

Extraction efficiency of PCDD/Fs in mineral feed additives

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1. Introduction

Today, a number of dioxin contamination episodes occurred all over the world have demonstrated the feasibility for these toxicants to reach the food chain^{1,2,3,4,5}. In consequence, some measures to protect and improve the quality of human health have been enforced. As an example, the EU set maximum levels for PCDD/Fs in food, feed as well as in a large number of additives included as a feed intermediates^{6,7}. Some of the most employed feed additives are constituted by mineral matrices such as sepiolite, bentonite, kaolin, etc. These minerals are composed by a variable content of natural magnesium and aluminum silicates and other minor components. In general terms, they are characterized by their micron sized particles, swelling properties (i.e. bentonite, vermiculite), large surface areas (i.e. sepiolite, zeolite), high cation exchange capacity (i.e. bentonite, vermiculite), chemical stability (i.e. sepiolite, talc, kaolin) and charge distribution. Other minerals participating in feed manufacture are also halite, calcium carbonate, sodium sulphate, etc.

The literature of dioxin levels in feed ingredients is scarce and guidelines related to PCDD/F levels in particular cases such as sepiolite are still under evaluation⁸. Recently, the evaluation of methodologies used for the analysis of mineral origin feed additives has become an interesting issue due to the complexity of these matrices. In some cases, classical extraction methods do not allow to achieve the required extraction efficiency. Moreover, they can lead to different results and thus can affect the final decision on setting maximum and action levels for these kinds of materials. For these reasons, an harmonization of the methodologies for the analysis of PCDD/Fs in mineral matrices is mandatory. In this way, Hosseinpour et al. proposed the use of accelerated solvent extraction (ASE) instead of Soxhlet extraction for the analysis of caolinitic clays and copper oxide with good results⁹.

The objective of the present work is to evaluate an alternative method to Soxhlet extraction for the analysis of PCDD/Fs in mineral feed additives and to show the variability of the results depending on the sample treatment. In addition valuable data on PCDD/F concentrations in a wide range of mineral additives is also reported.

2. Materials and Methods

A sepiolite sample was analyzed by two different analytical methods.

1) In the first method, dioxins were removed from the mineral samples by Soxhlet extraction (24 h) using toluene:cyclohexane (1:1, v/v).

2) The second method consisted on an acid attack combined with a simultaneous extraction during aprox. 16 h, using 20 g of the sample, 200 ml of HCl (1M, 6M or 12 M) and 200ml of n-hexane/dichloromethane (1:1, v/v). After the acid treatment, the aqueous phase was extracted twice with 25 ml n-hexane and the organic phases were recombined and stored at 4°C. The solid residue was filtered, neutralized and re-extracted by Soxhlet (24 h) with toluene. Next, before the clean-up procedure, the organic extracts from the Soxhlet and the acid treatment were recombined and concentrated until 5 ml c.a.

Finally, the extracts obtained from both methods were concentrated and transferred to n-hexane prior to the purification step. Details on the clean-up procedure based on the use of the Power PrepTM system (FMS Inc., MA, USA) and instrumental analysis performed by HRGC/HRMS (Autospec Ultima NT, Waters, Manchester, UK) are described elsewhere¹⁰. The criteria for ensuring the quality of dioxin analysis include the application of some quality control (QC) and quality assurance (QA) measures, such as a continuous monitoring of laboratory contamination based on the determination of a blank sample covering the whole analytical procedure, including extraction, clean-up and quantification⁸.

3. Results and Discussion

In this work, the influence of the extraction procedure for the analysis of PCDD/Fs has been evaluated over a number of mineral matrices currently employed as feed additives. The study is primarily focused in sepiolite since the particular properties of this mineral revealed dissimilar behavior depending on the extraction treatment. In addition, other minerals such as halite, sodium sulphate, calcite, bleaching earths, kaolin, ferric chloride, all of them with remarkable differences in terms of physicochemical properties, were also considered in order to achieve valuable information on these materials. Thus, PCDD/PCDF analyses were performed over 16 different mineral feed additives. Compiled data including type of matrix, methodology employed, recovery rates and PCDD/Fs concentration are summarized in Table 1.

Table 1. Summary of PCDD/PCDF found in different mineral matrices studied.

