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RISK CHARACTERIZATION OF PERFLUOROALKYL ACIDS EXPOSURE OF AQUATIC ORGANISMS IN LAKE SHIHWA, KOREA

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Hoon Yoo

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John P Giesy
Major Professor's Signature

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RISK CHARACTERIZATION OF PERFLUOROALKYL ACIDS EXPOSURE OF AQUATIC ORGANISMS IN LAKE SHIWHA, KOREA

By

Hoon Yoo

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ABSTRACT

RISK CHARACTERIZATION OF PERFLUOROALKYL ACIDS EXPOSURE OF AQUATIC ORGANISMS IN LAKE SHIWHA, KOREA

By

Hoon Yoo

The Shihwa-Banweol Industrial Complexes (SBIC), located on the west coast of Korea, is one of the main national industrial complexes with a wide range of industries currently operating. Recently, significant quantities of perfluoroalkyl acids (PFAs) were observed in the waters of Lake Shihwa receiving wastewaters from SBIC. Thus, it was deemed timely to determine concentrations of PFAs in aquatic animals of Lake Shihwa and assess the potential risks that these compounds might pose to aquatic wildlife in Lake Shihwa

Aquatic samples (fish, blue crab, mussel, and oyster) and bird eggs were collected in May to June 2006 from the Lake Shihwa area. All biotic samples contained measurable concentrations of significant level of PFAs in their tissues. PFOS was the predominant PFAs in fish species (mullet, rockfish, and shad), followed by the longer chain perfluorocarboxylic acids (PFCAs), PFUnA > PFDA > PFDoA > PFNA > PFOA. In the egg yolks of birds (little egret, little ringed plover, and parrot bill), measured concentrations of PFOS were similar to birds eggs from other urban areas, but greater than those from remote regions.

The spatial distribution of PFOS concentrations in marine organisms demonstrates that biota samples from sites close to the outlets of inland creeks were more contaminated than those sampled at sites away from the release sources. This observation is consistent to the distribution of water-borne PFOS in Lake Shihwa. It

could be said that wastewaters from SBICs are at least one identified source of PFAs into Lake Shihwa, consequentially contributing the elevated PFAs concentrations in the marine wildlife.

In pharmacokinetic study, PFOS has a half-life of almost four months in male chickens; in contrast, greater than half of introduced PFOA was eliminated within a week. Thus, combined with a greater PFOS and a lesser PFOA in birds from the Lake Shihwa area, at least current PFOA concentrations are unlikely to cause acute effects to birds.

For the hazard assessment of fish, PFOS body residues were compared to a benchmark tissue concentration that would not be expected to cause acute effects in fish. The calculated hazard quotients (HQs) were less than 1.0 for all species. Even a HQ estimated from the greatest PFOS in fish was only to be 6×10^{-4}. Thus, at least current concentrations of PFOS in fish living in Lake Shihwa are not likely to cause acute lethality.

Multiple lines of evidence were used to assess the PFAs associated-risks on birds, which are one of the top-predators in the food web of the Lake Shihwa region. From a bottom-up approach using fish as a sole diet for birds, a range of HQ (8.0×10^{-3} - 9.0×10^{-3}) generated was hundred-folds less than the least observable adverse effect level (LOAEL) for PFOS. Similarly, the calculated HQs based on residue concentrations in egg yolk were 8.0×10^{-3} for PFOS only and 9.0×10^{-3} for a mixture of PFAs, respectively, when the LOAEL used as a benchmark dose. Although there are many uncertainties in deriving these risk values, similar risk estimates from two opposite but complimentary approaches indicate that current concentrations of PFOS and a mixture of PFAs would not be expected to pose adverse effects to the avian population around the Lake Shihwa area.

To my parents, Hanoh Yoo and Even Shin, and my brother, Hyun Yoo, and my wife, Boram Yoo, for their love and support.

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LIST OF ABBREVIATIONS

PFA: Perfluoroalkyl acid

SBIC: Shihwa-Banweol Industrial Complex

PFOS: Perfluorooctanesulfonic acid

PFHS: Perfluorohexanesulfonic acid

PFAS: Perfluoroalkyl sulfonates

FOSA: Perfluoroalkyl sulfonamide

PFCA: Perfluorocarboxylic acid

PFDoA: Perfluorododecanoic acid

PFUnA: Perfluoroundecanoic acid

PFDA: Perfluorodecanoic acid

PFNA: Perfluorononanoic acid

PFOA: Perfluorooctanoic acid

PFO: Perfluorooctanoate

PFHA: Perfluorohexanoic acid

FTOH: Fluorotelomer alcohol

BCF: Bioconcentration factor

HQ: Hazard quotient

TRV: Toxicity reference value

GJIC: Gap junction intercellular communication

LOAEL: Lowest observable adverse effect level

Chapter 1

Introduction: Perfluoroalkyl acids

PFAs in the Environment

PFAs as emerging contaminants

Perfluoroalkyl acids (PFAs) are fully fluorinated synthetic compounds that have been used for a wide range of industrial applications and in the manufacture of commercial products since their introduction in the late 1940's. PFAs are used in the production of refrigerants, surfactants, polymers, pharmaceuticals, wetting agents, pesticides, and fire-fighting formulations (Kissa, 2001). The popularity of PFAs in industrial areas comes from their favorable physico-chemical properties, such as strong chemical/biological/thermal resistance, special surface-activity, and both lipid- and water-repellency. However, the scientific community became widely aware that there could be potential environmental problems with PFAs when one of the major manufactures, 3M, announced the voluntary phase-out of perfluorooctanesulfonyl fluoride-based products from the market in 2000 (Renner, 2001). At that time, there was limited information on the toxicity of the PFAs and due to a lack of standards and methods, even less information on the concentrations of these compounds in the environment. Since then, more toxicological tests have been conducted and revealed that PFAs, which were once considered to be biologically inert, are bioactive at the cellular level and are toxic to laboratory animals. Increasing numbers of peer-reviewed papers have pinpointed the potential environmental hazards posed by PFAs. Drinking water contamination near DuPont's Washington Works facility in West Virginia made the

public aware of these emerging contaminants (Hogue, 2005). Among the family of PFAs and related compounds, two eight-carbon PFAs, perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), have received much of attention from environmental scientists, toxicologists, and legislators than other PFAs. These two compounds are considered to be the ultimate degradation products of PFAs and related compounds, and exposure levels are great in the environment (Giesy and Kannan, 2002; OECD, 2002; US EPA, 2003). PFOS was the primary active ingredient in 3M Scotchgard® stain repellent, and PFOA is used in DuPont's Teflon® products.

PFAs are ubiquitous global contaminants that are considered to be PBT (persistent, bioaccumulative, and toxic) contaminants, a category which also includes compounds such as DDTs, PCBs, and dioxins. Their presence has been confirmed in the serum or blood of the general public from various countries, different ethnicities, and all ages (Kannan et al., 2004). They have also been found in wildlife in remote regions of the globe such as Arctic marine mammals (Giesy and Kannan, 2001; Bossi *et al.*, 2005). Food web studies and laboratory experiments have shown that PFAs are bioaccumulative to some degree and able to biomagnify in fish-eating top predators, such as mink, eagles, and polar bears (Martine *et al.*, 2003a & 2003b; Giesy *et al.*, 2006). Generally, monitoring has shown that PFA levels in abiotic matrices, wildlife, and humans are higher in urban and industrialized areas than in rural and less industrialized areas. This observation implies that, although they are globally ubiquitous, releases of PFAs into the environment are closely related to human activities. As a consequence, legal measures and management programs have been initiated and developed in order to reduce the

environmental release of PFAs (See the section for Regulation of PFAs). Replacements for PFOS- or PFOA-based chemicals are already on the market.

At present, it is quite challenging to analyze the risks of PFA exposure to humans and wildlife. First of all, now there are vast numbers of known PFAs and unidentified fluorinated chemicals used in the world (Kissa, 2001). Analytical chemists are still trying to develop new technology such as efficient and reliable analytical methods and instruments (Martin *et al*, 2004a). Although sufficient toxicological data are available for mammals, toxicologists have not yet pinpointed the mechanisms underlying the toxic responses. Data generated prior to development of proper analytical techniques, are somewhat unreliable (Susan *et al.*, 2006). Most toxicological studies have investigated short-term exposure scenarios and may not be reliable for predicting long-term effects. Distinct pharmacokinetic characteristics between humans and other mammals also prevent risk assessors from extrapolating animal-based outcomes. The contamination sources and environmental fates of PFAs are not fully identified with few proposed hypotheses, which make it difficult to properly manage exposures to living organisms.

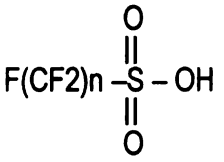
Physical/chemical properties

In PFAs or poly-fluorinated chemicals, a number of fluorine atoms are substituted for hydrogen atoms along their carbon backbone. This replacement with strongly electronegative fluorine brings unique chemical properties to PFAs, such as lowering pK_a. The overlapping of the 2s and 2p orbitals of fluorine with the corresponding orbitals of carbon results in effective shielding of carbon chains, and contributes to the rigidity of perfluorocarbon chains (Key, 1997; Kissa, 2001). Generally, the structure of PFAs

consists of a non-polar perfluoroalkyl tail, and an ionic or neutral functional group (Figure 1).

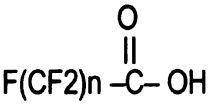
The tail of PFAs is characterized by both oleophobic (oil-repelling) and hydrophobic (water-repelling) properties, which are in contrast to the hydrophobic hydrocarbon chain. Commonly, PFAs with sulfonic and carboxylic group are called perfluoroalkyl sulfonates (PFASs) and perfluoroalkyl carboxylates (PFCAs), respectively. PFAs with charged moieties are better surfactants than hydrocarbon-based surfactants (Giesy and Kannan, 2002). PFAs can lessen the surface tension of aqueous solutions and lower critical micelle concentrations more than hydrocarbon-based surfactants. In addition, these highly polarized, high-energy C-F bonds are thermodynamically stable; thus they are largely resistant to metabolism, microbial degradation, hydrolysis, and photolysis (OECD, 2002). Strong chemical stability to acids, alkalis, and oxidizing agents also makes them useful in operating conditions where hydrocarbon-based surfactants cannot be used, such as in metal plating or in fire extinguishers (UNEP, 2006). Plausible precursor molecules of PFASs and PFCAs are also gaining some interests, including perfluoroalkyl sulfonamides (FOSAs) and fluorotelomer alcohols (FTOHs).

PFASs
(Perfluoroalkyl sulfonates)



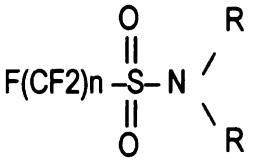
PFDS (Perfluorodecanesulfonate)
PFOS (Perfluorooctanesulfonate)
PFHxS (Perfluorohexanesulfonate)

PFCAs
(Perfluoroalkyl carboxylates)



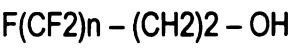
PFDoDA (Perfluorododecanoic acid)
PFUnDA (Perfluoroundecanoic acid)
PFDA (Perfluorodecanoic acid)
PFNA (Perfluorononanoic acid)
PFOA (Perfluorooctanoic acid)

FOSAs
(Perfluoroalkyl sulfonamides)



PFOSA (Perfluorooctanesulfonamide)
N-EtFOSA (N-ethyl FOSA)
N-MeFOSA (N-methyl FOSA)
N-EtFOSEA
N-MeFOSEA
N-EtFOSE alcohol
N-MeFOSE alcohol

FTOHs
(Fluorotelomer alcohols)



6:2 FTOH
8:2 FTOH
10:2 FTOH

Figure 1. Structure of perfluorinated acids (PFAs) of current interests.

The physico-chemical properties and persistence parameters of PFOA and PFOS are summarized in Table 1&2. However, these estimates should be used with caution, since they were not measured under well-controlled experimental conditions, and many essential data are still lacking. PFOS is extremely persistent. PFOS does not hydrolyze, photolyze or biodegrade in any environmental condition tested (OECD, 2002). It is not expected to volatilize based on an air/water partitioning coefficient of $<2\times10^{-6}$ Pa m^3/mol and it was classified as a type 2, nonvolatile chemical by OECD. PFOS is moderately water soluble (680 mg/L in pure water).

Table 1. Physical/chemical properties of PFOS and PFOA [a]

Parameter	PFOS (Potassium salt)	PFOA
Melting point	≥400 °C	45-50 °C
Boiling point	Not calculable	189-192 °C at 736mm Hg
Specific gravity [b]	~ 0.6 (7-8)	-
Vapor pressure	3.31×10^{-4}Pa at 20 °C	1.33×10^{-5} Pa at 25 °C (PFOA ammonium salt) 1.33×10^{4} Pa at 25 °C (PFOA)
Water solubility		
Pure water	680mg/L at 20°C	3.4g/L
Fresh water	370mg/L	
Sea water	12.4mg/L	
Octanol solubility	56mg/L	
Log K_{ow} [d]	-1.08	
Henry's law constant [d] (atm.m^3/mol)	4.34×10^{-7}	

[a] Data were collected from OECD (2000) and Giesy et al. (2006)
[a] pH values in parentheses
[b] Log Kow calculated with solubility of PFOS in water and n-octanol
[c] Henry's law constant calculated at 20 with the solubility for pure water

Table 2. Persistence parameters available for PFOS and PFOA

PFA	Media	Study	Degradation half-life	Reference
PFOS	Water	Hydrolysis	≥ 41yr at 25°C [a]	3M Company 2001b
	Activated-sludge	Biodegradation	No loss after 20 wk	Gledhill&Markely,2000
		Photolysis	≥ 3.7yr at 25°C [b]	3M Company 2001a
PFOA	Water	Photolysis	>349 days	Hatfield, 2001
	Water	Photolysis	No loss after 35 days	Oakes et al., 2004
		Hydrolysis		
		Biodegradation	~235 days	
	Water	Hydrolysis	~90.1 days	US EPA, 2002
		OH Reaction		Hurley et al., 2004

[a] This estimate was influenced by the analytical limit of quantification and that no loss of PFOS was detected in the study.
[b] No evidence of direct or indirect photolysis of PFOS in the study. The indirect photolytic half-life was estimated using an iron oxide photoinitiator matrix model

However, the solubility of PFOS decreases with increasing dissolved solids as a result of a salting-out effect. Available data indicate PFOA also does not significantly photolyze, hydrolyze, or undergo abiotic or biotic degradation under environmental conditions. The solubility of PFOA is reported to be as much as 3.4 g/L. PFOA as the free acid is capable of escaping from water to air; however, this is unlikely to be a significant process due to the strong acidity of PFOA with a pKa of ~2.5, thereby the virtually non-volatile conjugate base (PFO, $C_8F_{15}O_2^-$) of PFOA acid ($C_8HF_{15}O_2^-$) is dominant at environmental conditions (Prevedouros et al., 2006). Accurate prediction of the environmental fate and transport of PFAs is very difficult due to the lack of accurate physico-chemical parameters for most PFAs. For example, the octanol-water partitioning coefficient (K_{ow})

is commonly used to describe the bioavailability of organochlorine contaminants (e.g., PCBs, dioxins, and organochlorine pesticides). In biotic samples, however, PFAs are often accumulated in blood and organs related to enterohepatic circulation rather than in lipids. This behavior is attributed to the lipophobic and hydrophobic properties of PFAs, which form three immiscible layers when they are experimentally added to octanol (a lipid surrogate) and water. Thus, the utility of estimated K_{ow} values for PFAs is questionable.

Sources to the environment

There are both direct and indirect sources of PFAs emission to the environment. Direct sources include the manufacture and application of PFAs, while indirect sources result from the liberation of impurities in finished goods containing PFAs or their precursors which can be degraded to the final metabolites, PFOS and PFOA (UNEP, 2006; Prevedouros *et al.*, 2006).

Direct sources – During manufacturing processes, PFAs may be discharged in sewage effluent and the volatile precursors of PFSAs and PFCAs may be released to the atmosphere. Currently, these manufacturing processes are thought to be a major source of PFAs to the local environment. For example, the estimated emissions of PFCAs via direct sources accounted for almost ~80% (3.2×10^3-6.9×10^3 tones) of total global emissions, and fluoropolymer manufacturing facilities were the single largest known source (2.4×10^3-5.4×10^3 tones) of PFCA emissions (Prevedouros *et al.*, 2006). As a consequence, effluents from fluorochemical manufacturing facilities have elevated concentrations of perfluoroalkylated substances. Concentrations of PFOS and PFOA

measured downstream of 3M's fluorochemical manufacturing facility in Decatur, AL, were as great as 0.11 μg/L and 0.39 μg/L, respectively (Hansen *et al.*, 2002), while these PFAs were not detected upstream of the facility. A variety of different uses of PFAs are reported in the surface treatment, paper protection, and performance chemicals. The PFOS concentration in effluents collected from representative industry in Australia ranged from 0 to 2.5 μg/L (2.5 μg/L for leather, 0.12 μg/L for metal, 0.14-1.2 μg/L for paper, 1.2 μg/L for photographic, not found in textiles or electronics) (Hohenblum *et al.*, 2003). PFAs are present at elevated concentrations, not only in the industrial wastewaters, but also in public wastewater. In a multi-city study by the 3M Company, PFA concentrations in publicly-owned treatment works effluents ranged of $0.05\text{-}4.98\times10^2$ μg/L for PFOS and 0.04-2.28 μg/L for PFOA (3M, 2001). The occurrence of PFAs in corresponding sludge samples was also observed in that study. Application of commercial products containing PFAs, such as fire-fighting foams, is another source to the environment. Aqueous film-forming foams (AFFF), which are used to extinguish liquid-fuel fires, contain PFAs as a key ingredient. As a result of historic fire-fighting training exercises at Air-Force Bases, elevated levels of PFOS (8 to 1.10×10^2 μg/L) and PFOA (not detected to 1.05×10^2 μg/L) have often been detected in groundwater in the US close to fire-training areas (Moody *et al.*, 2003).

Indirect sources – Liberation of PFSA or PFCA residual impurities in products as well as precursor degradation are assumed to increase the levels of PFOS and PFOA in the environment, although these processes are of lesser importance than direct sources. For example, fluorotelomer-based products may contain trace levels of PFCAs (<1-100 ppm) as unintended reaction byproducts (Prevedouros *et al.*, 2006). Significant amount

of precursor molecules unbound to fluorinated materials have been measured in commercially available carpet and rug protectors (Joyce *et al.*, 2006). For example, air samples from Griffin, GA, USA had the greatest airborne FOSAs and FTOHs concentrations, perhaps, because Griffin is located in the midst of western Georgia and eastern Alabama, a major carpet manufacturing and treatment zone. Indoor air concentrations of FOSAs were between 10 and 20 fold greater than outdoor concentrations in Canadian houses (Shoeib *et al.*, 2005). Laboratory experiments have suggested that these precursor molecules can be broken down into PFOS and PFOA via biotic and abiotic pathways (See the section for Environmental transport). Both FOSAs and FTOHs, which carry a PFOS and a PFOA moiety, respectively, are widely distributed throughout the North American troposphere (Stock *et al.*, 2004).

