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Perfluorinated compounds in minke whales (*Balaenoptera acutorostrata*) and long-beaked common dolphins (*Delphinus capensis*) from Korean coastal waters

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ABSTRACT

This is the first study to report the concentrations and accumulation profiles of PFCs in marine mammals from Korea. The concentrations and profiles of 10 PFCs in the liver of minke whales and common dolphins from Korean coastal waters were recorded in this study. The mean concentrations of PFOS and PFUnDA were 3–20 times higher than that found for other PFCs analyzed. The concentrations of PFOS in cetaceans from Korea were relatively lower than those reported in other countries. Inter-species differences in the concentrations of PFOS, PFOSA and PFNA were found between two cetacean species, while no difference was observed in the concentrations of PFDA, PFUnDA and PFDoDA between the species. The dominant PFC compounds found in cetaceans were PFUnDA and PFOS, accounting for 70–80% of the PFCs. The accumulation profiles and correlation analysis indicated that two cetacean species have different exposure routes and metabolic capacity for PFCs.

Perfluorinated compounds (PFCs) have been used since the late-1940s in a variety of industrial and commercial products, such as polymers, stain repellents, lubricants, paper coatings, and cosmetics (Giesy and Kannan, 2001, 2002). A global monitoring study in the early 2000s showed the widespread presence of perfluorooctanesulfonate (PFOS) and related compounds in wildlife tissues as well as bioaccumulative properties of these compounds (Giesy and Kannan, 2001; Kannan et al., 2005; Houde et al., 2006; Sinclair et al., 2006). Toxicological studies on PFOS and perfluorooctanoic acid (PFOA) showed adverse effects on intercellular communication, membrane transport, and developmental and neuroendocrine anomalies in laboratory animals (Berthiaume and Wallace, 2002; Hu et al., 2002; Austin et al., 2003; Lau et al., 2004; Yoo et al., 2008).

PFCs have been detected in both biotic and abiotic compartments from most locations worldwide (Kannan et al., 2001; Houde et al., 2005; Sinclair et al., 2006; Senthilkumar et al., 2007; Dorneles et al., 2008; Tao et al., 2008; Yamashita et al., 2008). Some PFCs have been shown to biomagnify in the marine foodweb (Giesy and Kannan, 2001; Martin et al., 2004; Tomy et al., 2004; Kannan et al., 2005; Houde et al., 2006; Sinclair et al., 2006). Marine mammals occupy a high trophic status in the food chain, and because marine mammals possess relatively low metabolic activities, they accumulate high levels of PFCs (Kannan et al., 2001, 2002a; Houde et al., 2005; Van de Vijver et al., 2007; Dorneles et al., 2008). In Korea, traditionally, cetaceans were hunted by fishermen for subsistence before the ban on commercial whaling was imposed by the moratorium of the International Whaling Commission (IWC) in 1986. However, many cetaceans are by-caught in fishing nets and/or stranded. A total of 587 cetaceans belonging to nine species were collected from Korean coasts in 2006 (An and Kim, 2008). The major species that are by-caught or stranded are minke whales (*Balaenoptera acutorostrata*) and long-beaked common dolphins (*Delphinus capensis*), which collectively accounted for 75% of the total number of individuals.

The worldwide production of perfluorooctane sulfonyl fluoride (POSF) was estimated to be 96,000 tons during the period 1970–2002, and the current inventory of PFOS in ocean surface waters is estimated to be 235–1770 tons (Paul et al., 2009). However, only limited information is available on contamination by PFCs in Korean coastal waters (So et al., 2004; Yoo et al., 2009). A few studies have reported accumulation of PFCs in birds and in human blood from Korea (Kannan et al., 2002b, 2004; Yoo et al., 2008). Studies on distribution and sources of PFCs in the marine ecosystem are essential for the conservation and management of cetaceans. The

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objective of this study was to investigate the contamination status and accumulation profiles of PFCs in the liver of two species of cetaceans collected from Korean coastal waters.

