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Species-specific concentrations of perfluoroalkyl contaminants in farm and pet animals in Japan

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ABSTRACT

The persistent metabolites of perfluorinated compounds (PFCs) which have been detected in the tissues of both humans and wildlife, and human contamination by PFCs suggest differences in the exposure patterns to these compounds. However, studies focused on identifying human exposure pathways to PFCs are scarce. To provide a preliminary assessment of PFCs in farm animals such as chicken, cattle, pigs, goats and horses, blood and liver samples were collected from various regions in Japan. Additionally, dog sera samples representing pet animals were also employed for analysis. Perfluorooctane sulfonate (PFOS) was the most prominent contaminant found in farm and pet animals, with mean sera PFOS concentrations (in decreasing order) of: chicken (5.8 ng/ml) > cattle (3.0 ng/ml) > goat (2.4 ng/ml) > horse (0.71 ng/ml) > pig (0.37 ng/ml). Chicken livers (67 ng/g) contained the highest mean PFOS concentration among the farm animals, followed by those of pigs (54 ng/g) and cattle (34 ng/g). In comparison to PFOS levels in farm animals, the detected levels of other PFCs were not significant. The high levels of PFOS found in cattle fetal livers suggest that PFOS crosses the placental barrier to enter fetal circulation. The consumption of chicken by humans might produce higher PFOS exposure in humans compared to that in farm animals; however, the current levels of PFOS in farm animals in Japan were lower than those reported in fish and wild animals. Elevated concentrations of both PFOS (25 ng/ml) and perfluorohexane sulfonate (PFHxS; 10 ng/ml) were found in dog sera, indicating that further studies are needed to identify PFC sources in the human environment.

1. Introduction

Perfluorinated compounds (PFCs), which have a wide range of industrial and consumer applications, have been used for over 50 years. The strong carbon–fluorine (C–F) covalent bonds present in PFCs account for the thermal and chemical stability of these compounds, which thus resist hydrolysis, photolysis, biodegradation and metabolism (Kissa, 2001). It has been reported that PFCs are found in several species of wildlife and fish from various locations, including remote areas (Kannan et al., 2001a,b,2002). These compounds bind to serum albumin and are found in the protein fraction of blood (Han et al., 2003). Some studies have reported the occurrence of PFCs in the body fluids of non-occupationally exposed humans in various geographical locations (Olsen et al., 2003; Kannan et al., 2004; Guruge et al., 2005a). Potentially serious health effects of certain PFCs have been reported, including liver damage and detrimental effects on development, thyroid and pancreatic functions and reproduction and even mortality in animals (Seacat et al., 2002; Thibodeaux et al., 2003; Kennedy et al., 2004; Lau et al., 2004). Therefore, the occurrence of these chemicals in the human body has raised considerable public health concerns about the effects of these chemicals. Since these compounds are found in various concentrations in humans, it is important to focus on pathways of exposure. Human exposure to organochlorine pollutants through foodstuffs originating from animals, for example, is considered to be greater than exposure through other pathways. Therefore, monitoring PFCs in domestic animals will provide insight not only into animal contamination, but also into potential human exposure through ingestion of animal products. PFC contamination in edible fish, wild animals and drinking water has been reported worldwide, however, data are scarce for farm animals. In the present study, the concentrations of several PFCs in the sera, plasma and livers of six species of farm and pet animals were measured to determine current contamination levels in animals from several locations in Japan.

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2. Materials and methods

2.1. Samples

The blood and liver samples used in the present study were collected from farm animals in 2003 and 2004. Alphabetical codes were used to identify sample locations in order to avoid any conflict of interest. Animal samples of six species (cattle, pigs, chickens, goats, horses and dogs) were categorized into five prefectures according to sample locations (Table 1). A small number of samples were also collected from animals employed for various research programs at two national institutes in Tsukuba, Japan (Area A in Table 1), where the animals were treated under strict guidelines for animal experiments. Cattle samples were included from three strains: Japanese black, Holstein and F1 (crossbred from Japanese black and Holstein cattle). Dog sera were obtained from a veterinary clinic in Area C. All animals were healthy and professional veterinary officers conducted the sampling. Sera and plasma were prepared by centrifugation at 3000 rpm for 15 min and all samples were kept at −20 °C until analysis.

2.2. Sample preparation and analysis

Samples were analyzed for 10 PFCs: perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), and perfluorooctane sulfonylamide (PFOSA). Analysis was carried out using an ion-pairing method, which has been described elsewhere (Taniyasu et al., 2003; Kannan et al., 2004; Guruge et al., 2005a). Briefly, 1 ml of serum was mixed with 1 ml of 0.5 M tetra-*n*-butylammonium hydrogen sulfate solution and 2 ml of buffer (pH 10, 0.25 M) in a polypropylene (PP) tube. The sample mixture was extracted with 5 ml of methyl *tert*-butyl ether (MTBE) by shaking for 20 min followed by centrifugation. Four milliliter of MTBE was then removed from the tube and placed in a second PP tube. Extraction was performed two more times, removing 5 ml of MTBE each time and combining it in the second PP tube. The combined extracts were concentrated under nitrogen after adding 0.5 ml of methanol. The sample was passed through a 0.1 μm nylon filter before injection. For the extraction of liver samples, a homogenate of 1 g of liver in 5 ml of distilled water was prepared. A 1 ml aliquot of the sample homogenate was extracted according to the procedure described above.

