

Interlaboratory study on harmonization of extraction procedures for determination of PCDD/F and PCB in feedingstuff

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Introduction

The process of harmonization of extraction methods for the determination of PCDD/F in mineral feed, which was initiated with the interlaboratory study on extraction techniques for sepiolite in 2006, was continued with an interlaboratory comparison on extraction methods for fullers Earth and manganese oxide^{1,2,3}. The study on the harmonization of extraction techniques for PCDD/F in fullers earth and manganese oxide comprises the interlaboratory study with 23 participating laboratories and additional comprehensive tests at the CRL Freiburg. As test materials, fullers earth, which is a mixed hormite-smectite mineral, and manganese oxide were analysed. Fullers Earth is comparable to other mineral clays, which are used as feed additives, and can also be used as bleaching earth for food contact. Manganese oxide is added to compound feed as trace elements in concentrations up to 150 mg/kg (calculated as total Mn).

Materials and Methods

Structure of the study

The comprehensive study organized by the CRL Freiburg comprised two parts: first, evaluation of the influence of different parameters on extraction of fullers earth and manganese oxide (at the CRL Freiburg) and second, an interlaboratory study with National Reference Laboratories (NRLs) and qualified laboratories with outstanding experience in the field of PCDD/F- and PCB-analysis in feeding stuff (see table 1), testing selected parameters for extraction of fullers earth and manganese oxide.

Table 1: Participating laboratories of the interlaboratory study on extraction methods for the determination of PCDD/F in fullers earth and manganese oxide

NRLs	
Austria	Umweltbundesamt
Belgium	CART
	Federal Agency for the Safety of Food
	NIPH - National Institute of Public Health
Czech Republic	Institute of Public Health
Denmark	National Food Institute
Greece	NCSR Demokritos
Hungary	Central Laboratory of National Institute for Agricultural Quality Control
Italy	Istituto Zooprofilattico dell' Abruzzo
Luxembourg	Okometric
Malta	Central Science Laboratory (CSL)
Netherlands	RIKILT
Portugal	Instituto Nacional de Engenharia, Tecnológica e Inovação (INETI)
Slovakia	National Reference Centre for Dioxins and Related Compounds
Slovenia	Institute for Public Health
Other laboratories	
France	CARSO
Germany	CVUA Münster
	Eurofins GfA
	LAVES Oldenburg
	SGS Institut Fresenius
Spain	Institut Quimic de Sarria (IQS)
	University A Coruna
USA	Analytical Perspectives

Test material

Two different test materials were provided as test material:

- Fullers earth: a mixed hormite-smectite mineral
- Manganese oxide

Interlaboratory study

The participants of the interlaboratory study received one sample of fullers earth and manganese oxide, a fullers earth extract and an analyte solution. The laboratories were asked to perform duplicate analyses and analyze reagent blanks using the following extraction methods:

- Soxhlet extraction with toluene and a mixture of toluene and a polar solvent
- Other routinely used extraction methods
- Soxhlet extraction with toluene or a mixture of toluene and a polar solvent after pre-treatment with 3 M HCl at room temperature

For the fullers earth extract, the material was extracted with ethanol/toluene (70+30; v/v) without pre-treatment. No additional unlabeled PCDD/F congeners were added.

The analyte solution EDF-5008-50 provided by Cambridge Isotope Laboratories (CIL) contained PCDD/F at concentrations of 2, 5 and 10 pg/ μ l for tetra-, penta- to hepta- and octachlorinated PCDD/Fs.

Comprehensive study at the CRL Freiburg

The second part of the study on the influence of extraction parameters was a comprehensive test at the CRL Freiburg. The influence of the following parameters were checked:

- Extraction methods: Twisselmann or pressurized liquid extraction
- Extraction solvents and solvent mixtures
- Pre-treatment with different concentrations of hydrochloric acid

Sample preparation and instrumental method

Various extraction methods were tested using different solvents and solvent mixtures for extraction. Additionally, pre-treatment of fullers earth and manganese oxide before extraction was checked. The applied extraction methods are shown in figure 1 and 2.

