Perfluoroalkyl Acids in the Egg Yolk of Birds from Lake Shihwa, Korea

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Concentrations of perfluoroalkyl acids (PFAs) were measured in egg yolks of three species of birds, the little egret (Egretta garzetta), little ringed plover (Charadrius dubius), and parrot bill (Paradoxornis webbiana), collected in and around Lake Shihwa, Korea, which receives wastewaters from an adjacent industrial complex. Mean concentrations of perfluorooctanesulfonate (PFOS) ranged from 185 to 314 ng/g ww and were similar to those reported for bird eggs from other areas of Korea have been reported on limited extents and are able to biomagnify to top predators such as marine mammals and fish-eating birds (6–9). PFAs, in particular PFOS and perfluorooctanoic acid (PFOA), were once considered to be biologically inert. However, they are relatively bioactive at the cellular level, causing diverse effects including blockage of cell–cell communication (10) and initiation of hepatic peroxisome proliferation (11). These two PFAs have also been shown to cause developmental toxicities in experimental animals including rodents, birds, and amphibians (12–15).

The widespread occurrence of perfluoroalkyl acids (PFAs) in wildlife has spurred monitoring efforts and regulatory concerns regarding these emerging contaminants (1–4). The physicochemical properties of PFAs make them very useful for application in various commercial products such as surface protectors for carpets and leather, active components in fire-fighting foams, and processing aids in the production of fluoropolymers (5). Food web studies and an examination of concentrations in biota suggest that perfluorooctanesulfonate (PFOS) and other PFAs are bioaccumulative to some extent and are able to biomagnify to top predators such as marine mammals and fish-eating birds (6–9). PFAs, in particular PFOS and perfluorooctanoic acid (PFOA), were once considered to be biologically inert. However, they are relatively bioactive at the cellular level, causing diverse effects including blockage of cell–cell communication (10) and initiation of hepatic peroxisome proliferation (11). These two PFAs have also been shown to cause developmental toxicities in experimental animals including rodents, birds, and amphibians (12–15).

Birds from urbanized areas contain greater concentrations of PFAs in their tissues or eggs than those from more remote areas (6, 16–18). Concentrations of PFAs in osprey eggs were also correlated with the concentrations of other persistent contaminants in the environment, suggesting local sources of exposure (19). Furthermore, guillemot eggs collected from 1968 to 2003 showed an increase in PFOS concentrations by 30-fold from 1968 (25 ng/g ww) to 2003 (614 ng/g ww), which corresponds to a greater use of fluorochemicals (17).

Lake Shihwa, located on the west coast of Korea, is an artificial saltwater lake that has received industrial wastewater discharges from bordering Shihwa and Banweol Industrial complexes (SBICs, approximate total industrial area = 31 km²) since the completion of dike construction in 1994 (Figure 1). Investigations of trace metal and persistent organic pollutants, including PCBs, PAHs, organochlorines, and alkylphenols in water and sediment from Lake Shihwa and its neighboring industrial complexes, have suggested a moderate-to-high degree of contamination (20, 21). Concentrations of PFAs of waters of Lake Shihwa and creeks running through the SBICs and lake organisms such as fish and marine invertebrates have recently been reported (22, 23). These earlier studies found relatively great concentrations of PFOS in the water column and certain fishes and invertebrates. Concentrations of PFAs in the livers of birds from other areas of Korea have been reported on limited sample sizes (24). However, no attempt had been made to investigate the concentrations and effects of PFAs in the upper-trophic organisms, such as fish-eating birds in this region.

The aim of this study was to measure concentrations of PFOS and other PFAs in the eggs of birds collected in the vicinity of Lake Shihwa. Egg yolks were selected for this study because the concentrations of contaminants in eggs are often used in exposure and risk assessments (17). Assessments of the potential risks of PFOS and a mixture of PFAs to birds

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in the Lake Shihwa area were made on the basis of the currently available toxicological benchmark doses for birds (25). Regional specific concentrations of PFAs in the water and fish diet of birds were also incorporated to provide multiple lines of evidence for assessing risk. The gap junction intercellular communication (GJIC) cell bioassay was used to obtain relative toxic potencies of individual PFAs to calculate PFOS-equivalent concentrations in egg samples for the risk assessment of PFA mixtures (26).

