A NEW EPISODE OF PCDDs/PCDFs FEED CONTAMINATION IN EUROPE: THE CHOLINE CHLORIDE.

J. J. Llerena, E. Abad, J. Caixach and J. Rivera.

Laboratory of Dioxins, Dept. of Ecotechnologies, IIQAB-CSIC, C/ Jordi Girona 18, 08034 Barcelona (Spain). Fax: 34-932045904, E-mail: jraeco@iiqab.csic.es

Introduction

The choline chloride (2-hydroxyethyl-trimethyl ammonium hydroxide), also known as vitamin B4, is a widely used feed additive, due to its importance on the metabolism of livestock. It plays a central role in several metabolic ways (i.e. choline is a structural component of acetylcholine). Commercial formulations employed in feed's elaboration include the use of a variety of some vegetable carriers such as sawdust, almond's shell or corncob at different proportions, which are called as pre-mixed choline chloride.

In June 2000, analysis of PCDDs/PCDFs performed by some German labs within the framework of routine feed controls, detected remarkable levels of dioxins in some batches of pre-mixed product. Further research indicated that the origin of these products was Spain. Immediately the German authorities communicated to the Spanish government. Afterwards, the Spanish Ministry of Agriculture, Fisheries and Food, jointly with the Autonomic Governments and the Spanish industry conducted a deep plan to detect and to isolate the contaminated point source. Administration services identified the elaboration plant, stopped the production and blocked the distribution of the already prepared pre-mixes. Then a sample collection episode of a variety of products were collected from different sites and shipped to the Laboratory of Dioxins of the CSIC for dioxin characterization. First sample was received on June 8th, 2000, and last one on July 7th, 2000. References of the samples can be found in Table 1. Samples can be divided as follows:

Animal Feed (6 samples): Six different feeds were analyzed.
Choline Chloride (20 samples) in different formulations: in liquid form (78 % and 70 % w/w) and in vegetal carrier (60 % and 50 % w/w).
Carriers (10 samples): Five samples of mixed vegetal carriers (without choline chloride) were analyzed. In addition, samples of the individual compounds (corncob, almond's shell, and pine sawdust) were also analyzed.

The analysis of those samples allowed to dismiss the contamination of pure choline chloride, and that the point source of dioxin was the vegetal carrier formed by wood sawdust which presented remarkable content of PCP and dioxin levels up to 224.41 pg WHO-TEQ/g.

The aim of this paper is to present the results of those analysis and to show the importance of a quick answer to avoid the distribution of contaminated ingredients to the market.

Methods and Materials

Owing to their lipophilic nature, the analyses of PCDDs/PCDFs were carried out on the assumption that major content of these compounds was placed in the lipid fraction. Extraction of solid samples was carried out using a soxhlet for 12 h with toluene-ciclohexane; for choline chloride solution (concentration of 78 %) a liquid-liquid extraction with dichloromethylene was performed.

Afterwhich, the samples were spiked with known amounts of a $^{13}C_{12}$-PCDDs/PCDFs. Organic matrix was removed by a sulphuric acid treatment, if needed, whereas PCDDs/PCDFs remained in the n-hexane fraction. Finally, the extracts were rotary concentrated prior to the clean up process.

The clean up was based on the use of multilayer silica, basic alumina and PX-21 carbon adsorbents as described in reference 1. 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). Quantification was carried out by the isotopic dilution method[1]. The results are expressed in pg I-TEQ/g and pg WHO-TEQ/g[2]. TEQs values were calculated using the limit of detection (LOD) value for non-detected compounds or values below the LOD.

Results and Discussion

For each type of sample, average value of WHO-TEQ and I-TEQ as well as the standard deviation (STD) for both values are shown in Table 2. For the calculation of those values, samples were grouped by formulation.

The analysis of choline chloride showed that the most concentrated formulations presented the lowest dioxin levels (0.02 pg WHO-TEQ/g for a concentration of 78 %, and 0.23 pg WHO-TEQ/g for 70 %). Increased dioxins levels were related with decreased choline chloride : vegetable carrier ratio (11.48 pg WHO-TEQ/g at 60 % and 53.67 pg WHO-TEQ/g at 50 %). Different values on "Choline chloride 50 %" can be explained by the differences in the composition of the vegetal carrier mixture.

The study of the results from the blended vegetal carriers confirmed the first hypothesis that the choline chloride was uncontaminated, and that the origin of the contamination was mainly due to one of the compounds used during the elaboration of the carrier. The most contaminated samples (CC28, CC30, CC33 and CC34), contained both corncob and pine sawdust.

Nevertheless, it was observed that highest levels of dioxins and furans were found in pine sawdust, with a equivalence toxicity of 224.41 pg WHO-TEQ/g (232.46 pg I-TEQ/g). Both neither corncob nor almond's shell showed significant amounts of dioxins and furans.

