible. Therefore, it seems appropriate to give guidance for the extraction of feed matrices and in particular the extraction solvents and solvent mixtures, which mainly influence the extraction efficiency of the analytes of interest.

The summarizing conclusions of the EURL recommendation document of 2007 [3] developed within the EURL/NRL network and based on extraction experiments for certain feed matrices still apply:

- For mineral feeds such as clays (except oxides of trace elements) and compound feeds, a mixture of toluene and polar solvents (e.g. ethanol, acetone, isopropanol; in substantial proportion) is the best extraction medium.
- For oxides of trace elements, toluene is the most suitable solvent.
- There is no need to pretreat samples with hydrochloric acid (HCl).
- For an unknown premixture or compound feed or a premixture or compound feed with problematic composition, it is recommended to combine two extraction steps, e.g. first with toluene and then with a toluene / polar solvent mixture.

Based on these recommendations and the above-mentioned aspects regarding extraction of feed samples the following is concluded:

> **For the extraction of feed samples, in particular for samples with unknown composition, two consecutive extractions using toluene as a first step and a mixture of toluene and a polar solvent as a second step should be applied.**

When applying these combined extraction steps the risk of an insufficient extraction of a feed matrix could be minimized and comparability of results of different laboratories applying different extraction techniques and solvents or solvent mixtures can be improved. If, for particular feed matrices, information on the extraction efficiency for certain extraction solvents and the corresponding evaluation of results is available (e.g. from PTs, CRMs) or if the result of the first extraction is significantly (> 50 %) below legal limits, the above-mentioned two-step extraction procedure may be replaced by a single-step extraction applying a suitable solvent or solvent mixture.

4. Confirmation of results for PCDD/Fs

4.1 Background

Commission Regulation (EC) No 152/2009 [1] states that a high selectivity (specificity) in particular for the “distinction between PCDD/Fs and dioxin-like PCBs and a multitude of other, co-extracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest [is required]. For GC-MS methods, a differentiation among various congeners is required, such as between toxic (for example, the seventeen 2,3,7,8-substituted PCDD/Fs, and twelve dioxin-like PCBs) and other congeners.”

Additionally, specific criteria for removal of interfering substances, gas chromatographic separation and addition of internal standards are set. The removal of interfering substances in particular by application of suitable chromatographic techniques during the clean-up procedures as well as during gas chromatographic separation is required. Regarding gas chromatographic separation it is stated that “the separation of the isomers shall be sufficient (< 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF)”, but no specific recommendations as regards the type of the capillary column for GC analysis are given. Furthermore, for specific criteria for confirmatory methods, reference is made to internationally recognised standards, e.g. EN 16215 [2], EPA 1613 [4] and EPA 1668 [5].

EN 16215:2020 [2] mentions the use of a non-polar stationary phase or an alternative column in case of problematic separations of critical congeners for GC analysis.

ISO 13914:2023 (Soil, treated biowaste and sludge - Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass selective detection (HR GC-MS)) [6] states that for PCDD/Fs “there is no capillary column available at present that allows the separation of all 2,3,7,8-substituted congeners from all other non-2,3,7,8-substituted congeners. Complete separation can only be achieved by analysing a sample on different capillary columns of different polarity.”

EPA method 1613, revision B (Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS) [4] defines that for “confirmatory analysis isomer specificity for 2,3,7,8-TCDF cannot be achieved on the DB-5 column. Therefore, any sample in which 2,3,7,8-TCDF is identified by analysis on a DB-5 column must have a confirmatory analysis performed on a DB-225, SP-2330, or equivalent GC column.”

4.2 Recommendations for confirmation of results for PCDD/Fs

The chromatographic separation of relevant 2,3,7,8-substituted PCDD/F congeners from non-2,3,7,8-substituted PCDD/Fs and other interfering substances is critical for the correct quantification of PCDD/Fs in feed.

Specific criteria regarding the application of certain stationary phases for gas chromatographic separation are not defined in legislation. Also, EN 16215:2020 [2], ISO 13914:2023 [6] and EPA method 1613 rev. B [4] emphasize the necessity to separate non-2,3,7,8-substituted PCDD/F congeners from the analytes of interest.

In particular, for the analysis of feed samples for PCDD/Fs interferences of non-2,3,7,8-substituted PCDD/Fs may be relevant due to the source of contamination of these matrices (e.g. by open drying processes). Depending on the contamination and therefore the resulting congener pattern, interferences with different non-2,3,7,8-substituted PCDD/F congeners are possible.

In order to reduce the influence of interferences on the analytical results for 2,3,7,8-substituted PCDD/F congeners and as a consequence on the WHO-TEQ sum parameter the following is concluded:

> **In case of exceedance of legal limits, for confirmation of results in duplicate analysis two different stationary phases with different polarity, different elution order or different separation of the analytes of interest from interferences should be applied for the GC analysis.**

In this way the duplicate analysis does not only exclude the possibility of internal cross-contamination or accidental mix-up of samples, but also reduces the risk of false non-compliant results due to the different chromatographic separation of the analytes of interest.

5. References

[1] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

[2] EN 16215: 2020: Animal feeding stuffs: Methods of sampling and analysis - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

[3] CRL for Dioxins and PCBs in feed and food, Determination of dioxins in mineral feed, trace elements, premixtures and compound feed: Recommendation for extraction procedures, 2007 (https://eurl-pops.eu/user/pages/05.news/06.download-eurl-recommendation/EURL_Recommendation_for_extraction_procedures.pdf?g-b7b30d77)

[4] EPA Method 1613 Revision B Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, October 1994

[5] EPA 1668 Revision C Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, April 2010

[6] ISO 13914:2023: Soil, treated biowaste and sludge - Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass selective detection (HR GC-MS)