Environmental transport

The ubiquitous occurrence of the least volatile PFAs at often elevated levels in Arctic mammals has been an interesting issue, in that PFOS and PFO (perfluorooctanoate, the dominant form of PFOA at environmentally relevant pH) have a relatively low tendency to partition to the atmosphere and undergo long-range transport (Giesy and Kannan, 2002). Two transport mechanisms have been proposed to explain this unexpected observation. Atmospheric chemists hypothesized that more volatile precursors can be transported to the Arctic region by air currents and subsequently degraded to form PFOS and PFCAs via atmospheric reactions or metabolism (Ellis *et al.*, 2004; Wallington *et al.*, 2006). For example, the vapor pressures of PFOS precursors (ca 0.5 Pa for N-EtFOSEA and N-MeFOSEA) are almost 1000 folds greater than PFOS (Giesy and Kannan, 2002).

The relatively great solubility of PFAs in water, in particular of PFCAs, supports an aquatic transport route to the Arctic. The development of more accurate analytical techniques to analyze trace levels of PFAs allowed the determination of PFAs in open oceans, indicating the oceanic movement of certain PFAs to remote regions via major currents. Seawater from the Atlantic and Pacific Ocean were contaminated with trace but measurable amounts of PFAs (pg/L) such as PFOS, PFHS, PFOA, and PFOSA (Yamashita *et al.*, 2004). The quantity of PFO transported through the ocean currents flowing to the Arctic was estimated to be 2-12 tones per year; this route contributes a greater amount of PFAs to the Arctic than the estimated atmospheric transport route (0.1-1 ton/year) in the northern hemisphere from FTOH degradation and subsequent atmospheric deposition (Prevedouros *et al.*, 2006).

Several studies have supported the widespread global presence of the least volatile PFAs using their semi-volatile precursors. The non-fluorinated parts of precursor compounds, such as FOSAs and FTOHs can be degraded into PFOS and PFCAs via atmospheric reactions, metabolism, and microbial activity (Ellis *et al.*, 2004; Tomy *et al.*, 2004; Dinglasan *et al.*, 2004). Of those mechanisms, atmospheric dispersion and degradation of FTOHs is thought to be a pathway for the global dissemination of PFCAs. In a smog chamber experiment, chlorine atom-initiated oxidation of FTOHs leads to the formation of small, but significant yields of PFCAs (Ellis *et al.*, 2004). In their experiment, the addition of 8:2 FTOH generated almost 4% of total PFCAs in the absence of NOx in the air. Hydrogen atom abstraction by OH radicals from the $-CH_2-$ group is suspected to be an initial reaction of FTOHs oxidation. Laboratory incubation tests showed that *N*-ethyl perfluorooctane sulfonamide (*N*-EtFOSA or a Sulfluramid®,

which is used as an insecticide) was biotransformed by rainbow trout (*Onchorhynchus mykiss*) into PFOS over time (Tomy *et al.*, 2004). In metabolic products of FTOHs in rat hepatocytes, PFOA and minor amounts of PFNA were confirmed. Similarly, in a mixed microbial system, FTOHs biodegradation yielded PFOA and telomer acids (Dinglasan *et al.*, 2004). The authors hypothesized that the main mechanism was the oxidation of the 8:2 FTOH to the telomer acid via the transient telomer aldehyde followed by the ultimate formation of the stable PFOA via a β-oxidation of the telomer acid.

The well-established equilibrium partitioning (Eq-P) theory predicts the phase-partitioning behavior of nonpolar organic contaminants among water, biota, and sediments in the aquatic environment (Di Toro *et al.*, 1991). Hydrophobic interactions and K_{ow} are a key theoretical basis of this concept, which has been employed to set environmental criteria for non-polar chemicals (e.g., PAHs and chlorinated organic chemicals). However, the perfluorinated tail of PFAs is not only hydrophobic, but also oleophobic (Kissa, 2001). Thus, Eq-P theory is inadequate to describe the environmental fate of PFAs (Giesy *et al.*, 2006). Most commercial perfluoro-surfactants are synthesized as salts and have intrinsically polar natures. As a result, they often exist as dissociated salts in aqueous environments rather than as the acid form at environmentally realistic pH. Therefore, ionic and electrostatic interactions between compartments may be important to describe environmental behaviors of PFAs discharged into the aquatic environment.

Toxicity of PFAs

Pharmacokinetics and toxicology

PFAs have distinct elimination kinetics and tissue disposition patterns compared to other persistent organic pollutants (POPs). Notably, they are bound to serum proteins such as albumin and lipoproteins, and are preferentially accumulated in the liver and gall bladder, not in fatty adipose cells (Luebker *et al.*, 2002; Jones *et al.*, 2003; Martin *et al.*, 2003a). Therefore, the target site of action would be the liver of an exposed receptor organism. The abilities to eliminate PFAs from the body are very different among species and sex. Epidemiological studies and medical monitoring of retired fluorochemical workers reveal that PFOS and PFOA are rarely eliminated from the body without notable sex differences. For example, the biological half-life of PFOS and PFOA in occupationally-exposed people is estimated to be 1428 and 1600 d, respectively (3M, 2000). However, salient differences in elimination kinetics of PFOS and PFOA have been found between males and females, and across experimental animals (Butenhoff *et al.*, 2004a). In general, both chemicals are quickly absorbed, but PFOS tends to be more persistent in the body than PFOA. For example, the elimination half-life for PFOS in male rats was more than 90 d, while that of PFOA was 4.4 to 9 d (Lau *et al.*, 2004). Sex differences are also obvious in some animals. For example, half of the blood-borne PFOA in female rat was removed within 24 hr after an oral dose, compared to 6 to 8 d for male rat (Kemper, 2003). This gender difference is also seen in exposed dogs. So far, scientists have found that organic anion transporters (OATs), which are regulated by sex hormones, play an important role in depurating PFAs, consequently resulting in different renal clearance rates between females and males. For example, OAT2 activity in the kidney of adult male rats is only

13% of that of female rats (Kudo *et al.*, 2002). The amount of OAT2 can be increased in the kidney of males by treatment with estradiol or castration.

With respect to their toxicology, mammalian studies have reported developmental and reproductive toxicities (Kennedy *et al.*, 2004; Lau *et al.*, 2004; Betts, 2007). The ammonium salt of perfluorooctanoate (APFO) and other PFAs (e.g., PFOS, and perfluorodecanoic acid (PFDA)) are well known peroxisome proliferators and inducers of hepatic CYP450 enzymes. The hepatotoxicity of PFAs is reversible on termination of dosing, and manifests as increased liver weight due to hepatocellular hypertrophy and proliferation of smooth endoplasmic reticulum and peroxisomes, increases in plasma transaminases, liver degeneration and necrosis in rodents (Butenhoff *et al.*, 2004b; Luebker *et al.*, 2005). Exposure to PFOS and *N*-ethyl-*N*-(2-hydroxyethly)-perfluorooctanesulfonate (which is converted metabolically to PFOS) impaired reproductive system function in rats, causing an increase in perinatal mortality (Lau *et al.*, 2003). Neonatal mortality was also observed following PFOS exposure in a two-generation reproduction study in rats (Luebker *et al.*, 2005). PFOS did not affect reproductive performance (mating, estrous cycling, and fertility), except at higher doses (3.2mg/kg/day) where impaired reproductive outcomes were found, including decreased length of gestation and number of implantation sites, and an increased number of stillborn pups. The NOAEL for offspring effects was 0.4mg/kg/day (oral dosing) based on mortality and decreased pup weight gain through lactation observed in the F1 generation.

In contrast to PFOS, the reproductive system was not the primary target of PFOA in a rat study. Reproductive performance was unaltered by dosing up to 30 mg/kg APFO, including fertility, sperm number and quality, number of liveborn/stillborn pups,

gestation lengths, and estrous cycling (Butenhoff *et al.*, 2004b). No evidence of embryo-fetal toxicity or gross developmental abnormalities was noted in the studies that evaluated the oral and inhalation routes of APFO in rats, even at the doses that produced maternal toxicity (Stapels *et al.*, 1984). Changes in body weight, which is the most sensitive indicator of APFO exposure, showed an interesting outcome across multiple phases of sexual maturation and gender. Decreased pup weights, increased pup mortality, and delayed sexual maturation in F1-generation offspring were seen at a higher dose (30 mg/kg) in rats. Sprague-Dawley male rats of either the parent or F-1 generation exposed orally to APFO showed statistically lower body weight compared to vehicle control rats, but this reduction was not observed in female rats. In addition, weight decrements of sexually mature male rats (%-control) were more severe than sexually immature animals at a given concentration.

PFAs are a known surface-active agents and are structurally analogous to endogenous fatty acids (e.g., hormones), meaning that they are potentially toxic at (sub)-cellular levels. Studies have shown that they are inhibitors of gap junction intercellular communication (GJIC) and disruptors of membrane integrity (Yoo *et al.*, 2005; Hu *et al.*, 2002). Cellular communication through gap junctions is critical to maintain cellular homeostasis, growth, differentiation, and apoptosis. Interference with cellular communication may lead to disease and disruption of cellular integrity. PFOS and PFOA are known to be potent PPAR-α agonists and to increase peroxisome proliferation in adult rodents (Ikeda *et al.*, 1985; Seacat *et al.*, 2003). Microarray analysis suggested that PFAs can modulate gene expression, and provided insight into the effects of PFAs on many biochemical pathways (Hu *et al.*, 2005). Exposure of Sprague-Dawley rats to PFOA and

PFOS resulted in the common up-regulation of genes involved in fatty acid and lipid metabolism such as peroxisomal fatty acid beta-oxidation. PFOA treatment suppressed genes related to lipid transport, inflammation, immunity, and especially cell adhesion, while PFOS treatment down-regulated genes involved in signal transduction and neurosystem regulation.

Regulation of PFAs

In a recent risk assessment aimed for the public exposure to PFOA that is one of the predominant PFAs in human and wildlife, the margin of exposure (MOE) was estimated to range from 1600 (liver-weight increase) to 8900 (Leydig cell adenoma), and the assessment concluded that the MOE values represented substantial protection of children, adults, and the elderly at the present exposure levels (Butenhoff *et al.*, 2004a). This study derived a conservative MOE value using multiple biological responses from broad toxicokinetic and toxicodynamic studies by comparison of serum PFOA concentrations in a general or non-occupationally exposed population.

In addition to voluntary actions by PFA manufactures to stop or minimize the release of these chemicals, especially with regard PFOS and PFOA, into the environment, some legislative measures and management programs are now being developed in countries including US, Canada, and UK, and in international organizations including OECD, EU, and UNEP.

The US EPA finalized two Significant New Use Rules (SNURs) in 2002, requiring companies to inform the EPA before manufacturing or importing 88 listed PFOS-related substances with the exception of a few chemicals with essential uses in

aviation, photography, and microelectronics (US EPA, 2002). Four years after the initial withdrawal of PFOS-based products, the EPA proposed an additional SNUR under the Toxic Substances Control Act (TSCA) in March 2006, which included another 183 perfluoroalkyl sulfonates with carbon chain lengths of five or more carbons in regulation (US EPA, 2006). EPA further proposed an amendment to the Polymer Exemption rule in 2006 which would remove polymers containing perfluoroalkyl moieties (CF_3-) or longer chains from exemption.

A preliminary draft Hazard Assessment of PFOA released by the US EPA in August 2002 found that PFOA and its salts are of similar concern due to their structural analogy with PFOS. Currently, human health effects of long-term exposure to PFOA and its salts are the subject of an EPA risk assessment. The EPA is taking action to help minimize the potential impact of PFOA on the environment. In January 2006, EPA initiated the PFOA Stewardship Program, in which the eight major companies in the fluoropolymer and telomere industry committed voluntarily to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95% no later than 2010, and to work toward eliminating emissions and product content of these chemicals by 2015. Participants in this program include, 3M/Dyneon, Arkema,Inc., AGC Chemicals/Asahi Glass, Ciba Specialty Chemicals, Clariant Corporation, Daikin, E.I. duPont de Nemours and Company, and Solvay Solexis.

The final Environmental Canada/Health Canada assessments of PFOS, its salts and its precursors were released in July 2006. The ecological risk assessment has concluded that PFOS and its salts are persistent and bioaccumulative. PFOS, its salts and its precursors have immediate or long-term harmful effects on the environment

(Environmental Canada, 2006b).

The hazard assessment of PFOS prepared by the OECD in 2002 following the 34th Joint Meeting endorsed a draft assessment of PFOS and its salts by the United States (US) and the United Kingdom (UK). The Hazard Assessment concludes that PFOS and its salts are persistent, bioaccumulative and toxic to mammalian species. The US EPA made a request for an international measure to be passed on PFOS, but the OECD did not recommend action for a ban (UNEP, 2006). Instead, they recommended that governments contact PFOS manufactures in their country to determine whether the companies have plans to stop PFOS production. In 2003, OECD also sent a questionnaire to OECD member and non-member countries requesting information on the production and use of PFOS, PFOA and perfluoroalkyl sulfonates and products/mixtures containing these chemicals.

An environmental risk assessment prepared by the UK-Environmental Agency and discussed by the EU member states under the umbrella of the existing substances regulation (ESR DIR 793/93) shows that PFOS is of concern (UNEP, 2006). In June 2004, the UK government announced unilateral action to phase out PFOS and related compounds because of their persistence and bioaccumulative potential. The EU has recently decided on restrictions on the marketing and use of PFOS (EU, 2006). The measures cover PFOS acid, its salts and PFOS derivatives, including PFOS polymers. The decision prohibits the sale and use of these compounds as a substance or constituent of preparations in a concentration equal to or higher than 0.005% by mass. Furthermore, semi-finished products and articles containing PFOS at more than 0.1% by mass are prohibited

PFAs in the Aquatic Environment

Exposure levels: Water and aquatic organisms

Chemodynamic values of PFOA and PFOS indicate they are likely to exist in the dissolved phase rather than in the gaseous phase (Table 1&2). For example, the low Henry's Law constants of PFOA and PFOS estimated indirectly from high water solubility and negligible volatility predict that all PFOA and PFOS species are expected to partition primarily to water. PFOA is the major contaminant measured in water samples from freshwater to seawater (Table 3). In general, levels of PFAs in inland waters were greater than those in coastal waters. Other PFAs such as PFHS and PFNA are also present, but often at insignificant levels (data not shown). PFOS concentrations in the Great Lakes ranged from 6 to 1.2×10^{2} ng/L for Lake Ontario and from 1.1×10^{1} to 3.9×10^{1} ng/L for Lake Erie.

Table 3. Concentrations of PFOS and PFOA in waters (ng/L)

Freshwater	PFOS	PFOA	Ref.
Lake Ontario, USA/Canada	6-121	15-70	Boulanger et al., 2003
Lake Erie, USA/Canada	11-39	21-47	Boulanger et al., 2003
Tennessee River, USA	17-144	<25-598	Hansen et al., 2002
Pearl River, China	1-94	<1-13	So et al., 2007
Yangtze River, China	<1-13	2-245	So et al., 2007
Effluent Water			
New York, USA	3-68	58-1,050	Sinclair, 2006b
Coastal Water			
Korea	<0.1-3	0.2-11	So et al., 2004
Tokyo Bay, Japan	0.3-58	1.8-192	Yamashita et al., 2004
Hong Kong, China	<0.1-3	0.2-5	So et al., 2004
Nordic seawater, Norway	<0.3-22	3.5-8.5	
Open Ocean			
Atlantic Ocean	<0.1	0.1-0.4	Yamashita et al., 2004
Pacific Ocean	<0.1	<0.1-0.1	Yamashita et al., 2004

A mean concentration of PFOS upstream of a fluorochemcial manufacturing facility in Tennessee River, AL, USA was 3.2×10^{1} ng/L; however, PFOS concentrations were almost four-fold greater downstream of the plant, with a mean concentration of 1.1×10^{2} ng/L. PFOA showed a contamination profile similar to PFOS in this river. In the Tennessee River, the elevated levels measured did not change downstream of the facility, suggesting the absence of physical removal mechanisms from water, such as volatilization or adsorption to soil or sediment. In effluent collected from wastewater treatment plants in New York, PFOA concentrations were as great as 1.05×10^{3} ng/L, but the greatest PFOS concentration measured was 6.8×10^{1} ng/L. PFCAs with a chain length of 9 to 11 carbons were also present in effluents. In coastal waters, Tokyo Bay was more

contaminated with PFAs than other East Asian coastal waters. Surprisingly, trace but measurable levels of PFAs were detected in open oceans. Interestingly, sediments play a less important role in PFA accumulation than water. While other persistent organic contaminants often occur at the parts-per million level in sediment, sedimentary PFA concentrations are at the low parts-per-billion level.

In a contrast to a composition profile of PFAs in water columns, PFOS predominates in the tissues of all aquatic organisms, while in most cases PFOA comprises only a minor portion of total PFAs analyzed (Table 4). Generally, concentrations of PFOS in tissues increase with the trophic status of aquatic animals. Thus, top predators in the aquatic food web, such as polar bears and piscivorous birds, contain the greatest PFOS body burden up to low parts-per-million levels (Giesy and Kannan, 2001). Together with the frequent prevalence of PFOA in the water column, the bioavailability of PFOS seems to be greater than that of PFOA. A known precursor of PFOS, PFOSA, was also ubiquitous in aquatic food web, and a large proportion of PFOSA to total PFAs was observed in certain fish and invertebrates, indicating that these organisms had limited capacities to biotransform PFOSA, possibly to the presence of PFOS (Table 4). Longer chain PFCAs such as PFUnA and PFDA occur as the dominant PFCAs in the tissues of aquatic organisms (Martin *et al.*, 2004b). Increasing field-based biomagnification factors (BMFs) and bioaccumulation factors (BAFs) with increasing perfluoroalkyl chain-length were observed in laboratory bioaccumulation studies (Martin *et al.*, 2003a & 2003b; Martin *et al.*, 2004b).

