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Highlights

- DL-compound TEQ levels in the livers of ewes were up to 5-fold higher than in cows.
- No significant differences in levels of NDL-PCBs were found between the two species.
- DL-compound levels were remarkably lower than those reported in previous studies.
- Data are consistent with the expected low DL-contamination level in sampling areas.
- Previous fat-related MLs for ovine livers were more precautionary than current MLs.

Abstract

Products of animal origin represent the main route of human exposure to dioxins and dioxin-like PCBs (DL-compounds). Recently, concerns have been raised about ovine products, particularly the liver, in which relatively high levels of DL-compounds have been reported. We surveyed ovine and bovine livers in areas with no known sources of dioxin or DL-PCB contamination, in order to assess accumulation patterns for both DL-compounds and non-DL (NDL-) PCBs. None of the ovine and bovine samples exceeded the current Maximum Limits (MLs) for DL-compounds. Liver DL-compound TEQ concentrations were up to 5-fold higher in sheep than in cows. No statistically significant differences in total NDL PCBs levels were found. The main contributors to TEQ levels were the Penta- and Hexa-chlorinated PCDFs and PCB 126. The results confirm the increased bioaccumulation in ovine liver towards specific DL compounds even in ewes reared in areas with no known sources of PCDD/Fs or DL-PCBs contamination.

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are widespread persistent environmental contaminants. One group of PCB congeners, referred to as dioxin-like (DL-)PCBs, share the property of interacting with the intracellular aryl hydrocarbon receptor (AhR). This is considered to be a key factor for causing a large array of adverse effects affecting liver, thyroid and immune functions, along with reproduction and neuro-development; DL-compounds are also recognized as carcinogenic and teratogenic agents (Mandal, 2005). Based on the chemical structure and the inability to bind the AhR, another group of PCB congeners are known as non-DL PCBs (NDL-PCBs) and are characterized by a different toxicological profile (Elabbas et al., 2013). Six NDL-PCBs are used as indicators for regulatory purposes, not because of their specific toxicity but owing to their easy quantification when compared to other NDL-PCBs and the fact that their sum represents about 50% of the total NDL-PCBs in food (EFSA, 2005). Among the chemical residues that may occur in animal products, DL-compounds have recently been ranked as being of the highest concern for human health (EFSA, 2013). This is of particular concern, as products of animal origin represent the main route of exposure to these compounds for humans (Jensen and Bolger, 2001). A renewed interest into DL-compound food chain contamination has been triggered by several incidents in which both scientific investigations and specific knowledge on production processes were necessary to identify the source of contamination (Malisch and Kotz, 2014). Identifying contamination events, possibly by tracing back the source of pollution, is the key to the consequent development of suitable actions to reduce human exposure to DL-compounds, which, in studies on different group populations, has been calculated to exceed the Tolerable Weekly Intake (TWI) of 14 pg TEQ kg-1 body weight (b.w.) in a percentage of individuals between 1.0 and 52.9% (EFSA, 2012). Based on a number of published surveys conducted in

different European countries, concerns have been raised about ovine products, particularly the liver. Indeed, an average DL-compound burden higher than that recorded in other livestock species (including ruminants) has been reported in this food commodity, with single values often exceeding the action levels and, in some cases, also reaching the maximum tolerance levels (MLs) set by Regulation (EC) No. 1881/2006 (European Commission, 2006). The tendency of sheep to accumulate high levels of PCDD/Fs and DL-PCBs in the liver was further pinpointed by an opinion of the European Food Safety Authority (EFSA, 2011). According to data submitted by eight European countries, EFSA reported that more than 50% of the sheep livers analyzed exceeded the MLs in force at that time (4.5 pg TEQ g-1 fat for PCDD/Fs and 10 pg TEQ g-1 fat for the sum of PCDD/Fs and DL-PCBs) and concluded that "the frequent consumption of sheep liver, particularly by women of child-bearing age and children, may be a potential health concern".

Although grazing habits, breeding characteristics and other physiological factors may predispose sheep to accumulate DL compounds to a higher extent than other herbivore species (Rose et al., 2010), it should be noted that meat TEQ values appear to be of the same order of magnitude in all livestock species (Fernandes et al., 2010; EFSA, 2011), thus pointing to a specific accumulation of such compounds in the ovine liver.

The MLs for sheep liver (European Commission, 2011) have been recently set at 1.25 pg TEQ g-1 w/w for PCDD/Fs, 2.00 pg TEQ g-1 w/w for the sum of PCDD/Fs and DL-PCBs, and 3.0 ng g-1 w/w for NDLPCBs (European Commission, 2013). Most importantly, these MLs are no longer expressed on lipid content but on a wet weight basis (w/w); the shift to current MLs was chosen in order to account for the known interindividual variability in fat content within the same species and among the different tissues analyzed for dioxin determination such as liver, perirenal fat, muscle, and others, as underlined by many authors (Irigaray et al., 2005; Brambilla et al., 2011).

Moreover, this choice is expected to solve the analytical problems arising from possible differences in fat extraction methods adopted by Official European Laboratories, which are thought to substantially affect the accuracy of PCDD/Fs and DL-PCBs TEQ determination in such a food commodity (Kotz et al., 2012). However, assuming a mean fat content of 5% in the liver, the new MLs (pg TEQ g-1 w/w) would result in approximately four-fold higher levels than those previously reported on a fat basis (Hoogenboom et al., 2015b), being therefore less conservative than the previous ones.

