CONCENTRATION AND DISTRIBUTION of DIOXINS and PCBs IN SHEEP GRAZING NEAR A WASTE INCINERATOR

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Introduction

This report is concerned with an investigation on dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) contamination in a farm sheep near to an industrial area and a waste incinerator (Lecce province, Apulia Region, Italy). In November 2009, the Local Health Authority ordered to slaughter all animals in the farm due to non-compliant samples of sheep milk and meat. In cooperation with the Local Health Authority, the Italian National Reference Laboratory (NRL) for dioxins and PCBs in feed and food collected samples from farm sheep in order to evaluate levels and distribution patterns of PCDD/Fs, dioxin-like PCBs (DL-PCBs) and non dioxin-like PCBs (NDL-PCBs) in several animal tissues. The present study is a part of a larger investigation that is still ongoing.

Materials and methods

Sampling

Samples of blood, liver, muscle and perirenal fat from a farm sheep were collected at slaughter by the official veterinarian officers.

Before slaughtering, animals were categorized by gender (male - female), age (lambs under 3 months - lambs between 3- 8 months) and lambings (first, second and third lambing), each group counted 8 to 17 individuals. Four matrices (blood, liver, perirenal fat and muscle) were taken from each animal.

On a total of 356 samples collected (i.e. 89 animals), 45 samples of liver, muscle and perirenal fat have been analyzed for PCDD/Fs, DL-PCBs and NDL-PCBs, in order to evaluate the contamination levels and congener profiles in different animal tissues. The number of animals and samples are provided in Table 1.

Table 1. Animals and samples analyzed

Groups	N. of animals	N. of samples
Lamb < 3 months	3	9
Lamb 3 - 8 months	3	9
First lambing ewe	3	9
Second lambing ewe	3	9
Third lambing ewe	3	9
Total	**15**	**45**

Analysis of samples

Samples were homogenized and analyzed by a validated method routinely used for PCDD/Fs, DL-PCBs and NDL-PCBs analysis in food and feed, the method was also endorsed to perform a number of proficiency tests with successful results.

All samples were spiked with the specific PCDD/Fs, DL-PCBs and NDL-PCBs standard solution, a mixture of $^{13}C_{12}$-labelled congeners (Wellington Laboratories, Ontario, Canada).

The extraction and clean-up procedures, as well as the analytical determination, were previously reported[1].

PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.10 μm film thickness, J&W Scientific, California) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10,000 operating with electron ionization (EI) at 40 eV in the selected ion monitoring (SIM). The HRGC/HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XP (Thermo Fisher, Bremen, Germany). DL-PCBs and NDL-PCBs were separated by HRGC on a HT-8 capillary column (60 m x 0.25 mm, 0.25 μm film thickness, SGE Analytical Science Pty, Ltd. Victoria, Australia) and determined by HRMS, under conditions described for PCDD/Fs.

PCDD/Fs and DL-PCBs toxic equivalent (TEQ) values were calculated using the World Health Organization Toxic Equivalency Factors (WHO-$TEFs_{2005}$), while the sum of six indicator congeners was calculated for NDL-PCBs. WHO-TEQs and the amount of six NDL-PCBs were expressed as upper bound (UB) concentrations,

assuming that all values of specific PCDD/F and PCB congeners below the limit of determination (LOD) are equal to their respective LOD.

Results and discussion

Table 2 reports the analytical results, gathered by the age of animals, the number of lambings and the matrices collected. The lipid content and the contamination levels of PCDD/Fs, DL-PCBs and NDL-PCBs were determined, for all samples. At the same time, the mean concentration and the corresponding standard deviation was calculated for each parameter. Following European legislation, the contamination levels have been expressed on fat basis for perirenal fat and muscle, while it has been expressed on product basis for liver. Twenty four out of 45 analyzed samples (i.e. 53%) exceeded the maximum limits prescribed in the Commission Regulation 1881/2006 (as amendments) for at least one of the three regulated parameters (WHO-TEQ PCDD/Fs, WHO-TEQ PCDD/Fs + DL-PCBs and sum of NDL-PCBs). Thirteen animals (87% of the total) exceeded the maximum limit for one or more matrices.

Table 2. Lipid content and UB levels of PCDD/Fs, DL-PCBs and NDL-PCBs detected in different lambs and sheep matrices. For each investigated parameter, the mean concentration levels and the corresponding standard deviations are reported.