Table 5. Toxicity to Aquatic Organisms (mg/L)

Species	PFAs	Duration	Media	Endpoint	NOEC	LOEC	EC50	LC50	Ref
Aquatic plants									
Duckweed	PFOS	7-d	Freshwater	Growth	6.6		31.1		Boudreau et al., 2003a
(*Lemna gibba*)	PFOA	7-d	Freshwater	Growth			80		Boudreau et al., 2004
Freshwater algae	APFO	96-h	Freshwater	Growth	12.5				Tompson et al., 2004
Invertebrates									
Chironomustentans	PFOS	10-d	Freshwater	Survival	0.049		>0.15		MacDonald et al., 2004
	PFOS	10-d	Freshwater	Growth	0.049		0.087		MacDonald et al., 2004
	PFOS	20-d	Freshwater	Survival	0.095		0.092		MacDonald et al., 2004
	PFOS	20-d	Freshwater	Growth	0.022		0.094		MacDonald et al., 2004
Daphnia magna	PFOS	21-d	Freshwater	Mortality		5.3			Boudreau et al., 2003a
	PFOS	28-d	Freshwater	Reproduction	7.0		11.0		OECD, 2002
	PFOA	48-h	Freshwater	Mortality				400	Boudreau et al., 2004
	PFOA	48-h	Freshwater	Mortality				269	Ward et al., 1996a
Zooplankton	PFOS	35-d	Freshwater	Community	3.0				Boudreau et al., 2003b
Mysid shrimp	PFOS	96-h	Seawater	Mortality	1.1			3.6	OECD, 2002
Eastern oyster	PFOS	96-h	Seawater	Mortality	1.9		>3.0		OECD, 2002
Mysid shrimp	PFOS	35-d	Seawater	Survival	0.55				OECD, 2002
Amphibians									
Leopard frog	PFOS	16-wk	Freshwater	Partial life cycle	0.3	3		6.2	Ankley et al., 2004
Xenopus	PFOS	96-h	Freshwater	FETAX	4.82	7.97	12.1	13.8	OECD, 2002
Fish									
Fathead minnow	PFOS	28-d	Freshwater	Mortality	0.3	3.0		7.2	Oakes et al., 2005
Fathead minnow	PFOS	96-h	Freshwater	Mortality	3.3			9.5	OECD, 2002
Rainbow trout	PFOS	96-h	Freshwater	Mortality	6.3	13.0		22	OECD, 2002
Bluegill sunfish	PFOS	96-h	Freshwater	Mortality	4.5			7.8	OECD, 2002
Sheephead minnow	PFOS	96-h	Seawater	Mortality	<15			>15	OECD, 2002
Rainbow trout	PFOS	96-h	Seawater	Mortality				13.7	OECD, 2002
Fathead minnow	PFOS	42-d	Freshwater	Mortality	0.3				OECD, 2002
Fathead minnow	PFOS	21-d	Freshwater	Fecundity			0.23		Ankley et al., 2005
Fathead minnow	PFOS	21-d	Freshwater	Grwth/Sur.	0.3			?	Ankley et al., 2005
Rainbow trout	APFO	96-h	Freshwater	Mortality	500			710	Thompson et al., 2004
Fathead minnow	APFO	96-h	Freshwater	Mortality	400			740	Ward et al., 1996b
Bluegill sunfish	APFO	96-h	Freshwater	Mortality				569	3M Company, 1978

Archived biological samples are useful to evaluate temporal trends of PFA emission to the environment, assuming tissue data are a surrogate of PFA exposure. PFOS concentrations in liver tissues of ringed seals (*Phoca hispida*) from east and west Greenland showed a significantly increasing trend starting in 1986 (Bossi *et al.*, 2005). This annual increase was observed in PFDA and PFUA as well. Archived guillemot (*Uria aalge*) eggs collected from 1968 to 2003 also revealed steady increases in PFA concentrations by 7 to 11% annually (Holmström *et al.*, 2005). The results of lake trout (*Salvelinus namaycush*) analysis were consistent with these observations in marine mammals and birds, reporting a 4-fold increase between 1980 and 2001 (Martin *et al.*, 2004b). Future monitoring studies are warranted to see if early reduction efforts by governments and relevant industries will result in a decline of PFA emission and exposure.

Aquatic toxicology: Algae, invertebrates, amphibians, and fish

The adverse effects of water-borne PFAs, in particular PFOS or PFOA, have been investigated in common test species (Table 5). Freshwater has been used most commonly as the test medium, in part because the maximum test concentrations attained in seawater are often lower than the expected effect concentrations. For example, the solubility of PFOS in salt water is approximately 12.4 mg/L as compared to 680 mg/L in freshwater. Toxicities of PFOA have been tested using different salts, mostly the ammonium salt of PFOA (APFO).

Table 5. Toxicity to Aquatic Organisms (mg/L)

Species	PFAs	Duration	Media	Endpoint	NOEC	LOEC	EC50	LC50	Ref
Aquatic plants									
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Daphnia magna	PFOS	21-d	Freshwater	Mortality		5.3			Boudreau et al., 2003a
	PFOS	28-d	Freshwater	Reproduction	7.0		11.0		OECD, 2002
	PFOA	48-h	Freshwater	Mortality				400	Boudreau et al., 2004
	PFOA	48-h	Freshwater	Mortality				269	Ward et al., 1996a
Zooplankton	PFOS	35-d	Freshwater	Community	3.0				Boudreau et al., 2003b
Mysid shrimp	PFOS	96-h	Seawater	Mortality	1.1			3.6	OECD, 2002
Eastern oyster	PFOS	96-h	Seawater	Mortality	1.9		>3.0		OECD, 2002
Mysid shrimp	PFOS	35-d	Seawater	Survival	0.55				OECD, 2002
Amphibians									
Leopard frog	PFOS	16-wk	Freshwater	Partial life cycle	0.3	3		6.2	Ankley et al., 2004
Xenopus	PFOS	96-h	Freshwater	FETAX	4.82	7.97	12.1	13.8	OECD, 2002
Fish									
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Rainbow trout	PFOS	96-h	Seawater	Mortality				13.7	OECD, 2002
Fathead minnow	PFOS	42-d	Freshwater	Mortality	0.3				OECD, 2002
Fathead minnow	PFOS	21-d	Freshwater	Fecundity			0.23		Ankley et al., 2005
Fathead minnow	PFOS	21-d	Freshwater	Grwth/Sur.	0.3			?	Ankley et al., 2005
Rainbow trout	APFO	96-h	Freshwater	Mortality	500			710	Thompson et al., 2004
Fathead minnow	APFO	96-h	Freshwater	Mortality	400			740	Ward et al., 1996b
Bluegill sunfish	APFO	96-h	Freshwater	Mortality				569	3M Company, 1978

Literature values show that PFOS is moderately toxic under acute exposure conditions and slightly toxic under chronic exposure conditions to aquatic plants, invertebrates, and fish (Table 5.). In contrast, PFOA is rarely toxic to aquatic organisms and adverse effects were only observed at concentrations of several hundred parts-per-million (mg/L). Among commonly used test organisms, the aquatic insect *Chironomus tentans* was the most sensitive species to PFOS exposure. Toxic effects such as mortality and growth inhibition were observed at tens of part-per-billion levels (μg/L) in this species, which were almost 100-fold less than effect concentrations of PFOS in freshwater fish (LC50: 7.8-22mg/L). PFOS also exhibited comparable toxicity to marine rainbow trout. Of the endpoints selected for fish species, fecundity was the most sensitive. The 21-d 50% effect concentration (EC50) on fecundity of fathead minnow (*Pimephales promelas*) was determined to be 0.23 mg PFOS/L. The acute lethal concentration which generates 50% mortality of an exposed population (LC50) for PFOA was quite comparable among fish species at a range of 569-740 mg/L, including rainbow trout, fathead minnow, and bluegill sunfish. In amphibians, a partial life-cycle toxicity test from early embryogenesis through complete metamorphosis was conducted using the northern leopard frog (*Rana pipiens*). Time to metamorphosis was delayed and growth was reduced in the 3 mg PFOS/L treatment.

A freshwater microcosm study was conducted for 42 d to evaluate the effects of PFOS on zooplankton community structure and dynamics. A community-level no-observable-effect concentration (NOEC) of 3.0 mg PFOS/L was determined for a 35-d exposure. The most sensitive taxonomic groups, Cladocera and Copepoda, were virtually eliminated at the greatest test concentration (30 mg PFOS/L) after 7 d. A threshold level

for the 35-d exposure was estimated to be between 0.3 and 3.0mg PFOS/L in this study. Species richness was also negatively influenced. The total number of individual species over the 35 d exposure in controls was 23.8, whereas only 8.2 species were found in the 30 mg PFOS/L treatment.

Biochemical and histological studies have been also conducted for PFAs using various aquatic species. PFOS increased hepatic fatty acyl-CoA oxidase activity and oxidative damage in fathead minnow after a 28-d PFOS exposure (Oakes *et al.*, 2005). A dose-dependent decrease in cell viability by PFOS and PFOA was found after a 24-h exposure in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*). In that study, significant induction of reactive oxygen species was also found at an exposure concentration of 15 mg PFOS/L and 15 mg PFOA/L, while antioxidant enzymes activities and GSH content decreased. Though PFOS is a known peroxisome proliferator in rat and mouse, the low sensitivity of fish species towards peroxisome proliferators have been reported. For example, peroxisomal catalase and palmitoyl CoA oxidase activities did not change significantly in PFOS-exposed carp (*Cyprinus carpio*) (Hoff *et al.*, 2003). A slightly increased incidence of thyroid follicular cell atrophy was observed in metamorphs of the northern leopard frog exposed to 3 mg PFOS/L (Ankley *et al.*, 2004). Overall, it seems that acute or chronic effects are unlikely to occur at reported environmentally relevant concentrations of PFOS and PFOA in freshwater.

Study Design for Environmental Risk Characterization in Lake Shihwa

The Lake Shihwa area

Lake Shihwa, located on the west coast of Korea, is an artificial lake with a 12.7 km long sea-dike (Figure 2). The lake receives both industrial and municipal wastewaters from the neighboring Shihwa-Banwol Industrial Complexes (SBIC) and residential areas, respectively. About seven thousands companies, largely steel/mechanical (48.9%), electric/electronic (14.6%), and petrochemical companies (10.1%) currently operate in SBIC. Initially, Lake Shihwa was constructed to supply freshwater for industrial and agricultural purposes. Despite government efforts, gradual deterioration of lake water quality has been reported as well as the massive death of certain bivalve species, due to low annual precipitation and lack of enough tributaries in this region. For this reason, pollution status in this area has been relatively well-documented compared to other regions in Korea, and surveys indicated that lake seawater and sediments in upper inland areas were contaminated with metals and trace organic contaminants (alkyl phenols, PCBs and PAHs). Westerly winds dominate the lake at an annual average velocity of 1.5 m/s, and they typically flush the atmospheric contaminants emitted from SBIC to the residential area on the east side of the lake. Since new management action allowing daily exchange of lake waters with outer Kyounggi-bay seawaters in 2001 has been exercised, water quality is reported to have improved compared to when the sea-dike was firmly closed. In addition, a number of wildlife species, particularly birds, including migratory species that use Lake Shihwa, are now observed in this region. Thus, it is timely and imperative to determine the potential ecological risks of PFAs posed to Lake Shihwa wildlife.

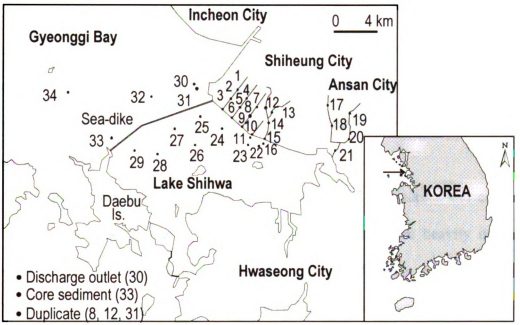

Figure 2. The water sampling locations in Lake Shihwa areas (withdrawn from Rostkowski et al., 2006).

PFA Concentrations in Lake Shihwa Waters

Only a small number of publications are available for PFAs pollution in Korean waters. The first peer-reviewed report dates back to 2004 as a part of screening study to survey PFA contamination in coastal waters of highly industrialized East Asian regions, including Hong Kong, South China, and Korea. Surprisingly greatest concentrations of PFAs were measured at some locations in Korea. One sampling location situated within Kyeonggi Bay had 0.33 ng PFOSA/L, 730 ng PFOS/L, 13 ng PFNA/L, 320 ng PFOA/L, and 52 ng PFHS/L. These concentrations were 5 to 63-fold greater than the maximum concentrations determined in other locations in that international joint study. Except for this hotspot, PFA concentrations in Korean coastal waters were comparable to other East Asian locations.

This survey result raised the urgent question of the sources and contamination status in Kyeonggi Bay and Lake Shihwa, both of which are heavily influenced by industrial effluents from SBIC. As a result, the Lake Shihwa region was revisited in December 2004 and a more systematic monitoring plan was made to ensure the quality of data, including sampling locations, QA/QC for chemical analysis, and the latest instrumentation. Water samples were collected from streams discharging industrial effluents into Lake Shihwa (n=21), from Lake Shihwa (n=8), and Gyeonggi Bay (n=5). In that study, a broad range of PFAs were monitored, including four sulfonic acids (PFOS, PFHxS, PFBS, and FOSA) and five carboxylic acids (PFDA, PFNA, PFOA, PFHpA, and PFHxA). Concentrations of all target PFAs were greater in inland streams than in the lake itself or in Kyeonggi Bay waters (Table 6.). Of the PFAs investigated, PFOS and PFOA were detected at all sampling sites and occurred at the greatest concentrations.

Concentrations of PFOS were in the range of 2-651 ng/L, while PFOA was present at 10-fold lower concentrations with a range of 0.9-62 ng/L. An interesting finding was a decreasing concentration gradient of PFAs as a function of distance from inland waters, implying that industrial effluents were a possible release source into Lake Shihwa and Kyeonggi Bay.

Table 6. Concentrations of PFAs in water samples from streams, Lake Shihwa, and Gyeonggi Bay, Korea (Rostkowski et al., 2006)

Map ID	Perfluoroalkylsulfonic acids (ng/L)				Perfluoroalkylcarboxylic acids (ng/L)				
	PFOS	PFHxS	PFBS	FOSA	PFDA	PFNA	PFOA	PFHpA	PFHxA
Streams									
1	20.15	2.02	0.89	<0.50	1.00	1.18	7.32	0.91	0.77
2	47.68	4.27	1.55	2.36	6.01	4.75	20.19	2.17	2.94
4	45.21	7.94	3.83	0.59	0.63	1.83	7.53	1.28	3.88
5	283.44	18.32	18.11	1.19	3.18	5.29	19.39	3.58	10.50
7	20.79	2.90	1.61	<0.50	0.56	1.30	6.20	1.03	1.13
8	64.59	5.10	1.50	<0.50	2.34	5.58	20.98	2.20	2.00
9	46.45	5.03	1.29	0.60	2.41	4.18	13.39	2.18	2.58
10	651.35	84.61	24.03	1.69	2.40	6.95	61.69	7.10	27.27
12	45.47	8.93	<0.50	0.58	<0.50	2.81	20.46	3.50	6.05
13	47.45	5.25	1.18	<0.50	2.69	5.42	33.62	3.54	6.39
14	48.52	6.97	2.74	<0.50	0.86	2.75	19.69	2.58	3.48
15	60.58	8.91	2.45	0.79	1.53	3.71	21.11	3.21	5.06
16	59.45	7.51	<0.50	<0.50	2.25	3.87	29.36	2.44	2.52
18	8.03	2.25	0.58	<0.50	<0.50	1.36	11.63	1.27	1.16
20	10.38	3.18	0.79	<0.50	<0.50	0.83	5.21	1.14	1.26
21	17.51	1.94	0.64	0.58	0.76	1.69	9.44	1.40	1.60
Lake Shihwa									
22	18.32	2.04	1.03	<0.50	0.90	2.10	10.86	1.10	1.37
23	16.09	1.74	<0.50	<0.50	0.56	1.65	9.18	0.98	0.98
24	12.59	0.63	<0.50	<0.50	<0.50	1.29	4.52	<0.50	0.70
25	7.33	0.89	0.58	<0.50	<0.50	0.63	1.67	<0.50	<0.50
27	9.92	1.26	0.54	<0.50	<0.50	0.93	4.47	0.60	0.54
Gyeonggi Bay									
31	8.26	0.55	<0.50	<0.50	<0.50	0.83	3.29	<0.50	0.60
32	5.31	0.55	<0.50	<0.50	<0.50	0.51	2.51	0.50	0.49
33	2.58	0.99	<0.50	<0.50	<0.50	<0.50	0.94	0.44	<0.50
34	2.24	0.79	2.53	<0.50	<0.50	<0.50	1.07	<0.50	<0.50

Hazard Assessment Strategy

Intensive monitoring efforts for PFAs around the globe have generated a lot of useful data, which can be used by regulatory agencies to take the proper management actions to prevent or reduce the risks of PFA exposure to humans and wildlife. However, such efforts are rarely found in Korea, one of the most highly industrialized countries in the world. Despite the recent study on PFAs in lake waters, contamination status of these emerging chemicals in living animals are not understood at all. This study was designed to conduct a preliminary risk assessment of PFA exposure to marine species and their top predators (birds) in the Lake Shihwa region, where one of the more industrialized complexes in Korea is located. In order to achieve this goal, a research framework was developed (Figure 3).

In the first chapter, the peer-reviewed literature on PFAs was studied from toxicology to chemistry and regulation. The available toxicological benchmark values protective of fish and avian species were also determined. In Chapter II, the pharmacokinetics of PFAs were investigated using implanted white leghorn chickens (*Gallus gallus*) as model species under laboratory conditions for an exposure and elimination period of 8 weeks in order to assess the risk of PFAs to bird species. This exposure study was collaborated with the National Institute of Animal Health, Tsukuba, Japan. Briefly, capsules containing PFOS and PFOA were subcutaneously implanted in an experimental animal for 4 weeks and they were allowed to depurate for a further 4 weeks. Blood samples were taken at an interval of two or three days over the entire experimental period. Organ samples such as brain, kidney, and liver were collected at the

termination of the exposure and after depuration, respectively. Uptake and depuration kinetics were studied separately in blood and organs and the results were discussed.