Following the cited EFSA opinion (EFSA, 2011), a survey was started in Piedmont (North-western Italy) in order to compare the liver accumulation patterns of both PCDD/Fs, DL-PCBs and the six marker NDL-PCBs also including a parallel sampling program on cattle liver. All the examined livers were from animals reared in carefully selected areas, that are located in a small mountain valley where the only industrial settlements consist in the stone quarries. In such areas known sources of dioxin contamination have never been identified.

2. Materials and methods

2.1. *Sample collection*

Planning an effective sampling program to compare ovine and bovine specimens had to address the difficulties in finding suitable samples, as well as making efficient use of the available resources for expensive analytical determinations. The statistical power to detect a relevant difference between the two species was taken into account. Assuming a population standard deviation of 0.2 pg TEQ g-1 w/w, a sample size of 30 (28) was calculated in order to estimate the population mean with 95% level of confidence and a precision ranging between ±0.075 pg TEQ g-1 w/w. The results were expressed as mean ± standard deviation (SD) and median with range as appropriate.

A sample size of 10 bovine animals was calculated in order to detect a difference of 0.25 pg TEQ g————————————1 w/w when comparing this reference group with the 30 subjects of the "experimental" group (sheep), assuming the use of a power of 80%, equal standard deviations (a conservative 0.22) in the two groups and a two-sided test with 5% significance level.

Liver samples from 37 ewes and 16 cows were collected at slaughterhouses between May 2012 and June 2013. Immediately after collection, livers specimens were ID labeled and stored at -20 °C until being processed for the analytical determinations.

Selected animals were clinically healthy, multiparous, at the end of their production cycle (mean age for ewes 9 years, and 10 years for cows) and exclusively reared in farms located in a limited area of the Piedmont Region, North-Western Italy, where no dioxin contamination episodes have occurred to our knowledge. All selected animals were from known meat production breeds, namely Piemontese breed cows, and Biellese, Sambucana, and crossbred Frabosana ewes.

To avoid possible biases linked to a lower rate of hepatic elimination of the investigated compounds, livers with manifest macroscopic alterations, which, according to the veterinarian officers would have been unfit for human consumption, were not included in the study and discarded; using these criteria, a final selection of 30 liver samples from ewes and a minimum of 10 samples from cows, for correct statistical comparison was fulfilled.

2.2. *Analytical determinations*

All PCDD/Fs, DL- and NDL-PCBs standards were purchased from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA), all solvents were of gas chromatography grade. Quantitative determinations of PCDD/Fs were performed with a 7-point calibration curve ranging from 0.05 to 200 pgmL-1 for tetra-CDD/Fs, from 0.25 to 1000 pg mL-1 for

penta-, hexa- and hepta-CDD/Fs, and between 5 and 2000 pg mL-1 for octa-CDD/Fs. DL-PCBs and NDL-PCBs were detected by an 8-point calibration curve ranging from 2 to 200 pg mL-1 for both of these compounds. Calibration solution and samples were added with $^{13}C_{12}$-labeled and $^{37}C_{l4}$-labeled congeners, as internal, recovery and clean-up standards, as shown in previous study (Olanca et al., 2014).

Liver portions (mean weight 300 g) were blended using a blade grinder (Retsch, Düsseldorf, Germany) and then lyophilized (Criofarma, Turin, Italy). Starting wet weight and lyophilized amount obtained for each samplewas recorded in order to relate fat fraction extracted from the dried processed sample to initial wet weight. Fat extraction from 45 g of lyophilized liver was performed by a Soxhlet Extraction System B-811 (Buchi, Cornaredo, Italy), configured for 260 cycles overnight in toluene/ethanol 70/30 v/v solvent mixture, after adding $^{13}C_{12}$ labeled dioxin and PCB internal standards.

Following steps of digestion, serial drying steps in Rotavapor ® (Heidolph, Schwabach, Germany) and oven, HCl digestion overnight and NaOH/KOH washing steps were carried out. Final purification of hexane re-suspended extracts was performed using a Power-PrepTM automated cleanup system (Fluid Management Systems,Waltham, USA), configured with acid silica column, a basic alumina column and an activated carbon column: purified fractions were then reconstituted, prior to instrumental analysis, with nonane containing $^{13}C_{12}$ labeled recovery standards.

Finally, extracts were analyzed for DL-PCBs, PCDD/Fs NDL and NDL-PCBs content by GCeHRMS, based on the internationally recognized method EPA1613-revision B (EPA,1994) and method EPA 1668-revision C (EPA, 2010): analysis was carried out using a GC Trace Series 2000 (ThermoQuest), with a DB-5 MS capillary column (60 m 0.25 mm, 0.1 mm), coupled with a MAT 95 XL (Thermo-Finnigan), with a resolution of 10,000 in selected ion monitoring (SIM)mode (Squadrone et al., 2015). Quantificationwas based on the

isotope dilution method. As required by EPA methods, the Limit of Quantification (LOQ) and recovery of each compound were evaluated for each sample in each analytical session. Laboratory performances were monitored every year by the proficiency tests organized by the European Union Reference Laboratory for Dioxins and PCBs in Feed and Food. The calculations of the Upper Bound TEQ level for PCDD/Fs and DL-PCBs sums were performed according to Regulation (EU) No. 589/2014 (European Commission, 2014). Data were adjusted for both the current World Health Organization Toxicity Equivalent factors (TEF-WHO2005), as required by the Regulation (EU) No. 1259/2011 (European Commission, 2011), and the previous TEF-WHO1998, in order to compare DL-compound concentrations found in our study to the data set mentioned in the EFSA opinion (EFSA, 2011).