Group	**Tissue**	**Lipid content** (%)	**PCDD/Fs** (pg WHO_{05}-TEQ/g)	**DL-PCBs** (pg WHO_{05}-TEQ/g)	**NDL-PCBs** (ng/g)
Lamb < 3 months	Liver*	4.55 ± 0.52	0.89 ± 0.20	1.10 ± 0.57	1.44 ± 0.51
	Perirenal fat**	---	0.70 ± 0.11	3.72 ± 0.97	11.00 ± 4.66
	Muscle**	7.34 ± 5.19	0.58 ± 0.38	3.65 ± 1.41	11.14 ± 6.37
Lamb 3-8 months	Liver*	3.08 ± 0.99	1.74 ± 0.34	2.68 ± 0.80	5.20 ± 0.84
	Perirenal fat**	---	1.52 ± 0.80	7.12 ± 4.36	38.37 ± 31.88
	Muscle**	3.53 ± 2.34	1.31 ± 0.73	5.53 ± 4.46	30.14 ± 14.06
First lambing ewe	Liver*	6.80 ± 2.42	1.53 ± 1.05	3.47 ± 2.15	5.56 ± 5.80
	Perirenal fat**	---	0.44 ± 0.37	2.41 ± 1.39	9.04 ± 6.82
	Muscle**	8.71 ± 9.49	0.53 ± 0.28	2.24 ± 0.72	17.30 ± 11.31
Second lambing ewe	Liver*	3.97 ± 0.39	0.98 ± 0.31	2.03 ± 0.09	2.07 ± 0.98
	Perirenal fat**	---	0.42 ± 0.02	2.84 ± 0.19	11.33 ± 4.27
	Muscle**	6.28 ± 1.88	0.65 ± 0.22	2.43 ± 1.63	10.96 ± 5.90
Third lambing ewe	Liver*	6.02 ± 2.01	2.09 ± 0.96	3.36 ± 1.23	1.78 ± 1.01
	Perirenal fat**	---	2.79 ± 1.80	9.10 ± 3.74	29.90 ± 21.79
	Muscle**	6.84 ± 4.65	1.29 ± 0.01	3.05 ± 2.05	13.99± 6.11

* PCDD/Fs, DL-PCBs and NDL-PCBs concentrations reported on product basis

** PCDD/Fs, DL-PCBs and NDL-PCBs concentrations reported on fat basis

The high levels of dioxins and PCBs detected in all matrices confirmed the presence of a contamination source in the area. In fact, aerial fall-out is one of the main sources of dioxins and PCBs contamination of vegetation, recently highlighted by EFSA, in particular pasture[2]. Certainly, soil is a natural reservoir of dioxins and PCBs, sheep can involuntary intake soil through the ingestion of particles deposited on vegetation or directly when feeding on pasture herbage close to the ground surface.

PCDD/Fs concentrations in liver ranged from 0.67 to 3.12 pg WHO-TEQ/g, their levels in perirenal fat and muscle were between 0.14 and 4.79 pg WHO-TEQ/g fat. DL-PCBs levels in liver ranged from 0.74 to 5.90 pg WHO-TEQ/g, in perirenal fat and muscle between 0.75 and 13.28 pg WHO-TEQ/g fat. The concentrations of NDL-PCBs in liver ranged from 0.61 to 12.25 ng/g, while their levels in perirenal fat and muscle varied from 3.20 to 60.91 ng/g fat. In most cases, lamb 3-8 months and third lambing ewe recorded the highest contamination levels.

When calculated on fat basis, PCDD/Fs+DL-PCBs cumulative WHO-TEQs and the amount of NDL-PCBs for liver samples recorded the highest values, whereas perirenal fat and muscle showing similar contamination levels.

PCDD/Fs+DL-PCBs concentrations in liver were between 6 and 60 times higher than those found in perirenal fat and muscle tissue. For the sum of NDL-PCBs, liver was more contaminated than perirenal fat and muscle by a factor of up to 12. This can be explained by referring to a preferential binding of dioxin-like compounds in liver tissue. According to the literature, these results confirm the differences in contamination levels in liver and meat or fat probably due to the physiological function of the liver[3,4,5]. Furthermore, dioxin-like compounds are known to bind with different affinities to aryl hydrocarbon receptors of the proteins present in liver.

Contamination levels showed a high variability among the groups of animals as well as animal tissues, and no relationship was observed between the age and dioxins and PCBs levels. Most likely, these outcomes have been influenced by the restricted number of sample collected. However, similar results were reported by other authors[5,6].

As for the age, no evidence for a correlation between the number of lambings and the levels of contaminants was found, probably due to the presence of multiple parameters influencing the levels of contaminants: e.g. the concentration of lipophilic contaminants increases with age as a result of bioaccumulation, while a reduction during lactation is related to the excretion in milk.