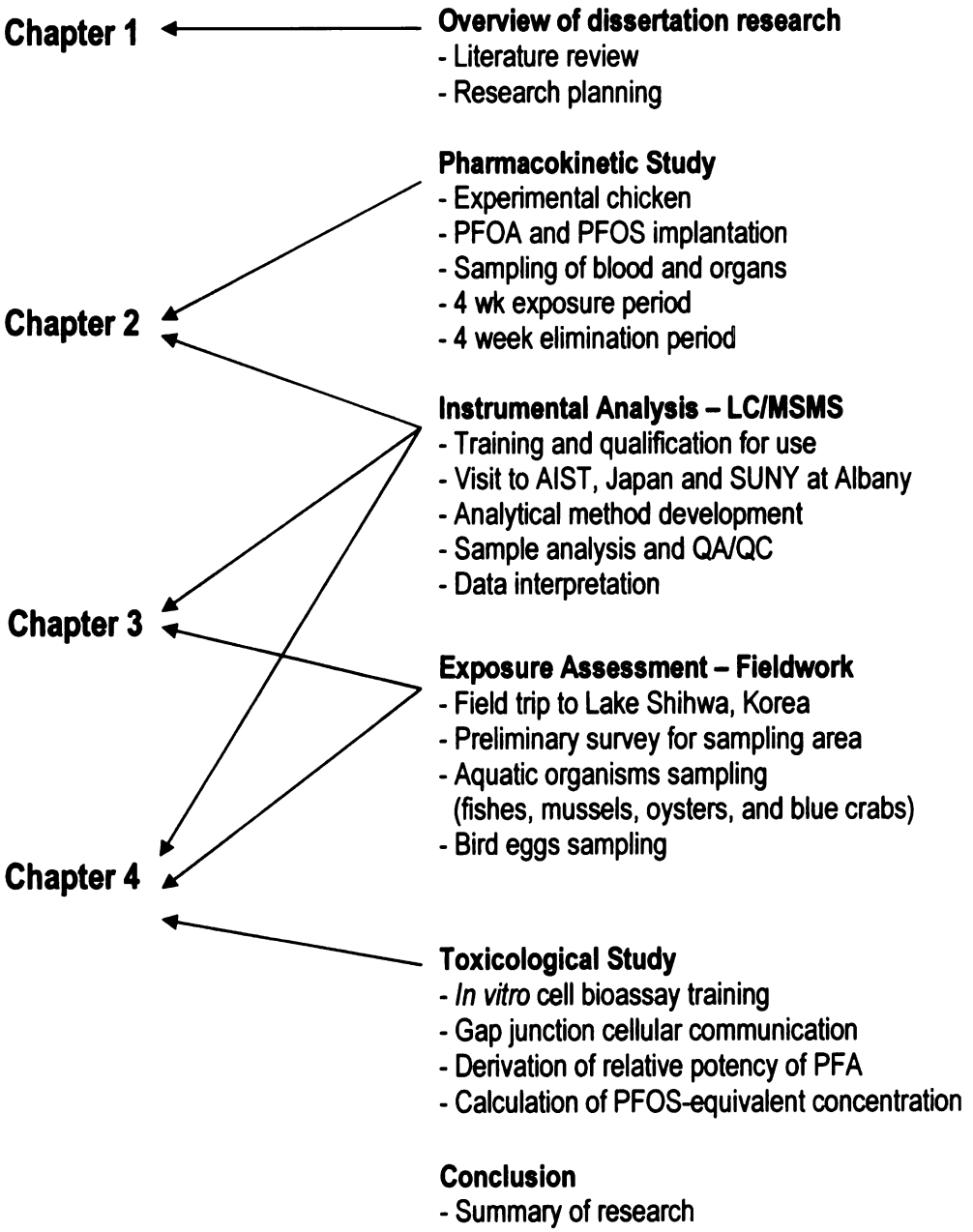

Figure 3. Dissertation research scheme and assigned works in each chapter

In Chapters III and IV, PFA concentrations were determined and associated risks were characterized in marine organisms such as fish, mussel, oyster, and blue crab, and in egg yolks of birds, little egret *(Egretta garzetta)*, little ringed plover (*Charadrius dubius*), and parrot bill (*Paradoxornis webbiana)*. A preliminary field survey and field sampling were conducted from May to June 2006 in the Lake Shihwa area. Stationary fish nets were installed for 4 days to collect marine species. After a preliminary survey for nests with a help of an avian expert, birds' eggs were sampled. Nests of little ringed plover were found at islands in Lake Shihwa and eggs of parrot bill were collected in the wetland of Lake Shihwa away from known pollution sources. One large little egret colony was surveyed in a section of the city of Ansan, Korea.

In biota, PFAs were present as a mixture of PFAs rather than a single congener. As discussed earlier, unfortunately most toxicological studies targeted PFOS and PFOA. Currently, water quality guideline values that are protective of aquatic organisms are only available for PFOS. Recently, toxicity reference values (TRVs) of PFOS for blood, liver, and egg yolk were developed for avian species. Thus, the risk assessment approach used for exposure to complex mixtures of PCBs was benchmarked for a mixture of PFA in biotic samples.

Because PFAs are the newest halogenated contaminants, the use of the proper analytical techniques as well as the latest instruments are essential to the success of the present research. To analyze the biological samples from the pharmacokinetic study and fieldwork campaign, I visited the National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan for a total of nine months. The potential pollutants group at AIST is one of the renowned laboratories for the analysis of PFAs. They

developed high-resolution liquid-chromatography (HPLC) tandem mass-spectrometry (LC/MSMS) solely devoted to PFAs analysis. For some remaining samples, I also visited the State University of New York at Albany, NY, and completed sample analysis for a month with a help of Prof. Kannan.

Chapter 2

Depuration Kinetics and Tissue Disposition of PFOA and PFOS in White Leghorn Chickens (*Gallus gallus*) Administered by Subcutaneous Implantation

Abstract

To address the elimination kinetics and tissue disposition of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) in avian species, an exposure study was designed with low-exposure conditions using a subcutaneous implantation in male chickens (*Gallus gallus*). Chickens were exposed to two levels of PFOA or PFOS for 4 weeks and allowed to depurate for additional 4 weeks. Over the entire experimental periods, body index, clinical biochemistry and histopathological assessments were not statistically different among treatments relative to the controls (p>0.05), except significant decreases in total cholesterol and phospholipids in PFOS-exposed chickens. The elimination rates for PFOA (0.150±0.010 d^{-1}) were almost six-fold faster than those of PFOS (0.023±0.004 d^{-1}). After an exposure, kidney had the highest concentration of PFOA followed by liver and brain. In contrast, liver was found to be a central accumulation site for PFOS with lesser concentrations in kidney and brain. The estimated biological half-life of PFOA (4.6±0.3 d) and PFOS ($t_{1/2}$ = 125 d) in chickens was simlar to mammals.

Introduction

The use of perfluorinated acids (PFAs) in a variety of products has resulted in them being ubiquitous in the environment, occurring globally in humans and wildlife (Giesy and Kannan, 2002; Olsen *et al.*, 1999; Lau *et al.*, 2004). PFAs have been used as surface protectors for carpets and leather, and as surfactants in cosmetics as well as processing aids in the production of fluorinated polymers and active-component in fire-fighting foams (Kissa, 2001). To date, due to their widespread use in industrial and commercial applications the two PFAs that have received the greatest attention are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). In a monitoring study of human serum collected from the general public, PFOS was determined to be the most abundant PFA followed by PFOA (Kannan *et al.*, 2004). Similar profiles of PFAs have also been observed in the tissues of terrestrial and marine wildlife with PFOS being the dominant PFA in these samples with concentrations in some cases exceeding those measured in human populations (Houde *et al.*, 2006). Several food web studies have shown that some PFAs have the potential to bioaccumulate into the lower trophic organisms and through tropic transfer and biomagnification, can accumulate in to upper trophic level organisms (Vijver *et al.*, 2003; Martin *et al.*, 2004). While exposure pathways of PFOA, PFOS and related PFAs to humans have not yet been fully elucidated, the consumption of fish (Falandysz *et al.*, 2006) and farm animals (Guruge *et al.*, 2005) have been suggested as major contributors of PFAs to exposed human populations. Nevertheless, efforts reducing PFAs environmental emission are now being initiated in both governmental and industrial areas.

To effectively control exposures to these compounds, it is necessary to understand their pathways of exposure and to develop models to predict the rates of movement in the environment. However, due to their amphiphilic properties, PFAs do not behave in the same manner as the more studied organochlorine contaminants. Specifically, PFAs have fewer tendencies to partition into lipids, but rather are preferentially bound to proteins and retained in the blood and liver of wild animals. To date, only few pharmacokinetic studies with PFOA and PFOS have been conducted with mammals that have included rats, dogs, and monkeys (Seacat *et al.*, 2002; Kudo *et al.*, 2002; Lau *et al.*, 2004). In these studies, administered PFOA has shown to be readily absorbed but has a relatively short half-life with notable species- and gender- differences in rates of elimination when compared to PFOS. For example, in male rats the half-life for PFOA of blood is approximately one week while in female rats the half-life is approximately one day (Butenhoff *et al.*, 2004). In contrast, PFOS is also readily absorbed but is poorly eliminated from blood and other tissues with biological half-lives that can range from several weeks to months depending on species and sex.

Concentrations of PFAs in wild birds have been studied globally, and data suggest that birds from urban areas are more contaminated with PFAs than those from rural areas (Kannan *et al.*, 2001; Verreault et al., 2005). Other studies have quantified PFOS in several farm animals including chickens and livestock. In that study, concentrations of PFOS in blood plasma and liver were greater in chickens than in other farm animals that were evaluated (Guruge *et al.*, 2005). While pharmacokinetic studies with PFOS have not been conducted with avian species, some kinetic data are available from acute and chronic dietary studies that been conducted with two species, northern bobwhite quail

(*Colinus virginianus*) and the mallard (*Anas platyrhynchus*) (Newsted *et al.*, 2005). The results of those studies indicated that the half-life of PFOS in the blood and liver of juvenile birds ranged from approximately 7 to 18 d while in adult birds, the half-life blood ranged from 14 to 21 d. Based on the results of those avian studies, the elimination rate of PFOS from birds has been assumed to be faster than those observed in mammalian species. However, since treatment levels used in these studies were greater than that usually used in kinetic studies, the overall pharmacokinetics of PFOS in those species may have been influenced. Therefore, additional kinetic studies with PFOA and PFOS for birds are needed under low-exposure levels that would not be expected to affect pharmacokinetic parameters.

To address the above questions, an exposure study was designed with low-exposure conditions to better understand the pharmacokinetic behavior of PFOA and PFOS in male chickens (*Gallus gallus*). First, elimination kinetic parameters from chicken blood were measured for PFOA and PFOS administered subcutaneously. Second, tissue disposition patterns of introduced PFOA and PFOS were examined in tissues of brain, kidney, and liver following an exposure and a elimination period. In addition to pharmacokinetic evaluations, biochemical and histopathological parameters were also evaluated. Finally, the pharmacokinetic results from this study were compared with findings from other studies that have been conducted with alternative study designs and/or other species. A subcutaneous implant-exposure scheme, which is widely exercised in veterinary science as an efficient drug delivery system, was used to introduce the PFOA or PFOS into the chickens. For this reason, the uptake rate kinetics were not be determined in this study. To date, chickens have been shown to be among the most

sensitive avian species to PFOS and, accordingly these data will aid in future ecological risk evaluations of PFAs exposure for avian species (Molina *et al.*, 2006).

Materials and methods

Test substances and reagents

Two perfluorinated chemicals, perfluorooctanoate (PFOA, purity 95%, CAS number 335-67-1) and perfluorooctane sulfonate (PFOSK, purity >98%, CAS number 2795-39-3) were purchased from Wako Chemicals, Japan and Fluka, Italy, respectively. Pesticide-grade methanol, ammonium acetate, and ammonium solution (25%) were purchased from Wako Chemicals, Japan. Milli-Q water was used in the whole experiment. Nylon filters (0.1 μm, 13 mm i.d.) were purchased from Iwaki, Japan. Additional clean-up for tissue extracts was carried out using Oasis weak-anion exchange (WAX®) cartridges purchased from Waters Corp., Milford, MA, USA.

Animals and Exposure

This experiment followed the guidelines for animal experiments of the National Institute of Animal Health, Tsukuba, Japan. White leghorn (*G. gallus)* PDL-1 strain were obtained from a flock for which performance of 3 successive generations of specified-pathogen-free chickens had been maintained to insure the health of the birds used in the study. Eggs were hatched and male chickens were housed until six-weeks of age at temperature and humidity-controlled facilities located at the National Institute of Animal Health, Japan. Hatchlings were fed a standard experimental diet (SDL-1) while chickens greater than 4 wk of age, were fed SDL-4 (Nippon Formula Feed Co., Ltd). Six-week-

old, male chickens (347.4 ± 15.7 g, n = 30) were randomly selected and placed into cages, one cage per treatment with six chickens per cage. Experimental treatments consisted of chickens exposed to either PFOA (0.1 mg/mL or 0.5 mg/mL) or PFOS (0.02 mg/mL or 0.1 mg/mL) or a saline vehicle control. All stock solutions were prepared in 0.9% NaCl in Milli-Q water. Exposure of the chickens to these concentrations was done via the subcutaneous implantation of a 2 mL osmotic pump (ALZET® 2ML4), which has a releasing rate of 2.5 μL/hr for 4 wk. Under sodium pentobarbital anesthesia, an osmotic pump was implanted surgically into hypodermal tissue at right side of trunk of each chicken. Chickens were fed with the standard SDL-4 diet during the exposure and the elimination phase of the study. At intervals of 2 to 3 d, one to two milliliter of blood was drawn from the wing vein of chickens during experimental periods using a heparinated needle and stored in polypropylene (PP) centrifuge tubes until analyzed for PFOA or PFOS. To avoid the potential influence from the hypodermal pump operation, blood was sampled from the wing vein opposite to the wing where the pump had been implanted. At the end of an initial 4 wk-exposure period, half of the chickens from each treatment group were anesthetized and blood was collected for determination of blood chemistries. Following a blood collection, the organs of the euthanized chickens, such as brain, liver, and kidney were placed in PP bags for tissue analysis and histopathology examination. The remaining chickens were maintained for an additional 4 weeks and then euthanized when blood and other tissue were then processed as given above. In an elimination phase, implanted capsules were not retrieved from experimental chickens in order to avoid additional surgery; therefore it was assumed that all perfluorinated chemicals in implants

had been in the proceeding 4-wk period. All biological samples (blood and organs) were kept at negative 20 C^{o} until instrumental analysis.

Sample extraction

Blood samples were extracted with an ion-pairing method with some modifications (Kannan *et al.*, 2004). Briefly, 0.5 mL of blood sample was diluted 10 times with saline buffer (0.9% NaCl in Milli-Q water) while organ tissues were mechanically homogenized with a vortex mixer and 0.5g of the homogenate was diluted with 2 mL Milli-Q water. One milliliter of diluted blood or tissue-water mixture was then transferred into a 15 mL PP tube, and 1 mL of 0.5 M ion-pairing agent (Tetrabutyl ammonium adjusted to pH 10) was added to the mixture. Two milliliter of 0.25 M extraction buffer (Sodium carbonate + Sodium bicarbonate) was then added followed by the addition of 5 mL of methyl *tert*-butyl ether (MTBE). Samples were shaken for 20 min then centrifuged for 15 min (2000 rpm). The organic phase was removed (4 mL) and put in a clean 15 mL PP centrifuge tube. The extraction was then repeated twice and the organic phase from all extractions were combined and then evaporated to near dryness under a gentle stream of nitrogen. The sample was then re-dissolved in 1 ml methanol and then filtered through 0.1 μm nylon filter. For the tissue analysis, a solid-phase extraction (SPE) step was employed as an additional clean-up with some modifications (So *et al.*, 2006). Briefly, a half milliliter aliquant of unfiltered organ extract obtained from ion-pairing extraction was diluted with 100 mL Milli-Q water and then the water-extract mixture was passed through an Oasis WAX® cartridge (0.2 g, 6 cm^3) at an elution rate of 1 drop/sec. At the completion of sample loading, the cartridge was washed with buffer adjusted to pH 4 (25 mM acetic

acid 170 mL + 25 mM ammonium acetate 30 mL) and methanol. Then, a target fraction containing PFOA or PFOS was collected with 0.1% NH_4OH dissolved in methanol. Teflon or glassware was avoided in extraction procedures to remove possible contamination of samples and sorption of analytes.

Matrix recoveries

To evaluate overall extraction efficiencies, either PFOA or PFOS was fortified into blood and organs tissue homogenates prior to extraction. Recoveries from blood (n=10) were 81.3±6.7% and 87.0±5.3% for PFOA and PFOS, respectively. In tissues, comparable efficiencies were obtained in brain, kidney, and liver. Recoveries from PFOA-spiked samples ranged from 86.9 to 94.3% (n=4), while extractions efficiency ranged from 81.4 to 88.2% (n=4) for PFOS-spiked tissues. Reported concentrations of PFOA and PFOS were not corrected for recoveries of matrix spikes. The limit of quantification (LOQ) for both compounds varied from 1 to 5 ng/mL or ng/g wet wt., depending on the sample type.

Instrumental analysis and data analysis

Quantification of PFOA and PFOS in blood or tissues was conducted using HPLC with high resolution, electrospray tandem mass spectrometry (HPLC-MS/MS). Separation of analytes was performed by an Agilent HP 1100 liquid chromatography (Agilent, Palo Alto, California) interfaced with a Micromass Quattro II mass spectrometer (Waters Corp., Milford, Massachusetts) operated in electrospray negative mode. Ten μL aliquot of extract was injected onto a Keystone Betasil C_{18} column (2.1 mm i.d. × 50 mm length, 5 μm) with 2 mM ammonium acetate and methanol as mobile phase, starting at 10%

methanol. Ions were monitored using selected reaction monitoring at m/z 413 and 369 for PFOA and at m/z 499 and 99 for PFOS. Concentration of PFOA or PFOS in extracts was quantified using calibration curves constructed by external standards (0.01, 0.05, 0.2, 1, 10 ng/mL). Acquired data were deemed acceptable if QC standard included in sample batch fell within 30% of the theoretical value, otherwise samples were run again with a new calibration curve.

Clinical chemistry and histopathology

Thirteen biochemical parameters were analyzed in plasma using a Hitachi 7020 auto-analyzer with standards from Wako Pure Chemical Industries Ltd, Japan. All standards were used in accordance with the manufacturer's instruction and stated expiration date. Parameters included total cholesterol (T-Cho), free total cholesterol (F-Cho), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), , phospholipids (PL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH). The following tissues were fixed in 10% phosphate–buffered formalin and processed for histopathological examination: liver, kidney, spleen, heart, lung, thymus, testis, bursa of fabricius, and brain.

Data and statistical analysis

A one-compartment model was used to describe the elimination behavior of PFOA or PFOS from blood in male chickens (Eq. 3).

$$C_t = C_0 \exp(-kt) \qquad \text{(Eq. 3)}$$

Where C_t is the concentration of PFOA or PFOS at the time (t) in an elimination phase, C_0 is the concentration at the onset of depuration (ng/mL).