2.3. *Statistical analysis*

D'Agostino-Pearson and Shapiro-Wilk tests were performed, assuming an α error equal to 0.05, in order to verify the normality of the distributions of the collected data; based on these results, the non-parametric Manne-Whitney test for rank comparison (α error of 0.05) was chosen to detect significant differences in median TEQ concentrations and also at the level of a single congener (17 PCDD/Fs, 12 DL-PCBs, and 6 NDL-PCBs), comparing ovine and bovine groups (GraphPad Prism 6.01 software).

3. Results and discussion

3.1. *PCDD/Fs and DL-PCBs TEQ*

Mean and median TEQ levels of DL-compounds in ovine and bovine liver samples are reported in Table 1. As expected, there were 3.5-5-fold higher TEQ levels ($p < 0.05$) for PCDD/Fs in sheep compared to cows (median value 0.27 pg WHO-TEQ_{2005} g-1 w/w vs 0.07

pg WHO-TEQ_{2005} g-1 w/w) and for the sum of PCDD/Fs and DL-PCBs (median value 0.77 pg WHO-TEQ_{2005} g-1 w/w vs 0.15 pg WHO-TEQ_{2005} g-1 w/w). To compare our data with those provided by Member States and reported by EFSA in 2011, a conversion from liver w/w to its fat content as well as a correction with previous WHO-TEF1998 were performed (Table 2), resulting in mean contamination levels in the examined ewe livers of 5.92 pg WHOTEQ_{1998} g-1 fat for PCDD/Fs and 11.24 pg WHO-TEQ_{1998} g-1 fat for PCDD/Fs þ DL-PCBs, respectively. For comparison, both values were approximately 3-fold lower than those reported in ovine livers in the mentioned survey by EFSA (EFSA, 2011), and about 4-fold lower than those measured in monitoring investigations of sheep hepatic samples from lower Saxony (Germany) (Bruns-Weller et al., 2010).

More to the point, even livers from young ewes fed “clean” grass pellets (containing 0.33 ng TEQ kg-1 PCDD/Fs þ DL-PCBs) for 56 days had PCDD/Fs and PCDD/Fs þ DL-PCBs levels roughly twice those measured in our study (Hoogenboom et al., 2015a).

As regards cows, the mean liver WHO-TEQ1998 levels (1.14 pg WHO-TEQ_{1998} g-1 fat for PCDD/Fs and 2.28 pg WHO-TEQ_{1998} g-1 fat for PCDD/Fs þ DL-PCBs) were comparable to those reported in 2008 by the Nordic Countries survey on feed and food (Wiborg et al.,) where, in the absence of known DL-compound contamination outbreaks, hepatic concentrations amounted to 0.92 pg WHOTEQ_{1998} g-1 fat for PCDD/Fs and 1.98 pg WHO-TEQ_{1998} g-1 fat for the sum of PCDD/Fs and DL-PCBs. Likewise, liver concentrations of five selected PCDD/Fs (TCDD, PeCDD, HxCDD, PeCDF, and HxCDF) from 10 month-old Holstein Friesian heifers fed for 31 weeks with hay displaying concentrations of the selected PCDD/Fs below LOD, were three-to tenfold higher than those detected in our survey (Thorpe et al., 2001). It can therefore be reasonably concluded that the relatively low TEQ values we found in both cow and ewe livers are consistent with the expected low dioxin background contamination level of the area selected for the present survey. This conclusion is also

supported by the advanced age of the sampled animals, that is normally associated with higher accumulation rates of such persistent contaminants. In line with what is outlined in the EFSA opinion (EFSA, 2011), data from the present study further confirm the unlikelihood that the environmental exposure may have accounted for the almost 4-fold higher DL-compound accumulation in ovine vs. bovine livers.

3.2. *Regulatory aspects concerning liver DL compound contamination*

If we consider the current liver MLs (1.25 pg TEQ g-1 w/w for PCDD/Fs and 2.0 pg TEQ g-1 w/w for the sum of PCDD/Fs and DLPCBs), none of the analyzed ewe samples would have resulted as noncompliant (Table 3). By contrast, if expressed on lipid content, almost 20% of the samples would have exceeded the previous MLs (4.5 pg TEQ g-1 fat for PCDD/Fs and 10 pg TEQ g-1 fat for the sum of PCDD/Fs and DL-PCBs, data not shown).

Even in that case, however, the rate of non-compliant samples would have been much lower than that reported by EFSA in 2011 (more than 50% of samples exceeding lipid based MLs), thus confirming the expected low level of DL-compound contamination in sampling areas. It is worth noting that none of the bovine samples exceeded the MLs, irrespective of the basis for calculating the TEQ.