Figure 1: Applied pre-treatment and extraction methods of fullers earth; left side: extraction methods with different solvents and solvent mixtures without pre-treatment with HCl; right side: extraction with ethanol/toluene (70+30; v/v) after pre-treatment with HCl.

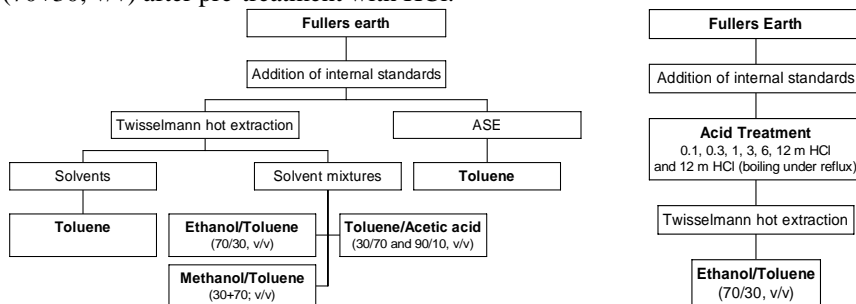
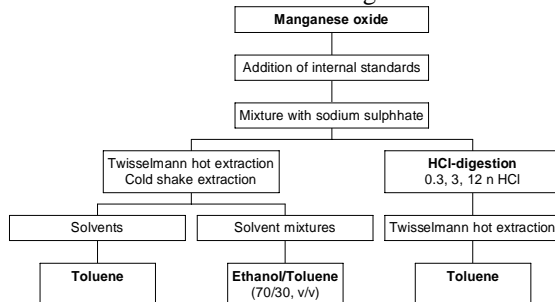


Figure 2: Applied pre-treatment and extraction methods of manganese oxide



These extraction steps were followed by a further clean-up step for mineral feed. The detailed clean-up steps are described elsewhere^{3,4}.

High-resolution gas chromatography (HRGC) coupled to high-resolution mass spectrometry (HRMS) was employed. In brief, separation for PCDD/Fs was carried out on a fused silica capillary column (DB-5 MS, 60 m, 0.25 mm i.d., 0.25 µm film thickness, J&W scientific, Folsom, USA). The MS resolution was 10'000.

Results and Discussion

Fullers earth

Interlaboratory study

The average WHO-PCDD/F-TEQ results in pg/kg product for the applied extraction methods and the CVs are shown in Figure 3.

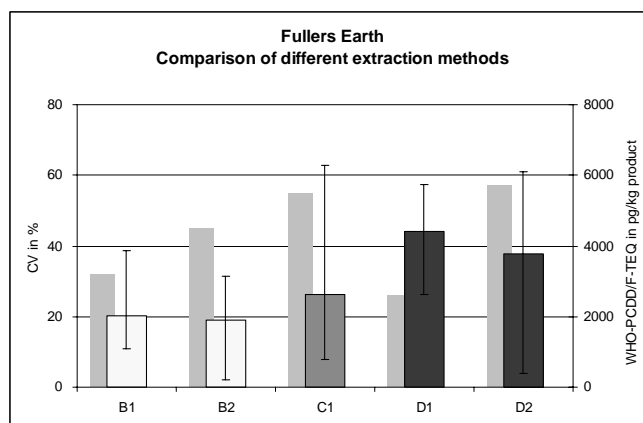


Figure 3: Comparison of WHO-PCDD/F-TEQ results on pg WHO-PCDD/F-TEQ/kg product. B1: Soxhlet extraction with toluene + polar solvent, B2: Soxhlet extraction with toluene, C1: PLE with toluene + polar solvent, D1: HCl-digestion (3 M) and Soxhlet extraction with toluene, D2: HCl-digestion (3 M) and Soxhlet extraction with toluene + polar solvent. Coefficient of variation (CV) in % in light grey

With concentrations of about 2 ng WHO-PCDD/F-TEQ/kg product, the mean and median for extraction with toluene and a mixture of toluene and a polar solvent were comparable. Mean levels found by extraction with PLE were about 40 % higher. The Soxhlet extraction with a mixture of toluene and a polar solvent had a considerably lower CV (32 %) compared to Soxhlet extraction with toluene (CV 45 %) or PLE with a mixture of toluene and a polar solvent (55 %).

