# Perfluorinated compounds (PFCs) in cetaceans from Korean coastal waters

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**ABSTRACT**

No information is available on the occurrence and concentrations of perfluorinated compounds (PFCs) in marine mammals from Korean coastal waters. This paper presents data on the concentrations and accumulation features of 10 PFCs (PFHS, PFOS, PFDS, PFOSA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA) in the livers of minke whale and common dolphin collected in Korea in 2006. PFOS and PFUnDA were detected in all of the liver samples from two cetaceans species collected from Korean coastal waters, indicating the widespread distribution of these contaminants in Korea. The overall concentrations of PFOS and PFUnDA were higher about 3−20 times than concentrations measured in the livers of other PFC. Concentrations of PFOS measured in the livers of cetaceans from Korean coastal waters were lower than those reported for a number of cetacean species from several other locations worldwide. PFOS, PFOSA and PFNA were significantly greater than in the dolphin liver samples compared to those of minke whale, while PFDA, PFUnDA and PFDoDA were not significantly different between both cetacean species. Although the average concentrations of PFCs in male samples were higher than those of female samples, only PFOS and PFDoDA of minke whale had significant differences between male and female. PFUnDA was the predominant compound, which accounted for 49 ± 11% in minke whale and 35 ± 8% in dolphin to total PFC concentrations. The next contributor of total PFCs was PFOS, which accounted for 29 ± 13% in minke whale and 33 ± 8% in dolphin. These contamination patterns of PFCs in cetaceans were different with other countries, suggesting a specific source of PFCs in Korean coastal waters.

KEYWORDS: COMMON DOLPHIN; MINKE WHALE; PFCS; PFOS; KOREAN COASTAL WATERS

## INTRODUCTION

Perfluorinated compounds (PFCs) have been used in a variety of industrial and commercial products, such as polymers, stain repellents, lubricants, paper coatings, and cosmetics since mid-1940s (Giesy and Kannan, 2001; 2002). Global monitoring of these contaminants in wildlife has found that perfluorooctanesulfonate (PFOS) and related salts are widely distributed, environmentally persistent, and bioaccumulative (Giesy and Kannan, 2001a; Kannan *et al*., 2005; Houde *et al*., 2006; Sinclair *et al*., 2006). The toxicity assessment of PFOS and perflurooctaonic acid (PFOA) has shown to adversely affect intercellular communication, membrane transport, 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 biotic and abiotic compartments from most locations worldwide (Kannan *et al*., 2001a; Houde *et al*., 2005; Nakata *et al*., 2006; Sinclair *et al*., 2006; Kim and Kannan, 2007; Senthilkumar *et al*., 2007; Dorneles *et al*., 2008; Tao *et al*., 2008; Yamashita *et al*., 2008). Some PFCs have been shown to biomagnify to higher trophic level 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 such as cetaceans have high trophic level in food chain and relatively low metabolic activities, thus these species have increased concentrations of PFCs (Kannan *et al*., 2001a; 2002; Houde *et al*., 2005; Van de Vijver *et al*., 2007; Dorneles *et al*., 2008).

Total worldwide production of perfluorooctane sulfonyl fluoride (POSF) was recently estimated to be 96000 tons during 1970–2002 and current inventory of PFOS in ocean surface waters was estimated to be

235–1770 tons (Paul *et al*., 2009). However, only limited information is available on contamination of PFCs in Korean coastal waters (So *et al*., 2004; Yoo *et al*., 2009). A few studies have reported the accumulation by PFCs in birds and human blood in Korea (Kannan *et al*., 2002a; 2004; Yoo *et al*., 2008). Studies on the potential health risks associated with exposure to PFCs in marine ecosystem are essential for conservation and management plans of marine mammals such as cetaceans. Therefore, the major objective of this study was to elucidate the contamination status of PFCs in the livers of two cetacean species collected from Korean coastal waters and to investigate accumulation features of these contaminants according to species and gender in cetaceans.