It must be remarked that the pattern of the PCDDs/PCDFs in sample from pine sawdust is in agreement with the pattern of pentachlorophenol treated wood, which seems to be the key for the determination of the origin of the contamination (Figure 1).

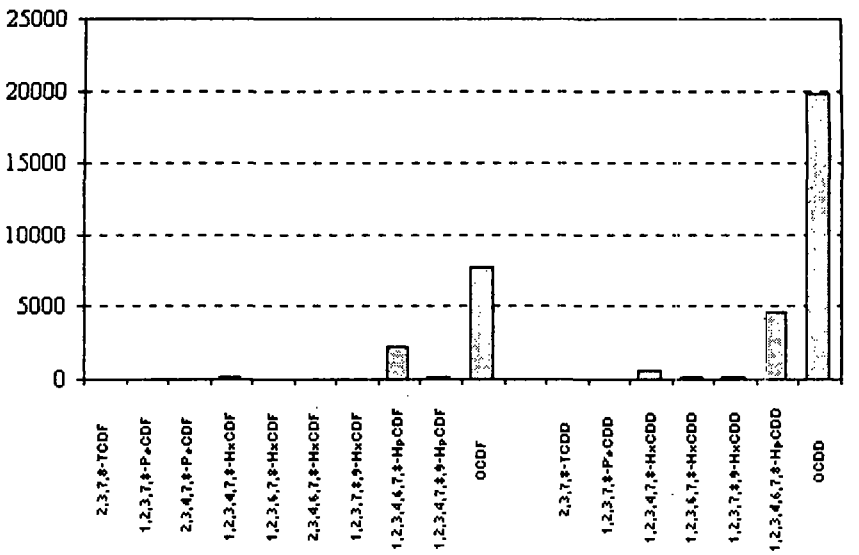
Table 1. List of samples and results (in pg WHO-TEQ/g, upperbound)

Ref	Sample	WHO-TEQ	Ref	Sample	WHO-TEQ
CC01	Choline Chloride 78 %	0.02	CC21	Feed	0.14
CC02	Choline Chloride 78 %	0.02	CC22	Feed	0.22
CC03	Choline Chloride 78 %	0.03	CC23	Feed	0.18
CC04	Choline Chloride 70 %	0.23	CC24	Feed	0.10
CC05	Choline Chloride 70 %	0.28	CC25	Feed	0.26
CC06	Choline Chloride 70 %	0.19	CC26	Feed	0.34
CC07	Choline Chloride 60 %	11.10	CC27	Carrier (blend)	0.60
CC08	Choline Chloride 60 %	11.85	CC28	Carrier (blend)	83.30
CC09	Choline Chloride 50 %	34.97	CC29	Carrier (corncob 100%)	0.26
CC10	Choline Chloride 50 %	108.34	CC30	Carrier (blend)	50.24
CC11	Choline Chloride 50 %	121.95	CC31	Carrier (blend)	2.80
CC12	Choline Chloride 50 %	7.30	CC32	Carrier (wood 100%)	224.41
CC13	Choline Chloride 50 %	50.45	CC33	Carrier (blend)	37.72
CC14	Choline Chloride 50 %	33.02	CC34	Carrier (blend)	45.60
CC15	Choline Chloride 50 %	54.82	CC35	Carrier (corncob 100%)	1.54
CC16	Choline Chloride 50 %	37.83	CC37	Carrier (almonds' shell)	0.04
CC17	Choline Chloride 50 %	61.44			
CC18	Choline Chloride 50 %	64.38			
CC19	Choline Chloride 50 %	69.30			
CC20	Choline Chloride 50 %	0.26			

Table 2. Summary of results expressed as pg TEQ /g (upperbound).

Matrix	No. of samples	pg WHO-TEQ/g				pg I-TEQ/g			
		Max	Min	Avg	STD	Max	Min	Avg	STD
Choline Chloride 78 %	3	0.03	0.02	0.02	0.01	0.03	0.04	0.03	0.01
Choline Chloride 70 %	3	0.19	0.28	0.23	0.05	0.17	0.21	0.21	0.05
Choline Chloride 60 %	2	11.10	11.85	11.48	0.53	14.19	14.89	14.54	0.49
Choline Chloride 50 %	12	0.26	121.95	53.67	35.82	0.32	179.20	64.49	49.91
Carrier (blend)	6	2.80	83.30	36.71	31.27	3.22	97.61	41.90	36.55
Carrier (almonds' shell)	1	0.04	n.p.	0.04	n.p.	0.04	n.p.	0.04	n.p.
Carrier (corncob 100%)	2	0.26	1.54	0.90	0.91	0.26	1.81	1.04	1.10
Carrier (wood 100%)	1	224.41	n.p.	224.41	n.p.	232.46	n.p.	232.46	n.p.
Feed	6	0.10	0.34	0.21	0.09	0.10	0.41	0.24	0.12

Figure 1. Congener profile of pine sawdust (concentration in pg/g)



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