**Materials and Methods**

**Egg Collection and Sample Extraction.** Eggs of three species of birds were collected from the area in and around Lake Shihwa during the breeding season of May 2006 (Figure 1). One colony of little egrets (*Egretta garzetta*) was found in a section of the city of Ansan, while eggs of the little ringed plover (*Charadrius dubius*) were sampled at various locations, including the islands (Figure 1). Nests of the parrot bill (*Paradoxornis webbiana*) were found in the constructed wetland located upstream of Lake Shihwa. One or two eggs per nest were collected, and a total of 10, nine, and four nests were surveyed for the little egret, little ringed plover, and parrot bill, respectively. The mean shell length (mm) and egg weight (g) of each species are summarized (Table 1).

![Map showing sampling locations in this study. Little egret eggs (star) were collected at one colony located at the hills in the city of Ansan. Little ringed plover eggs (circles) were collected at various sites, including islands and walkways in Lake Shihwa, while parrot bill eggs (squares) were sampled at locations in upstream wetlands of Lake Shihwa.](image)

**TABLE 1. Bird Egg Sample Descriptions**

<table>
<thead>
<tr>
<th>species</th>
<th>no. of nests</th>
<th>no. of eggs</th>
<th>shell length (mm)</th>
<th>egg weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>little egret&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>20</td>
<td>44.3 ± 3.5</td>
<td>27.0 ± 5.8</td>
</tr>
<tr>
<td>little ringed plover&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>17</td>
<td>30.8 ± 1.7</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td>parrot bill&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4</td>
<td>7</td>
<td>13.4 ± 0.7</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> One or two eggs per nest were collected. *Egretta garzetta.* <sup>b</sup>Charadrius dubius. * Paradoxornis webbiana.

Bird egg samples were stored individually in a polypropylene container and kept at −20 °C until instrumental analysis. Egg yolks were extracted with an ion-pairing liquid extraction method described elsewhere (6). Briefly, an aliquot of homogenized egg yolk (1 g ww) was diluted 4-fold (w/v) with Milli-Q water before extraction. A total of 1 mL of a diluted yolk–water mixture was mixed with 1 mL of 0.5 M tetrabutyl ammonium hydrogensulfate solution and 2 mL of 0.25 M sodium carbonate buffer in a 15-mL polypropylene tube. This mixture was shaken for 20 min at 250 rpm with 5 mL of methyl tert-butyl ether. After centrifugation, the organic phase was removed and transferred to a clean 15 mL polypropylene tube. The extraction was repeated twice as described above. The resulting three extracts were combined and concentrated under a gentle stream of nitrogen and reconstituted in 1 mL of methanol. Samples of 5 ng of 1<sup>37</sup>C<sub>7</sub>-PFOS and 1<sup>37</sup>C<sub>6</sub>-PFOA from Wellington Laboratories (>98%, Guelph, ON, Canada) were spiked as internal standards. All solvents were high-performance liquid chromatograph (HPLC)-grade, and all reagents were ACS-grade (J. T. Baker, Phillipsburg, NJ).

**Instrumental Analysis and Quality Assurance/Quality Control.** Target PFAs in extracts were separated using an Agilent 1100 HPLC. An aliquot of 10 μL of the extract was injected onto a 50 × 2 mm (5 μm) Keystone Betasil C18 column, separately for sulfonates and carboxylic acids. A gradient HPLC with a mobile phase consisting of 2 mM ammonium acetate and methanol was used. For sulfonic acids, at a flow rate of 300 μL/min, the amount of methanol was increased from 10% to 100% methanol in 10 min, held at 100% methanol for 5 min, and then was reverted to 10% methanol. For carboxylic acids, methanol was ramped up from 10% to 100% in 7 min, held for 3 min at 100%, and then ramped down to 10%. The HPLC was interfaced with an Applied Biosystems API 2000 tandem mass spectrometer (MS/MS; Foster City, CA). The MS/MS was operated in electrospray negative ionization mode. Analyte ions were monitored in the multiple-reaction monitoring mode. Parent and daughter ion transitions used for identification and quantification were 399 > 80 (perfluorohexane sulfonate, PFHS), 499 > 99 (PFOS), 599 > 99 (perfluorodecanesulfonate, PFDS), and 498 > 78 (perfluorooctane sulfonamide, PFOSA) for sulfonic acids and 413 > 369 (PFOA), 463 > 419 (perfluorononanoic acid, PFNA), 513 > 469 (PFDA), 563 > 519 (PFUnDA), and 613 > 569 (perfluorododecanoic acid, PFDoDA) for carboxylic acids. Two internal standards of 503 > 99 (1<sup>37</sup>C<sub>7</sub>-PFOA) and 417 > 372 (1<sup>37</sup>C<sub>6</sub>-PFOA) were also monitored for analysis recovery of sulfonates and carboxylic acids, respectively. Concentrations of PFAs in extracts were quantified using a matrix-matched calibration curve constructed with a chicken egg from the market. The chicken egg was found not to contain measurable concentrations of target compounds. The coefficient of determination (r<sup>2</sup>) for each constructed curve was greater than 0.99. Acquired data were deemed acceptable if the QC standard measured after...
TABLE 2. Concentrations of Perfluoroalkyl Acids in the Egg Yolks of Birds Collected around Lake Shihwa (ng/g wet wt)

