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Organochlorine Pesticide and Polychlorinated Biphenyl in Calves from North-West Spain

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Abstract The aim of the present study was to assess the levels of organic pollutants in cattle from the NW of Spain. The livers of 101 animals from two regions (Galicia and Asturias) were analysed for seven organochlorine pesticides (HCB, α-HCCH, φ-HCCH, HEOD, DDT, TDE) and 34 PCBs congeners (8, 18, 28, 29, 31, 52, 77, 101, 105, 114, 118, 123, 126, 128, 138, 141, 149, 153, 156, 157, 167, 169, 170, 171, 180, 183, 187, 189, 194, 199, 201, 205, 206 and 209). The influence of different factors (type of farm and proximity to industrial areas) on pesticide accumulation in cattle was also studied. Overall, the frequency of occurrence and concentrations of OC pesticides and PCBs was low in calves from both regions. HEOD was detected most frequently and was found in more than half of the calves from each region. PCBs 141 and 153 were the most frequently detected PCB congeners but typically occurred in less than 20% of animals. The exposure of calves to the more abundant contaminants differed significantly between the regions and may be related to past usage patterns. Differences between farms in terms of their livestock management did not have a significant effect on pesticide accumulation by calves. The liver concentrations in calves were below maximum residues levels (RMLs) for human intake and there is no indication of any risk from these contaminants through eating calf liver.

Keywords Organochlorine pesticides · Polychlorinated biphenyls, calves · Farm-type industrialization

Organisms, including cattle, are exposed worldwide to a variety of persistent organic pollutants (POPs). Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are considered to be amongst the most important POPs because of their global use, environmental persistence, and their bioaccumulative and toxic properties (Hoffman et al. 2001; Kunisue et al. 2003). Exposure can be through all routes (inhalation, ingestion and dermal absorption). Because these compounds are lipophilic, they increase in concentration along food chains and bioconcentrate in animal and human tissues where they are associated with a wide range of toxic effects (Brouwer et al. 1995).

During the last few decades, much attention has been given to POPs internationally after it became apparent they were transported through the environment (Garrido Frenich et al. 2006) and could induce or aggravate certain health problems in humans. Several polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDDs/Fs) and PCBs have been shown in experimental animals to induce biochemical and toxicological responses that are similar to those induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic congener within these groups of compounds. Bocio and Domingo (2005) reported that meat, particularly that with a high fat content (Guruge et al. 2005), dairy products, and fish make up >90% of the intake of PCDDs/Fs and PCBs for the general human population. Dietary intake of these compounds is considered to be the most important exposure route in humans (Falandysz and Kannan 1992;

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Guruge et al. 2005) and maximum residue limits (MRLs) have now been established for human foodstuffs; in meat, they are typically in the 0.01–0.1 mg/kg range (Garrido Frenich et al. 2006). In addition, the high level of fat in meat products such as offals has emphasized the need for sensitive and fast methods for pesticide residue analysis for the control of POPs in fatty matrices (2004/61/EEC).

The north-west (NW) of Spain is an important area for bovine production and cattle are typically reared on small farms. There are two readily distinguished regions in this part of Spain. Galicia is predominantly a rural agricultural area with relatively little industry and low recorded levels of environmental contamination. Asturias has a large central industrial area, mainly metal industry and coal mining, that has resulted in the release of pollutants into the environment where animals graze (EPER 2005). While the exposure of cattle (and of humans eating locally reared cattle) in this region has been assessed for toxic metals (López Alonso et al. 2000, 2002), exposure and assimilation of cattle in NW Spain to POPs has not, as far as we are aware, been measured. Indeed, to date, there appear to be no published data for OC pesticide and PCB concentrations in cattle and scant published data for cattle-derived meat products for anywhere in Spain (Herrera et al. 1994; Lázaro et al. 1999). The aim of our study was to quantify OC and PCB concentrations in cattle from NW Spain and to assess whether various different factors (type of farm and proximity to industrial areas) affected the accumulation of POPs by cattle.

Material and Methods

A total of 101 calf livers (72 from Galicia, 29 from Asturias) from 35 different farms were collected from six slaughterhouses between April and June 2006. Information on each calf was obtained from farm documentation that accompanied the calves to slaughter. In Galicia, most cattle are pure Galician blonde breed and Galician-blonde × Holstein-Friesian crosses. Cattle are reared either on small extensive farms where they graze on local pasture and are largely kept outdoors, or on intensive farms where they are kept indoors and are maintained on commercially produced feed that is not produced locally. In contrast, most cattle reared for meat production in Asturias are crosses of different breeds. They are reared on small traditional farms that have an outdoor management regime and animals eat local forage.

Liver samples were taken from the caudal lobe, placed on ice immediately after collection, transported to the laboratory, and stored at −18°C until processed. Each liver was later defrosted and chopped into small pieces that were mixed together so that each liver was thoroughly homogenised.