In line with what reported by other authors (Hoogenboom et al., 2015b), taken together, our results confirm that the application of the wet weight-based MLs result in an appreciably less conservative approach than the previous fat-related MLs, particularly for ovine livers. Although a very limited fraction of the European population is reported to consume regularly such an offal (EFSA, 2011), further data are needed, also in the light of illegal slaughtering, which may be linked to ethnic and/or religious practices (Pointing et al., 2008).

3.3. *Congener patterns of PCDD/Fs and DL-PCBs in liver*

The differences in congener profiles expressed as median values per g of fresh tissue are presented in Fig. 1A/B and 2A/B. In monitoring investigations like the present one, based on sample collection at the slaughterhouse, very limited information is usually available concerning data other than breed, age and the location of the farm. In addition, there is a lack of specific information concerning the diet of the animals enrolled in the study and on the occurrence of farming/agricultural practices prone to increase the dioxin burden of the investigated individuals (e.g. illegal bonfires, waste burning, use of wastelands for grazing, etc.). As a consequence, caution should be exercised in drawing conclusions about species-related differences in congener profiles.

Regarding PCDD/Fs, the highest upper bound median concentrations in bovine liver samples were recorded for octachlorodibenzodioxin (OCDD), while in sheep specimens 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF, see Fig. 1A) displayed the highest median levels. A high liver OCDD concentration was also reported in cattle bred in an area that hosted a PCB production plant in Italy (Turrio-Baldassarri et al., 2009). After converting the concentrations to TEF values for PCDD/Fs group and expressing results on a wet weight basis, the major contribution to Upper Bound total TEQ concentrations in ewes and cows was mainly from the highly chlorinated PCDFs, specifically 2,3,4,7,8-PeCDF, followed by 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, and 2,3,4,6,7,8-HxCDF (Fig. 1B). A relatively high accumulation of the same highly chlorinated PCDD/Fs has also been reported in previous studies involving dairy cows and beef calves fed uncontaminated diets (Feil et al., 2000; Richter and McLachlan, 2001). As regards sheep, in a study focused on the assimilation of DL-compounds in conventionally reared farm animals, relatively higher transfer factors were found for heavily vs. lower chlorinated PCDD/Fs in ovine and porcine livers (Fernandes et al., 2011).

The congener 2,3,4,7,8-pentachlorodibenzofuran, together with other PeCDFs and HxCDFs are often linked to a broad spectrum of human activities, ranging from burning waste for metal smelting

and cement kiln, to different chemical manufacturing or processing (e.g. pulp bleaching), reaching in some cases up to 20% of the total congener emission (Toren and Blanc, 1997; Karstensen, 2008). For this reason, identifying existing reservoirs of PCDD/Fs and other DLcompounds, which may often reflect older contaminations, cannot be easily achieved in areas where no overt episodes of pollution were reported or suspected (Chi et al., 2007).

Regarding DL-PCBs, PCB 118, followed by PCB 105, 156 and 167 were the most abundant congeners found in both ovine and bovine liver samples (Fig. 2A). In particular, sheep livers had PCB 126 concentrations more than five-fold higher than cow livers. After calculation of TEQ values for each congener, the main contribution to Upper Bound total TEQs in ewes and cowswas almost exclusively from PCB 126, in line with its TEF values up to 1000 times higher than that of the other DL-PCBs (Fig. 2B). A similar liver accumulation pattern, with a remarkable contribution to total TEQ by highly chlorinated PCDFs and PCB126 was recently reported in a study in which lambs were fed with DL-compound contaminated grass for 56 days (Hoogenboom et al., 2015a). A substantial contribution of PCB 126 to total liver TEQ was also reported in sheep bred in rural sites with no history of industrial activity that might have affected the background contamination by DL-compounds (Fernandes et al., 2011). Overall, this is of particular concern in the light of the recent

re-classification of PCB 126 by the International Agency for Research on Cancer (IARC) as a known human carcinogen, along with TCDD and 2,3,4,7,8 PeCDF (IARC, 2015).

The above results underline a clearly different kinetic behaviour between the various PCDD/Fs and DL-PCB congeners, pointing to specific accumulation mechanisms. As expected, most of the highest-contributing congeners to liver TEQ values in sheep also

accounted for the observed statistically significant differences ($p < 0.05$) with respect to cow specimens, and namely the Penta-, Hexa- and Hepta-chlorinated dibenzofurans, and PCB 126. Taken together, all the above differences found at the single congener level comparing the two animal groups, despite being statistically significant (Figs. 1 and 2A), should be adjusted to include only total PCDD/Fs TEQ and DL-PCBs TEQ concentrations, when additional data on soil/grass contamination and/or any information about exposure events over the long career of selected animals are not available.

3.4. *Marker NDL-PCBs in liver*

Median and range liver values of the 6 marker NDL-PCBs congeners, as well as their sum, are shown in Fig. 3. No statistically significant differences in total NDL-PCBs content were recorded between the two species, with values around 900e1000 pg g-1 liver (referring to wet weight), and mean concentrations of 13.2 ng g-1 in the ovine group and 10.3 ng g-1 in the bovine group related to fat fraction (Table 1). The mean content of NDL-PCBs in the ovine liver reported by the EFSA (2011) was about 2-fold higher (26.8 ng g-1 fat) than that found in the present study (Table 2).