Also the results of the Soxhlet extraction with toluene and a mixture of toluene and a polar solvent of each laboratory were investigated for significant trends. Seven laboratories reported higher results for a mixture of toluene and a polar solvent, seven reported higher results for toluene and seven rather unchanged results. A significant increase or decrease when using a mixture of toluene and a polar solvent compared to toluene was not observed.

Digestion with 3 M HCl showed higher concentrations of about a factor of two, compared to extraction without HCl pre-treatment. The levels for extraction with toluene (mean: 4.4 ng/kg product) and with a mixture of toluene and a polar solvent (mean: 3.7 ng WHO-PCDD/F-TEQ/kg product) after HCl-digestion were rather comparable with a higher CV for a mixture of toluene and a polar solvent.

Comprehensive study at the CRL Freiburg

For Twisselmann hot extraction without pre-treatment, the solvents/solvent mixtures toluene and toluene + acetic acid resulted in highest levels of about 3 ng WHO-PCDD/F-TEQ/kg product. Concentrations determined by extraction with toluene + ethanol or methanol were about a factor of 2 lower, levels determined by ASE with toluene were about one third lower if compared to Twisselmann extraction with toluene.

HCl-digestion before Twisselmann extraction with ethanol/toluene (70/30, v/v) showed increasing levels of WHO-PCDD/F-TEQ with increasing concentration of HCl ranging from about 1.5 ng/kg (without digestion) to

about 7 ng/kg (digestion with 12 M HCl and boiling under reflux). The digestion with 0.1 and 0.3 n HCl showed only slightly higher TEQ-levels compared to extraction without digestion. A change of the congener pattern for increasing strength of HCl was not observed. All relevant PCDD congeners increased in the same way. Recoveries of the internal standards were acceptable for all extraction techniques.

Manganese oxide

Most laboratories, including the CRL, reported only LOQs or “not analyzable” for nearly all congeners for extractions with and without acid pre-treatment. Due to the low levels of the congeners (high differences between upper- and lower-bound-results) and the low recoveries, focus was set on the recoveries of the ¹³C₁₂-labeled standards for the different extraction techniques.

The highest recoveries (mean between 50 and 60 %, but still high variations) were found for the extraction with toluene (with and without digestion with HCl). For extractions using mixtures with polar solvents, the recoveries were considerably lower. In both cases recoveries decreased with increasing number of chlorine substituents at the dibenzo-p-dioxin and dibenzofuran structure.

Conclusions and recommendations

Mean and median results for Soxhlet extraction with toluene and toluene + polar solvent were comparable. The digestion with up to 0.3 M HCl showed no significant difference compared to the extraction without digestion. Higher concentrations of HCl than 0.3 M do not reflect physiologic conditions any more and are therefore not relevant. The recommendations given for the extraction for sepiolite - using toluene + a substantial fraction of polar solvents - are also applicable for fullers earth.

When analyzing manganese oxide, highest recoveries of ¹³C₁₂-labeled standards were found for extraction with toluene. Digestion with HCl before extraction didn't improve the recoveries. Additional use of polar solvents resulted in considerably lower recoveries. Toluene is therefore the best suitable extraction solvent for manganese oxide, but further improvement of the methods applied is still necessary.

As a main conclusion from this comprehensive study and the sepiolite study in 2006 organized by the CRL, the best suitable extraction method for mineral feeds such as clays (except oxides of trace elements) and compound feed is toluene mixed with a substantial fraction of a polar solvent. For oxides of trace elements, toluene is the most suitable solvent. For an unknown premixture or compound feed with problematic composition it is recommended to combine two extraction steps, e.g. first with toluene and then with toluene / polar solvent mixtures. There is no need to pretreat the feed samples with HCl.

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References

1. Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs
2. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed
3. Kotz A, Malisch R, Hädrich J, Adamovic K, Gerteisen I, Tritschler R, Winterhalter H, 2007. *Organohalogen Compd* 69, 130-133
4. Malisch, R., Bruns-Weller, E., Knoll, A., Thoma, H., Peichl, L., 1997. *Organohalogen Compd* 31, 83-88