<table>
<thead>
<tr>
<th>species</th>
<th>PFHS</th>
<th>PFOS</th>
<th>PFDS</th>
<th>PFOSA</th>
<th>PFOA</th>
<th>PFNA</th>
<th>PFDOA</th>
<th>PFUnA</th>
<th>PFDoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>little egret (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max</td>
<td>9.5</td>
<td>1205.5</td>
<td>4.7</td>
<td>1.4</td>
<td>4.0</td>
<td>68.6</td>
<td>89.6</td>
<td>260.0</td>
<td>32.2</td>
</tr>
<tr>
<td>min</td>
<td>&lt;LOQ</td>
<td>30.4</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>3.9</td>
<td>14.6</td>
<td>26.0</td>
<td>6.2</td>
</tr>
<tr>
<td>mean</td>
<td>2.3</td>
<td>185.4</td>
<td>0.8</td>
<td>0.7</td>
<td>1.7</td>
<td>25.4</td>
<td>43.6</td>
<td>95.1</td>
<td>19.5</td>
</tr>
<tr>
<td>% detected*</td>
<td>85%</td>
<td>100%</td>
<td>45%</td>
<td>40%</td>
<td>60%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>max</td>
<td>9.5</td>
<td>889.3</td>
<td>5.4</td>
<td>1.1</td>
<td>25.2</td>
<td>106.7</td>
<td>114.9</td>
<td>314.6</td>
<td>47.3</td>
</tr>
<tr>
<td>little ringed plover (n = 17)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>2.3</td>
<td>215.1</td>
<td>1.4</td>
<td>NA</td>
<td>8.4</td>
<td>51.0</td>
<td>52.7</td>
<td>153.9</td>
<td>21.4</td>
</tr>
<tr>
<td>% detected*</td>
<td>82%</td>
<td>100%</td>
<td>76%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
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<td></td>
</tr>
<tr>
<td>max</td>
<td>5.3</td>
<td>761.7</td>
<td>2.2</td>
<td>NA</td>
<td>1.4</td>
<td>97.3</td>
<td>287.7</td>
<td>675.0</td>
<td></td>
</tr>
<tr>
<td>parrot bill (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>1.3</td>
<td>314.1</td>
<td>1.1</td>
<td>NA</td>
<td>0.8</td>
<td>40.0</td>
<td>114.2</td>
<td>201.0</td>
<td>25.6</td>
</tr>
<tr>
<td>% detected*</td>
<td>57%</td>
<td>100%</td>
<td>71%</td>
<td>0%</td>
<td>42%</td>
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<td>100%</td>
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<tr>
<td>LOQ</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

*% detected represents a portion of samples greater than LOQ (=0.8 ng/g). Below LOQ was arbitrarily assigned as a half of LOQ to calculated mean concentrations.