**MATERIALS AND METHODS**

Liver samples were collected from 66 minke whales and 47 common dolphins caught incidentally in fishing nets along the Korean coasts in 2006. After biometric measurement for collected cetaceans, the specimens were dissected and immediately transported to the laboratory of the Cetacean Research Institute (CRI). All of the samples were kept in a freezer at $-20^0$C until further analysis.

Concentrations of 10 perfluorochemicals, such as perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHS), perfluorodecanesulfonate (PFDS), perfluorooctane sulfonamide (PFOSA), perfluoroheptanoic acid (PFHpA), perfluoroooctanonic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA) were determined in 113 liver samples of two cetacean species. In the present study, potassium salts of PFOS (>95%), PFOA (98%), PFHS (99.9%), and PFOSA (95%) were provided by the 3M Company (St. Paul, MN, USA). PFHpA, PFNA, PFDA, PFDoDA, and PFUnDA were obtained from Fluorochem Ltd. (>95%, 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 solvents were HPLC grade, and all reagents were ACS grade (J.T. Baker, Phillipsburg, NJ, USA).

PFCs in livers were analyzed following the method described elsewhere (Kannan *et al*., 2001a; Tao *et al*., 2006). The liver samples (~ 1 g) were homogenized and then 5 mL of Milli-Q water was added to a liver homogenate. One milliliter 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 added to a PP tube. After being thoroughly mixed, the samples were extracted with 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 into another PP tube. The sample mixture was extracted with 3 mL of MTBE by shaking for 20 min. The MTBE layer was combined with first MTBE extract, and evaporated to near-dryness under gentle stream of nitrogen. The samples were adjusted to 1 mL of methanol, and vortexed for 30 s and finally filtered through 0.2 µm nylon filter into an autosampler vial.

Analyses of 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 2 mM ammonium acetate/methanol starting at 10% methanol at a flow rate of 300 µL/min. The gradient increased to 100% methanol at 10 min and was held for 2 min and then reversed back to 10% methanol. The MS/MS was operated in electrospary negative ion mode. 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. Samples were injected twice, to monitor 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. Recoveries of internal standards of $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA and $^{13}C_2$-PFDA were 102 ± 10% (average ± standard deviation), 101 ± 13%, 151 ± 19% and 129 ± 14%, respectively. Concentrations were not corrected for the recoveries of internal standards. Matrix spikes were performed for 4 liver samples. Known amounts of mixed PFC standards (each 10 ng) were

spiked into the samples before extraction and were processed in the same way as the samples. Recoveries of 10 PFCs spiked onto 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 they were less than limit of detection of target compounds. The limit of quantification (LOQ) was determined as the lowest acceptable standard in the calibration curve that is defined as a standard within ± 30% of the theoretical value and that has a peak area twice as great as the analyte peak are in blanks. LOQs of individual chemicals of PFCs ranged from 0.5 to 2 ng/g wet weight (wt).

Student's *t*-test was performed to assess significant differences of the PFC concentrations according to species and sex in cetaceans. Pearson correlation analysis was performed to investigate the relationships among chemical concentrations and biological data such as body size. These statistical analyses were done by using SPSS software for Windows 11.0.

**RESULTS AND DISCUSSION**

PFOS was detected in all of the liver samples from two cetaceans species collected from Korean coastal waters (Table 1). 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. PFUnDA was found to be dominant PFC in the livers of the cetaceans at concentrations ranging from 2.6 to 129 ng/g wet wt in minke whale and from 17 to 193 ng/g wet wt in common dolphin. The overall concentrations of PFOS and PFUnDA were higher about 3−20 times than concentrations measured in the livers of other PFC. The hepatic concentrations of PFOSA showed relatively higher concentration compared to other PFCs except for PFOS and PFUnDA. The concentrations of PFDA, PFDoDA and PFOSA were similar to each other and they were found in almost all of the liver samples from both cetacean species. PFOA was measurable in 1.5% of minke whale livers and in 23% of common dolphin livers, although the PFOA concentrations were higher than the PFOS concentrations measured in human serum samples from Korea (Kannan *et al*., 2004). PFHS and PFDS were measured in only a few samples and their frequencies of detection were less than 5% for both cetacean species. PFHpA was not detected in any of the samples.