These results confirm once again a low background contamination of PCB compounds in the area selected for the present survey and further support the specific accumulation of PCDD/Fs and DL-PCBs in the ovine liver (see section 3.1).

In contrast to the comparable total contents of the 6 marker NDL-PCBs, statistically significant differences between bovine and ovine liver specimens were found at the single congener level. In particular, PCB 138 content was about 3-fold higher in cows than in sheep, while the oppositewas true for PCB 153, displaying nearly 2-fold higher values in sheep than in cows. While the biological relevance of these differences - if any - remains to be established, the recorded differences among congener patterns, as stated for DL-compounds

(see section 3.3, Fig. 1A and B), should be associated, through a multidisciplinary approach, with animal physiology, environment and history of human activities in the related areas (Panton et al., 2013).

In conclusion, this study indicates that, even though the survey was conducted in an area where no known episodes of dioxin contamination had occurred, ovine livers are characterized by a DLcompound burden much higher than that characterizing livers from cows reared in the same area. Therefore, our data provide further evidence of the occurrence of a species-related mechanism of hepatic sequestration, strictly limited to DL-compounds, and further research is warranted to gain insight into liver-related factors affecting the kinetics of these chemicals in ruminant species.

Regarding risk assessment and regulatory issues, our results confirm the less conservative nature of the new Regulation considering liver TEQ expression on a tissue basis rather than on a lipid basis.

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Figure captions

Figure 1. A: Median PCDD/Fs concentrations with interquartile range (25-75%) of single congeners in ovine and bovine liver samples, expressed as pg/g wet weight. B: Median TEQ_{2005} concentrations with interquartile range (25-75%) of single congeners and sum of PCDD/Fs TEQ Upper Bound (after the x-axis gap). Concentrations are expressed as pg TEQ/g wet weight. Congeners with low TEQ concentrations are enlarged in the nested figures (C and D).

Figure 2. A: Median DL-PCBs concentrations with interquartile range (25-75%) of single congeners in hepatic samples from sheep and cows, expressed as pg/g wet weight. B: Median TEQ2005 concentration with interquartile range (25-75%) of single congeners and sum of DL-PCBs TEQ Upper Bound (after the x-axis gap). Concentrations are expressed as pg TEQ/g wet weight. Congeners with low TEQ concentrations are enlarged in the nested figure (C).

Figure 3. Median concentrations with interquartile range (25-75%) of single congeners and total NDL-PCBs content in ovine and bovine liver samples, expressed as ng/g wet weight.

Figure 1.

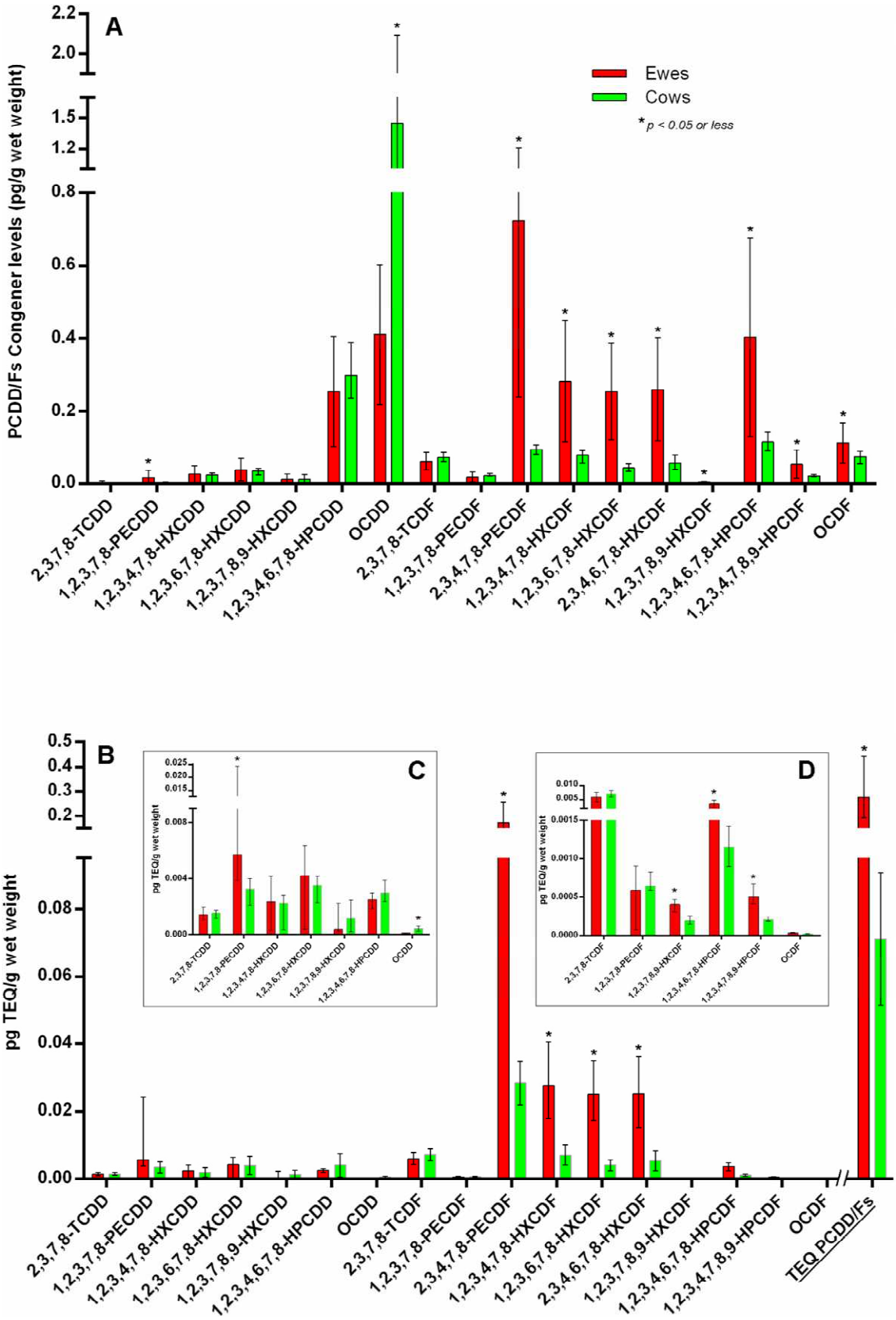
A
Ewes
Cows
*p < 0.05 or less
PCDD/Fs Congener levels (pg/g wet weight)
2,3,7,8-TCDD
1,2,3,7,8-PECDD
1,2,3,4,7,8-HXCDD
1,2,3,6,7,8-HXCDD
1,2,3,7,8,9-HXCDD
1,2,3,4,6,7,8-HPCDD
OCDD
2,3,7,8-TCDF
1,2,3,7,8-PECDF
2,3,4,7,8-PECDF
1,2,3,4,7,8-HXCDF
1,2,3,6,7,8-HXCDF
2,3,4,6,7,8-HXCDF
1,2,3,7,8,9-HXCDF
1,2,3,4,6,7,8-HPCDF
1,2,3,4,7,8,9-HPCDF
OCDF
B
C
D
pg TEQ/g wet weight
TEQ PCDD/Fs