To account for growth dilution as a factor in determining the elimination rate kinetics, elimination rate constant was determined by first estimating the overall elimination rate constant then adjusting the rate constant by the growth rate constant for each chicken used in the study (Eq. 2).

$$k = k' + k_g \qquad \text{(Eq. 2)}$$

Where k is the overall elimination rate constant (day^{-1}), k' and k_g are final first order elimination rate constant (day^{-1}) and the growth rate constant (day^{-1}), respectively.

To evaluate treatment effects on body index, serum chemistry, and tissue accumulation, one-way ANOVA was performed with SYSTAT® at the significance level set to p=0.05.

Results

Body index, clinical biochemistry and histopathology

No significant differences were observed for body-weight gains among doses (vehicle control, low-dosed and high-dosed) for PFOA and PFOS nor were there any statistical differences observed between PFOA and PFOS treatments over an entire experimental period (p > 0.05) (Table 7). Growth rates (20 ~ 21 g/d) were determined to be comparable between non-exposed and exposed chickens at the end of a 4-wk exposure phase. Following an exposure period, there were slight increases in the liver to body weight ratios from PFOA and PFOS treated groups (2.5 ~ 2.6), however these increases were not statistically different with the vehicle control group (2.2). This result was also

observed in chickens collected at the end of a depuration period of the study. Exposure to PFOA or PFOS also did not statistically affect either the kidney to body ratio or the testis to body ratios. Sampled amount of brain were so small that we could not use it for comparison. In chickens collected at the termination of an exposure period, most of clinical chemistry parameters were not significant different among treatments (Table 8) and no significant lesions were seen relative to those from vehicle controls (data not shown). However, after a depuration phase there were significant decreases in total cholesterol and phospholipids in chickens exposed to both low- (0.02 mg PFOS/mL) and high-dosed (0.1 mg PFOS/mL) treatment.

Table 7. Mean body-weight gain (s.d.) and organ to body weight ratio at the end of an exposure (A) and an elimination period (B).

A. Exposure	Body wt. (g)	Liver/Body (%)	Kidney/Body (%)	Testis/Body (%)
Vehicle control	465 (4)	2.24 (0.19)	0.96 (0.01)	0.039 (0.001)
Low PFOA	468 (38)	2.63 (0.23)	1.01 (0.09)	0.043 (0.012)
High PFOA	477 (18)	2.48 (0.12)	0.97 (0.05)	0.034 (0.005)
Low PFOS	495 (32)	2.51 (0.24)	1.03 (0.03)	0.045 (0.025)
High PFOS	505 (93)	2.51 (0.21)	0.95 (0.10)	0.047 (0.022)

B. Elimination	Body wt. (g)	Liver/Body (%)	Kidney/Body (%)	Testis/Body (%)
Vehicle control	1063 (4)	2.07 (0.14)	0.83 (0.10)	0.12 (0.11)
Low PFOA	1000 (100)	1.94 (0.21)	0.84 (0.01)	0.27 (0.14)
High PFOA	965 (71)	1.94 (0.01)	0.85(0.01)	0.10 (0.02)
Low PFOS	1038 (28)	1.97 (0.12)	0.87 (0.12)	0.19 (0.07)
High PFOS	1015 (135)	1.82 (0.10)	0.81 (0.05)	0.22 (0.16)

Note. Six week-old male chicks were exposed to either PFOA (total 0.2mg or 1.0mg) or PFOS (total 0.04mg or 0.2mg) via subcutaneous implantation for 4 weeks and were allowed to depurate for further 4 weeks (n=6 for exposure and n=3 for depuration).

Table 8. Mean serum lipids and biochemistry measurements for experimental chickens determined at the end of an exposure (A) and an elimination period (B) (n=3) (*: $p<0.05$)

Treatment	T-Cho[1] mg/dL	F-Cho[2] mg/dL	HDL[3] mg/dL	LDL[4] mg/dL	TP[5] g/dL	Alb[6] g/dL	BUN[7] mg/dL	NEFA[8] mEq/L	PL[9] mg/dL	AST[10] IU/L	ALT[11] IU/L	ALP[12] IU/L	LDH[13] IU/L
A. EXPOSURE													
Vehicle control	128.3	19.0	86.5	23.0	3.3	1.8	0.8	0.6	209.5	245.5	2.0	3108	528.5
Low PFOA	135.1	23.2	91.0	27.0	3.5	1.9	1.1	0.6	228.7	229.7	2.0	3080	539.3
High PFOA	134.4	21.7	86.3	27.3	3.4	1.8	1.1	0.8	215.0	239.0	2.3	3691	561.3
Low PFOS	122.8	18.8	89.0	23.5	3.5	1.9	1.0	0.9	217.0	215.0	2.0	3480	465.0
High PFOS	138.9	22.9	95.6	31.3	3.7	2.0	0.9	1.0	226.3	241.0	1.7	3177	464.7
B. ELIMINATION													
Vehicle control	126.7	18.8	82.6	20.5	3.6	2.0	1.0	0.7	207.0	225.0	1.5	1979	429.5
Low PFOA	137.4	19.0	96.2	30.0	4.1	2.2	1.0	0.6	220.7	242.3	1.0	2297	412.7
High PFOA	136.1	18.8	98.9	26.5	3.9	2.1	1.0	0.9	233.0	207.5	1.0	2260	350.5
Low PFOS	107.2*	15.2	79.6	18.0	3.4	1.9	1.1	0.6	172.0*	209.0	1.5	2053	443.5
High PFOS	113.3*	16.2	84.2	21.7	3.4	1.8	1.0	0.6	172.3*	215.3	1.7	1957	386.7

[1] Total cholesterol [2] Free cholesterol [3] High density lipoprotein [4] Low density lipoprotein [5] Total protein
[6] Albumin [7] Blood urea nitrogen [8] Non-esterified fatty acid [9] Phospholipid [10] Aspartate aminotransferase
[11] Alanine aminotransferase [12] Alkaline phosphatase [13] Lactate dehydrogenase

Uptake profiles and elimination behaviors in blood

The time-course concentrations of PFOA and PFOS released from implanted capsules demonstrated different uptake profiles in chicken blood over an exposure period. Introduced PFOA in both low- (0.1 mg/mL) and high-dosed (0.5 mg/mL) treatment behaved similarly with a rapid increase (t_{max}=7 d) followed by sustained blood levels in later periods, while blood-borne PFOS fluctuated over a 4wk-uptake period (Figure 4). Concentrations of PFOA and PFOS in blood reflected the dose from capsules containing target PFAs. For example, the maximum PFOA concentrations in blood taken from low-dosed and high-dosed treatment were 103 and 333 ng/mL, respectively. In non-exposed chickens during an uptake period, PFOA blood concentrations were below the limit of quantification, while measured PFOS concentrations (3.5±0.7 ng/mL) were at least 8 times lower than those in any given PFOS treatment.

Rates of elimination from blood in chickens were significantly different for PFOA and PFOS (Figure 5). Concentrations of PFOA in blood decreased rapidly during a depuration phase, with PFOA concentrations at the termination of depuration only being 2~3% compared to those in the onset of a depuration. In contrast, only 48~52% reduction of PFOS concentrations in the blood compartment was found during the same experimental period (Figure 5). The first-order elimination rate constant was similar between treatments administered with the same compound (PFOA: 0.150±0.010 d^{-1} and PFOS: 0.023±0.004 d^{-1}). The overall elimination half-life ($t_{1/2}$) of treatment-averaged PFOA (4.6 d) was almost six-fold shorter than that of treatment-averaged PFOS (31.1 d). Growth dilutions that occurred in a depuration period did not influence on the final $t_{1/2}$ of PFOA (5.2 d) on average. However, it increased the elimination half-live of PFOS such

that high-dosed treatment ($t_{1/2}$ = 72.2 d) had smaller value than low-dosed treatment ($t_{1/2}$ = 177.7d). In a vehicle control group, PFOA and PFOS blood concentrations over a depuration period were comparable to those concentrations in an exposure period.

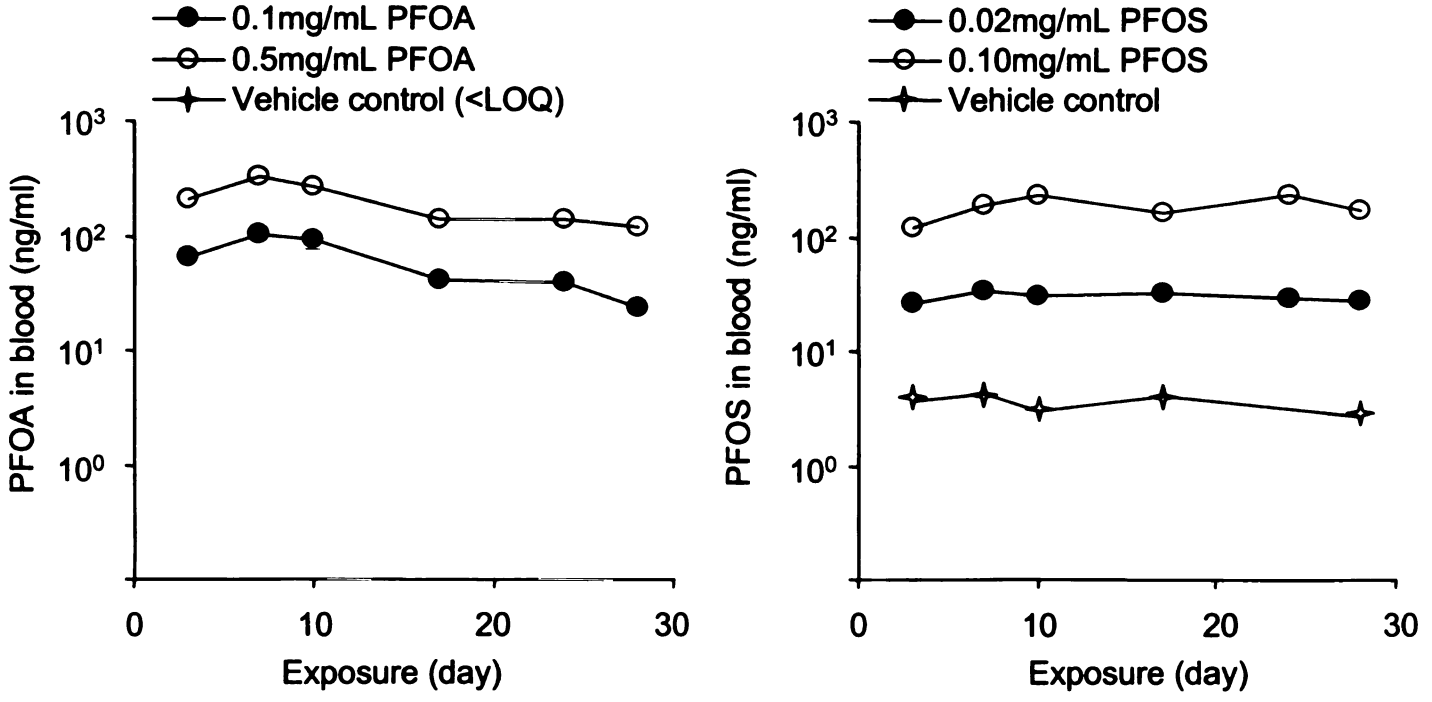

Figure 4. Uptake profiles of PFOA and PFOS (ng/mL) introduced into male chickens using an implantation for a 4-wk exposure period (n=2). Closed circles and open circles symbolize low-dosed and high dosed-treatment, respectively. The implants deployed in low-dosed and high-dosed PFOA treatment contained 0.1 mg/mL and 0.5 mg/mL, respectively, while a fifth of concentrations were used in low-dosed (0.02 mg/mL) and high-dosed (0.1 mg/mL) PFOS treatment. The saline buffer was used in a vehicle control treatment. All PFOA measurements from vehicle control were less than LOQ (1ng/mL)

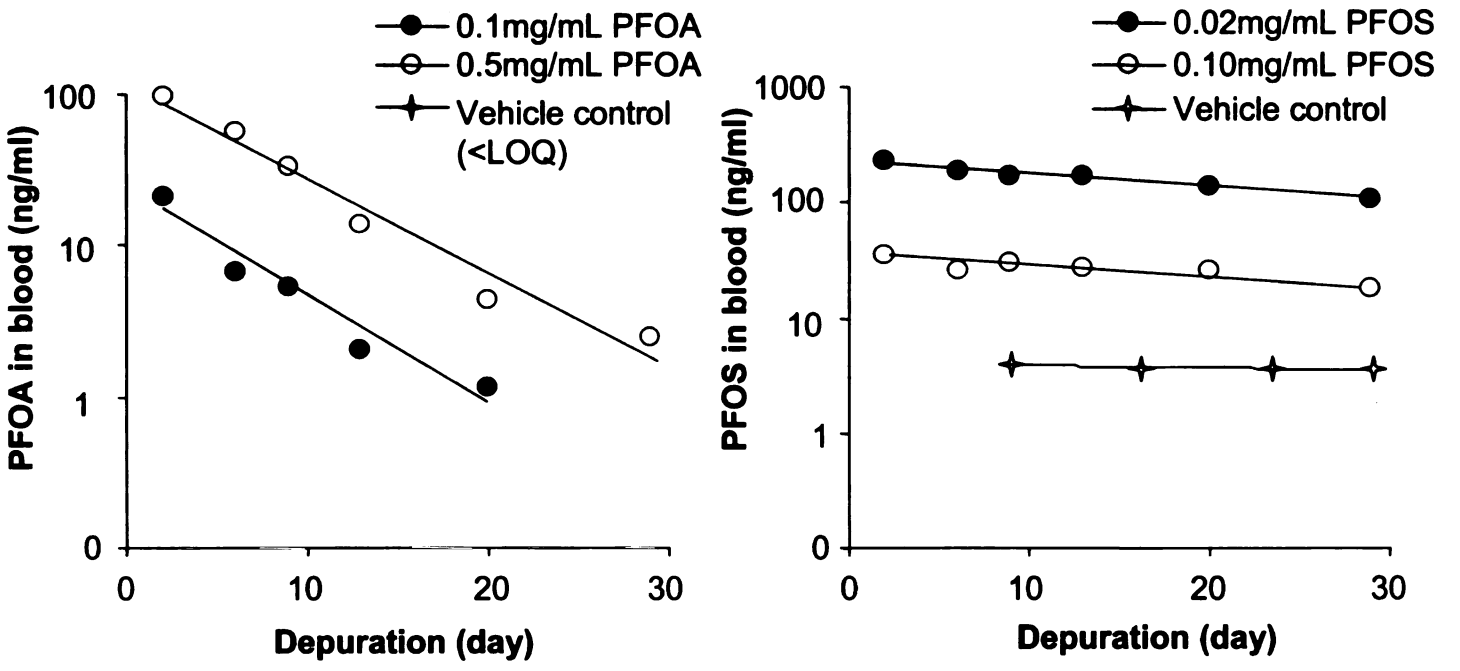

Figure 5. Blood concentrations of PFOA or PFOS (ng/mL) in male chickens over a 4-wk depuration period (n=2). PFOA concentration in Day 28 in low-dosed group was lower than LOQ (1 ng/mL). Symbols are the same as in the Fig. 1. All PFOA measurements from vehicle control were less than LOQ (1ng/mL)

Organ concentrations

Distinctive accumulation features of these two perfluorinated compounds were observed in organs (brain, kidney, and liver) of experimental chickens following an exposure and a depuration period (Figure 6). After a 4-wk dosing period, the greatest concentration of PFOA was measured in kidney tissue, followed by liver and brain tissue (Fig. 6A~C). In the high-dosed treatment (total 1.0mg PFOA dosed), the concentration of PFOA in kidney was as high as 186 ± 40 ng/g ww. A measurable concentration of PFOA was also detected in brain homogenate of PFOA-treated chickens. Concentrations of PFOA in all tissues rapidly decreased proportionately to that in blood. By the completion of a depuration phase, an average of approximately 92% of organ PFOA was cleared from all tissues investigated. Organs of unexposed chickens did not have any contamination of PFOA in entire experimental periods. Interestingly, tissue disposition and elimination pattern of PFOS were significantly different than those observed for PFOA. Following a cessation of exposure, the greatest concentration of PFOS was determined in liver tissues, with lesser concentrations in kidney and brain (Fig. 6D~F). Hepatic samples of chicken from the high-dosed treatment (total 0.2mg PFOS dosed) accumulated up to 769 ng/g ww, which was 5- and 55-fold greater than those in kidney and brain, respectively. Even after a 4-wk depuration period, concentrations of PFOS in organs were not significantly changed, while PFOS level increased in brain tissue of the least dosed chickens ($p < 0.05$). Experimental chickens incubated with only saline buffer also contained detectable amounts of PFOS in liver tissues in all experimental periods, and brain and kidney tissues only after a depuration phase.

