COMPREHENSIVE STUDY ON DIOXIN CONTENTS IN BINDER AND ANTI-CAKING AGENT FEED ADDITIVES

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Introduction

The aim of this work is focussed on the evaluation of the presence of PCDDs/PCDFs in some binder and anticaking agent feed additives such as bentonite, vermiculite, sepiolite clays, Kaolins with an especial attention to the sepiolite.

These minerals are composed by a variable content of a natural magnesium silicate and other minor components such as aluminum, iron silicates, carbonates, feldspars or quartz. In example, the sepiolite (EC number: E-562) consists of a sedimentary hydrated magnesium silicate (60% minimum) with a chemical formulae: $Mg_4Si_6O_{15}(OH)_2 \cdot 6(H_2O)$, montmorillonite (30% maximum) and free amianthum. These natural clays are characterized by their micron sized particles, swelling properties large surface areas, high cation exchange capacity, chemical stability and charge distribution. Other related substances also include sepiolite clays (40% hydrated magnesium silicated minimum), kaolins or zeolites. Owing to its physical and chemical properties these products are widely employed as additives (blinders, anti-caking agents and coagulants) in feedingstuffs. The annual production of sepiolite in Spain exceeded 762 ktonnes in 1996 and 1000 ktonnes including attapulgite and bentonite.

After the dioxin contamination episode of animal feed in Belgium in may 1999 some measures to protect and improve the quality of human health were enforced by the European Union (EU). In particular, a regulation on the conditions for the authorisation of some additives in feedingstuffs was adopted in which a limits values for PCDDs/PCDFs content of 500 pg WHO-TEQ/kg was established in the light that any investigation could give rise a possible re-examination of the provisions adopted [1]. Afterwhich, in order to provide updated information on dioxin contamination a surveillance programme was undertaken by some European member countries. As a results, the Commission concluded that the data provided on the presence of dioxins in natural mixtures of steatites and chlorites, sepiolite and sepiolitic clays indicated that these additives were not contaminated with dioxins or contain levels of dioxins below the threshold of the analytical method [2].

A summary of the data reported in this work was also gathered by the Spanish Sepiolite Industry to the Spanish Delegation to provide information to the European Commission as indicated by the regulation previously mentioned.

Materials and Methods

All the pollutants were removed from the samples by Soxhlet extraction using toluene for 24 h. Prior to the extraction process, the samples were spiked with labeled PCDD/PCDF standards described in EPA 1613.The clean up was based on the use of multilayer silica, basic alumina and PX-21 carbon adsorbents, prepacked in teflon columns and hermetically sealed (FMS Inc., MA).

Final separation, identification and quantification of PCDDs/PCDFs was performed by HRGC/HRMS analysis. Purified extracts were analyzed by HRGC-HRMS/EI(+)-SIM on a GC 8000 series gas chromatograph (Carlo Erba Instruments, Milan, Italy) coupled to an Autospec *Ultima mass spectrometer (Micromass, Manchester, UK) equipped with a CTC A 200S* autosampler, at 10000 resolving power (10% valley definition). Chromatographic separation was achieved with a DB-5 (J&W Scientific, CA, USA) fused-silica capillary column (60 m x 0.25 mm ID, 0.25 μm film thickness) with helium as the carrier gas at a linear velocity of 35 cm/s (T: 100°C) in the splitless injection mode (1-2 μL). The temperature program was: 140 °C (1min) to 200 °C (1min) at 20 °C/min, then at 3°C/min to 300 °C and held isothermally for 20 min at 300 °C for the DB-5 GC column and 140 °C (1min) to 200 °C (1min) at 20 °C/min, then at 2°C/min to 280 °C. Quantification was carried out by the isotopic dilution method [3]. The results are expressed in pg I-TEQ/Kg and pg WHO-TEQ/kg [5,6]. TEQs values were calculated using the limit of detection (LOD) value for non-detects compounds or values below to the LOD [1].

Results and Discussion

The surveillance program on dioxin content in additives included mainly the analysis of sepiolite samples and other related substances such as sepiolitic clays, kaolins, vermiculites, zeolites and bentonites. In a preliminary study a total of 16 different samples were analyzed (Table 1). In all cases, the results revealed values below to the limit of 500 pg WHO-TEQ/kg. Interesting cases were focused on the kaoline samples whereas the results were 10000 times higher. The remarkable presence of PCDD respect to PCDF was dominated by OCDD which levels were up to 132 μg/kg. The profile expressed in pg WHO-TEQ/kg indicated an important contribution of the 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD versus the others congeners (figure 1).

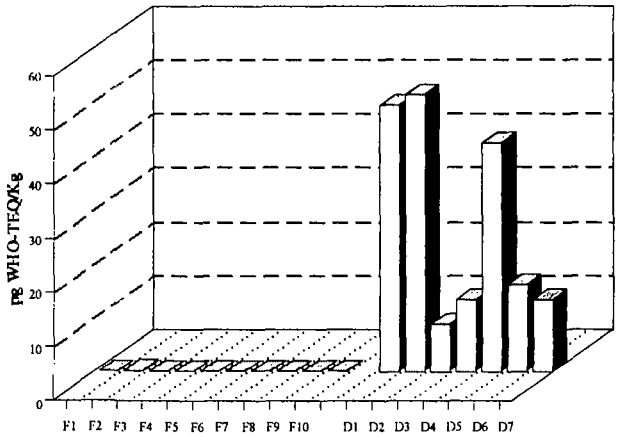
Table 1. Preliminary results on the study of feedingstuffs additives.

