gmp.GALIS: a self-monitoring system for feed producers. PCDDs/Fs and dl-PCBs results from 2011 to 2014

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Introduction

The European laws about food safety, Regulation (EC) nº 178/2002[1], Regulation (EC) nº 183/2005[2] and Regulation (EC) nº 882/2004[3]; have enforced higher feed requirements, including production and use when that feed is intended for food-producing animals. These strict new requirements are difficult to fulfill by a single producer. Therefore, Galician producers of feedingstuffs grouped in AGAFAC (compound feedingstuffs producers association), have joined their strengths creating the gmp.GALIS program, that is developed by SANOR, S.L.U., a company created by AGAFAC.

The gmp.GALIS program is a pioneering initiative in Spain. It was set up in 2004 with the aim of increasing the safety standards for animal feed in Galicia (13% of Spanish production), in agreement with HACCP standards, following existing models in other countries. gmp.GALIS Lab area coordinates aspects related with analytical laboratories carrying out two types of analysis: analysis of nutriments and analysis of pollutants. Among pollutants, GALIS included PCDDs/Fs and dl-PCBs because they are listed on directive 2002/32/EC[4] as undesirable substances.

PCDDs/Fs analyses started in 2005 and dl-PCBs were included in the methodology by mid 2006. Since that moment 265 raw material samples were analyzed for determination of PCDDs/Fs and dl-PCBs. In this study we present the results obtained for 106 samples provided by 52 suppliers that were collected and analyzed along the 2011-2014 period. These samples corresponded to 27 different raw materials, which were initially grouped in 5 families: vegetal and mineral samples, animal and vegetal fats and animal protein concentrates.

Materials and methods

Dry solid samples were ground up to a fine powder, spiked with ^{13}C labeled PCDDs/Fs and dl-PCBs standards (EPA1613-LCS and WP-LCS, Wellington Laboratories, Canada) and then Soxhlet extracted with toluene (8 h). Sample extracts with a lipid content over 1 g were treated with H_2SO_4 to remove fats. Oil and fat samples were dissolved in n-hexane after spiking with labeled standards and then treated with sulfuric acid. In both cases dried extracts were dissolved in hexane and cleaned-up by solid/liquid adsorption chromatography using a Power Prep FMS system (Fluid Management Systems, USA) equipped with a set of disposable columns: multilayer silica columns, basic alumina columns and PX-21 carbon. Two extracts (1: PCDDs/Fs+non-ortho PCBs; 2: ortho-PCBs) were obtained and concentrated up-to 2 mL using a rotary evaporator (Büchi, Switzerland) and then a gentle stream of N_2.

Prior to HRGC/HRMS analysis, recovery standards (EPA1613-ISS and WP-ISS Wellington Laboratories, Canada) were added. Analyses were carried out using a Thermo Finnigan MAT 95XP mass spectrometer coupled to two gas chromatographs (Thermo Scientific, Germany). PCDDs/Fs were separated using a DB-5MS capillary column (60 m x 0.25 mm x 0.10 μm; Agilent, USA), the temperature program was from 140ºC (2 min) to 200ºC at 11 ºC/min, and then to 300ºC at 3 ºC/min. dl-PCBs analyses were performed on a Rtx-2330 (60 m x 0.25 mm x 0.1 μm; Restek, USA). The temperature program was from 80ºC (2 min) to 225ºC at 20 ºC/min, and then to 300ºC (5 min) at 2.5 ºC/min. Transfer line temperature was set at 290 ºC in both cases. The mass spectrometer was operated in EI mode (45 eV), using multiple ion detection (MID). Source temperature was set at 260 ºC and the spectrometer was tuned to a minimum resolution of 10,000 (10% valley) using FC-43. The two most abundant isotope peaks (M^+ and $[M+2]^+$ or $[M+4]^+$) for each PCDDs/Fs or PCBs congener were monitored. Identification was carried out using chromatographic retention times and isotopic ratios. Quantification was achieved by isotopic dilution method using relative response factors (RRF) obtained from the analysis of standard solution mixtures (EPA1613 CVS and WP CVS solutions; Wellington Laboratories, Canada). This

method was accredited according to the standard UNE-EN ISO/IEC 17025:2005 since 2009. LDD for each compound was calculated applying IUPAC criteria to a set of representative samples.
Statistical analysis was carried out using R software[5] (R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion
Samples can be divided in 5 big groups according to their origin: vegetal fats (n=32), animal fats (n=31), vegetal samples (n=18), mineral samples (n=19) and animal protein concentrates (n=5). A glycerin sample was also analyzed but it was not included in any group due to its special characteristics.
Results were evaluated using upper-bound concentrations and total equivalent concentrations were calculated using 2005 WHO-TEF. Since all samples were destined to animal feed, results are reported in pg/g, relative to a feed with a moisture content of 12%. In terms of WHO-TEQ PCDDs/Fs+dl-PCBs (total WHO-TEQ) all samples were under the legal limits[4], but they showed important differences in concentration levels and in the respective contribution of PCDDs/Fs and dl-PCBs to the total WHO-TEQ.