A remarkable difference was observed between dioxins and furans congeners; the age, the number of lambings and the type of tissue seemed to influence the PCDD/Fs pattern. In liver, a higher relative abundance of furans compared to dioxins was observed in all groups, a predominant class was not always found in muscle and perirenal fat. On the contrary, the NDL-PCBs and DL-PCBs profiles were very similar in all samples.

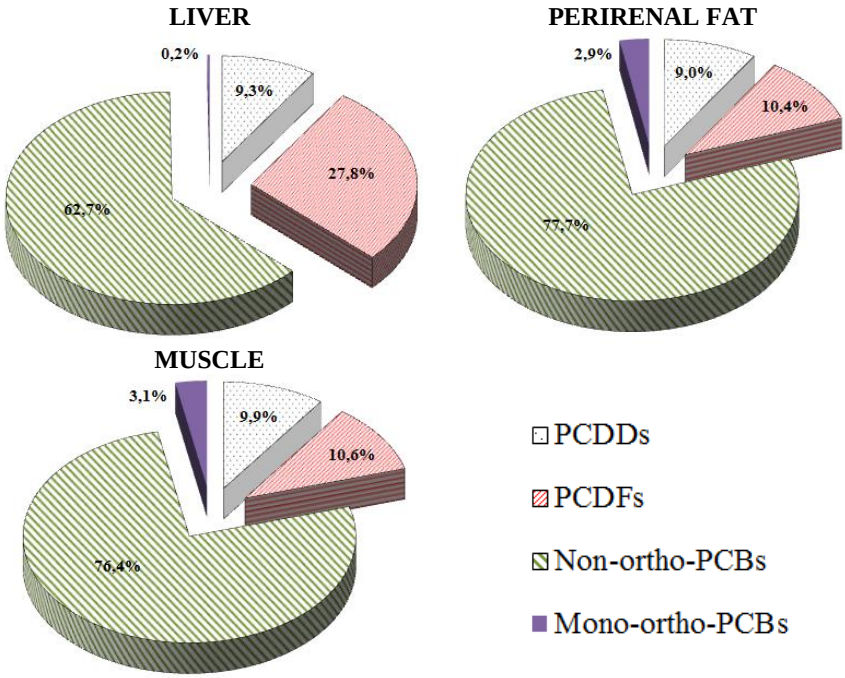
Generally, the congener profile is related to the different abundance of congeners in the environment originated from the contamination sources, and the toxicokinetic processes taking place after exposure of animals to contaminants through the diet. As reported above, the samples were taken from sheep exposed to the same source of PCDD/Fs and PCBs. Therefore, the differences in PCDD/Fs and PCBs concentrations and patterns between the analyzed matrices should be principally influenced by the physiological function of the liver.

Regarding PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs contribution to the total TEQ, some differences were observed between liver on one hand, and perirenal fat and muscle on the other hand (Figure 1). In particular, the main differences are related to PCDFs and non-*ortho* PCBs. The most important contribution to total TEQ was given by non-*ortho* PCBs, even if in different percentages for the investigated tissues. In fact, the contribution of non-*ortho* PCBs was 62.7% in liver, and increased to 76.4% and 77.7% in muscle and perirenal fat respectively. The involvement of PCDFs was about 10% in perirenal fat and muscle, increasing to 27.8% in liver, while PCDDs percentage was similar in all matrices (9.3% – 9.9%). The mono-*ortho* PCBs were quite low in perirenal fat and muscle (range 2.9 – 3.1%) and almost negligible in liver (0.2%).

Considering the specific contribution of each of the PCDD/Fs and DL-PCBs to the cumulative TEQ, the distribution was nearly the same for all matrices: 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were the main contributors for PCDDs and PCDFs respectively, while PCB-126 was the most relevant among DL-PCBs in terms of toxic equivalents, as reported in literature[3,6,7].

In conclusion, sheep of the same flock exposed to an emission source of dioxins and PCBs, show differences in tissue levels of contaminants; substantial differences were found in the distribution of congeners among liver, perirenal fat and muscle for animal. The highest concentrations were recorded in liver, perirenal fat and muscle showing lower contamination levels. No correlation was found between the age of animals, the number of lambings and the contamination levels. The non-*ortho* PCBs were the major contributors to overall TEQ, mono-*ortho* PCBs the lower. As regards dioxins, PCDDs provided a similar contribution to TEQ for all matrices, while PCDFs contribution in the liver was greater than in the muscle and perirenal fat. These outcomes have been influenced by the low number of animals analyzed for each group and more consistent results could be achieved by increasing the number of samples.

Figure 1. Mean contribution (%) of PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs to total TEQ in liver, perirenal fat and muscle in sheep



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