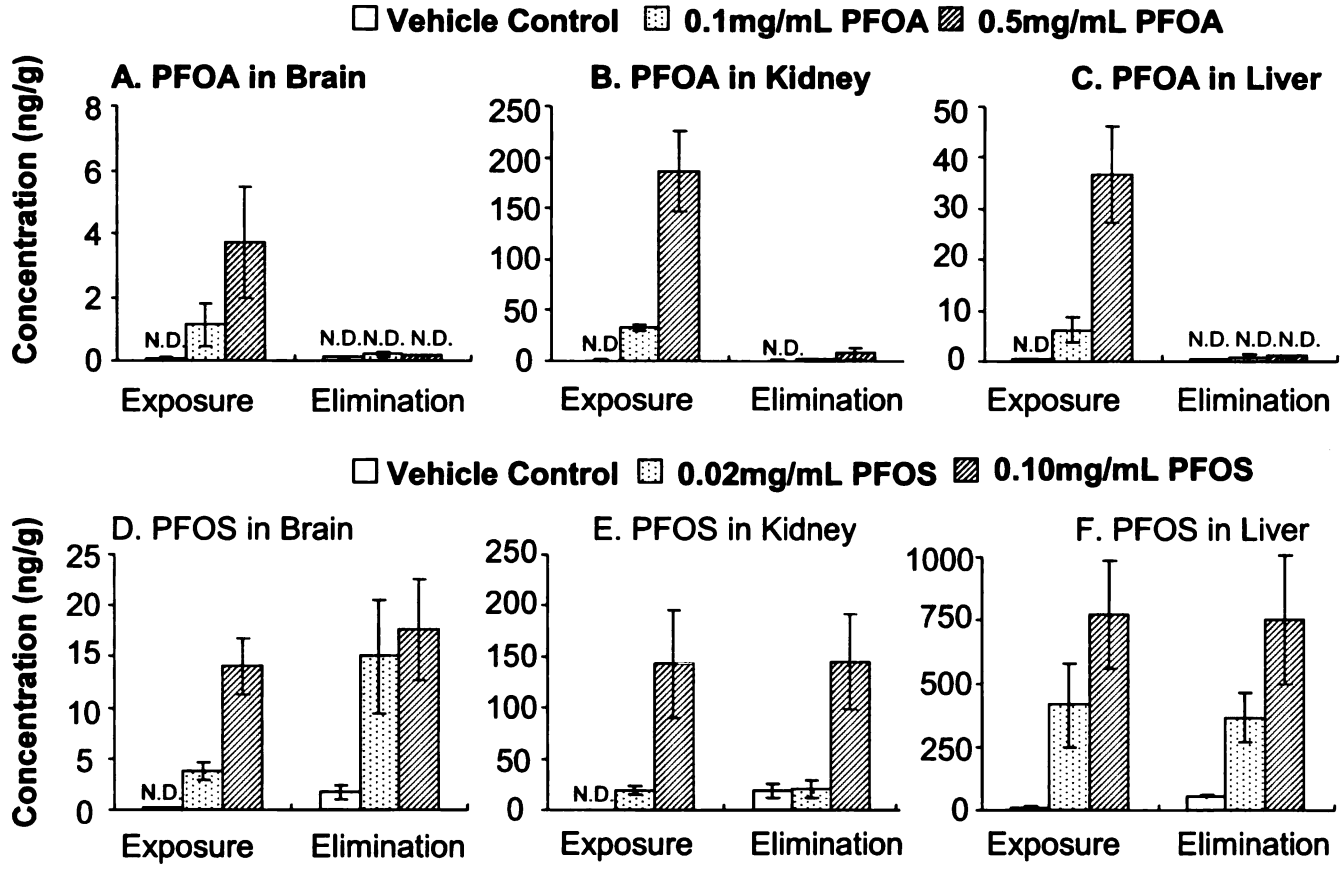

Figure 6. Concentrations (ng/g ww) of PFOA (A~C) or PFOS (D~F) in organs retrieved from chickens at the completion of an exposure and a depuration period (n=3). (N.D. < LOQ)

Discussion

PFOA vs. PFOS elimination kinetics

The observation that introduced PFOS was retained longer in the *G. gallus* than PFOA is consistent with the empirical results from other animals (Butenhoff, et al., 2004; Lau et al., 2004). This different elimination kinetics is interesting, in that these two compounds are structurally analogous anionic surfactants and possess comparable physico-chemical properties such as a low *p*Ka (presumably <3), a low Henry's Law constant, and similar hydrophobicity based on critical micelle concentration (CMC for $C_7F_{15}COOH$ = 8.7 ~ 10.5 mmol/L; CMC for $C_8F_{17}SO_3K$ = 8 mmol/L) (Kissa, 2001). In contrast, they can be distinguished with their hydrophilic carboxylic and sulfonic functional group that endow them with surfactant properties and the fact that overall, PFOS contains one additional carbon in the perfluorinated portion of the molecule.

To date there has been no clear mechanistic explanation of the dissimilar elimination behaviors among PFAs with few suggested mechanisms. At present, urinary excretion is known to a major pathway for clearing PFOA from mammals, most likely by the process of renal tubular secretion (Harada *et al.*, 2005). Following an intra-peritoneal administration, 55% of dosed PFOA was eliminated in both male and female rates within 120 h after an injection, while only less than 1% was eliminated in the feces (Kudo *et al.*, 2001). The level of organic anion transporters (OATs) mRNA present in kidney of the male rat was only 13% of that present in the female rat whose clearance rate is greater than the male rat (Kudo *et al.*, 2002). Surprisingly, castrated male rats showed the increased expression of OAT2 mRNA and competent eliminating ability of PFOA to that of female rats, which suggests an important role of these transporter proteins for PFOA

removal via the kidney and also influences of sex-hormones (Kudo *et al.*, 2001; Kudo *et al.*, 2002). In a case of PFOS, removal mechanisms such as an active transport via OATs have not been well examined. Rather than facilitating of PFOS removal, retardation processes of PFOS removal could be hypothesized such as a well-known enterohepatic-recycling. In addition, greater binding strength of PFOS for serum proteins may also contribute the increased retention of PFOS than PFOA, by reducing renal excretion efficiency by mitigating water-soluble nature of its conjugate base ($C_8F_{17}SO^{-3}$) dominant species at physiological pH. For example, displacement potency of PFOS for native hormones in chicken serum was greater than that of PFOA (Jones *et al.*, 2003). It may be useful to study comparatively the modulation of expression of OATs or other transporters in kidney after a PFOS exposure, which would help to elucidate the observed differences between the clearance rates of PFOA and PFOS in birds.

Organ distribution

Tissue disposition of these persistent and anionic PFAs are different from that of conventional persistent, but nonionic organic contaminants such as PCBs and PAHs, which tend to accumulate in fat tissue. In contrast, the primary sites of accumulation for PFAs are found to be in blood, liver, and kidney depending on which species are investigated. One of the reasons for the favored accumulation in those sites is the binding of PFAs to protein albumin, which is abundant in blood and liver (Jones et al., 2003; Martine et al., 2003a). Though the octanol-water partitioning coefficients (K_{ow}) are incorporated in a bioaccumulation modeling for hydrophobic compounds, the amphiphilic nature of PFCs makes calculation of K_{ow} impossible. Even though a K_{ow} for

PFOA or PFOS could be driven using its solubility in water and octanol determined separately, an estimate predicts less potential to accumulate in adipose tissue.

Among the organs and blood, kidney accumulated the greatest concentrations of PFOA with its relative concentration being intensified toward the end of a depuration period (Figure 7). As already discussed, renal uptake processes by the OAT family for removal of PFOA may also contribute accumulation of PFOA in kidney tissues as an intermediate step before urinary excretion. Efficient exclusion of PFOA organ residue once an exposure source was removed may also imply the dynamic redistribution of organ-accumulated PFOA into the blood compartment where subsequent active elimination processes follow. Liver is recognized as a central site for PFOS storage, thus this tissue has been frequently analyzed in monitoring studies of PFAs (Houde *et al.*, 2006). Recirculation of PFOS via enterohepatic system has been suggested as one reason for extended retention of PFOS in the body (Johnson *et al.*, 1984; Lau *et al.*, 2004). In aquatic exposure studies, it was shown that relatively great amounts of PFOS were accumulated in organs related to enterohepatic cycling such as the gall bladder and liver (Martin *et al.*, 2003a). Dietary PFOS was more efficiently absorbed into trout intestine than food-borne PFOA, supporting the enterohepatic recirculation hypothesis (Martin *et al.*, 2003b). In all tissues examined, PFOS concentrations were not significantly varied between a cessation of exposure and depuration, rather a statistically significant increase of PFOS level was observed in brain tissue. The ratio of PFOS concentration in liver to that in blood from low-dosed treatment ($PFOS_{liver:blood}$ = 15) was three times greater than that determined in high-dosed treatment ($PFOS_{liver:blood}$ = 5) following an exposure period, but was similar at the end of a depuration phase with increased ratios ($PFOS_{liver:blood}$ = 33

and 29 for low- and high-dose treatment, respectively). This implies that either the mechanism of elimination of PFOS from liver is a slow process compared to that from blood, or blood-borne PFOS may be redistributed to liver. This liver to blood ratios could be different in wildlife, where precursors of PFOS either identified or not also contribute overall bioaccumulation of PFOS which is the ultimate degradation product of perfluorooctane sulfonyl fluoride based products (Giesy and Kannan, 2002).

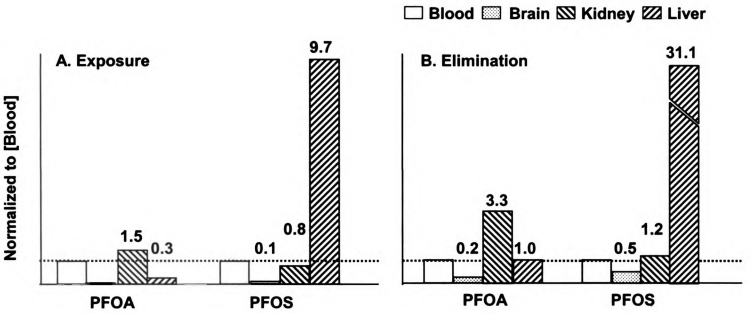

Figure 7. Normalization of individual organ concentration to blood concentration after an exposure (A) and a depuration period (B). Number on top of the bar represents fold difference of concentration on average of two exposure levels

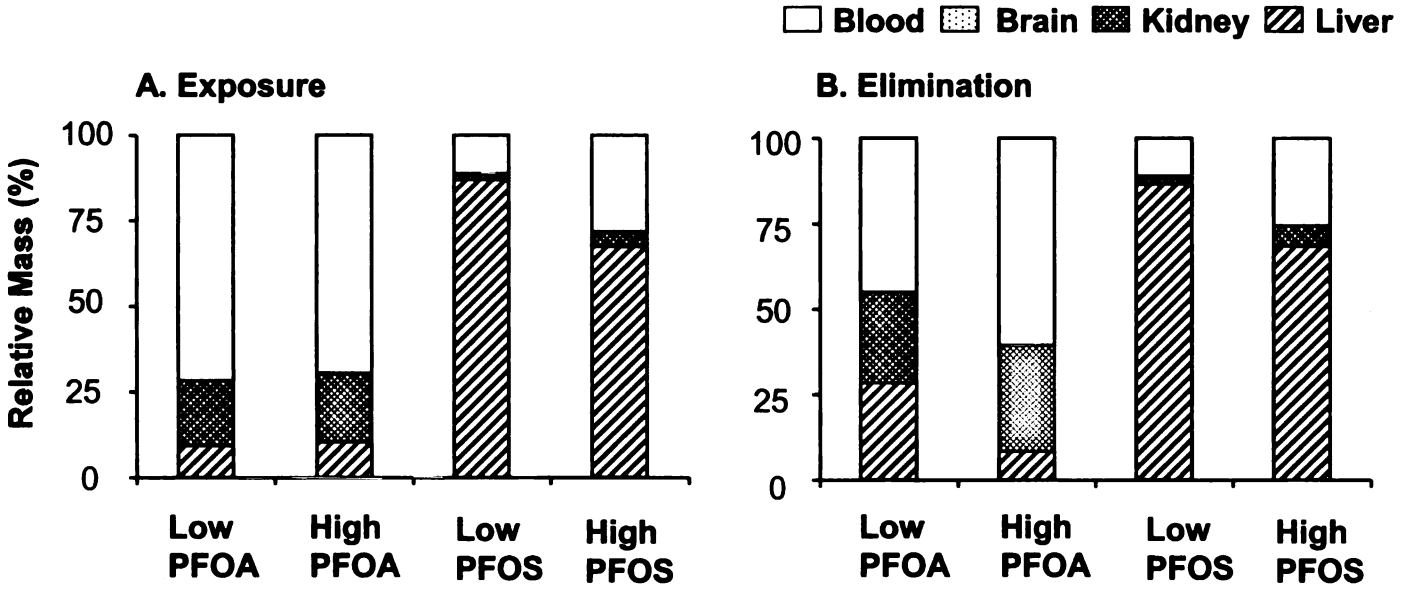

Figure 8. Relative mass (%) of PFOA or PFOS in body reservoirs (blood, brain, kidney, and liver) after an exposure and a depuration period. Low- and high-dosed PFOA treatment had total 0.2mg and 1.0mg of PFOA for a 4wk exposure, respectively, while one fifth of amounts were administered into low- and high-dosed PFOS treatment. A 5% of body weight was assumed to be total volume of blood in the experimental chickens

Analysis of the relative mass of PFOA or PFOS in each organ following an exposure and a depuration phase demonstrated that blood and liver are the largest reservoirs controlling PFOA and PFOS body-burden in exposed chickens, respectively (Figure 8). The estimated total quantity of PFOA or PFOS that remained in bodies (= blood + brain + kidney + liver) of chickens at the end of a depuration period was only 0.7% and 20.4% of total PFOA and PFOS administered via subcutaneous implantations. This small PFOA body residue is in part attributed to rapid removal characteristics of PFOA from the body. However, this mass balance of introduced PFOA and PFOS has some uncertainty. First of all, we did not retrieve the introduced capsule containing PFOA or PFOS for residual determination at the completion of treatments. We did not measure PFAs concentrations in chicken flesh that accounts for the most of body weight due to the lack of proper extraction method.

Age-dependent organ accumulation of PFOS was observed in non-exposed chickens in this study (Figure 6D-F). Until the first 4-wk exposure phase was over, all the organ PFOS concentrations were not measurable; however significant PFOS levels were measured in brain, kidney, and liver tissue following a 4-wk extended incubation. The effects of age on PFOS accumulation were observed in studies with domestic farm animals (Guruge et al., 2005). In general, PFOS concentrations in liver and blood increased with ages of farm animals investigated. PFOS concentration in the diet of experimental chickens was not determined in this study. However, the influences of supplementary food as a likely exposure pathway to livestock need to be further evaluated, since consumption of domestic animals could be an exposure route of PFAs to human population. Detection of PFOA and PFOS in brain of the exposed chickens

suggests that in birds which are exposed to PFAs, these anionic compounds may cross the blood-brain barrier that is vital to inhibit entry of xenobiotic contaminants into the central nervous system. Similar to this laboratory scale exposure study, measurable concentrations of PFOS were reported in brain tissues of wild birds such as glaucous gulls and pelicans (Verreault *et al.*, 2005; Olivero-Verbel *et al.*, 2006). However, careful interpretation should be taken with brain data, because anatomical complexity of brain introduces PFOS contamination through extraction errors such that blood capillaries are mistakenly included in tissue homogenate. In addition, the fact that only the free anion can travel into brain mitigates the possibility of PFOS or PFOA passage, presumably bound to plasma proteins (Kaassen *et al.*, 2001).

Sublethal effects

The observation of statistically significant lesser total cholesterol and phospholipid levels in plasma after the PFOS exposure is consistent with other PFOS exposure studies (Seacat *et al.*, 2003). Inhibition of the rate-limiting enzyme, HMG-CoA reductase in cholesterol synthesis was observed in PFOS-exposed rats (Seacat *et al.*, 2003). Interestingly, the significant decline of total cholesterol and phospholipids at the end of a depuration phase rather than at the end of an exposure phase suggests the current exposure levels and/or 4-wk exposure duration were not enough to cause altered lipid metabolism in chickens, but prolonged PFOS exposure until the depuration phase pronounced the reduction of those lipids levels. However, these changes did not affect histopathology of the exposed chickens with any significant lesions being not seen in examined organs of chickens from treated groups. It has been suggested that high PFOA

exposure could interfere with genes involved in fatty acid metabolism and cholesterol syntheses in rats (Guruge *et al.*, 2006), however fatty acid related parameters were not changed in any PFOA treatments in this experiment.

Comparison with other studies

The biological half-life of PFOA and PFOS in *G. gallus* were compared with other male species and summarized in Table 9. The half-life of PFOA from the chicken ($t_{1/2}$ = 5.2 d) were comparable to that of the rat ($t_{1/2}$ = 5.6 d), and about 4 times faster than that of the dog ($t_{1/2}$ = 20 ~ 23 d) and monkey ($t_{1/2}$ = 21 d). Thus, the half-life seems to be a function of body size and/or physiological parameters, such that fish ($t_{1/2}$ = 4.5 d) have the fastest clearance of PFOA, followed by the chicken=rat>dog=monkey> human (Harada *et al.*, 2005). In contrast, the biological half-life of PFOS in our treated chicken ($t_{1/2}$ = 125 d) was similar to mammals including the rat ($t_{1/2}$ > 90 d) and the monkey ($t_{1/2}$ = 100 ~ 200 d). This suggests, unlike PFOA body behavior, that a number of vertebrates share common accumulation mechanisms for PFOS, through binding to plasma proteins as a governing process that is unlike in invertebrates (e.g., fish PFOS $t_{1/2}$ = 12 d).

The only available pharmacokinetic study with two bird species reported a shorter biological half-life for PFOS than those obtained in our experiment (Newsted et al., 2005). Several factors may have influenced the pharmacokinetics in the two experimental animal studies. First, in our study we used a subcutaneous implantation method, while they employed a dietary exposure dosing (Newsted *et al.*, 2005). Metabolism of target compound introduced often affects pharmacokinetic behavior in animals, but virtually non-metabolized property of PFOA or PFOS ignores chances that

may result in observed different pharmacokinetics in two studies. Secondly, exposure levels were far different in the two studies, which could modify removal kinetics. At the highest treatment in a current study, blood PFOS concentrations were realized at most tens of ppb level that were approximately three order less than those measured at tens of ppm level in the previous study. That high exposure scheme was attributed to the fact that they designed their experiment to determine the acute toxicity of dietary PFOS in bird species. A biphasic type of PFOS elimination was seen in a mammalian study with PFOS-treated cynomolgus monkeys (Seacat *et al.*, 2002). In their study, the elimination rate in first 50 days after cessation appeared to be faster than that at the end of a 1-year depuration period. Similarly, high PFOS-treated chickens eliminated blood-borne PFOS almost twice as faster as did low PFOS-dosed chickens in this study. These observations may indicate that the biological mechanism of PFOS elimination is acting differently at lower- and higher- body burdens.