Liver samples were obtained from 66 minke whales (*B. acutorostrata*) and 47 long-beaked common dolphins (*D. capensis*) that were found entangled in fishing nets in Korean coastal waters in 2006. Minke whales were collected throughout the Korean coast, and the long-beaked common dolphins were collected from the east coast of Korea. After biometric measurement, the cetaceans were dissected and transported to the laboratory of Cetacean Research Institute. All of the samples were kept in a freezer at −20 °C until extraction.

Concentrations of 10 perfluorochemicals, PFOS, perfluorohexanesulfonate (PFHS), perfluorodecanesulfonate (PFDS), perfluorooctane sulfonamide (PFOSA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA) were determined in 113 samples of liver from the two cetacean species. Potassium salts of PFOS (>95%), PFOA (98%), PFHS (99.9%), and PFOSA (95%) used were provided by the 3 M Company (St. Paul, MN, USA). PFHpA, PFNA, PFDA, PFDoDA, and PFUnDA were obtained from Fluorochem Ltd. (>95%, Glossop, Derbyshire, UK). PFDS, $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA, and $^{13}C_2$-PFDA were purchased from Wellington Laboratories (>99%, Guelph, ON, Canada). All the solvents used were HPLC grade, and all the reagents used were ACS grade (J.T. Baker, Phillipsburg, NJ, USA).

The PFCs in liver samples were analyzed using the method described elsewhere (Kannan et al., 2001; Tao et al., 2006). The liver samples (~1 g) were homogenized with 5 mL of Milli-Q water. A volume of 1 mL of the homogenate was transferred to a 15 mL polypropylene (PP) tube. Five nanograms of internal standards ($^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA, and $^{13}C_2$-PFDA), 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate solution (adjusted to pH 10), and 2 mL of 0.25 M sodium carbonate buffer were then added to the PP tube. After being mixed thoroughly, the samples were extracted using 5 mL of methyl *tert*-butyl ether (MTBE) by shaking vigorously for 40 min. The MTBE layer (~4 mL) was separated by centrifugation at 4000 rpm for 5 min, and then transferred to another PP tube. The sample mixture was extracted again using 3 mL of MTBE by shaking for 20 min. The MTBE layer was combined with the first MTBE extract, and evaporated to near dryness under a gentle stream of nitrogen. The samples were adjusted to a volume of 1 mL with methanol, and vortexed for 30 s. Finally, the samples were filtered through a 0.2 μm nylon filter into an autosampler vial.

PFCs were quantified using an Agilent 1100 series high-performance liquid chromatography (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple quadruple mass spectrometer (ESI-MS/MS). Ten microliters of the extract was injected onto a 100 mm × 2.1 mm (5 μm) Keystone Betasil C8 column. The mobile phase was a solution of 2 mM ammonium acetate and methanol, starting at 10% methanol at a flow rate of 300 μL/min. The gradient increased to 100% methanol after 10 min, and was held at this level for 2 min, and then reversed back to 10% methanol. The MS/MS was operated in the electrospray negative ion mode. The target compounds were determined by multiple reaction monitoring (MRM). The MRM transitions were 399 > 80 for PFHS, 499 > 99 for PFOS, 503 > 99 for $^{13}C_4$-PFOS, 599 > 99 for PFDS, 498 > 78 for PFOSA, 363 > 319 for PFHpA, 413 > 369 for PFOA, 417 > 372 for $^{13}C_4$-PFOA, 463 > 419 for PFNA, 465 > 420 for $^{13}C_2$-PFNA, 513 > 469 for PFDA, 515 > 470 for $^{13}C_2$ PFDA, 563 > 519 for PFUnDA, and 613 > 569 for PFDoDA. The samples were injected twice to monitor for sulfonates and carboxylates separately.

