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Geographic variation in tissue accumulation of endocrine disrupting compounds (EDCs) in grazing sheep

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Muscle concentrations of few of the endocrine disrupting compounds, measured in the muscle of sheep from regions exposed to greater pollution, were elevated.

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ABSTRACT

Muscle tissue was collected from ewes and lambs derived from farms throughout Scotland and sample concentrations of five endocrine disrupting compound groups were determined. Farms of origin were categorised according to geographic region. There were few statistically-significant differences with region or distance from cities. However, the magnitude of the difference between the highest and lowest mean values in ewe muscle from different regions exceeded 30% for 13 of the 15 compounds that were consistently detected in muscle, with animals derived from the industrialised region having the highest mean values for 11 of the 13 compounds. A less marked trend was apparent in the lamb muscle (8 of 13 highest were in the industrialised region). The physiological effects of such small differences in exposure to mixtures of pollutants remain to be determined.

1. Introduction

Endocrine disrupting compounds (EDCs) comprise a range of anthropogenic chemicals, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs), all of which are highly persistent and remain in the environment for periods of years (Smith, 1996), and phthalates and alkyl phenols which are more readily degraded but are also continually produced and released into the environment (Clark et al., 1992; ECPI, 1995). These chemicals are present in low concentrations throughout the environment but accumulate in animal tissue. For some, such as the PCBs and dioxins, it is thought that this is primarily through ingestion in food (Fries, 1995; Norstrom, 2002), while for others, such as the PAHs and di(2-ethylhexyl) phthalate (DEHP), inhalation may be an important route of exposure (Whyatt et al., 1998; Kavlock et al., 2002; Zanieri et al., 2007).

These pollutants have the potential to disrupt human and animal health and reproductive function (Toppari et al., 1996; IEH, 1999; Paul et al., 2005; Fowler et al., 2008), even at concentrations normally considered harmless (Myers et al., 2009). Thus, if risk is to be managed, both with respect to the health of domestic animals and to that of human consumers of their muscle tissue or milk, knowledge of tissue accumulation in animals with potentially different rates of environmental exposure is essential.

Since environmental concentrations of pollutants are likely to be greater around sites of production and use (Stevens et al., 2003; Zanieri et al., 2007), at least for classes of chemical that are currently manufactured, it was postulated that higher muscle concentrations would be recorded in animals reared near to cities and industrial areas than in rural areas.

While the importance of aerial transfer to herbage of pollutants such as PCBs is recognised (Welsch-Pausch et al., 1995), the processes that govern the long range transport and bio-accumulation potential of at least some EDCs are poorly understood (Gouin and Harner, 2003). However, it was further postulated that in view of their diverse characteristics, the patterns of distribution of and accumulation would differ between the compounds measured, making prediction of overall risk more difficult.

The aim of this study was to determine muscle tissue concentrations of selected, representative EDCs in sheep reared in different locations in Scotland and to relate these to likely sources of pollutants and to EDC class. It is known that the effects of exposure to EDCs on animal health and physiology can be exacerbated by increased age and therefore duration of exposure (Colborn et al., 1993) and so animals of contrasting ages were included in the study. Muscle was the tissue of choice for chemical analysis because

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ruminant meat represents a significant component of the diet of many people and so exposure to EDCs through this route may be significant.

2. Material and methods

2.1. Sources of animals and muscle tissue collection

At two abattoirs serving different regions of Scotland, samples of muscle tissue from the upper neck (little economic value) were collected, between late October and January, from 14 groups of 8 lambs and 7 groups of 8 mature, multiparous ewes, primarily of hill and upland breeds. All samples were wrapped in aluminium foil and stored at −20 °C until analysis.

The animals sampled were derived from farms representing many regions within Scotland, including some that were close to major conurbations (less than 40 km) and/or in the "Central Belt" of Scotland which contains the great majority of the population and of the manufacturing industry in the country, including oil refining and chemical processing. Others were located at distances up to 250 km from the main sources of pollution (Fig. 1). The prevailing wind direction is south-westerly and so the sources of animals were both up- and downwind of the main industrial area.

2.2. Analytical protocols

Muscle tissue concentrations of selected compounds from a number of EDC classes commonly present in the environment were determined:

a) Diethylhexyl phthalate (DEHP) – the phthalate which is one of the most commonly used plasticizers and is therefore one of the most significant environmental phthalates (Sharman et al., 1994).
b) Nonyl phenol (NP) – this is widely used in industry and domestically and, while it has a short half life, it is present in the environment (Rhind et al., 2002).
c) PCB congeners (The ICES 7) – this is a set of congeners (28, 52, 101, 118, 138, 153, 180) that is internationally recognised as an appropriate index of PCB pollution (ICES, 1986).
d) Polybrominated diphenyl ethers (PBDE) – congeners measured include a range of degrees of bromination (28, 47, 99, 100, 153, 154, 183).
e) Polycyclic aromatic hydrocarbons (PAH) – 16 compounds that have been defined by the US EPA as priority pollutants (http://www.epa.gov/waterscience/criteria/wqcriteria.html).