Table 9. Elimination half-life (days) of PFOA and PFOS in blood or serum from other studies

PFOA	Sex	Dose Form	Half-life	Sources
Chicken	Male	Implant	5.2	This study
Rat	Male	i.v.	5.6	Ohmori et al. (2003)
Dog	Male	i.v.	20 and 23	Hanhijärvi et al. (1988)
Monkey	Male	i.v.	21	Noker (2003)
Human	Both	Occupational	1600	Burris et al. (2002)

PFOS	Sex	Dose Form	Half-life	Source
Chicken	Male	Implant	125	This study
Mallard	Both	Dietary	6.9	Newsted et al. (2005)
Rat	Male	i.v.(^{14}C-labelled)	>90	Jonhson et al. (1979)
Monkey	Male	Oral	100~200	Seacat et al. (2002)
Human	Both	Occupational	1428	3M (2000)

Chapter 3

Perfluoroalkyl Acids in Marine Organisms from Lake Shihwa, Korea

Abstracts

Concentrations of eight perfluoroalkyl acids (PFAs) were determined in three marine fishes (mullet, shad, and rockfish) and marine invertebrates (blue crab, oyster, and mussel) from Lake Shihwa, Korea, where great concentrations of PFAs in waters of Lake Shihwa and adjacent industrial effluents were reported. Perfluorooctanesulfonate (PFOS) was the dominant compound of PFAs analyzed in these marine organisms and most PFOS concentrations were greater than the sum of all other PFAs monitored (PFHS + ΣPFCAs + N-EtFOSAA). The mean concentrations of PFOS were 8.1×10^{1} and 3.6×10^{1} ng/g ww in liver and blood of fish, respectively. Perfluorocarboxylic acids (PFCAs) were also found in fish at ten-fold lower concentrations. Longer chain PFCAs, such as perfluorododecanoic acid (PFDoA, C12) and perfluoroundecanoic acid (PFUnA, C11) occurred in fish at greater concentrations than did perfluorononanoic acid (PFNA, C9) and perfluorooctanoic acid (PFOA, C8). A spatial comparison of blue crab data showed that PFOS concentrations in soft tissues decreased as a function of distance from the shore and the inputs from the industrialized areas where wastewaters are discharged into Lake Shihwa. Only PFOS was a detectable PFA in mussel and oyster with a mean of 0.5±0.2 ng/g and 1.1±0.3 ng/g ww, respectively. A significant positive correlation was observed between concentrations of PFUnA and PFDA in both liver and blood of fish, implying the same sources of PFCAs in this area. The calculated hazard quotients (HQs) using the estimated whole body PFOS residue were less than 1.0 for all fishes. For

example, an HQ calculated from the greatest PFOS in fish was estimated to be only 6×10^{-4}. Current concentrations of PFOS in fish in Lake Shihwa are not expected to cause acute lethality of the fish.

Introduction

Perfluoroalkyl acids (PFAs) have been commonly used in industrial applications since the mid 1940's, as surface protectors for carpets and leather, active-component in fire-fighting foams and as processing aids in the production of fluorinated polymers (Kissa, 2001; Giesy and Kannan, 2002). Recent global PFAs monitoring of biota have found that the perfluorooctanesulfonate (PFOS) and other related PFAs are ubiquitously found in various tissues of animals ranging from invertebrates, fishes, birds, and mammals globally irrespective of locations and some of them are biomagnified in the upper tropic level organisms (Vijver *et al.*, 2003; Sinclair *et al.*, 2006). To date, PFOS has been found to be the predominant compound among PFAs analyzed with current analytical techniques, while another toxicological counterpart of interest, perfluorooctanoate (PFOA) is not persistent in tissues of wildlife except human serum (Houde *et al.*, 2006; Kannan et al., 2004). While there have been some assessments of the ecological risks of these newly emerged organic contaminants based on accumulated PFAs exposure data around the globe (OECD, 2002; U.S. EPA, 2003), few data are available for assessing current potential risks of PFAs in wildlife from Korea (Kannan et al., 2002).

Lake Shihwa, located on the west coast of Korea, is an artificial saltwater lake that has been receiving industrial wastewater discharges from the Shihwa and Banweol Industrial Complexes (SBICs, approximate total industrial area = 31 km^2) since its

construction in 1994 (Figure 9). Although this man-made lake's function was originally planned to supply waters to SBICs and nearby agricultural areas, gradual deterioration of lake water quality prompted scientific studies evaluating the environment quality of Lake Shihwa (Jung et al., 1997). Those research efforts indicated moderate-to-relatively great concentrations of trace metals and persistent organic pollutants such as PCBs, PAHs, organochlorine insecticides, and alkylphenols in waters and sediments from this region (Khim et al., 1999; Li et al., 2004). Recently, concentrations of PFAs in waters of the lake and tributary streams were reported with greatly elevated water-borne PFOS, suggesting that local sources discharge PFAs or their precursors into the lake (Rostkowski et al., 2006).

Lake Shihwa (surface area = 56.5 km^2, drainage basin = 476.5 km^2) provides valuable habitats for diverse aquatic animals and birds, particularly migratory species. Because significant quantities of PFAs were observed in Lake Shihwa, it was deemed prudent to determine concentrations of PFAs in aquatic animals of Lake Shihwa and assess the potential risks that these compounds might pose to those organisms and the organisms that might eat them. In the present study, concentrations of PFOS and other PFAs were measured in various marine organisms (fish, crab, mussel, and oyster) from Lake Shihwa. Since PFAs have a propensity to bind to proteins and be retained in the blood and liver, fish blood and samples of liver and or hepatopancreas were collected at various locations in Lake Shihwa for instrumental analysis. Second, the current status of PFOS contamination in this region was assessed by comparing results from this study to concentrations of PFOS that have been reported to occur in fish from other parts of the world. Third, the sources of PFAs in this region are discussed based on the current

understanding of the environmental fate of PFAs. Finally, a screening level hazard assessment was conducted using PFOS concentrations in the organisms.

Materials and Methods

Sample Collection

Fishes and blue crabs were collected in May 2006 at two locations near to the SBICs and at two locations near the inland discharges from Lake Shihwa (Figure 9). Stationary fish nets were installed at sampling locations for three days before collection. Immediately after net fishing, all samples were kept in iceboxes and transported to the laboratory where samples of blood and liver were taken. Unfortunately, large amounts of entrapped jelly fish caused mortality of several fish species and resulted in only one or two fish species being available at each location. Composite liver and blood samples (two fish per composite, number of composite = 3) of mullet (*Chelon haematochelia*, mean fish weight = $3.5\times10^{1}\pm4.5$ g wet weight, ww), rockfish (*Sebastes schlegeli*, mean fish weight = $8.5\times10^{1}\pm1.1\times10^{1}$ g ww), and shad (*Konosirus punctatus*, mean fish weight = $3.8\times10^{1}\pm6.0$ g ww) were prepared. Fish blood was collected by use of a heparinated syringe and transferred in a 15mL polypropylene (PP) tube. Mussel and oyster were collected at one location from a barge ship located in the middle of the lake (Figure 9). Soft tissues of blue crab (*Portunus trituberculatus*, three individuals at each location) and marine invertebrates (mussel for n=6 and oyster for n=4) were used for PFAs analysis. Tissues were then mechanically homogenized with a vortex mixer and kept at negative 20°C until the extraction. To avoid cross contamination, the homogenizer probe and parts were thoroughly rinsed with Milli-Q water and methanol (MeOH) between homogenizing.

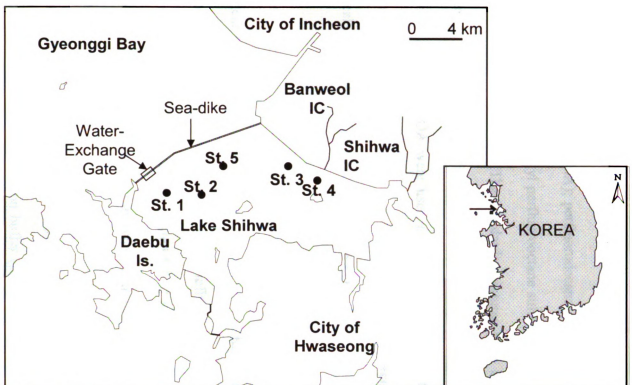

Figure 9. Map showing sampling locations in Lake Shihwa study for marine fishes and invertebrates. Fish and blue crabs were collected at four locations (Stations 1 to 4) and mussels and oysters were collected only at one location (Station 5). Seawaters of Gyeonggi Bay are now allowed to flow in the lake through a water-exchange gate during flood tides. IC = industrial complex

Chemicals and Standards

A mixture of PFAs was used as an external standard and contained two perfluoroalkyl sulfonates that included PFOS and perfluorohexanesulfonate; PFHS) as well as five perfluoroalkyl carboxylic acids including perfluorododecanoic acid (PFDoA); perfluoroundecanoic acid (PFUnA); perfluorodecanoic acid (PFDA); perfluorononanoic acid (PFNA); PFOA, and N-ethyl perfluorooctane sulfonamide acetate (N-EtFOSAA) at 10 ng/mL for each standard. The standards mixtures were supplied by the National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan. [^{13}C]PFOS and [^{13}C]PFOA were used as internal standards. Pesticide-grade MeOH, ammonium acetate, and ammonium solution (25%) were purchased from Wako Pure Chemicals, Japan. Milli-Q® (Bedford, MA) water was used in the whole experiment.

Sample Preparation

Biological samples were extracted with an ion-pairing liquid extraction method with some modifications (Kannan *et al.*, 2004). Briefly, an aliquant of blood (0.5 mL) or tissue homogenate (0.5 g wet wt) was diluted 5-fold with Milli-Q water. One milliliter of diluted blood or tissue-water mixture was then transferred into a 15mL PP tube. Extraction solutions and solvent were pipetted into the mixture in the following sequence; 1mL of 0.5M ion-pairing agent (tetrabutyl ammonium hydrogensulfate) adjusted to pH 10, two milliliter of 0.25M extraction buffer (sodium carbonate + sodium bicarbonate), and 5mL of methyl *tert*-butyl ether (MTBE). Samples were rigorously shaken for 20 min followed by centrifugation for 15 min (2×10^3 rpm). The isolated organic phase was transferred to a clean 15 mL PP centrifuge tube. This extraction was repeated twice and

all combined MTBE supernatants were evaporated to near dryness under a gentle stream of nitrogen. The sample was then re-dissolved in 1 ml MeOH. Further clean-up of resulting extracts were conducted using solid-phase extraction (SPE) with some modifications (So *et al.*, 2006). Briefly, a half milliliter aliquant of unfiltered extracts was diluted with 100mL Milli-Q water and consequential water-extract mixture was passed through an Oasis WAX® cartridge (0.2g, 6 cm^3) at an elution rate of 1 drop/sec. After sample loading was completed, cartridge was washed in the sequence of buffer (25mM acetic acid 170mL + 25mM ammonium acetate 30mL) adjusted to pH 4 and MeOH. The fraction containing PFAs of interest was retrieved by eluting 0.1% NH_4OH dissolved in MeOH. Teflon or glassware was avoided in whole extraction procedure to remove possible contamination of samples and sorption of analytes.

PFAs Analysis by LC/MS/MS and QA/QC

Separation and quantification of PFAs in biological samples were performed using an Agilent HP 1100 liquid chromatography (HPLC) (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro II mass spectrometer (MS/MS) (Waters Corp., Milford, Massachusetts) operated in electrospray negative ionization mode. Ten microliters aliquot of extract was injected onto a Keystone Betasil C_{18} column (2.1 mm i.d. $\times$ 50 mm length, 5 μm) with 2 mM ammonium acetate and MeOH as mobile phase, starting at 10% MeOH. Detailed instrumental parameters can be found in Taniyasu et al., 2003. Concentrations of PFAs in extracts were quantified using external calibration curves (1×10^{-2}, 5×10^{-2}, 2×10^{-1}, 1×10^{0}, 1×10^{1} ng/mL). Acquired data were deemed to be acceptable if QC standard measured after every 10 injections fell within 30% of its

theoretical value, otherwise samples were run again with a newly constructed calibration curve. The limit of quantification (LOQ) in this study was determined to be 0.25 ng/g wet wt for all analytes in all sample types.

To ensure the quality of analytical procedures, procedural blank, procedural recovery were performed with each set of extraction batch, and matrix-spiked recovery were tested for each type of biota. All target PFAs in procedural blanks were below LOQ. Mean recoveries (n=2) from each biota homogenate sample (0.5 g or 0.5 mL) spiked with 10 ng PFAs were 90.1% for PFOS, 87.5% for PFHS, 81.1% for PFDoA, 97.0% for PFUnA, 105.5% for PFDA, 100.9% for PFNA, 80.4% for PFOA, 79.5% for [^{13}C]PFOA and 78.1% for N-EtFOSAA. Mean matrix-spike recoveries for two internal standards [^{13}C]PFOS and [^{13}C]PFOA were 90.6% and 78.1%, respectively. Concentrations reported in this paper were not adjusted for matrix recoveries. For calculation of mean concentrations, values determined to be less than the LOQ were assigned as one half of LOQ.

Data and Statistical Analysis

The normality of the concentration data was analyzed by means of a Kolmogorov-Smirnov test. Differences in PFOS concentrations in tissues of animals among locations were tested by one-way ANOVA (Type I error level of 5%). The Tukey test was used as post-hoc criterion. Because equal variance was not obtained for PFOS concentrations from Lake Shihwa, Japan, and other locations, to which concentrations determined in this study, were compared by use of the non-parametric Kruskal-Wallis test without data transformation. Associations between PFA concentrations in fish were investigated using

a Pearson correlation analysis. All statistical analyses were performed with the SYSTAT® 11 Package (SYSTAT Software Inc., Richmond, CA).

Results

PFAs Concentrations in Marine Fishes

Concentrations of two sulfonate PFAs (PFOS and PFHS), and homologous carboxylic PFAs with carbon-chain lengths between 8 and 12, and N-EtFOSAA were determined in blood and liver of three marine fish species (rockfish, shad, and mullet) (Table 10). As has been reported in previous studies of PFA in biota, PFOS was the predominant PFA in fish caught in Lake Shihwa. Concentrations of PFOS in all individual fish were greater than the sum of all other PFAs monitored (PFHS + ΣPFCAs + N-EtFOSAA). In general, fish liver contained greater concentrations of PFOS than did blood (12 out of 18 individuals). A sample of liver from a mullet captured at station 4 had 2.5×10^{2} ng PFOS/g ww, which was the greatest concentration of PFAs determined in this study; meanwhile blood samples from mullet at station 3 contained a concentration as great as 9.3×10^{1} ng PFOS/mL. Analyzed by species, mullet ($1.9\times10^{2}\pm3.2\times10^{1}$ ng/g, ww) accumulated 6.8 to 8.2-fold greater concentrations of PFOS in their livers than did rockfish or shad (Figure 10). In contrast, the fold-difference in concentration of PFOS in blood ranged from 1.9- (mullet to rockfish) to 3.0-fold (mullet to shad). PFHS was detectable in most samples and concentrations in liver (<LOQ-2.0 ng/g ww) and blood (<LOQ-1.6 ng/mL) were similar. Detectable concentrations of N-EtFOSAA were measured in livers of mullet (14.1±4.3 ng/g ww), however in rock fish and shad, N-

EtFOSAA was in the range of <LOQ to 1.8 ng/g ww in liver and <LOQ to 4.2 ng/mL in blood.

Table 10. Mean PFAs concentrations (ng/g ww.) in the tissues of fish collected from Lake Shihwa, Korea[a]

Location	Species	PFOS	PFHS	PFDoA	PFUnA	PFDA	PFNA	PFOA	N-EtFOSAA
				Fish Liver					
St. 1	Rockfish	2.20×10^{1}	1.10	1.07	1.42	0.67	0.67	1.09	1.70
	Shad	2.79×10^{1}	0.13	0.64	1.83	1.29	0.45	0.32	0.45
St. 2	Rockfish	2.47×10^{1}	1.47	0.78	1.24	0.59	0.60	1.66	1.41
St. 3	Shad	2.85×10^{1}	0.13	0.49	1.12	1.27	0.57	0.59	0.36
	Mullet	1.76×10^{2}	0.96	1.35×10^{1}	2.38×10^{1}	1.04×10^{1}	2.89	1.72	1.32×10^{1}
St. 4	Mullet	2.08×10^{2}	1.31	1.57×10^{1}	2.60×10^{1}	1.34×10^{1}	3.82	1.74	1.51×10^{1}
Min		1.77×10^{1}	<0.25	0.34	0.89	0.46	<0.25	<0.25	0.27
Max	all (n=18)	2.52×10^{2}	1.61	1.89×10^{1}	3.06×10^{1}	1.50×10^{1}	4.23	2.16	1.95×10^{1}
Mean		8.12×10^{1}	0.85	5.37	9.23	4.61	1.50	1.19	5.36
				Fish Blood					
St. 1	Rockfish	2.83×10^{1}	1.02	2.16	3.60	1.90	1.38	1.61	2.64
	Shad	2.15×10^{1}	0.37	1.33	1.88	1.42	0.78	0.77	0.83
St. 2	Rockfish	3.14×10^{1}	1.35	2.82	3.62	1.86	1.86	3.12	3.33
St. 3	Shad	1.72×10^{1}	0.18	0.20	0.82	1.01	0.42	0.51	0.17
	Mullet	7.45×10^{1}	0.52	5.62	11.39	6.80	1.38	0.94	3.27
St. 4	Mullet	4.05×10^{1}	0.39	5.72	11.28	6.25	1.51	0.57	2.96
Min		1.48×10^{1}	<0.25	<0.25	0.72	0.81	0.25	0.33	<0.25
Max	all (n=18)	9.32×10^{1}	1.96	8.74	15.13	8.86	2.68	5.15	5.27
Mean		3.55×10^{1}	0.64	2.98	5.43	3.21	1.22	1.26	2.20

[a] Values are given in the mean of three replications and values below the limit of quantification (LOQ) were assigned as one half of LOQ in calculation of the means.

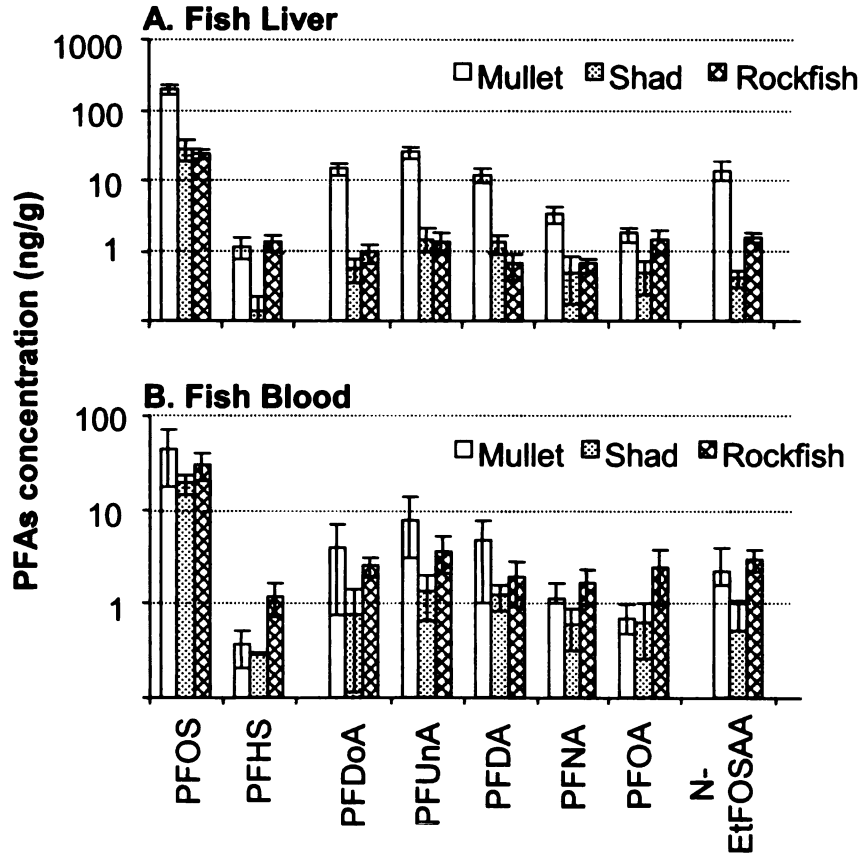

Figure 10. Species comparison of PFA concentrations in mullet, shad, and rockfish collected from Lake Shihwa. Mean concentrations were location-averaged (n=6). Error bars represent one standard deviation.