Results and Discussion

Concentrations in Bird Eggs. Mean concentrations of PFHS, PFOS, PFDS, PFOSA, PFOA, PFNA, PFDA, PFUnA, and PFDoA in egg yolks were summarized in Table 2. Great amounts of PFOS were detected in all of the egg yolk samples. PFOS was measurable in 32 of 44 samples, but at concentrations 2 orders of magnitude less than those of PFOS. Of the long-chain PFCAs, PFUnA was the most prevalent acid and second-most abundant PFA behind PFOS in the egg samples. The mean concentration of PFOS in the egg yolk of the little egret, little ringed plover, and parrot bill was 185, 215, and 314 ng/g ww, respectively (Figure 2). The greatest concentration of PFAs in this study was 1206 ng PFOS/g ww, determined in an egret’s egg yolk. In general, concentrations detected in bird eggs from the Lake Shihwa region were similar to those seen in bird eggs and yolks from the Norwegian Arctic, Great Lakes, and Delaware Bay, except for guillemot eggs from the Baltic Sea (561–871 ng PFOS/g ww during 2000–2003 sampling campaigns) (6, 16, 17, 19). In addition to PFOS, short-chain PFHS (35 of 44 eggs) and long-chain PFDS (27 of 44 eggs) were also detected in egg yolk samples, at concentrations as great as 9.5 ng/g and 5.4 ng/g ww, respectively. PFOSA was frequently found in the diet of birds (fish samples) but were only quantifiable in egg yolks of the little egret.

Concentrations of PFCS in egg yolks of the birds analyzed were some of the greatest ever found in wildlife species. In particular, the concentrations of long-chain PFCS were elevated in all three species (Table 2). Among the PFCS measured in this study, PFUnA (C11) was the dominant compound, followed by PFDoA (C12), PFDA (C10), PFNA (C9), and PFOA (C8). Mean concentrations of PFUnA in the little egret, little ringed plover, and parrot bill were 95, 154, and 201 ng/g ww, respectively. The predominance of PFUnA was also observed in osprey eggs from the Delaware River and Bay (19). PFOSA was detected in 60%, 100%, and 43% of the little egret, little ringed plover, and parrot bill eggs, respectively. In the parrot bill, the concentration of PFOA (ANOVA) was conducted with the Bonferroni posthoc criterion. Prior to analysis, values for concentrations of PFOS and total PFCS were log-transformed to attain normality (one-sample Kolmogorov–Smirnov test) and equal variances (Levene’s test). Probable associations among PFCS were tested using a Pearson correlation analysis. A probit analysis was used to derive the EC50 of inhibition of cellular communication (GJIC) using the Excel program. Statistical significance was set at the level of p ≤ 0.05, unless otherwise noted. All statistical analyses other than EC50 determinations were performed with the SYSTAT 11 statistical package (SYSTAT Software Inc., Richmond, CA).

Statistical Analysis. To evaluate the differences in accumulation of PFOS and total PFCS in the egg yolk of the bird species of interest, a one-way analysis of variance (ANOVA) was conducted with the Bonferroni posthoc criterion. Prior to analysis, values for concentrations of PFOS and total PFCS were log-transformed to attain normality (one-sample Kolmogorov–Smirnov test) and equal variances (Levene’s test). Probable associations among PFCS were tested using a Pearson correlation analysis. A probit analysis was used to derive the EC50 of inhibition of cellular communication (GJIC) using the Excel program. Statistical significance was set at the level of p ≤ 0.05, unless otherwise noted. All statistical analyses other than EC50 determinations were performed with the SYSTAT 11 statistical package (SYSTAT Software Inc., Richmond, CA).

Gap Junction Intercellular Communication (GJIC) Cell Bioassay. Rat liver epithelial cells (WB-F344 cells) were used to measure the inhibition potential of cellular communication by individual PFAs. WB-F344 cells were obtained from Drs. J. W. Brisham and M. S. Tsao of the University of North Carolina. The cells were cultured as described previously (10). GJIC was measured using the scrape loading dye transfer technique (27). Briefly, confluent cells were separated and removed from the plate by use of 1× trypsin–EDTA, and the cell solution was harvested. A total of 2 mL of the diluted cell solution was seeded to 35 mm tissue culture plates, and cells were allowed 48 h for attachment before the PFA dosing. Four PFAs (PFHS, PFOS, PFNA, and PFOA) were tested. At day 2, cells were exposed to each PFA dissolved in acetonitrile (0, 3.125, 6.25, 12.5, 25, 50, and 100 ng/mL) for 15 min. Following exposure, cells were rinsed with phosphate-buffered saline (PBS), and 1 mL of 0.05% Lucifer-yellow dye (Sigma, St. Louis, MO) was added to each plate. A surgical steel blade was used to make three scrapes through the monolayer of cells. After 5 min of incubation at room temperature, the dye was discarded, and the cells were rinsed with PBS and then fixed with 0.5 mL of 4% formalin. Dye migration was photographed at 200× using a Nikon epi-fluorescence phase contrast microscope illuminated with an Osram HBO 200W lamp and equipped with a COHU video camera. The area of dye migration from the scrape indicates the ability of cells to communicate with each other through the gap junction (10). The migrated area was calculated using the Gel Expert program (Nucleotech, San Mateo, CA). Each PFA concentration was tested in triplicate.