Detailed instrumental analysis and quantification has been described elsewhere (Yamashita et al., 2004; Guruge et al., 2005a). Briefly, analysis of PFCs was performed using a high performance liquid chromatograph-tandem mass spectrometer (HPLC-MS/MS), comprising an Agilent HP1100 liquid chromatograph interfaced with a Micromass® (Beverly, MA, USA) Quattro Ultima Pt mass spectrometer operated in the electrospray negative ionization (ESNI) mode. A 10 μl aliquot of the sample extract was injected into the system equipped with a guard column (XDB-C8, Agilent Technologies, CA) connected sequentially to a Betasil C18 column (Thermo Hypersil-Keystone, Bellefonte, PA) with 2 mM ammonium acetate/methanol as mobile phase, starting at 10% methanol. At a flow rate of 300 μl/min, the gradient was increased to 30% methanol at 0.1 min, 75% methanol at 7 min, and 100% methanol at 10 min. The system was switched back to its original conditions at 12 min, and kept at these conditions for 20 min. The capillary was held at 1.2 kV. Cone-gas and desolvation-gas flows were kept at 60 and 650 L/h, respectively. Source and desolvation temperatures were kept at 120 and 420 °C, respectively. MS/MS parameters were optimized to transmit the $[M-K]^-$ or $[M-H]^-$ ion before fragmentation to one or more product ions. Eight calibration curve points bracketing the concentrations in samples were prepared routinely, to check for linearity. Concentrations in procedural blanks of the target analytes were in the range from <0.01 to 0.05 ng/ml. The limit of quantification (LOQ), which ranged from 0.01 to 0.05 ng/ml for each target analyte, was determined as the lowest standard injected which could meet the acceptance criteria for a particular run, a signal-to-noise ratio of 3, and the maximum blank concentration.

2.3. Quality control

Procedural recovery and blank tests were carried out to determine the precision of the extraction and analytical protocol. The accuracy of the analyses was determined using matrix spikes. The mean recoveries of PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA and PFOSA spiked into the serum and liver samples ($n = 4$) prior to extraction were 68–103% (standard deviation 2–14%), and 54–129% (standard deviation 2–34%), respectively, and the recovery of ^{13}C-PFOA (Perkin–Elmer, Boston, MA) was 97 ± 5% and 98 ± 5% for serum and liver ($n = 4$). The matrix-spike studies suggest that the data are quantitatively acceptable. Concentrations were not adjusted for the purity of standards or recovery. Moreover, to reduce high background levels, all accessible polytetrafluoroethylene (PTFE) tubes and solvent inlet filter units in the high performance liquid chromatography (HPLC) system were replaced with stainless steel or peek tubes while no degasser or solvent selection valves were used. Additionally, suitable PP tubes and septa were selected after thorough blank checking. The details of analytical improvements to eliminate contamination sources have been discussed elsewhere (Yamashita et al., 2004).

3. Results and discussion

3.1. Concentrations of PFCs in animals

The mean and range concentrations of selective PFCs in serum, plasma and liver samples from various animal species are shown in Table 1. PFOSA was not detected, and PFHpA and PFHxA were rarely found in farm animals. PFOSA was detected in only a few dog serum samples (<0.01–0.06 ng/ml), hence, these data were not included in Table 1. The detection frequencies of PFHpA and PFHxA were 100% in dog sera and the mean concentrations were 0.32 (0.18–0.71 ng/ml) and 0.73 ng/ml (0.46–1.6 ng/ml), respectively. Concentrations of less than the LOQ were considered to be zero for calculating mean concentrations, thus mean values diverge somewhat from actual values, particularly in the case of goat plasma concentrations of perfluoro acids, in which one animal showed elevated concentrations for most analytes (Smirnov–Grubbs' test, $p < 0.01$).

Among fluoro chemicals, PFOS was detected in all samples used in the present study. The highest PFOS concentration found in dog serum was 57 ng/ml, and the mean concentration of PFOS in dog sera was 4.3-, 8-, 10-, 36- and 83-fold greater than the levels found in the sera and plasma of chickens, cattle, goats, horses and pigs, respectively (ANOVA with multiple comparison Tukey's test; $p < 0.01$). The highest serum PFOS concentration in farm animals was found in chickens at 19 ng/ml, which was nearly 1.8-, 4.8-, 19- and 21-fold greater than that in cattle, goats, pigs and horses, respectively ($p < 0.01$). In chickens, compared to pigs, greater bioaccumulation of other organochlorines such as dioxins, furans and polychlorinated biphenyls (PCBs) have been reported (Guruge et al., 2005b). Species differences for the elimination half-life of PFOS in biota have been reported to vary significantly (Environment Canada, 2004), hence, species specificity in PFOS