PCDD/F AND PCB CONTENT IN DIFFERENT PARTS OF SHEEP

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Introduction
Four different matrices from the same sheep have been analysed for content of PCDD/F (polychlorinated dibenzo-*p*-dioxin and -furan) and PCB (polychlorinated biphenyl). The content in leaf fat and two meat cuts (shank and flank) were comparable expressed as TEQ pr gram of fat whereas the content in liver was much higher also expressed as TEQ pr gram of fat. The maximum residue level (MRL) in the European Union (EU)[1] for PCDD/F in sheep is 3 pg TEQ/g fat for meat and fat and 6 pg TEQ/g fat for liver. None of the leaf fat or meat cuts had content above the MRL whereas 3 of the 5 liver samples had a PCDD/F-TEQ content significantly above the MRL. Although only 5 different sheep were sampled, it raises the question whether sheep liver should be considered non-suited for human consumption or if the MRL in sheep liver is too low.

Materials and Methods
Samples
From 5 sheep (A-E), the following matrices were taken at the slaughterhouse: liver, leaf fat, flank and shank. The sheep were from different farms in Denmark and the laboratory had no knowledge of their feed. Sheep A, B, C and E were females, 24 month old, weighing 30 kg. Sheep D was male, 8 month old, weighing 20 kg.

Standards
Standards were purchased from Cambridge Isotope Laboratories, INC. (USA) and Dr. Ehrenstorfer (Germany) and further diluted in toluene. The following substances were analysed: the seven 2,3,7,8-chloro substituted PCDDs, the ten 2,3,7,8-chloro substituted PCDFs, the four non-ortho PCBs (PCB77, 81, 126 and 169), eight mono-ortho PCBs (PCB105, 114, 118, 123, 156, 157, 167 and 189) and seven marker PCBs (PCB28, 52, 101, 138, 153, 170 and 180).

Analysis
The requirements for the analysis of dioxins and PCBs laid down in the Commission Directive 2006/1883/EF[2] were followed.
Typical sample size: Liver 23 g resulting in about 1 g of fat; leaf fat 3 g; flank and shank 20 g of meat so that a maximum of 3 g of fat (minimum 0.4 g) was analysed.

Fat extraction was performed with accelerated solvent extraction on ASE300 (Dionex) using acetone/pentane (12/88), 2 cycles, a temperature of 80 ^{o}C and static time 10 min. 100 ml extraction cells filled with sample, sea sand (minimum 10 g – void volume filled with sand) and Hydromatrix (10 g) were used. For liver 10 g of Polyacrylic acid-polyethylene oxide was used instead of Hydromatrix, as it is our experience that this is a more suitable drying material for wet matrices like liver. The extracted fat was weighed (fat percent determination).

Leaf fat or fat from ASE were dissolved in hexane and further cleanup and fractionation was done on Power Prep (FMS, USA) using multilayer silica (part no CLDS-ABN-STD), alumina (part no CLDA-BAS-011) and carbon columns (Part no CLDC-CCE-034). In addition, for fat sample sizes above 1g a Jumbo silica column (Part no HCDS-ACD-STD) was used. The Power Prep program was based on the method used at the University of Liege[3].

Detection and quantitation was done using a gas chromatograph – high resolution mass spectrometer (GC-HRMS, Trace GC ultra and Finnigan MAT95). The GC was equipped with split/splitless injector and DB5MS-DG column (10m pre-column, 60 m, 0.25 mm I.D, film thickness 0.25 µm). For the dioxin fraction from Power Prep containing the 17 PCDD/F and 4 non-ortho PCBs the following GC-program was used: 140 ^{o}C hold 2 min, 15 ^{o}C/min up to 240 ^{o}C, 1 ^{o}C/min up to 255 ^{o}C hold 10 min, 10 ^{o}C/min up to 325 ^{o}C hold 11 min. For the

fraction containing the 15 other PCBs the following GC-program was used: 90 °C hold 2 min, 20 °C/min up to 180 °C, 2 °C/min up to 260 °C, 5 °C/min up to 310 °C, hold 8 min. FC43 was used as calibration substance for the mass spectrometric detection.
Quantification was done using the QuanDesk software (ThermoFinnigan, Germany).