Type of samples	Levels (pg I-TEQ/kg)	Levels (pg WHO-TEQ/kg)
Sepiolite 1	210	290
Sepiolite 2	260	331
Sepiolite 3	210	293
Sepiolite 4	170	222
Sepiolite 5	110	157
Sepiolite 5 (Duplicate)	180	229
Sepiolite 6	220	260
Sepiolite 7	410	470
Kaolin 1	388210	460590
Kaolin 2	193250	232314
Bentonite	190	198
Zeolite	48	50
Vermiculite	60	63
Damoline	135	138
Sepiolite clay 1	338	421
Sepiolite clay 2	466	383

Figure 1: Congener-specific 2,3,7,8-PCDD/PCDF distribution in kaolin samples

F1: 2,3,7,8-TCDF
F2: 1,2,3,7,8-PeCDF
F3: 2,3,4,7,8-PeCDF
F4: 1,2,3,4,7,8-HxCDF
F5: 1,2,3,6,7,8-HxCDF
F6: 2,3,4,6,7,8-HxCDF
F7: 1,2,3,7,8,9-HxCDF
F8: 1,2,3,4,6,7,8-HpCDF
F9: 1,2,3,4,7,8,9-HpCDF
F10: OCDF

D1: 2,3,7,8-TCDD
D2: 1,2,3,7,8-PCDD
D3: 1,2,3,4,7,8-HxCDD
D4: 1,2,3,6,7,8-HxCDD
D5: 1,2,3,7,8,9-HxCDD
D:6 1,2,3,4,7,8,9-HpCDD
D7: OCDD



However, the absence of consistent data on the analysis of PCDDs/PCDFs in these kind of additives or any existing reference material, the large differences between the findings obtained in the preliminary study in matrix with the same nature and the analytical difficulties due to the remarkable adsorbent properties of these materials gave rise to elaborate an accurate study on the performance of the analytical methodology applied to the analysis of PCDDs/PCDFs in natural clays.

For the characterization of the PCDDs/PCDFs measurement procedure a comparative trial was performed to evaluate its variability in sepiolite samples. Thus, a representative sample collection of sepiolite Exal H from a total production of 39600 kg was collected from a Spanish basin located in Madrid. Next, the homogenized sample was shipped to the laboratory and carefully stored.

A total of 12 analysis were carried out to evaluate the repetitivity and the repeatibility over three consecutive days. The first day, 6 intra-day analysis allowed the assessment of the repetitivity. At a mean concentration of 180 pg WHO-TEQ/kg , the variability was 22 pg WHO-TEQ/kg. Bearing in mind that these samples represented a dioxin low content, the results indicated a good correlation between the findings with an acceptable standard deviation.

Next, over the second and the third day, 3 more inter-day analysis were respectively carried out to examine the repeatibility. For the calculation of the variability 3 values obtained from the first day were also considered. 9 results were used for the calculation of the repeatibility. The average value was 200 pg WHO-TEQ/kg and variability was 42 WHO-TEQ/kg.

The robustness of the methodology against the sample weight was also studied. Analyses in triplicate were carried out over a sample weight of 12.5, 25, 37.5 and 50 g respectively. 12 values allowed to calculate the variability. The results indicated little variation between the findings when the analyses of 12.5 g sample weight are considered (average of 270 pg WHO-TEQ/kg with a variability of 62 pg WHO-TEQ/kg and a coefficient correlation r of 0.8). However, an improvement in the statistics were observed by increasing the sample weight whereas the mean

was 240 WHO-TEQ/kg and the variability was 31 pg WHO-TEQ/kg (r:0.9) when 25, 37.5 or 50 g of sample were analyzed. This is consistent with the fact that operating near the level of detection the results show larger standard deviation when compared to the average value. Overall, it can be concluded that the results obtained in the analysis of PCDDs/PCDFs in sepiolite samples on the basis of the methodology previously described showed good method accuracy.

In connection, a preliminary study on the influence of sepiolite in dioxin content in feedingstuff of laying hens fed was also undertaken. The proposal of the study included the analysis of PCDDs/PCDFs in feed with 0 (control group) and 3% sepiolite. The exceding level of 3% sepiolite was used only for experimental evidence whereas 2% is the maximum legally established. A potential increasing on dioxin levels in derived products was also assessed by the analyses of eggs samples collecting over 8 months. The findings indicated little differences between the control and samples containing sepiolite (Table 2).

Table 2. Preliminary results on the influence of sepiolite in dioxin content in feedingstuffs.

Sample	Control group (pg WHO-TEQ/kg)	3% sepiolite group (pg WHO-TEQ/kg)
Feed		
Sample 1	60	70
Sample 2	70	80
Average	***65***	***75***
Eggs		
Sample 1	270	320
Sample 2	300	340
Sample 3	330	380
Sample 4	400	340
Average	***305***	***345***

Acknowledgments
The authors wish to thank Mª Generosa Martrat, Jordi Sauló and Miguel Angel Adrados for their contribution to this work. The financial support from TOLSA is gratefully acknowledged.

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