Figure 2.

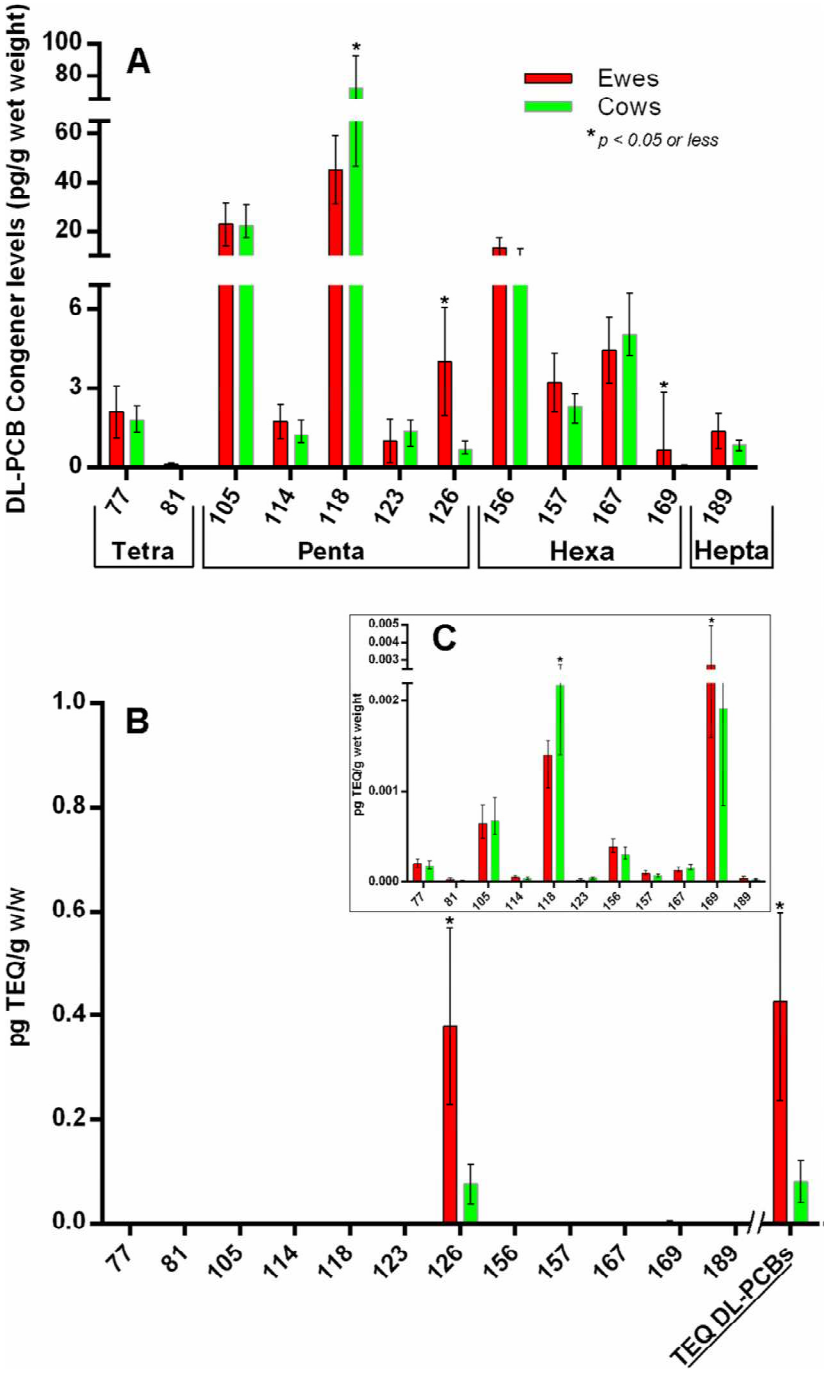
A
DL-PCB Congener levels (pg/g wet weight)
Ewes
Cows
* p < 0.05 or less
Tetra
Penta
Hexa
Hepta
77
81
105
114
118
123
126
156
157
167
169
189
B
pg TEQ/g w/w
TEQ DL-PCBs
C
pg TEQ/g wet weight