All TEQ (2,3,7,8-TCDD toxic equivalent) results are given as upper bound values[1] calculated using 1998 WHO-TEF (toxic equivalent factor) values[4].

Quality assurance
Each analytical series consists of 6-12 samples, one double determination of a sample, one reagent blank, one spiked sample and/or a natural contaminated control sample. Each sample was spiked with 13C-labelled standards at the beginning of the analysis.
The laboratory regularly participates with satisfactory results in the Norwegian Food Round Robin tests of different food items and FAPAS test on cod liver oil.

Results and Discussion
In table 1, all measured contents of PCDD/F-WHO-TEQ are shown for the four matrices (flank, shank, leaf fat and liver) in the five different sheep (A-E) together with the maximum residue level (MRL) in the EU. In general, liver contains much higher amounts of dioxins than the three other matrices. In 3 of the 5 liver samples the content of PCDD/F-TEQ is significantly above the MRL. However, the TEQ in sheep liver on fresh weight basis range from 0.3-1 pg PCDD/F-TEQ/g fresh weight, which is still much lower than the corresponding MRLs in fish (4 pg PCDD/F-TEQ/ g fresh weight)[1].

Table 1 Content of PCDD/F-TEQ in different matrices from 5 different sheep (A-E) including 7 duplicate analyses. Maximum residue levels (MRL)[1] for both PCDD/F-TEQ and the total-WHO-TEQ (sum of PCDD/F and PCBs) are shown.

	A	B	C	D	E	MRL PCDD/F	MRL Total-TEQ
	PCDD/F TEQ/g fat	PCDD/F TEQ/g fat	PCDD/F TEQ/g fat	PCDD/F TEQ/g fat	PCDD/F TEQ/g fat	PCDD/F TEQ/g fat	PCDD/F+PCB TEQ/g fat
Flank	0.26	0.72	0.69	1.2	0.41 0.61	3	4.5
Shank	0.63	0.55	1.2	N.A.*	1.9	3	4.5
Leaf fat	0.36	0.90	0.73 0.78	1.3	0.74	3	4.5
Liver	7.7 9.3	6.8 8.2	16.6 16.1	16.7 16.6	11.0 7.6	6	12

* Sheep D, shank was not analysed (N.A.) as it was not possible to extract enough fat.

Table 2 Content of PCB-TEQ in different matrices from 5 different sheep (A-E) including 7 duplicate analyses.

	A	B	C	D	E
	PCBTEQ/g fat	PCB TEQ/g fat	PCB TEQ/g fat	PCB TEQ/g fat	PCB TEQ/g fat
Flank	0.18	0.31	0.38	0.45	0.32 0.35
Shank	0.26	0.32	0.44	N.A.*	0.48
Leaf fat	0.25	0.42	0.66 0.58	0.51	0.38
Liver	1.6 2.0	1.1 1.0	3.0 2.8	1.9 1.7	1.4 1.3

* Sheep D, shank was not analysed (N.A.) as it was not possible to extract enough fat.

In table 2, all measured contents of PCB-WHO-TEQ are shown for the four matrices (flank, shank, leaf fat and liver) in the five different sheep (A-E). As for PCDD/F-TEQ, the content in liver is much higher than in the three other matrices although the difference is not as big as for PCDD/F-TEQ.

In figure 1, the average contribution of the different congeners to total-WHO-TEQ pr gram of fat is shown for the four different sheep matrices. The total-WHO-TEQ is the sum of the PCDD/F-TEQ and PCB-TEQ. Congeners contributing less than 2 percent to the total-WHO-TEQ are shown as "sum others".