A sub-sample of approximately 1 g wet weight (ww) was taken from each liver and sub-samples were processed and analysed separately. The mean (±SE) sub-sample weight was 1.061 (±0.030) g. Extraction, clean-up and analysis of the samples followed the methods described by Shore et al. (2001). In brief, each sub-sample was ground with acid-washed sand mixed with anhydrous sodium sulphate, and cold extracted in 50 mL of 50:50 hexane/acetone. The extract was dried to zero volume, re-suspended in hexane, clean-up by alumina column chromatography (0.8 g of 5% deactivated aluminium oxide) and analysed by gas chromatography with electron capture detection (GC-ECD). Seven organochlorine pesticides [hexachlo-robenzene (HCB), alpha-Hexachlorocyclohexane (α-HCCH), gamma-Hexachloro-cyclohexane (φ-HCCH), dieldrin (HEOD), dichlorodiphenyltrichloro-ethane (DDT), tetrachlorodiphenylethane (TDE)] and 34 PCBs congeners (8, 18, 28, 29, 31, 52, 77, 101, 105, 114, 118, 123, 126, 128, 138, 141, 149, 153, 156, 157, 167, 169, 170, 171, 180, 183, 187, 189, 194, 199, 201, 205, 206 and 209) were analysed. Dichlobenil was used as an internal standard. Peaks were identified by comparing their retention times with those of standards analysed at the same time. Analyte concentrations were calculated by internal standard techniques.

For quality assurance purposes, chicken liver spiked with a known concentration of each congener was analysed with each batch of samples. Average recovery figures for different compounds and congeners ranged between 66% and 94%. Concentration data in calf livers were not recovery corrected. Instrument LoD's (ng/mL) were calculated from the standard calibration curve as the y intercept of the curve plus three times the standard deviation of the curve, ranged from 0.021 ng/mL for φ-HCCH to 0.294 ng/mL for PCB 156.

All tissue contaminant concentration data are given as wet wt. (ww) concentrations. Values below the LoD were taken to be zero when calculating mean concentrations and congener-summed total PCB concentrations. Fisher's exact probability test and a two sample student *t*-test were used to compare differences between Galician and Asturian calves in the frequency of occurrence of liver contaminants and in liver contaminant concentrations, respectively. A general linear model was used to test for differences in contaminant concentrations between farm type (extensive, intensive) in Galicia and regression analysis was used to analyse the relationship between contaminant concentration in Asturias calves and distance to industry site.

Results and Discussion

In total, only two OC pesticides (HEOD, TDE) and six PCB congeners (29, 138, 141, 153, 183, 189) were detected in the livers of calves from either Galicia (Table 1) or Asturias (Table 2). The concentrations were below maximum residues levels (RMLs) (0.02–1 mg/kg range for OCs, (83/363/EEC) and 0.01–0.1 mg/kg range for PCDDs/Fs and PCBs; (396/2005/CEE). HEOD and PCBs 141 and 153 were detected more frequently than other compounds which were found only in single animals. The proportion of calves with HEOD residues was significantly higher for Galicia than Asturias (78.9% vs. 48.3%; Fisher's exact test, $p < 0.01$) whereas PCB 141 occurred in a higher proportion of Asturian than Galician calves (24.1% vs. 8.3%, Fisher's exact test, $p < 0.05$). Concentrations of HEOD and PCB 141 in those calves with detectable residues did not differ significantly between Galicia and Asturias (Student t test, $t \leq 1.8$, $p > 0.05$ in both cases). PCB 153 was detected in a small but similar proportion of animals (7%–10%) in both regions. These results suggest that previous use of dieldrin as a residual pesticide spray and to control insect vectors of disease may have been more prevalent in Galicia than Asturias. The reason why PCB 141 occurs in a greater proportion of calves in Asturias than in Galician is uncertain. PCB 141 contamination has not been reported in previous environmental studies in Asturias; furthermore, detectable liver PCB 141 concentrations increased with distance from the centre of the industrial area in Asturias ($R^2 = 0.817$; $F_{(1,7)} = 22.3$, $p < 0.005$, Fig. 1). This is not consistent with the industrial region being a source of these compounds to calves, and there was also no relationship between distance from the industrialised area and detectable liver concentrations of congener sum total PCBs ($F_{(1,12)} = 0.183$, $p > 0.05$).

We used liver HEOD concentrations in Galician calves to assess whether farm type may have any effect on pesticide accumulation by calves as most animals had detectable residues of this compound. Farming system can potentially affect exposure because pesticide deposited from the atmosphere onto the pasture surface can be consumed by cattle eating fresh grass, silage or hay (Fries and Paustenbach 1990; Lorber et al. 1994). In contrast, husks (which could potentially be contaminated) are removed from cereals fed directly to cattle kept indoors, although concentrate feed can be contaminated (Sharma et al. 2005). Soil ingestion also affects contaminant uptake by cattle and other ruminants (Hoffman et al. 2006, Skwarzec and Prucnal 2007) but this can occur in animals kept outdoors on pasture (Sharpe and Livesey 2005) and in those fed on grass silage indoors (Berende 1990). In the present study, we found no effect of farm management type on HEOD accumulation in either cross bred or Galician Blonde cattle ($R^2 = 0.021$, $F_{(1,72)} = 0.575$ $p = 0.451$, farm type; $F_{(1,72)} = 0.118$ $p = 0.732$ breed, and $F_{(1,72)} = 0.017$, $p = 0.896$ farm type × breed; Fig. 2).