The quantification of individual PFC compounds in liver samples was performed using quadratic regression fit analysis weighted by $1/x$ of the extracted calibration curve. All of the internal standards were detected with no interferences. The recoveries of the internal standards, $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA, and $^{13}C_2$-PFDA, spiked into each of the samples, were 102 ± 10% (average ± standard deviation), 101 ± 13%, 118 ± 12% and 129 ± 14%, respectively. The concentrations were not corrected for the recoveries of internal standards. Matrix spikes were performed for four liver samples. Known concentrations of mixed PFC standards (10 ng each) were spiked into the samples before extraction, and were processed in the same way as the samples. The recoveries of 10 PFCs spiked onto the liver samples ranged from 82% for PFHS to 143% for PFUnDA. Six procedural blanks were analyzed by passing water and reagents through the entire analytical procedure, and the results for these were below the limit of detection of the target compounds. The limit of quantification (LOQ) was determined as the lowest acceptable standard in the calibration curve that was defined as a standard within ±30% of the theoretical value that has a peak area twice as large as the analyte peaks are in the blanks. The LOQ values of the individual chemicals of PFCs ranged from 0.5 to 2 ng/g wet wt (wt).

Student's *t*-tests were performed to assess any significant differences in the concentration of PFC between the two species of cetaceans and gender. Spearman's correlation analysis was performed to investigate the relationships between the chemical concentrations and biological data, such as body size. These statistical analyses were performed by using the SPSS v. 11.0 software package for Windows.

PFOS and PFUnDA were detected in all the liver samples from the two species of cetaceans from Korean coastal waters (Table 1). The concentrations of PFOS in the livers of minke whales and common dolphins ranged from 2.8 to 162 ng/g wet wt and from 18 to 152 ng/g wet wt, respectively. The concentrations of PFUnDA ranged from 2.6 to 129 ng/g wet wt in minke whales and 17 to 193 ng/g wet wt in common dolphins. The overall concentration of PFOS and PFUnDA was 3–20 times higher than the concentrations of other PFCs measured in liver samples. Apart from PFOS and PFUnDA, PFOSA was detected relatively more frequently in cetaceans. The concentration of PFOSA ranged from <0.5 to 11 ng/g wet wt in minke whales and 2.2 to 35 ng/g wet wt in common dolphins. The concentrations of PFDA and PFDoDA were similar to each other, and these compounds were also found in most of the liver samples. The concentrations of PFNA in the livers of minke whales and common dolphins ranged from <1.0 to 11 ng/g wet wt and <1.0 to 45 ng/g wet wt, respectively. Although PFOA is the most abundant PFC in seawater samples (Yamashita et al., 2008), PFOA has been measurable in 1.5% of minke whales and 23% of common dolphin samples, which suggests low bioaccumulation potential of this compound (Conder et al., 2008). The concentration of PFOA in our minke whales and common dolphins ranged from <1.0 to 2.7 ng/g wet wt and <1.0 to 7.9 ng/g wet wt, respectively. PFHS and PFDS were only detected in a few samples, and their detection frequencies were <5% for the two cetacean species. PFHpA was not detected in any of the samples.

The concentrations of PFOS measured in the livers of minke whales (2.8–162 ng/g wet wt) and common dolphins (18–152 ng/g wet wt) from Korean coastal waters were compared with values reported for cetaceans from other locations worldwide (Fig. 1). PFOS has been found in all cetaceans, including those from the Arctic ocean (Tomy et al., 2004). Most studies have shown higher levels of PFOS in cetaceans than those found in the cetaceans from Korea. The highest concentration of PFOS was found in bottlenose dolphins from South Carolina in the USA (914 ± 515 ng/g wet wt, Houde et al., 2006). Harbor porpoises from the Baltic Sea (534 ± 357 ng/g wet wt, Van de Vijver et al., 2004) and the UK (524 ± 611 ng/g wet wt, Law et al., 2008) showed higher concentrations of PFOS compared with cetaceans from other locations. The