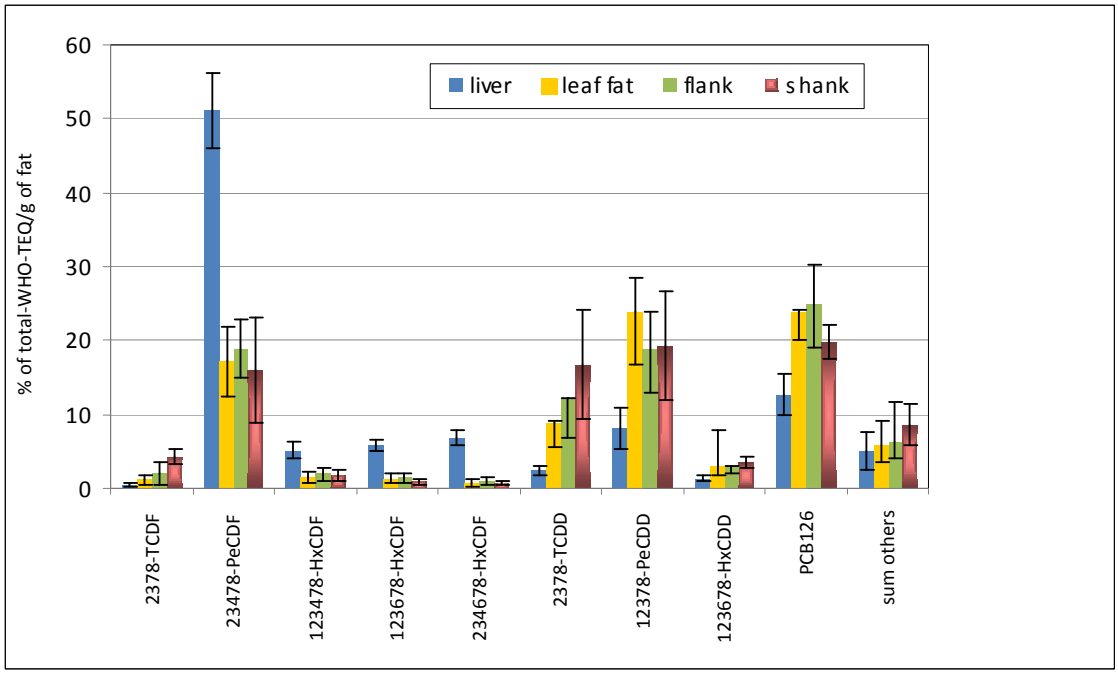

Figure 1 Contribution of different congeners to total-WHO-TEQ for four different sheep matrices. Mean of 5 samples. The vertical lines indicate ± 1 standard deviation.

The contribution to the total-TEQ for the different congeners is markedly different for liver compared to the other three matrices. 2,3,4,7,8-PeCDF accounts for more than 50 % of the total-TEQ for liver whereas it only accounts for approximately 20 % for the three other matrices. 1,2,3,7,8-PeCDD and PCB126 account for about 10 % of the total-TEQ for liver and about 20 % for the other three matrices.

The ability to accumulate PCDD/Fs and especially 2,3,4,7,8-PeCDF in liver compared to the other matrices seems specially pronounced for sheep. Preliminary results in our laboratory do not indicate the same marked trend for bovine and porcine.

Among the marker PCBs, PCB153 was present in the highest concentration in all four matrices (range 2 – 10 ng/g fat) except for one leaf fat where the content of PCB180 (36 ng/g fat) was much higher (analysed in duplicate). This was not found to be the case in the three other matrices from the same sheep (sheep C).

Our results are in line with those obtained by Schulz et al[5] who investigated fat tissue, meat and liver from five sheep. In their study, the content of PCDD/F-TEQ/g fat in liver was from 8 to 27 times higher than found in muscle. In addition, they analysed a number of cows and the PCDD/F-TEQ content in liver were only about twice the content in meat and fat[5].

Ferrario and Byrne[6] have analysed two chickens for content of PCDD/F and they found no marked difference in content between the liver and the meat/abdominal fat. Apart from the difference between chicken and sheep, the two chickens had eaten highly contaminated feed and this could influence the distribution in the various organs so as not to represent normal (steady-state) distribution.

In conclusion, leaf fat is a well-suited matrix for enforcement purpose as its dioxin and PCB content mirrors the content in the meat cuts well. In addition, leaf fat is faster to analyse because the first extraction step with ASE in general is not necessary.

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