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DIOXINS AND PCBS DISTRIBUTION IN LIVER, PERIRENAL FAT AND MUSCLE SAMPLES IN BOVINES FROM A CONTAMINATED LIVESTOCK

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Introduction

A case study on dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) contamination occurred in a beef cattle farm in South Italy is reported. In February 2015, after finding a number of non-compliant samples of bovine milk and meat, the Local Health Authority ordered to slaughter all animals in the farm. The Local Health Authority, in cooperation with the Italian National Reference Laboratory (NRL) for dioxins and PCBs in food and feed, collected samples from bovines belonging to the same livestock, in order to evaluate the levels and distribution of PCDD/Fs, dioxin-like PCBs (DL-PCBs) and non dioxin-like PCBs (NDL-PCBs) in different animal tissues, taking into account the age and gender of animals..

Materials and methods

Sampling

In 2015, samples of liver, muscle and perirenal fat, obtained from 16 animals belonging to the same beef cattle farm, were collected at the slaughterhouse by the official veterinarian officers on duty at the plant. Three different matrices (liver, perirenal fat and muscle) were taken from each animal, except for one calf at 9 months of age from which only liver and muscle samples were collected. Data on gender and age were also obtained for each animal. The sample under study consisted in 11 females and 5 males, while the age ranged between 9 and 176 months (Table 1).

A total of 47 samples were analyzed for PCDD/Fs, DL-PCBs and NDL-PCBs, in order to evaluate their levels and distribution in the different tissues of animals.

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. This method was also used to perform a number of proficiency tests with successful results.

Before analysis, 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 carried out as 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) mode. 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 the same operating conditions already described for PCDD/Fs.

For PCDD/Fs and DL-PCBs toxic equivalent (TEQ) values were calculated using the World Health Organization Toxic Equivalency Factors established in 2005 (WHO-$TEFs_{2005}$). For NDL-PCBs the analytical sum of six indicator congeners was calculated. WHO-TEQs and the sum 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 quantification (LOQ) are equal to their respective LOQ.

Results and discussion

The analytical results are summarized in Table 1. For all samples, the contamination levels of PCDD/Fs, DL-PCBs and NDL-PCBs were calculated, while the lipid content was determined for muscle and fat. According to the European Union (UE) legislation, the concentrations were expressed on fat basis for perirenal fat and muscle, while on product basis for liver. A fat content below 2% was found in 10

samples but the corresponding concentrations were reported on fat basis to be compared with the levels in perirenal fat.
With reference to the Commission Regulation 1881/2006 (and amendments), at least one of the three regulated parameters (WHO-TEQ PCDD/Fs, WHO-TEQ PCDD/Fs + DL-PCBs and sum of NDL-PCBs) was exceeded for all samples except one. All animals showed contamination levels above the corresponding EU maximum limits for one or more tissues.
PCDD/Fs concentrations in liver ranged from 0.04 to 0.19 pg WHO-TEQ/g, while their levels were between 0.14 and 0.81 pg WHO-TEQ/g fat for perirenal fat and from 0.14 to 0.89 pg WHO-TEQ/g fat for muscle. DL-PCBs levels in liver ranged from 0.57 to 4.73 pg WHO-TEQ/g, while they were between 7.21 and 48.19 pg WHO-TEQ/g fat for perirenal fat and from 6.49 and 54.16 pg WHO-TEQ/g fat for muscle. The concentrations of NDL-PCBs in liver were in the interval from 2.22 to 24.10 ng/g, while they were in the range from 27.81 to 330.90 ng/g fat for perirenal fat and from 30.38 to 283.30 ng/g fat for muscle.
Comparing PCDD/Fs and DL-PCBs WHO-TEQs, and the NDL-PCBs sum between perirenal fat and muscle, data showed a strong overlap, in particular for DL-PCBs and NDL-PCBs where the levels are higher and the contribution of not detected congeners is negligible. This correlation indicates that perirenal fat could be a good predictor of contamination in muscle and be used as eligible matrix to define the compliance of beef cattle. Similar outcomes on distribution of contaminants in different tissues of bovines have been reported by other authors [2,3,4].
Regarding the distribution of contaminants in liver versus muscle and perirenal fat, a positive correlation was obtained. As an example, figure1 A-C shows the relationship between the levels of PCDD/Fs, DL-PCBs and NDL-PCBs in liver and those found in muscle. A similar correlation was also found for liver and perirenal fat. The coefficient of determination (R^2) values were relatively high and ranged between 0.71 for PCDD/Fs and 0.97 for DL-PCBs.
Calculating all concentrations on fat basis (assuming a mean fat content of 5% in the liver 5), liver was the most contaminated matrix. The PCDD/F concentrations in liver were between 3 and 9 times higher than those found in perirenal fat and muscle. This ratio decreased to 1.1 – 2.7 for DL-PCBs and to 1.0 – 2.9 for NDL-PCBs. This can be explained by a preferential binding of dioxin-like compounds in liver tissue. These results agree with the findings of other studies, according to which the differences in contamination levels between liver and meat or fat are probably due to the physiological function of the liver [2,3]. Furthermore, dioxin-like compounds are known to bind with different affinities to aryl hydrocarbon receptors sited on proteins that are abundant in liver.
Considering the gender, no differences in the levels of contaminants were found; however these outcomes could be influenced by the low number of male cattle analyzed and more data on different gender groups of similar ages should be collected for further clarification.
In all analyzed samples, a negative relationship between the age of cattle and the levels of the three groups of contaminants was recorded. As reported in figure 1 D-E, the levels of NDL-PCBs in perirenal fat, muscle and liver decreased with animals age. This trend was the same for males and females, as reported above. In this case, the presence of a negative relationship can be due to multiple parameters influencing the levels of contaminants (e.g number of lactations or change of fat to body weight ratio).

