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Dioxins, DL-PCB and NDL-PCB accumulation profiles in livers from sheep and cattle reared in North-western Italy

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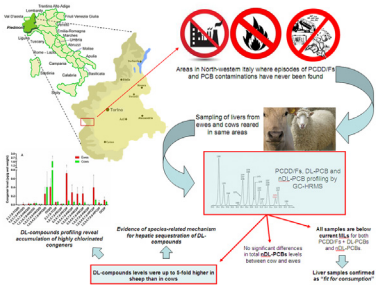
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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

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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 NDL-PCBs (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