Statistical Analysis. To evaluate the differences in accumulation of PFOS and total PFCS in the egg yolk of the bird species of interest, a one-way analysis of variance (ANOVA) was conducted with the Bonferroni posthoc criterion. Prior to analysis, values for concentrations of PFOS and total PFCS were log-transformed to attain normality (one-sample Kolmogorov–Smirnov test) and equal variances (Levene’s test). Probable associations among PFCS were tested using a Pearson correlation analysis. A probit analysis was used to derive the EC50 of inhibition of cellular communication (GJIC) using the Excel program. Statistical significance was set at the level of p ≤ 0.05, unless otherwise noted. All statistical analyses other than EC50 determinations were performed with the SYSTAT 11 statistical package (SYSTAT Software Inc., Richmond, CA).
was as great as 54.3 ng/g ww. In other studies, PFOA was not detectable in the eggs of guillemot and glaucous gulls from northern Europe (16, 17). Concentrations of PFAs in eggs from our study were 10-fold greater than those in glaucous gulls from the Norwegian Arctic (total PFAs was 42 ng/g ww with the dominance of PFUnA) (16).

Mean concentrations of PFOS in birds were inversely proportional to the length of eggs. The smallest parrot bill egg contained the greatest concentration, while the largest little egret egg contained the least concentration of PFOS in their respective yolks. However, this difference was not statistically significant (Figure 2; \( p > 0.05 \)). The occurrence of great mean concentrations of PFOS and total PFCA in the parrot bill is interesting because these eggs were sampled at the constructed wetland away from industrial complexes, whereas little ringed plover eggs were collected on islands and walkways in Lake Shihwa with a known PFA contamination. This constructed wetland was primarily designed to reduce the concentrations of suspended solids and eutrophic nutrients in streams from the residential areas before entering into Lake Shihwa. Thus, there could be a possibility of PFA contamination in these naturally treated municipal wastewater not yet investigated. The foraging ranges of the investigated birds have not been studied in the study area. Thus, further investigation of water contamination in this constructed wetland may help to explain unexpectedly great concentrations of PFAs in the egg yolks of the parrot bill.

**Contamination Sources to Birds and Relationship among PFAs.** The little egret and little ringed plover prey on aquatic organisms from Lake Shihwa (surface area = 56.5 km², drainage basin = 476.5 km²) and its watershed. The exposure concentrations in these two species of birds are presumably an indication of local PFA exposures. The existence of local sources of PFAs in Lake Shihwa has been suggested previously (22). Concentrations of PFA in the water were elevated in drains and streams receiving effluents from the nearby Shihwa Industrial Complex, and they gradually decreased in Lake Shihwa with distance from the industrial complex and were even less in the near-shore regions of Gyeonggi Bay. Concentrations of PFOS and total PFCA in fish and blue crabs also decreased as a function of distance from wastewater discharges to the water exchange gate in Lake Shihwa (23). In 2002, Korea had imported about 120 pounds used in the SBICs or their emissions from these areas, such as the Arctic, only a minor fraction of PFNA was accounted for 20% of the total concentration of PFAs. In contrast, PFNA, which is often a dominant PFCA in arctic marine mammals, accounted for only 10–20% of the total PFCA concentration. However, for birds from the remote areas, such as the Arctic, only a minor fraction of PFNA was also measured in eggs, similar to our eggs from urban areas (16). Considering the differences in habitat location, body size, and diets among birds surveyed, the similarity of composition of PFAs in eggs suggests that all three species have been exposed to a common source of PFAs. To properly answer this question, the study area needs to be revisited for dietary study and water sampling in the constructed wetland.