Figure 3.

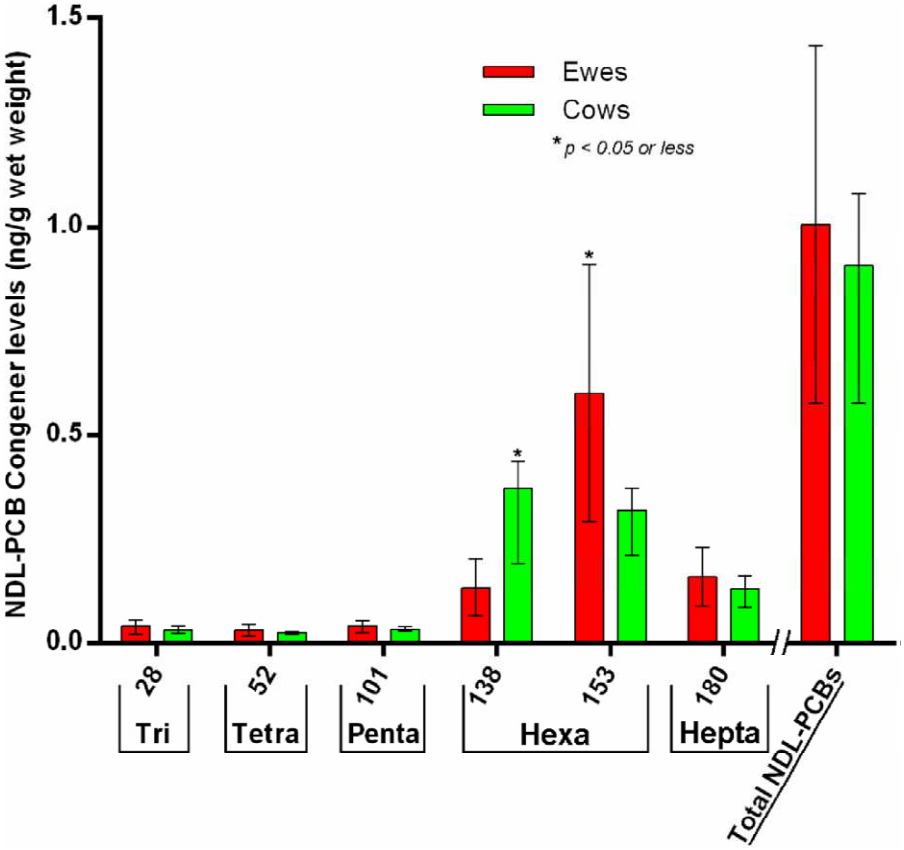
NDL-PCB Congener levels (ng/g wet weight)
1.5
1.0
0.5
0.0
Ewes
Cows
*p < 0.05 or less
*
*
28
52
101
138
153
180
Tri
Tetra
Penta
Hexa
Hepta
Total NDL-PCBs

Table 1: Comparison of mean and median upper bound TEQ levels (PCDD/Fs and sum of PCDD/Fs and DL-PCBs) and total NDL-PCBs concentrations in ovine and bovine liver samples; data are reported on a wet weight basis, as required by 2013 EU legislation, and are also related to fat content, considering current (2005) WHO-TEFs.

