Trend between PCDD/F concentrations in serum and muscle samples from different species of animals in FRANCE

Philippe Marchand[1], Anaïs Vénisseau[1], Aline Brosseaud[1], Christel Gadé[1], Bruno Le Bizec[1]

[1]LABERCA

Introduction

Two thousand animals were destroyed during the dioxin crisis that occurred in Savoie in France in 1999. Actual regulation regarding dioxins in food is based on maximal tolerable limits in various edible tissues or products. One disadvantage of this system fully justified on a consumer point of view, is the difficulty to perform an efficient and rapid control on living animals. For example the determination of PCDD/F levels in muscle clearly imply animal slaughtering. On the other hand, serum is a biological fluid recognized to be a good indicator of the organism exposure and this matrix is easy to collect on living animals. In this context, the purpose of the present study was to analyze serum and muscle samples from different animals and various species in order to investigate a possible correlation in between PCDD/F concentrations in these two matrices. This approach was also extended to dioxin-like and marker PCBs.

Materials and Methods

Samples

Serum and muscle samples from different species were analyzed including cows, hens and goats. For cow samples, three of them originated from the North of France and one from Savoie (France). They were animals which have been raised during several years in contaminated area before waste incinerators were stopped in these two spot. Considering hens, the animals were free-range chickens and came from the North of France. Some of the hens of this farm presented quite elevated concentrations of dioxins. Three chickens were analyzed individually, and the samples of 25 hens were pooled five by five. The three goats were part of a research project dedicated to the study of dioxin transfer from contaminated hay to milk in lactating animals (collaboration with ENSAIA, Nancy, France).

Extraction and clean-up

The sample preparation method used for human serum samples, has already been described elsewhere [1,2], and was adapted for the different species studied. The 35 ^{13}C-labelled standards (17 PCDD/Fs, 12 dioxin-like PCBs and 7 marker PCBs) were added to the serum sample before extraction. After spiking, the sample (20 mL for chicken serum or 40 mL for goat or bovine samples) was diluted with deionized water (20 or 40 mL), aqueous saturated ammonium sulphate (10 or 20 mL), ethanol (40 or 80 mL), and extraction was performed twice with hexane (160 or 250 mL). The total lipid content of the serum samples was determined using a colorimetric dosage (RANDOX® kit) on a 50-µL aliquot. Before extraction, muscle samples were freeze-dried and the internal standards were added. The extraction was performed using Accelerated Solvent Extractor (ASE) with a toluene/acetone mixture (70/30, v/v). The solvent was evaporated to dryness, permitting the estimation of the fat content. Clean up and separation processes were carried out using the classic liquid-solid adsorption chromatography with silica, Florisil and CarbopackC/Celite. The solvents used for the elution were hexane and toluene. The external standards were added for the recovery calculation ($^{13}C_{12}$-1, 2,3,4-TCDD for the PCDD/F, $^{13}C_{12}$-PCB #111 for the PCBs).

GC/HRMS analysis

GC/HRMS analysis of the 17 dioxins, 12 dioxin-like and 7 marker PCBs was performed as previously described by Fürst[3]. The congeners were separated by gas chromatography (GC) on a DB-5MS capillary column (30 m´0.25 mm, 0.25 µm) and determined by high-resolution mass spectrometry (HRMS) on a JMS 700D (Jeol), at a resolution of 10,000 in the Selected Ion Monitoring (SIM) and Electron Ionization (EI) modes. TEQ values were calculated using

WHO-TEFs.

Results and Discussion

The PCDD/F concentrations measured in serum and muscle samples from the different species are presented in Table 1. Bovine samples represent 4 points: 3 samples from the same farm in the North of France were collected at the same time; another one was a bull from the Savoie department. The contamination level in these samples was found relatively low. In the case of chickens, the 8 samples were collected in the same farm in the North department. Three of them were collected individually in the farm. The five others were pooled samples of 5 chickens. These chickens came from the same farm but were raised at the laboratory for a decontamination study. One of the individual muscle samples and the three first pooled samples were contaminated (PCDD/Fs above 2 pg WHO-TEQ/g fat) while the others were below the EU maximum authorized value for chicken muscle. Finally, the last point (in the center of the figure) represents the pooled goat samples. The animals were part of a study about the dioxin decrease in milk and tissues after a controlled intake with contaminated feeding stuffs. Except for chickens, where the lipid content was about 2.5%, in serums for all the other samples the lipid concentration was about 0.3%.

Table 1: WHO-TEQ for dioxins and PCBs in serum and muscle of different animals.