Concentrations of PFOS measured in the livers of cetaceans from Korean coastal waters were compared with those reported for a number of cetacean species from several other locations worldwide (Figure 1). PFOS was detectable in all of the cetacean species including Arctic area (Tomy *et al*., 2004), indicating the ubiquitous contaminant of this chemical in marine ecosystem. The highest concentration of PFOS was found in bottlenose dolphin from South Carolina, USA (Houde *et al*., 2006). Harbor porpoise from Baltic Sea (Van de Vijver *et al*., 2004) and UK (Law *et al*., 2008) showed higher concentrations of PFOS, compared to other locations and cetaceans. The PFOS concentrations in most of the cetacean samples worldwide showed higher than those in two species cetaceans from Korean coastal waters. The PFOS concentrations in melon-headed whale from Japan (Hart *et al*., 2008), harbor porpoise from Iceland (Van de Vijver *et al*., 2004) and sperm whale from North Sea (Kannan et al., 2002b) were similar to those measured in the present study. Some cetaceans collected from India (Yeung *et al*., 2009b), Brazil (Leonel *et al*., 2008) and Canadian Arctic (Tomy et al., 2004) showed lower levels than those in the present study.

The total concentrations of PFOS, PFOSA, PFNA, PFUnDA and PFDoDA in the livers of common dolphin (140 ± 70 ng/g wet wt) were higher than those in measured in the livers of minke whale (100 ± 60 ng/g wet wt). PFOS, PFOSA and PFNA were significantly greater than in the dolphin liver samples compared to those of minke whale. This result can be explained by the difference of inhabitation and diet. The common dolphin is a near-shore species feeding in coastal waters, whereas minke whale migrates both through near-shore and off-shore waters. The major diets of common dolphin are long-lived and larger predatory fish with high lipid content such as herring and mackerel. PFDA, PFUnDA and PFDoDA were not significantly different between both cetacean species.

The difference of PFCs for two cetacean species according to gender was investigated (Figure 3). Although the average concentrations of PFCs in male samples were higher than those of female samples, only PFOS and PFDoDA of minke whale had significant differences between male and female. Common dolphin samples did not show a gender difference concerning accumulation status. Previous studies have reported clear gender difference of PFCs in marine mammals (Kannan *et al*., 2002; Yeung *et al*., 2009b). Further investigation of these questions is needed.

Profiles of relative contribution of individual PFCs were compared between both cetacean species from Korean coastal waters (Figure 4). PFUnDA was the predominant compound, which accounted for 49 ± 11% in minke whale and 35 ± 8% in dolphin to total PFC concentrations. The second contributor of total PFCs was PFOS, which accounted for 29 ± 13% in minke whale and 33 ± 8% in dolphin. The accumulation pattern of PFCs in cetaceans in the present study was different what have reported in other studies. Almost all of the previous studies showed that the PFOS was the predominant PFC in cetaceans such as dolphin and porpoise livers (Yeung *et al*., 2009a). Other studies have reported PFNA to be the second most prevalent PFC after PFOS in marine mammals (Kannan *et al*., 2005; Nakata *et al*., 2006). However, the contribution of PFNA was low in the present study. Therefore, the contamination and accumulation of PFCs in abiotic (seawater and sediments) and biotic samples in Korean environment require further study.