Methods described previously were used to determine muscle concentrations of DEHP and NP (Rhind et al., 2005) and the selected PCB and PBDE congeners (Rhind et al., 2009). Experimental blanks were extracted with each batch of samples and corrected tissue values were determined by deducting the blank values from the measured values. Following extraction and clean up, concentrations of each were determined using gas chromatography linked to mass spectrometry (GC/MS).

Concentrations of selected PAHs (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno [1,2,3-cd]pyrene, dibenzo[a,h]anthracene and benzo[ghl]perylene) were determined using methods described by Rhind et al. (2009) except that the method of clean up was modified. Extracts were loaded on to a column comprising 1 g acid-modified silica plus 9 g of activated silica; the column was conditioned with 40 ml iso-hexane and then eluted by 50 ml iso-hexane:DCM(4:1 v/v) with the first 10 ml being discarded and the next 40 ml of eluate collected. The collected fraction was concentrated for GC–MS analysis.

Quality control samples were included with each batch of experimental samples analysed. Limits of detection (LOD) were 10 μg/kg for DEHP and NP, 20 ng/kg for all PCBs, 20 ng/kg for PBDE 28, 47, 99 & 100 and 50 ng/kg for PBDE 153, 154 & 183. The LOD for PAHs were 5 μg/kg for phenanthrene, fluoranthene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene, 15 μg/kg for pyrene and1 μg/kg for other compounds.

2.3. Statistical analysis

In order to assess the effects of location on lamb muscle EDC concentrations, the farms from which lambs (farms 1–14) and ewes (farms A to G) originated were classified into three geographic regions, based on topography and prevailing wind direction (Fig. 1):

a) North and East (NE; farms 1–6 and A and B) – an area of hill, upland and lowland coastal belt with a relatively low population and little industry, downwind of the Central Belt.
b) Central Belt (CB; farms 7–9 and C) – an industrialised and heavily populated, predominantly lowland, area, downwind of the South and West region.
c) South and West (SW; farms 10–14 and D, E, F and G) – an area of hill, upland and lowland coastal belt, with a relatively low population and little industry, upwind of the CB.

Farms were categorised, also, according to distance from the centre of the nearest city of more than 150,000 people (less than 40 km: farms 5–9 and C and D; more than 40 km: farm numbers 1–4, 10–14 and A, B, E, F and G).

All EDC values were $\log_{10}$ transformed before analysis to meet requirements for constant variance of residuals. Tissue concentrations of EDCs in sheep originating from each geographic region or at each distance from population centres were compared by ANOVA, blocking for farm, using Genstat for Windows release 10 (VSN International, Hemel Hempstead, UK). For some compounds, concentrations were below the LOD in some or all individuals; where more than 45% of values for one compound were below the LOD, it was excluded from the analyses; this limit was arbitrarily selected but allowed the inclusion of all data sets that were considered adequate for meaningful statistical analysis.

In addition to analyses of individual compounds, hierarchical analyses of variance of combined data were carried out, blocking for farm and animal within farm, either combining all detectable, individual compounds of PAHs, PCBs or PBDEs, respectively, or combining all classes of compound in a single analysis, with the main effects being region or distance and pollutant.

In addition to the comparisons of mean values at less than 40 km and more than 40 km from cities, mean concentrations of each compound, for animals of each farm, were regressed against distance from the centre of the closest city of more than 150,000 people. The analysis was not conducted where concentrations of compounds were at or below the LOD in more than 45% of individuals.

3. Results

EDC concentrations in ewe and lamb muscle tissues were not directly comparable, because the animals were derived from different farms and were not managed in an identical manner, but muscle concentrations of most of the EDCs measured were of similar orders of magnitude in the two groups (Tables 1 and 2). Concentrations of all classes of compound were highly variable with individual animals having values that differed by more than two orders of magnitude, both within and between farms (NP: 50–1070 μg/kg DM; DEHP: 30–4920 μg/kg DM; total PCB (sum of values for individual congeners): 70–8918 ng/kg DM; total PBDE (sum of values for individual congeners): 70–24,909 ng/kg DM; benzo[a]anthracene (PAH; selected for the purpose of illustration): <1.0–235 μg/kg).

Concentrations of PCB congeners 52 and 118, PBDE congeners 28, 153,154 and 183 and several PAHs (acenaphthalene, acenaphthene (lambs only), fluorene (lambs only), phenanthrene, anthracene (ewes only), fluoranthene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, dibenzo[a,h] anthracene and benzo[ghl]perylene) were at or below the LOD at all or most sites and so were not included in the analyses.

3.1. Lambs

There were no significant differences with geographic region in mean log concentrations of any of the compounds measured (Table 1), with the exception of PBDE 47 which was higher in the SW than either the NE or CB regions ($P = 0.027$). Mean concentrations of NP were significantly higher in animals from farms less than 40 km, compared to farms more than 40 km, from cities ($P = 0.028$) but mean concentrations of none of the other compounds differed statistically with distance (Table 2).

While few differences with region or distance were statistically significant, owing to the extreme variance in individual concentrations, there were marked trends in the mean concentrations associated with different regions or distances from cities for many compounds or classes. These trends were addressed using the arbitrary criterion of a 30% difference in mean values (expressed as a function of the lower/lowest value). On this basis, the highest mean values were observed in the CB for 8 of the 13 compounds in which there were differences of this magnitude, compared with 3