There was a significant positive correlation between concentrations of PFUnA and PFDA \( (r^2 = 0.54; p < 0.05) \) in egg yolks, but it was weak between PFNA and PFOA \( (r^2 = 0.17) \) (Figure SI-1 in the Supporting Information), suggesting that the birds studied share a common exposure source of PFCA, such as diet or ambient air. For example, a similar strong correlation between PFUnA and PFDA in fish collected from Lake Shihwa was also noted (Table S1 in the Supporting Information). The inhalation of precursors of PFCA could be another possible exposure route. There is evidence that...
fluorotelomer alcohols (FTOHs) could generate PFCA via atmospheric oxidation, aqueous photolysis, and biodegradation (28, 29). Studies have shown that FTOH could degrade into PFNA and PFOA, with an even number of PFCA being a major product. The degradation of FTOH has not been tested, but presumably its degradation pathways may lead to PFNA and PFOA. Unfortunately, air concentrations of semivolatile fluorinated organic compounds (SVFOCs) have not been surveyed in Korea. In a recent study in Okinawa, Japan, various SVFOCs were detected in air samples, including FTOHs and fluorotelomer olefin. Interestingly, FTOH had the greatest average concentration in the gas phase (30). More studies need to evaluate the relative contribution of the overall bioaccumulation of PFCA on birds from the inhalation of SVFOC-contaminated air.

In most samples of egg yolk, concentrations of PFCA with an odd number of carbons were greater than the adjacent even number of carbons. For example, the slopes of correlation were 1.7 for PFUnA and PFOA, and 2.1 for PFNA and PFOA, though the latter was not a significant relation in this study. This observation may be explained by differences in bioaccumulation potential among the PFCA. A laboratory-controlled dietary exposure experiment indicated that the increase of bioaccumulation potential among the PFCA increased with the chain length of the PFCA. Fish sampled in Lake Shihwa also had greater concentrations of PFUnA in the liver (mean, 24.9; range, 19.3–30.5 ng/g ww) than that of PFOA (mean, 11.9; range 7.3–15.0 ng/g ww) (Table SI-1 in the Supporting Information). Therefore, the accumulation pattern of PFCA in birds from Lake Shihwa is influenced by PFCA concentrations in prey items and the bioaccumulation potential of the individual PFCA.

PFOS-Equivalent Concentration. Each PFA tested elicited a concentration-dependent inhibition of cellular communication on rat liver epithelial cells (Figure 4). PFOS (EC50 = 12.5 µg/mL) was the most potent among the PFAs investigated, and the potency decreased in the order of PFUnA (16.6 µg/mL), PFOA (22.3 µg/mL), and PFDA (26.3 µg/mL). Previous studies have reported an EC50 for PFOS of 15 µg/mL (27). This observation demonstrates both the robust nature of the assay and the consistency of the results. A relative potency (RP) value for each PFA was obtained by normalizing the EC50 concentrations to PFOS EC50 (RPFA = EC50PFOS/EC50PFA), which showed RP values of 0.75, 0.56, and 0.47 for PFUnA, PFOA, and PFDA, respectively. A PFOS-equivalent concentration (PFOS-EQ) was calculated for each mixture of PFAs by multiplying RPFA by the corresponding concentration in egg samples and summing all PFOS-EQs (26). It should be noted that the present relative toxic potencies were derived from a mammalian cell bioassay, since methods for avian cells are not currently developed. It is conventional to use rat/laboratory animal data for risk assessment, in the absence of data from the species of interest. However, this cell bioassay allows the comparison of toxicities of individual PFAs. Furthermore, the relative potency of PFAs to alter GJIC is correlated with other biological end points (31, 32). Therefore, the results of this epigenetic test can be considered as being predictive of the potential toxicity of PFAs in the mixture.