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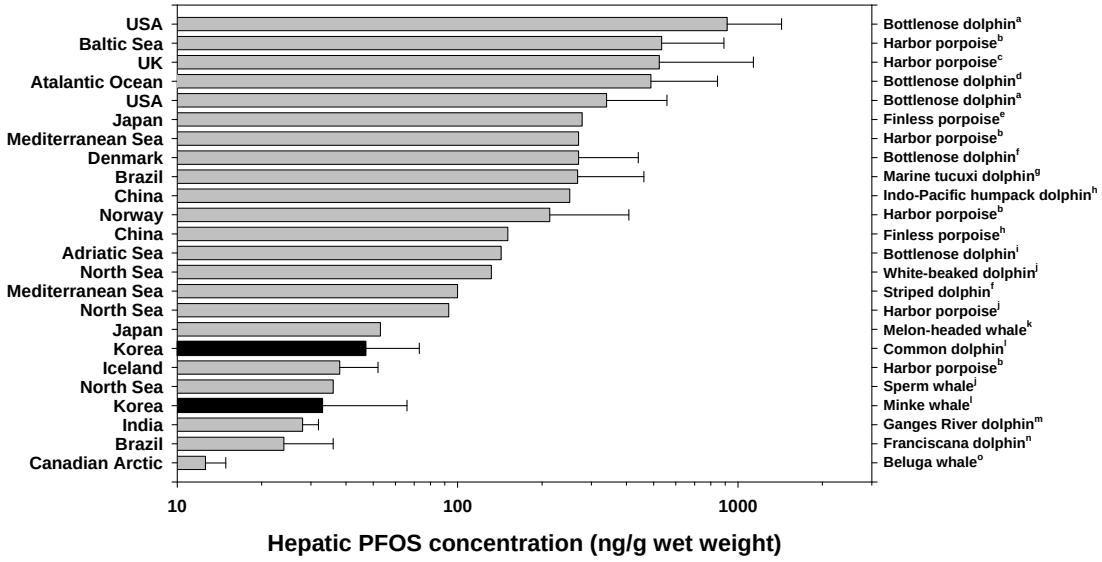
**Table 1**. Concentrations (ng/g wet weight) of perfluorinated compounds in the livers of minke whale and common dolphin from Korean coastal waters

| | Minke whale (*n* = 66) | | | | Common dolphin (*n* = 47) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | Median | Range | Detection frequency (%) | Mean ± SD | Median | Range | Detection frequency (%) |
| Body size (m) | 5.3 ± 1.1 | 5.1 | 3.2–7.7 | | 2.3 ± 0.2 | 2.3 | 2.0–2.5 | |
| PFHS | <1.0 | | <1.0–1.5 | 3 | 0.05 ± 0.26 | | <1.0–1.3 | 4.3 |
| PFOS | 33 ± 33 | 19 | 2.8–162 | 100 | 47 ± 26 | 40 | 18–152 | 100 |
| PFDS | 0.21 ± 1.0 | | <0.5–4.7 | 4.5 | <0.5 | | <0.5 | 0 |
| PFOSA | 2.7 ± 3.1 | 1.2 | <0.5–11 | 70 | 15 ± 9.2 | 17 | 2.2–35 | 100 |
| PFHpA | <1.0 | | <1.0 | 0 | <1.0 | | <1.0 | 0 |
| PFOA | <1.0 | | <1.0–2.7 | 1.5 | 0.58 ± 1.4 | | <1.0–7.9 | 23 |
| PFNA | 2.1 ± 2.4 | 1.8 | <1.0–11 | 60 | 8.6 ± 8.5 | 6.8 | <1.0–45 | 91 |
| PFDA | 8.5 ± 4.1 | 7.8 | <0.5–20 | 98 | 10 ± 6.4 | 8.3 | 2.4–36 | 100 |
| PFUnDA | 47 ± 24 | 43 | 2.6–129 | 100 | 50 ± 32 | 42 | 17–193 | 100 |
| PFDoDA | 8.0 ± 4.2 | 7.8 | <2.0–18 | 94 | 9.2 ± 4.4 | 8.5 | <2.0–27 | 98 |




a: Houde et al., 2006; b: Van de Vijver et al., 2004; c: Law et al., 2008; d: Kannan et al., 2001a; e: Nakata et al., 2006;
f: Giesy and Kannan, 2001; g: Dorneles et al., 2008; h: Yeung et al., 2009a; i: Kannan et al., 2002b; j: Van de Vijver et al., 2003;
k: Hart et al., 2008; **l: Present study**; m: Yeung et al., 2009b; n: Leonel et al., 2008; o: Tomy et al., 2004

**Figure 1**. Comparison of average PFOS concentrations (ng/g wet wt) in the livers of minke whale and common dolphin from Korean coastal waters with those in cetaceans from several other locations. Whiskers on the bars represent the standard deviation of PFOS concentration. The black boxes indicate the PFOS concentrations measured in the livers of cetaceans in the present study.