Wet weight (pg WHO-TEQ_{2005} g^{-1} w/w)			Lipid based (pg WHO-TEQ_{2005} g^{-1} fat)		
PCDD/Fs	**sheep**	**cow**	**PCDD/Fs**	**sheep**	**cow**
Minimum	0.09	0.04	Minimum	1.54	0.49
25% Percentile	0.19	0.06	25% Percentile	2.84	0.63
Median	**0.27**	**0.07**	**Median**	**3.76**	**0.86**
75% Percentile	0.43	0.08	75% Percentile	5.07	1.22
Maximum	1.04	0.11	Maximum	10.15	1.28
Mean	**0.34**	**0.07**	**Mean**	**4.15**	**0.90**
Std. Deviation	0.21	0.02	Std. Deviation	1.93	0.28
PCDD/Fs + DL	**sheep**	**cow**	**PCDD/Fs + DL**	**sheep**	**cow**
Minimum	0.21	0.05	Minimum	3.18	0.58
25% Percentile	0.45	0.12	25% Percentile	6.63	1.44
Median	**0.77**	**0.15**	**Median**	**9.9**	**2.21**
75% Percentile	0.96	0.19	75% Percentile	12.07	2.26
Maximum	1.89	0.22	Maximum	18.49	2.64
Mean	**0.76**	**0.15**	**Mean**	**9.45**	**1.90**
Std. Deviation	0.38	0.05	Std. Deviation	3.72	0.62
Wet Weight (ng g^{-1} w/w)			Lipid based (ng g^{-1} fat)		
NDL-PCB	**sheep**	**cow**	**NDL-PCB**	**sheep**	**cow**
Minimum	0.21	0.37	Minimum	2.81	4.26
25% Percentile	0.77	0.57	25% Percentile	8.89	7.88
Median	**0.97**	**0.90**	**Median**	**11.8**	**10.64**
75% Percentile	1.26	1.08	75% Percentile	17.14	12.85
Maximum	2.19	1.14	Maximum	26.9	15.09
Mean	**1.01**	**0.83**	**Mean**	**13.2**	**10.28**
Std. Deviation	0.43	0.28	Std. Deviation	5.95	3.35

Table 2: Comparison of mean upper bound TEQ levels (PCDD/Fs and sum of PCDD/Fs and DL PCBs) and total NDL-PCBs concentrations in ovine livers sampled in our study (n ¼ 30) and dataset taken from EFSA opinion (2011); to allow data comparison concentrations are reported on fat basis, considering old (1998) WHO-TEFs.

pg WHO-TEQ_{1998} g^{-1} fat			pg WHO-TEQ_{1998} g^{-1} fat			ng g^{-1} fat		
PCDD/Fs	This study n = 30	EFSA (2011) n = 332	**PCDD/Fs + DL**	This study n = 30	EFSA (2011) n = 332	**NDL-PCB**	This study n = 30	EFSA (2011) n = 257
Minimum	2.14	0.27	Minimum	3.86	0.47	Minimum	2.8	0.41
5% Percentile	2.62	0.98	5% Percentile	4.35	1.36	5% Percentile	7.4	1.38
50% Percentile	5.58	7.8	50% Percentile	11.63	14.26	50% Percentile	12.3	14.55
Mean	**5.92**	**14.9**	**Mean**	**11.24**	**26.12**	**Mean**	**13.2**	**26.78**
90% Percentile	8.63	36.14	90% Percentile	16.21	61.14	90% Percentile	19.5	52.69
95% Percentile	11.07	92.55	95% Percentile	17.37	98.06	95% Percentile	21.92	93.50
Maximum	14.95	116.3	Maximum	23.46	279.19	Maximum	26.9	350.45

Table 3: Upper bound TEQ level of PCDD/Fs and the sum of PCDD/Fs and DL-PCBs in sheep and bovine liver samples, considering 2005 WHO-TEF. Data are reported on wet weight and corrected, as required by legislation, for the uncertainty of the method (MU).

Animals	PCDD/Fs	MU	PCDD/Fs + DL-PCBs	MU
Ewes	ML (1.25 pgTEQ g^{-1} w/w)		ML (2.0 pgTEQ g^{-1} w/w)	
1	0.19	±0.04	0.40	±0.14
2	0.17	±0.03	0.30	±0.13
3	0.43	±0.09	0.90	±0.29
4	0.12	±0.02	0.24	±0.07
5	0.09	±0.02	0.21	±0.07
6	0.30	±0.06	0.74	±0.23
7	0.19	±0.04	0.54	±0.17
8	0.37	±0.06	0.96	±0.31
9	0.18	±0.03	0.23	±0.05
10	0.28	±0.06	0.82	±0.27
11	0.27	±0.05	0.62	±0.19
12	0.26	±0.05	0.55	±0.16
13	0.25	±0.05	0.87	±0.29
14	0.44	±0.09	1.13	±0.36
15	0.26	±0.05	0.72	±0.23
16	0.29	±0.06	0.81	±0.26
17	0.21	±0.04	0.45	±0.14
18	0.17	±0.03	0.44	±0.14
19	0.35	±0.07	1.03	±0.34
20	0.27	±0.05	0.98	±0.33
21	0.67	±0.13	1.34	±0.39
22	0.16	±0.03	0.36	±0.11
23	0.19	±0.04	0.48	±0.15
24	1.04	±0.21	1.89	±0.54
25	0.74	±0.15	1.25	±0.35
26	0.52	±0.10	0.88	±0.24
27	0.38	±0.08	0.80	±0.24
28	0.54	±0.11	1.27	±0.39
29	0.48	±0.09	0.70	±0.18
30	0.32	±0.06	0.89	±0.29
Cows	ML (0.3 pgTEQ g^{-1} w/w)		ML (0.5 pgTEQ g^{-1} w/w)	
1	0.04	±0.01	0.05	±0.01
2	0.11	±0.02	0.19	±0.05
3	0.08	±0.02	0.14	±0.04
4	0.06	±0.01	0.16	±0.05
5	0.07	±0.01	0.19	±0.06
6	0.06	±0.01	0.22	±0.07
7	0.07	±0.01	0.13	±0.04
8	0.05	±0.01	0.11	±0.03
9	0.09	±0.02	0.19	±0.06
10	0.07	±0.01	0.13	±0.04