Mineral Matrices	Spiked before acid treatment			Spiked after Soxhlet extraction ²			Spiked before Soxhlet extraction ²		
	Acid conc.	pg WHO TEQg	% recovery	pg WHO TEQg	% recovery	pg WHO TEQg	% recovery		
Sepiolite modified ¹	6M	0.96	40-76%	0.02-0.17	42-84%				
Sepiolite	6M	1.68	37-87%	0.04-0.62	70-90%	0.15-0.27	0-66%		
Kaolin	3M	193.25	-	0.02-388.21	91-98%				
Bentonite	6M	1.49	1-28%	0.03-0.11	59-74%	1.49	0-28%		
Mortmorilloite				2.38	81-93%				
Kieselguhr				137.5-114.5	89-102%				
Calcite						0.037-2.89	71-90%		
Halite						0.072-0.052	56-93%		
Periclase (MgO)						0.037-0.74	73-98%		
Sodium sulphate anhyd.						0.012	66-95%		
Ferric chloride						0.087	60-88%		
Bleaching earths						0.31-5.96	68-115%		

¹ Sepiolite with reological properties; ² No acid treatment

In general, the findings revealed satisfactory recovery rates using classical methods based on Soxhlet extraction or liquid-liquid extraction, without any

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pretreatment for matrices such as halite, periclase, ferric chloride or sodium sulphate with values between 60 and 115%. Moreover, concentration of PCDD/Fs kept below the limits established in the EU of 0.75 ng WHO-TEQ/kg in almost all the cases evaluated. On the contrary, poor recovery rates were achieved for matrices such as sepiolite or bentonite, following the same methodology. In concrete, the recovery dramatically decreases when increasing the degree of chlorination, being OCDF and OCDD the most critical congeners. This fact could be explained because of remarkable adsorption processes, which precisely are some of the most appreciated properties of these materials.

Taking into account these results, a comparison between the analysis of extractable PCDD/Fs by Soxhlet extraction and alternative methodologies based on acid attack was performed over a sepiolite sample. For this purpose, the matrix was treated with hydrochloric acid at 1M (slightly stronger conditions than in the digestive system in the animals), 6M and 12M (involving the full destruction of the crystalline structure, which does not occur to certain minerals during the digestion process). In figure 1 it can be observed the diffractograms obtained for the un-treated sepiolite and treated with hydrochloric acid at 0.3N as well as 12N overnight and how crystalline degradation is completely accomplished when the matrix is treated with 12N hydrochloric acid.

Table 2 summarizes the results obtained in a sepiolite sample using different extraction strategies above-mentioned. In general, the findings demonstrated increasing concentration of dioxins and furans when increasing the concentration of hydrochloric acid being 6M or 12M the optimum values. Moreover, better extraction efficiency was obtained with hydrochloric acid treatment 6M and 12M. As expected, no problems were observed regarding recovery rates for the Soxhlet spiking after the extraction. Figure 2 summarizes comparison values of concentrations of 2,3,7,8-PCDDs/Fs obtained during the experiment.

In the Soxhlet extraction, the dioxin concentration in terms of TEQ units was around 0.15-0.26 ng WHO-TEQ/kg (upperbound level). Comparable results were achieved when the matrix was treated with hydrochloric acid 1M. In contrast, hydrochloric treatment using 6M or 12 M resulted in an increase of the total WHO-TEQ four fold higher in comparison to those found following Soxhlet extraction procedure.

This point out whether or not these materials could retain 'non extractable' dioxins when traditional Soxhlet or other mild conditions extraction are applied. Moreover, these findings support the hypothesis that a complete destruction of the matrix combined with simultaneous extraction make those PCDD/Fs available from the sample and an exhaustive dioxin extraction can be accomplished. On the contrary, this scenario introduces another concept, the *bioavailability* of dioxins present in mineral matrices such as sepiolite. Ferrario et al. demonstrate the evidence of contamination in chickens that had been fed contaminated feed composed by ball clay¹¹. This means that digestive conditions would allow dioxins to be released and being metabolized by animals. However, is important to know witch percentage of total dioxins in the mineral would be released from the mineral under digestive conditions¹². In this sense, the harmonization of a methodology for the analysis of these toxicants in minerals or feed additives containing high amounts of minerals as well as the setting of maximum and action levels should take into account this situation in order to guarantee protection of public health and avoid an exposition of the animals and human beings to these contaminants.

Table 2. PCDD/PCDF concentrations in sepiolite analyzed by different methodologies.