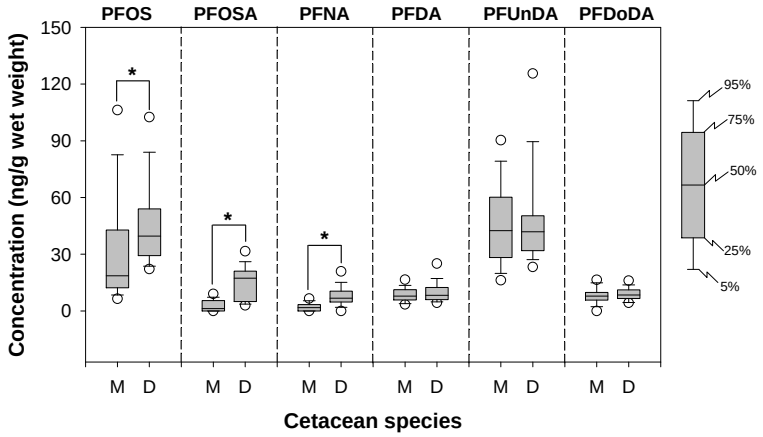



**Figure 2**. Comparison of PFC concentrations in the livers of minke whales and common dolphins collected from Korean coastal waters. M represents minke whale and D represents common dolphin. The symbol (*) indicates the significance at the level of $p < 0.05$ by Student *t*-test between two cetacean species.

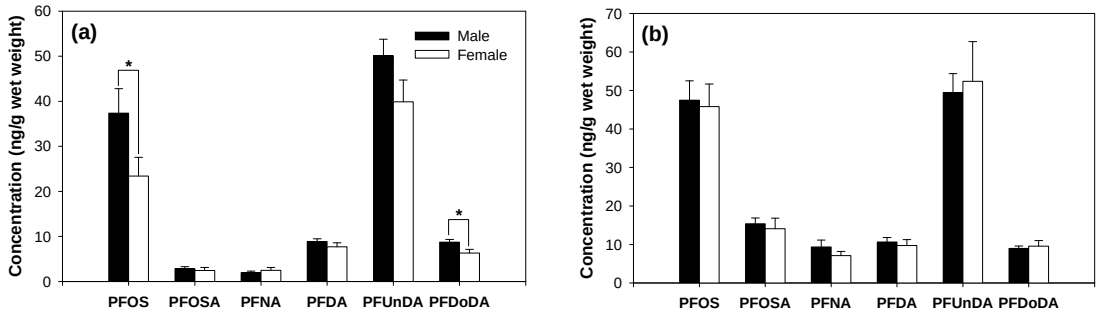



**Figure 3**. Comparison of PFC concentrations in the livers of male and female of minke whale (a) and common dolphin (b) collected from Korean coastal waters. Whiskers on the bars represent the standard error for individual PFC. The symbol (*) indicates the significance at the level of $p < 0.05$ by Student *t*-test between males and females of both cetaceans.

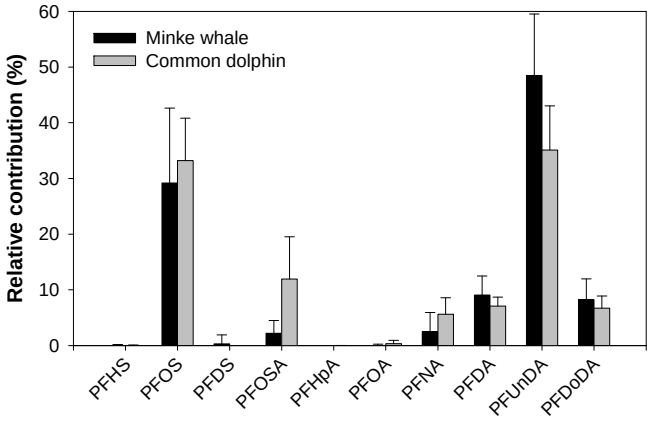



**Figure 4**. Relative contribution of PFCs to total PFC concentrations in the livers of minke whale and common dolphin collected from Korean coastal waters. Whiskers on the bars represent the standard deviation for individual contribution of PFC.