	PCDD/Fs (pg WHO-TEQ/g fat)		**DL PCBs (pg WHO-TEQ/g fat)**		**Marker PCBs (ng/g fat)**	
	Serum	**Muscle**	**Serum**	**Muscle**	**Serum**	**Muscle**
Bovine 1	1.30	0.75	1.39	1.93	13.48	10.62
Bovine 2	0.80	0.63	1.21	2.09	8.96	12.93
Bovine 3	0.98	0.61	1.11	1.63	13.13	10.61
Bull	2.35	0.88	3.57	5.22	37.88	18.16
Chicken 1	2.47	2.53	4.89	3.71	33.36	27.26
Chicken 2	1.09	0.90	2.18	1.92	14.32	15.62
Chicken 3	0.19	0.19	0.30	0.42	2.19	5.41
Pooled hens 1	7.91	5.23	3.78	5.63	44.77	55.33
Pooled hens 2	7.31	5.44	3.47	4.28	36.97	36.27
Pooled hens 3	7.33	3.69	3.27	3.10	33.35	26.02
Pooled hens 4	2.90	1.53	3.01	2.96	-	-
Pooled hens 5	2.51	1.27	2.18	1.67	18.57	13.81
Pooled goats	4.86	2.96	1.90	1.13	9.08	6.68

When all the species were gathered, a good correlation in between PCDD/Fs in serum and in muscle was observed (Fig.1). As a result of this correlation, it can be expected that a PCDD/F level found in the serum could be used as an indicator of the level of PCDD/Fs in muscle. The maximum authorized concentrations in PCDD/Fs in the muscle are 2 pg WHO-TEQ/g fat for poultry and 3 pg WHO-TEQ/g fat for ruminants(bovines and goats). The corresponding concentrations in serum are respectively roughly 3 and 5 pg WHO-TEQ/g fat (Fig.1). On the basis of these preliminary data, hypothetic action limits in serum could be suggested: 2 pg WHO-TEQ/g fat for poultry serum and 4 pg WHO-TEQ/g fat for bovine and goat serums. Of course this correlation is directly dependant of the determination of fat content that is determined in our lab with a colorimetric test for serum and gravimetric test for muscle.

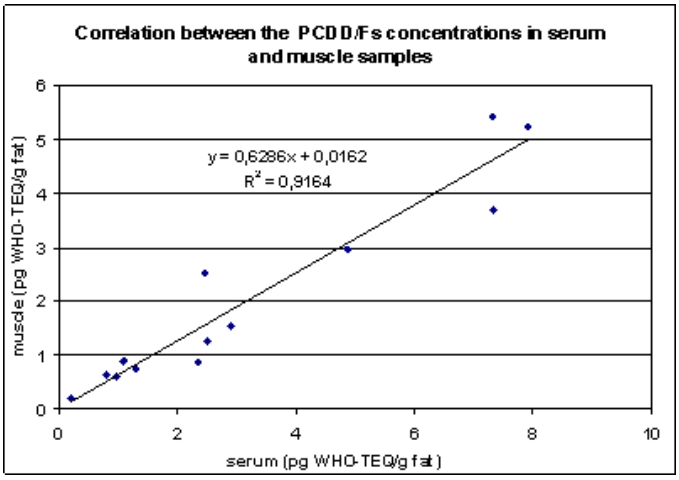

Figure 1: Observed correlation between the PCDD/F concentrations in serum and muscle

In the case of PCBs (dioxin-like and marker), the hypothesis of existing similar correlations in between serum and muscle was also investigated. The results obtained for the three species of animals studied are presented on figure 2. Correlation in between serum and muscle for marker PCBs was judged good (except one value). Compared with PCDD/Fs and marker PCBs, the results obtained for dioxin-like PCBs were not so good, but remained acceptable.

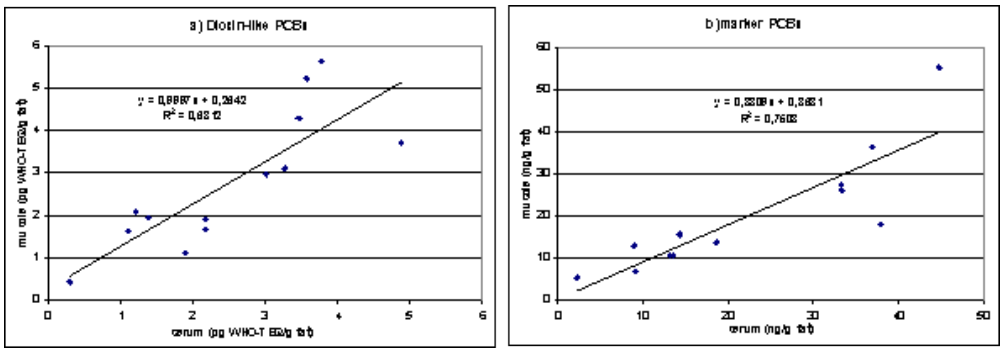

Figure 2: Observed correlation between the concentrations in serum and muscle: a) for dioxin-like PCBs and b) for marker PCBs.

Conclusion

In this restricted study, a relatively good correlation between serum and muscle concentrations for PCDD/Fs was evident. These observations suggest the possibility to build a predictive model permitting to approximate or to deduce PCDD/F concentrations in muscle sample from the concentration measured in serum. Thus, it would be possible to lay down limits for PCDD/F concentrations in serum. In case of contamination in an area due to industry or feeding stuff, local administration could choose the best way to regulate the crisis, due to the fact that animal decontamination studies have been already done. However, the study must be extended to a larger number of species and samples to validate these results.

It is also important to note that all the samples used in this study didn't result from a acute contamination or exposure due, for example to a feedingstuff with a high level of contamination, but a chronic exposure during several weeks or months.

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