COMPOUNDS	WHO-TEQ pg/g					Recovery (%)				
	Soxhlet ¹	Soxhlet ²	1M	6M	12M	Soxhlet ¹	Soxhlet ²	1M	6M	12M
2,3,7,8-TCDF	0.038	0.08	0.022	0.058	0.054	102	47	52	110	92
1,2,3,7,8-PeCDF	0.017	0.040	0.058	0.24	0.12	107	30	26	95	87
2,3,4,7,8-PeCDF	0.013	0.030	0.024	0.083	0.036	111	28	29	99	88
1,2,3,4,7,8-HxCDF	0.050	0.050	0.053	0.12	0.059	124	13	13	54	62
1,2,3,6,7,8-HxCDF	0.025	0.050	0.074	0.23	0.16	98	12	14	48	61
2,3,4,6,7,8-HxCDF	0.052	0.060	0.046	0.097	0.031	105	11	15	58	71
1,2,3,7,8,9-HxCDF	0.044	0.090	0.12	0.44	0.22	126	22	16	74	68
1,2,3,4,6,7,8-HpCDF	0.005	0.029	0.037	0.33	0.22	117	2	6	30	27
1,2,3,4,7,8,9-HpCDF	0.003	0.013	0.17	0.24	0.11	143	5	7	46	38
OCDF	0.0007	0.07	0.32	0.93	0.54	n.p.	n.p.	n.p.	n.p.	n.p.
2,3,7,8-TCDD	0.0033	0.03	0.014	0.056	0.041	111	34	40	99	92
1,2,3,7,8-PeCDD	0.081	0.018	0.12	0.43	0.31	114	18	22	92	86
1,2,3,4,7,8-HxCDD	0.020	0.010	0.12	0.15	0.038	136	6	12	56	62
1,2,3,6,7,8-HxCDD	0.082	0.013	0.21	0.56	0.36	104	8	13	51	63
1,2,3,7,8,9-HxCDD	0.35	0.49	0.35	1.69	1.11	n.p.	n.p.	n.p.	n.p.	n.p.
1,2,3,4,6,7,8-HpCDD	0.056	0.043	0.38	0.52	0.60	129	1	7	16	30
OCDD	0.095	0.042	1.45	2.31	2.56	139	0	3	26	16
TOTAL WHO-TEQ	0.15	0.26	0.27	0.69	0.63					

Soxhlet¹: spiking before the extraction; Soxhlet²: spiking after the extraction.

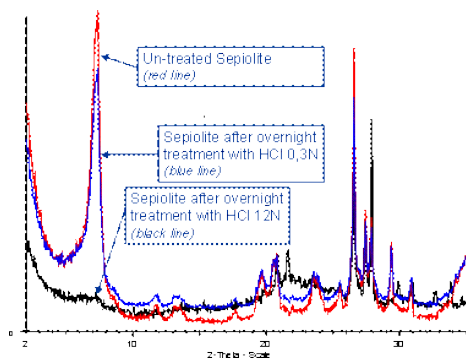
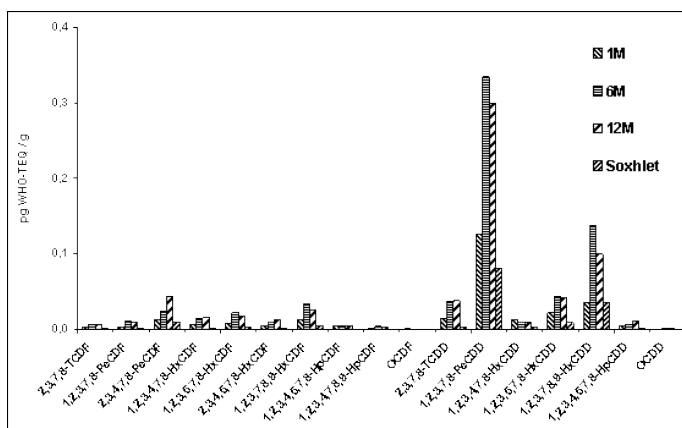


Figure 1. Diffractograms of the un-treated sepiolite and treated with hydrochloric acid at 0.3N as well as at 12N overnight.



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Figure 2.pg WHO-TEQ / g of PCDD/Fs in sepiolite analyzed with the Soxhlet and the acid treatment at three concentrations: 1M, 6M and 12 M.

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