Table 1 Number of calf livers from Galicia (total number analysed = 72) with detectable OC and PCB concentrations and summary statistics for detected concentrations (ng/g ww)

	HEOD	PCB 29	PCB 141	PCB 153	PCB 183	PCB 189	Congener summed total PCBs
Detected samples	56	1	6	7	1	1	13
Mean (±SEM)	13.8 ± 1.07	3.86 (–)	8.48 ± 1.14	13.8 ± 1.43	12.1 (–)	4.19 (–)	16.9 ± 8.90
Median	13.7	3.86	8.89	15.3	12.1	4.19	8.90
Q1–Q3	9.05–21.5	–	5.9–11.9	5.66–23.9	–	–	5.86–23.7
Range	4.12–45.8	–	5.31–12.4	5.42–71.5	–	–	5.31–71.5

ND = Nondetected, SEM = Standard error of the mean, Q1–Q3 = Interquartile range

All other compounds that were determined were not detected in any sample

Table 2 Number of calf livers from Asturias (total number analysed = 29) with detectable OC and PCB concentrations and summary statistics for detected concentrations (ng/g ww)

	HEOD	TDE	PCB 138	PCB 141	PCB 153	Congener summed total PCBs
Detected samples	14	1	1	7	2	10
Mean (±SEM)	19.7 ± 2.77	5.78 (–)	4.35 (–)	10.2 ± 1.20	10.5 (–)	9.69 ± 1.33
Median	18.7	5.78	4.35	9.57	10.5	8.66
Q1–Q3	12.8–24.6	–	–	7.34–10.5	–	6.04–11.7
Range	3.48–44.1	–	–	5.39–18.5	6.15–14.9	5.39–18.5

ND = Nondetected, SEM = Standard error of the mean, Q1–Q3 = Interquartile range

All other compounds that were determined were not detected in any sample

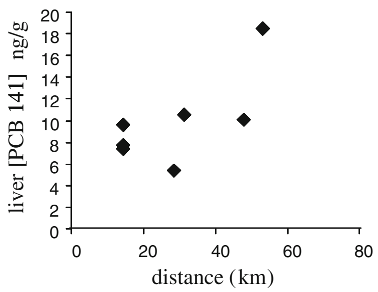

Fig. 1 PCB 141 plotted against distance—significant relationships ($F_{(1,7)} = 22.314$, $p = 0.005$) (tested by linear regression analysis), (NDs are not included)

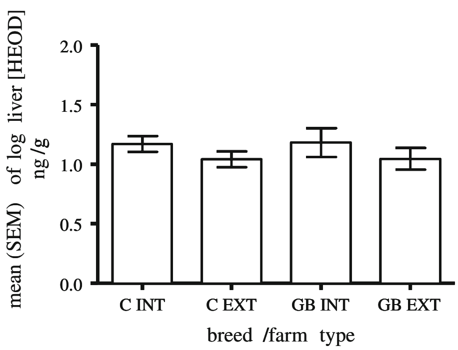

Fig. 2 Geometric mean ± GSE for HEOD in cross-breed calves from intensive farms (CINT), and extensive farms (CEXT), and in Galician Blonde calves from intensive farms (GB INT) and extensive farms (GBEXT)

There have been few other studies on organic contaminant concentrations in livestock in Spain. OCs were measured in meat and meat products purchased from stores and meat industries across Spain. HCB and HCH were detected in all the samples (in concentrations between 10 and 18 µg/g fat) but DDT, aldrin, endrin, heptachlor, heptachlor epoxide, chlordane, methoxychlor, endosulfan and trans-nonachlor were not detected (Herrera et al. 1994). In another study in NE Spain, congeners PCB 28, 52, 101, 138, 153 and 180 concentrations were quantified in human diet. PCB was not detected in any meat products (Lázaro et al. 1999). The levels of contamination in calves in the present study were therefore broadly consistent with concentrations detected in meat products elsewhere in Spain. The diversity and concentrations of the contaminants in the calves in our study were also generally lower than in wild predators in NW Spain (Carril González-Barros et al. 1997, López-López et al. 2001) but this is likely to reflect lower levels of exposure, as might be expected to occur in animals feeding at lower trophic levels and on diets with relatively low lipid content (Shore and Rattner 2001).

In conclusion, the frequency of occurrence and concentrations of OC pesticides and PCBs were low in calves from throughout NW Spain. The concentrations of those compounds that were detected were below RMLs and so no health risk is indicated for humans. There was also no evidence that differences between farms in terms of their livestock management had any significant effect on pesticide accumulation by the calves.

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