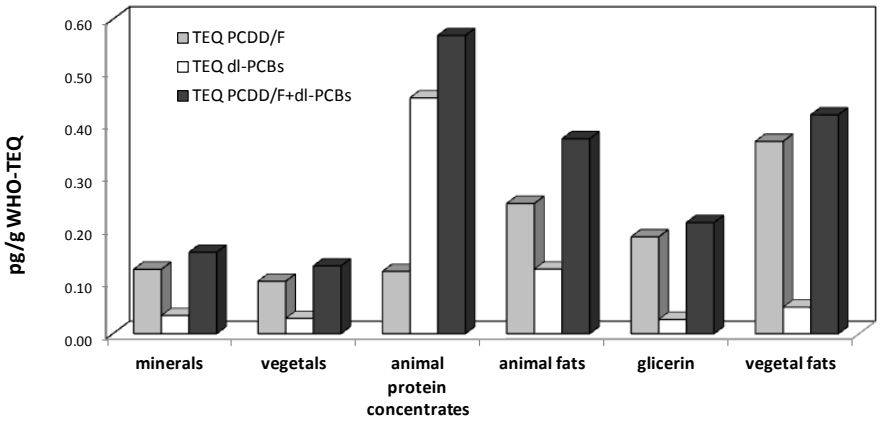

Figure 1. WHO-TEQ values obtained for PCDDs/Fs, dl-PCBs and PCDDs/Fs+dl-PCBs by group of samples.

As can be seen in Figure 1, vegetal samples (0.11-0.23 pg/g; average: 0.13 pg/g) and mineral samples (0.11-0.35 WHO-TEQ pg/g; average: 0.16 pg/g) showed the lowest total WHO-TEQ values, with concentrations slightly above the scope of the assay. Comparable WHO-TEQ values were obtained for both types of fats, vegetal fats ranging from 0.19 to 1.01 pg/g (average: 0.42 pg/g) and fats of animal origin ranging from 0.19 to 0.91 pg/g (average: 0.37 pg/g); however, the contribution of dl-PCBs to the total WHO-TEQ value was higher in the latter group.
The highest mean level of total WHO-TEQ was found in animal protein concentrates (average: 0.70 pg/g; 0.14-0.75 pg/g). These samples show a different behavior relative to the other groups, with concentrations of dl-PCBs accounting for more than 80% of the total WHO-TEQ. The group is comprised of 1 meat meal sample and 4 fish meal samples, and while the meat meal presented a profile similar to that of other groups, with PCDDs/Fs higher than dl-PCBs, the PCB contribution of fish meals dominates in the group profile.
Besides the classical approach a multivariate analysis was applied to samples. The concentration values for the 29 measured compounds were scaled to unit variance and subjected to principal component analysis (PCA). A scree test showed a large break after principal component 3, with the first three principal components together accounting for 59.6% of total variance in the scaled dataset. However, standard deviations greater than 1 (Kaiser criterion) appeared for components 1 to 7, which together accounted for 79.4% of total variance. In view of these results, two different rotated solutions, retaining respectively the first three (PC1-3) and the first seven (PC1-7) principal components, were computed and compared (Table 1).
As can be seen from the PCA results, most of the variation in the data can be explained by joint changes in the concentrations of structurally related compounds, with the different chemical classes displaying largely independent behavior. Both calculated solutions showed similar findings in this respect, but meaningful additional details could be extracted when seven instead of three principal components were taken into account.

Interestingly, 2378-TCDD, 12378-PeCDD, 2378-TCDF and 23478-PeCDF, compounds of high toxicological significance, all showed unique patterns of variation, different from those of their respective structural groups.

Table 1. Loadings for the Varimax rotated solutions obtained by retaining either 3 or 7 principal components (PC, principal components; RC, rotated components; loadings with an absolute value under 0.20 omitted for clarity).

	PC1-3 retained			PC1-7 retained						
	RC1	RC2	RC3	RC1	RC2	RC3	RC4	RC5	RC6	RC7
12378-PeCDD			0.25							-0.40
2378-TCDD									0.75	
123478-HxCDD			0.41			0.42				
123678-HxCDD			0.42			0.43				
123789-HxCDD			0.42			0.44				
1234678-HpCDD			0.39			0.45				
OCDD			0.31			0.31	0.31			0.26
23478-PeCDF	0.27									-0.31
123478-HxCDF	0.38			0.45						
123678-HxCDF	0.36			0.27						-0.30
123789-HxCDF	0.29									-0.46
234678-HxCDF	0.38			0.44						
2378-TCDF							0.58			-0.20
12378-PeCDF	0.24						0.26		0.36	-0.32
1234678-HpCDF	0.32			0.45						
1234789-HpCDF	0.37			0.36						
OCDF	0.34			0.33					0.35	
PCB126		-0.32			-0.27			0.33		
PCB169		-0.26						0.41		
PCB81								0.59		
PCB77							0.28	0.46		
PCB105		-0.28					0.46			
PCB114			0.28			0.26		0.27		
PCB118		-0.34			-0.28		0.31			
PCB123		-0.24			-0.34					0.21
PCB156		-0.36			-0.39					
PCB157		-0.36			-0.37					
PCB167		-0.37			-0.38					
PCB189		-0.33			-0.41					