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Table 1. Animal identification, age (month) and levels of PCDD/Fs, DL-PCBs and NDL-PCBs found in different cattle matrices (pg WHO-TEQ/g fat, except liver pg WHO-TEQ/g)

		Liver			*Perirenal Fat*			*Muscle*		
ID	**AGE**	**PCDD/Fs**	**DL PCBs**	**NDL PCBs**	**PCDD/Fs**	**DL PCBs**	**NDL PCBs**	**PCDD/Fs**	**DL PCBs**	**NDL PCBs**
134*	9	0,15	4,73	21,57	0,59	48,19	216,15	0,89	52,68	245,09
131*	11	0,12	3,89	20,38	0,81	43,09	330,90	0,59	50,47	255,53
122*	18	0,10	2,25	11,62	0,46	25,97	124,48	0,39	26,94	104,54
613*	31	0,11	1,58	7,04	0,30	29,06	110,72	0,25	24,21	110,00
537*	54	0,06	0,97	5,16	0,22	10,37	36,18	0,18	10,80	43,63
896*	67	0,05	0,57	2,72	0,17	8,95	27,81	0,15	6,49	30,38
583*	78	0,07	0,90	3,01	0,23	10,67	31,79	0,25	9,63	46,00
941*	106	0,06	0,67	4,17	0,14	7,21	34,69	0,27	9,95	40,49
012*	138	0,06	0,93	5,24	0,24	12,4	36,76	0,31	8,02	42,03
777*	146	0,06	1,06	5,26	0,24	11,11	41,40	0,20	13,46	50,27
601*	176	0,06	0,89	2,22	0,23	11,6	34,88	0,14	11,83	38,62
133**	9	0,12	3,85	17,66	---	---	---	0,66	49,15	283,30
132**	9	0,19	4,43	24,10	0,47	41,08	142,35	0,68	54,16	205,79
123**	18	0,13	1,71	14,52	0,29	12,74	88,61	0,51	17,69	116,66
614**	31	0,07	1,73	9,25	0,25	15,31	54,85	0,23	17,99	53,84
599**	41	0,04	1,11	3,13	0,18	13,31	45,95	0,32	19,55	62,19

* female
** male

Figure 1. Correlation between liver and muscle for PCDD/F-TEQ (A), DL-PCB-TEQ (B) and NDL-PCBs (C). Relationship between age and NDL-PCBs levels in liver (D), perirenal fat (E) and muscle (F)