Risk Characterization of PFOS and a mixture of PFAs. Ecological risks of PFOS and a mixture of PFAs to birds in the Lake Shihwa region were evaluated by using two approaches. First, concentrations of PFOS or the PFOS-EQs in eggs were compared with toxicological benchmarks that represent thresholds below which adverse effects on birds would not be expected. These benchmark values for avian species were determined using the most ecologically relevant end points with uncertainty factors assigned so that they are protective (33). Second, an average daily intake (ADI) value was determined for PFOS or a mixture of PFAs on the basis of the concentrations of PFAs in the diets of the birds. The ADI benchmark dose was then compared with the calculated dietary dose. To estimate the risk associated with the protection of 90% of the birds in a population inhabiting the region surrounding Lake Shihwa, a cumulative probability function was developed for PFOS concentrations in the egg yolks of the three species examined (Figure SI-2 in the Supporting Information).

Hazard quotients (HQ = sample concentration/benchmark dose) were calculated to provide preliminary estimates of risks associated with PFOS concentrations in birds. Two toxicological benchmarks for PFOS in the liver, serum, and egg yolk were reported from dietary exposure studies with mallards and bobwhite quail (25). The lowest observable adverse effect level (LOAEL) and toxicity reference value (TRV) for the birds were determined to be 62 000 ng PFOS/mL and 1700 ng PFOS/mL in egg yolk, respectively. In the present study, the 90th centile of PFOS concentrations in the yolks of bird eggs from the Lake Shihwa area was 481 ng/g ww, and the corresponding HQ was 0.008 on the basis of LOAEL and 0.283 on the basis of TRV. As discussed earlier, concentrations of PFCA measured in eggs from the Lake Shihwa area were greater than those in most other areas. Therefore, PFOS-EQs, based on relative potencies in the GJIC assay, were calculated to assess the risk of the PFA mixture in egg yolks. In this estimation, a slightly greater 90th centile (544 ng PFOS-EQ/g ww) resulted in greater HQs (LOAEL = 0.009 and TRV = 0.320), but none exceeded a value of 1.0.

Risk from dietary exposure to PFOS was evaluated for the population of little egrets. Little egrets feed on various aquatic organisms, but during egg sampling, only mullet carcasses were observed around the little egret colony. Thus, in this diet-based assessment, mullet caught in the shallow waters of Lake Shihwa were assumed to be the sole diet of the little egret. Toxicological doses for PFOS for a generic avian trophic level IV predator were used as the benchmark for these calculations (LOAEL = 0.77 mg PFOS/kg/d and TRV = 0.022 mg PFOS/kg/d) (25). The amount of food ingested (FI) per day relative to the body weight for wading birds was calculated for the little egret (500–5000 g ww; FI = 0.966 log (BW) − 0.64, BW = body weight of the bird) (34). Mean concentrations of PFOS in the carcasses of mullet were 39.1 ± 6.6 ng/g ww (Table SI-1 in the Supporting Information). The ADI determined for the little egret was 0.007 mg PFOS/kg/d. On the basis of this ADI value, HQs (=ADI/benchmark dose) were calculated to range from 0.008 to 0.009 on the basis of LOAEL and 0.305–0.330 on the basis of TRV. However, the HQs calculated for the PFA mixture here should be interpreted with caution, because the RP values are based
on a GJIC bioassay performed with rat cells, and no safety/uncertainty factors have been applied in calculating the HQs.

The estimated risks of PFAs in avian species based on the residue concentration in egg yolks (upper 10% of bird population) and based on dietary exposure approaches were quite similar. Although some assumptions have been made with limited toxicological data and other exposure routes such as inhalation were not considered, the comparable HQs derived allow us to evaluate the risks associated with current exposures from PFOS and PFA mixtures in Lake Shihwa at the screening level. Approximately a 100-fold difference between the PFA exposure levels and the threshold values suggest that immediate threats such as reproductive failure are unlikely to occur due to PFAs in the birds in the Lake Shihwa region. In addition, considering the conservative nature of TRV values (33), current concentrations of PFAs and the mixture of PFAs would not be expected to pose adverse effects to the avian population residing around Lake Shihwa. Nevertheless, further monitoring studies on the health of bird populations and sources of PFAs in this region are warranted as new data pertaining to toxicities of PFAs become available.

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Supporting Information Available
Details about (1) PFA concentrations in fish used as a diet of the birds of interest, (2) correlations between PFCAs in the white leghorn chicken (gallus domesticus) embryo. Environ. Toxicol. Chem. 2006, 25, 227–232.

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