Figure 2 shows plots of sample scores for selected pairs of rotated components, labeled according to type of raw material. Samples with high scores for RC1 (non-specific PCDF contamination) appeared for a limited number of matrices, namely zinc oxide (d), hydrogenated fat (w) and palm oil derived fat (u), while high scores for RC2 (corresponding mostly to mono-ortho PCBs) appeared only for fishmeal (n), mineral swine corrector (b) and one animal fat (o); some mixed fat (p) samples showed a mild level of mixed mode contamination. For RC3 (non-specific PCDD contamination) samples with high or medium scores appear only for hydrogenated fat (w), palm soap (v) and palm oil derived fat (u). Regarding components more related to specific compounds, high or medium scores for RC4 (2378-TCDF, 12378-PeCDF) appeared only for samples showing low to moderate RC1 scores (non-specific PCDF), and corresponding mostly to chicken fat (q), palm soap (v), corn DDGs (g), mixed fat (p) and fishmeal (n) matrices. Some fishmeal samples (n) show high negative scores for RC2 (mono-ortho PCB contamination) together with high scores for RC5 (non-ortho PCB contamination), whereas other matrices

showed different profiles of PCB contamination. Finally, relatively high scores for RC6 (strongly related to 2378-TCDD content) appeared only for one palm oil (s) sample and one palm soap (v) sample, both showing low to moderate scores for RC3.

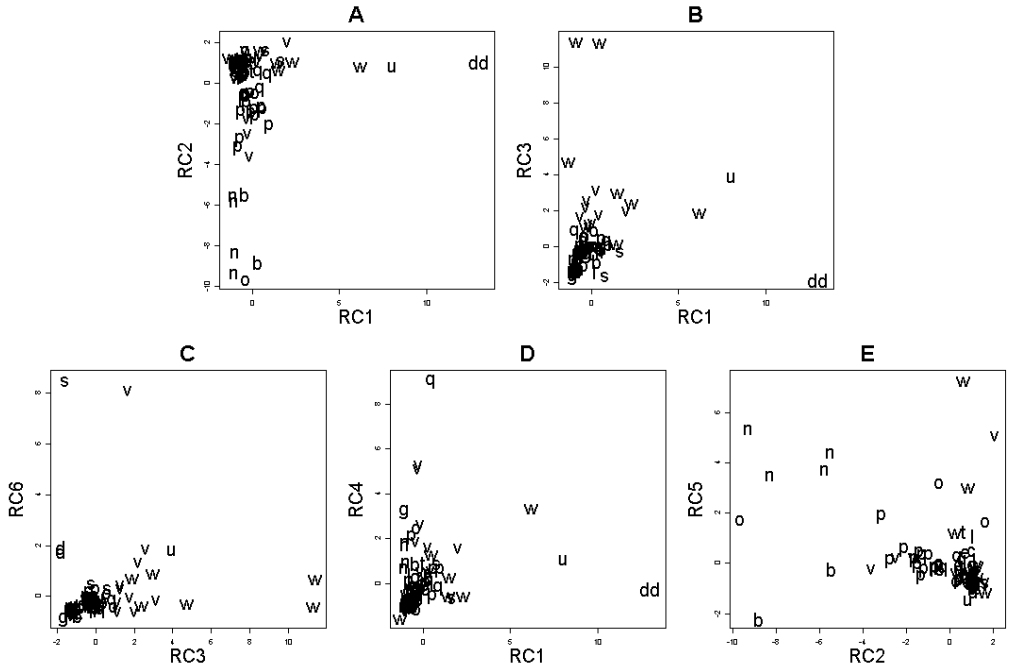

Figure 2. Plots of scores for selected pair of rotated components, from the solution retaining seven principal components (see text for annotation).

Thus, it can be concluded that i) a limited number of samples, corresponding also to a limited set of the analyzed matrices, account for the highest scores in the referred components, ii) the nature of the contamination occurring in each of the different matrices can be distinguished and related to different chemical families from the measured pollutants, and iii) contamination by some specific compounds, most notably 2378-TCDD, 12378-PeCDD, 2378-TCDF and 12378-PeCDF, is largely independent of contamination by other structurally related compounds. The PCA results indicate the convenience of a detailed classification of raw material families, and could be used in spotting critical matrices for the design of control and monitoring plans.

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References:
1. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.
2. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene.
3. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
4. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.
5. R Core Team (2013). R: A language and environmental for statistical computing. R Foundation for Statistical Computing, Vienna. Austria. URL http://www.R-project.org.