In general, longer chain carboxylated PFAs, such as PFDoA (C12) and PFUnA (C11) occurred in fish at greater concentrations than did PFNA (C9) and PFOA (C8) (Table 10). Liver of mullet contained relatively great concentrations of PFUnA ($2.5\times10^{1}\pm4.5$ ng/g ww) which was the greatest concentration of the PFCAs, followed by PFDoA > PFDA > PFNA > PFOA. Concentrations of PFCAs in rockfish and shad were only detectable in the liver (<1.5 ng/g ww). The mean hepatic PFOA concentration was 1.2±0.7 ng/g ww in all fishes. As observed for PFOS, lesser concentrations of PFCAs were measured in blood than liver of mullet. The greatest concentration of PFCA in fish blood was observed for mullet ($1.1\times10^{1}\pm2.7$ ng PFUnA/mL) with lesser concentrations in blood observed in decreasing of PFDA > PFDoA > PFNA > PFOA. In contrast, concentrations of PFCAs in blood of other fish species were less than those in mullett, with concentrations ranging from 1.6 to 3.6 ng/mL in rockfish and from 0.6 to 1.4 ng/mL in shad.

PFAs Concentration in Marine Invertebrates

Mean concentrations of PFOS, PFHS, PFDoA, PFUnA, PFDA, PFNA, PFOA and N-EtFOSAA in marine invertebrates are summarized in Table 11. While blue crabs were available at four locations (Station 1~4), mussels and oysters were only available in the middle of the lake (Station 5). Similar to PFAs accumulation patterns in fish, PFOS was the predominant PFA analyzed in these marine organisms. PFOS concentrations in the soft-tissue of crabs from Station 3 (9.6±1.0 ng/g ww) and Station 4 (8.3±0.1 ng/g ww) contained significantly greater PFOS concentrations than those from Stations 1 and 2 ($p<0.05$). PFOS concentrations in mussel and oyster collected from Station 5 were

0.6±0.2 ng/g ww and 1.1±0.3 ng/g ww, respectively and were less than those observed in blue crabs sampled in this study. In blue crab samples, concentrations of PFDoA ranged from 1.4 to 6.2 ng/g ww while PFUnA concentrations ranged from 1.4 to 1.9 ng/g ww. The concentrations of PFDoA and PFUnA were greater than the other quantified PFCAs, which contained concentrations ranging from 0.3 to 0.8 ng/g ww. PFOA was detected in all crab tissues but at quantifiable concentrations that ranged from 0.25 to 0.82 ng/g ww. With the exception of PFOA in oysters, all concentrations of the targeted PFCAs in mussel and oyster were less than their respective LOQ values. No quantifiable concentrations of either PFHS or N-EtFOSAA were observed in crabs, mussels or oysters (<LOQ).

Table 11. PFAs concentrations (ng/g wet wt) in the soft tissues of marine invertebrates collected from Lake Shihwa, Korea[a]

Location	Species	PFOS	PFHS	PFDoA	PFUnA	PFDA	PFNA	PFOA	N-EtFOSAA
St. 1	Blue crab	5.26	-[b]	1.52	1.89	0.82	0.42	0.63	-
St. 2	Blue crab	4.49	-	1.35	1.61	0.61	0.36	0.51	-
St. 3	Blue crab	9.60	-	1.48	1.63	0.80	0.27	0.29	-
St. 4	Blue crab	8.25	-	1.62	1.43	0.70	0.46	0.68	-
Min		4.60		1.30	1.41	0.60	<LOQ	0.25	
Max	all (n=12)	1.12×10^{1}		2.00	2.57	0.91	0.55	0.82	
Mean		7.39	-	1.63	1.80	0.80	0.41	0.58	-
St. 5	Mussel	0.55	-	-	-	-	-	-	-
St. 5	Oyster	1.10	-	-	-	-	-	0.31	-

[a] Values are given in the mean of four replications and values below the limit of quantification (LOQ) were assigned as one half of LOQ in calculation of the means. [b] All individual values below the LOQ (=0.25 ng/g wet wt).

Profiles of Relative Concentrations of PFAs in Marine Organisms

Profiles of relative concentrations of PFA in the three species of fish included in this study were similar to those reported in other studies (Houde *et al.*, 2006), with PFOS accounting for the greatest portion of the total mass of PFAs (Figure 11). The proportions of PFAs contributed by PFOS ranged from 72.0~85.2% in liver and 64.1~77.6% in blood. The next most prevalent PFAs were the longer chain PFUnA, which accounted for 5.4~12.9% and 4.2~9.3% of the total PFA concentrations in blood and liver, respectively.

While PFOA is ubiquitous in water samples and is also one of the perfluoroalkyl chemicals found in human blood (Yamashita et al., 2004; Kannan et al., 2004), its contribution to the total blood concentration of PFAs in fish was less than 2.5%. The ratios of PFOS to total PFCAs (ΣPFCA) concentrations in liver ranged from 3.2 in mullet to 6.5 in shad while for blood the ratios ranged from 2.1 (mullet) to 4.1 (shad). In blue crabs, the ratio of PFOS to total PFCA in ranged from 0.9 in samples collected from Station 2 to 2.0 in samples collected from Station 3.

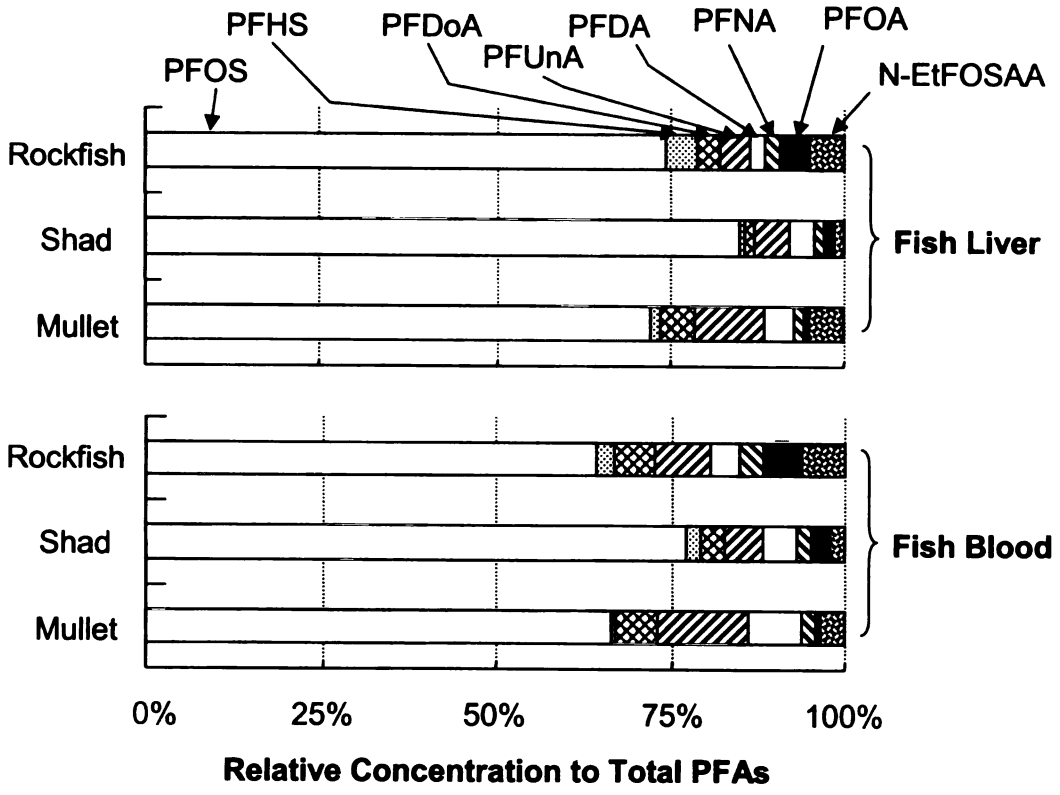

Figure 11. Composition profiles of PFAs in liver and blood of mullet, shad, and rockfish collected from Lake Shihwa

Discussion

Global Comparison of PFOS Concentrations

Extensive monitoring efforts have surveyed PFAs in biological matrices beginning in 2001 and delineated the extent of PFAs concentrations in wildlife, especially in marine mammals in north-America and northern Europe (Giesy and Kannan, 2001; Houde et al., 2006). In Korea, levels of PFAs in waters were first studied in 2005 in the streams of the Shihwa-Banweol industrial complex and Lake Shihwa (Rostkowski et al., 2006). To our knowledge, this is the first report regarding the concentrations of PFOS and other perfluoroalkyl compounds in marine organisms from Korea. Current concentrations of PFOS in the biota of Lake Shihwa were compared to concentrations of PFOS in fish from other locations around the world (Figure 12). In marine fishes collected around Japan, the median PFOS concentration was 6.4×10^{1} ng/g ww in livers (Taniyasu *et al.*, 2003). Global concentrations of PFOS in fish liver (excluding Japan) were also compiled and compared to livers of fish from Lake Shihwa. The global range in PFOS concentrations was 5.3 to 4.1×10^{1} ng/g ww with a median of 1.4×10^{1} ng/g ww. Fishes from Lake Shihwa had a median concentration of 3.1×10^{1} ng PFOS/g ww liver. This value was significantly greater than the median concentration of PFOS in fish liver globally, but was not statistically different from the median in Japan (Mann-Whitney U test, p=0.05). In the case of fish blood, there was no statistical difference between the median for Lake Shihwa (2.7×10^{1} ng PFOS/mL) and that for Japan (3.4×10^{1} ng PFOS/mL). Fishes collected from the Mississippi River in proximate to the 3M Cottage Grove contained far greater PFOS in blood than Lake Shihwa fishes did (MPCA, 2006). For example, a concentration of blood sample of smallmouth bass ranged from 1.7×10^{3} to 4.2×10^{3} ng

PFOS/mL. However, since we were not able to locate additional data on PFOS concentrations in blood of fishes from other global locations, meaningful comparisons could not be made. The relatively great PFOS concentrations observed in fish from Lake Shihwa when compare to the global data may be due to the large amounts of industrial and domestic wastes that Lake Shihwa receives on an annual basis.

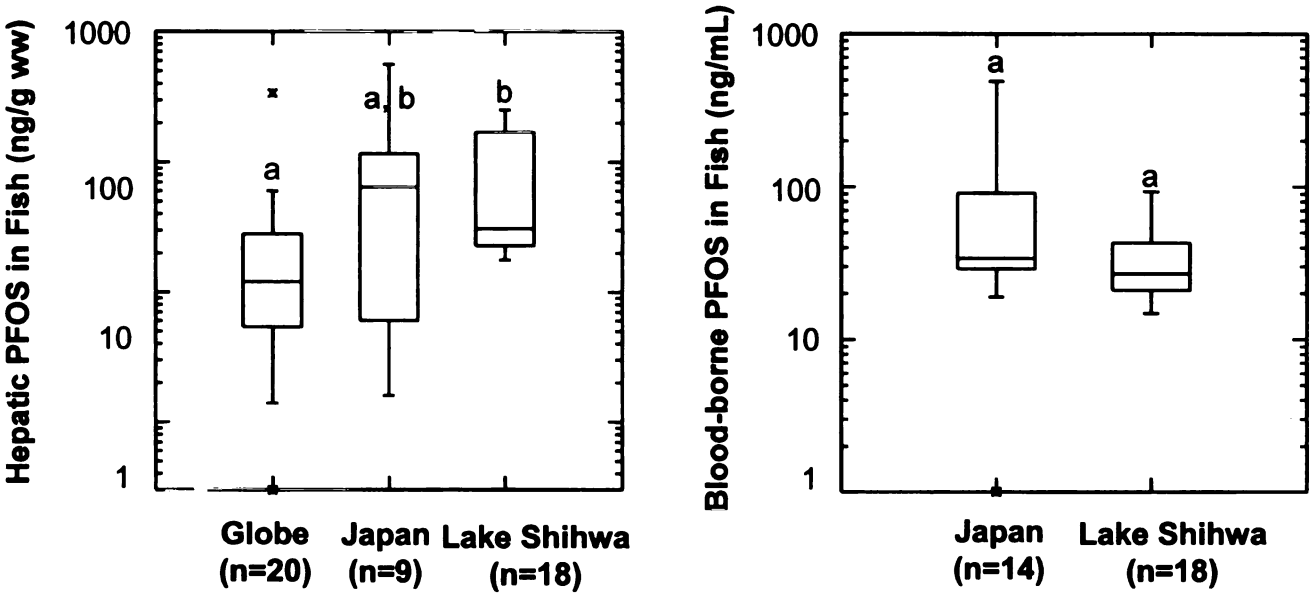

Figure 12. Comparison of PFOS concentrations in liver (ng/g wet wt) and blood (ng/mL) of fishes from Japan, Lake Shihwa and other global locations. The 25th and 75th percentiles define the box (asterisks are outliers). The letters "a" and "b" indicate the significant differences between groups (Mann-Witney U test: $p<0.05$).

Source Estimation of PFAs in Lake Shihwa

The spatial distribution of PFOS concentrations in marine organisms demonstrates that biota samples collected from sites close to the outlets of inland creeks (Stations 3 and 4) were more contaminated than those sampled at sites away from the release sources (Stations 1 and 2) (Table 10 & 11). The greatest mean concentration of PFOS in livers of fish from Lake Shihwa was measured in mullet sampled at Station 4 ($2.1\times10^{2} \pm 3.8\times10^{1}$ ng/g ww liver), while concentrations were approximately 10-fold less in livers of fish collected at Stations 3 and 4. Due to the nature of anionic organic compounds to generally be found in water column rather than to associate with particulates and sediments, elevated concentrations of PFOS in fish and blue crab tissue were primarily attributed to exposure to PFOS in the water column. Concentrations of PFOS in the waters at Stations 3 and 4 were reported to be 1.3×10^{1} and 1.8×10^{1} ng PFOS/L, respectively (Rostkowski et al., 2006). Although concentrations of PFOS in water around Station 1 and 2 are not available, previously reported concentrations of PFOS at locations further from the known effluents would indicate that they should be less at these two stations. In addition, Station 3 and 4 are close to wastewater releases from inland channels containing greater concentrations of PFOS (2.0×10^{1}-6.5×10^{2} ng PFOS/L in streams receiving effluents from SBICs). Direct comparison among locations is also complicated by the fact that different species of fish may have different rates of PFOS accumulation. For example, PFOS concentrations in shad did not differ between Stations 1 and 3. However, a comparison of blue crab data collected at all four locations showed that PFOS concentrations in soft tissues decreased as a function of distance from the

shore and the inputs from the urbanized and industrialized areas where wastewaters are discharged into Lake Shihwa (Table 11).

Even though PFOA was the second most abundant PFA in seawater samples (4.5-1.1×10^{1} ng/L) collected from Lake Shihwa, PFOA concentrations in fish were among the least of the targeted PFAs measured in this study. This observation was similar to that reported for other studies (Martin et al., 2004). In general PFOA has a lesser bioaccumulation potential than other longer chain PFCAs and sulfonated PFAs (Martin et al., 2003a, b). The occurrence of PFOSA in fish was reported elsewhere (Houde et al., 2006, Sinclair et al., 2006), but was not quantified in our samples. However, detection of N-EtFOSAA in marine biota suggests that precursor compounds for PFOS exist in this region and therefore, to a lesser degree, contribute to the overall accumulation of PFOS by marine fishes.

A significant positive correlation was observed between concentrations of PFUnA and PFDA in both liver and blood of fish (Figure 13). Fluorotelomer alcohols (FTOH) are known precursors of PFCAs via several mechanisms such as atmospheric degradation, hydrolysis, and biodegradation (Ellis et al., 2004; Gauthier and Mabury, 2005; Wang et al., 2005). For example, 8:2 FTOH can be degraded to PFNA and PFOA, and through the same processes, 10:2 FTOH could produce PFUnA and PFDA. Thus, significant relationships between PFUnA and PFDA in fish samples are indicative of the existence of the same source and in part, contamination of the semi-volatile 10:2 FTOH may be a source of PFUnA and PFDA in the Lake Shihwa area. The observation of greater concentrations of PFUnA than PFDA in fishes of Lake Shihwa are consistent with the

bioconcentration study results that PFUnA is more bioaccumulative than PFDA (Martin et al., 2003a).

BCFs for PFAs in Fish

Bioconcentration factors (BCF) were estimated for fish by dividing the concentrations of PFAs in fish by those in seawater, which had been previously determined for Lake Shihwa. Though bioaccumulation factor (BAF) is a more accurate estimate for describing the bioavailability from a field exposure, herein we used BCF terminology for convenience. However, because of a lack of information on PFA concentrations in seawater in the outer lake (Station 1 and 2), and the fact that the more bioaccumulative, longer chain PFCAs (PFDoA and PFUnA) were not measured in waters of Lake Shihwa, it was not possible to estimate BCFs for these PFAs. BCF values were determined only for PFOS, PFHS, PFDA, PFNA, and PFOA in mullet collected at location 3 and 4. Values of the BCF for PFOS were estimated to range from 2.2×10^3 to 5.9×10^3 and 1.1×10^4 to 1.4×10^4 in mullet blood and liver, respectively. These ranges are similar to those reported for fishes in Tokyo Bay (1.4×10^3-2.1×10^4) (Taniyasu *et al.*, 2003), but less than BCF values reported for fish from an area where a spill had occurred in Etobicoke Creek in Toronto (6.3×10^3-1.3×10^5) (Moody et al., 2002). BCF values for PFOS determined under laboratory conditions were estimated to be $4.3\times10^3\pm5.7\times10^2$ in blood and $5.4\times10^3\pm8.6\times10^2$ in liver of rainbow trout (*Oncorhynchus mykiss*) at the steady-state condition (Martin et al., 2003a). BCF for the six carbon PFHS was less than PFOS, with values ranging from 1.9×10^2 to 8.3×10^2 in blood samples and 6.4×10^2 to 1.5×10^3 in liver samples.

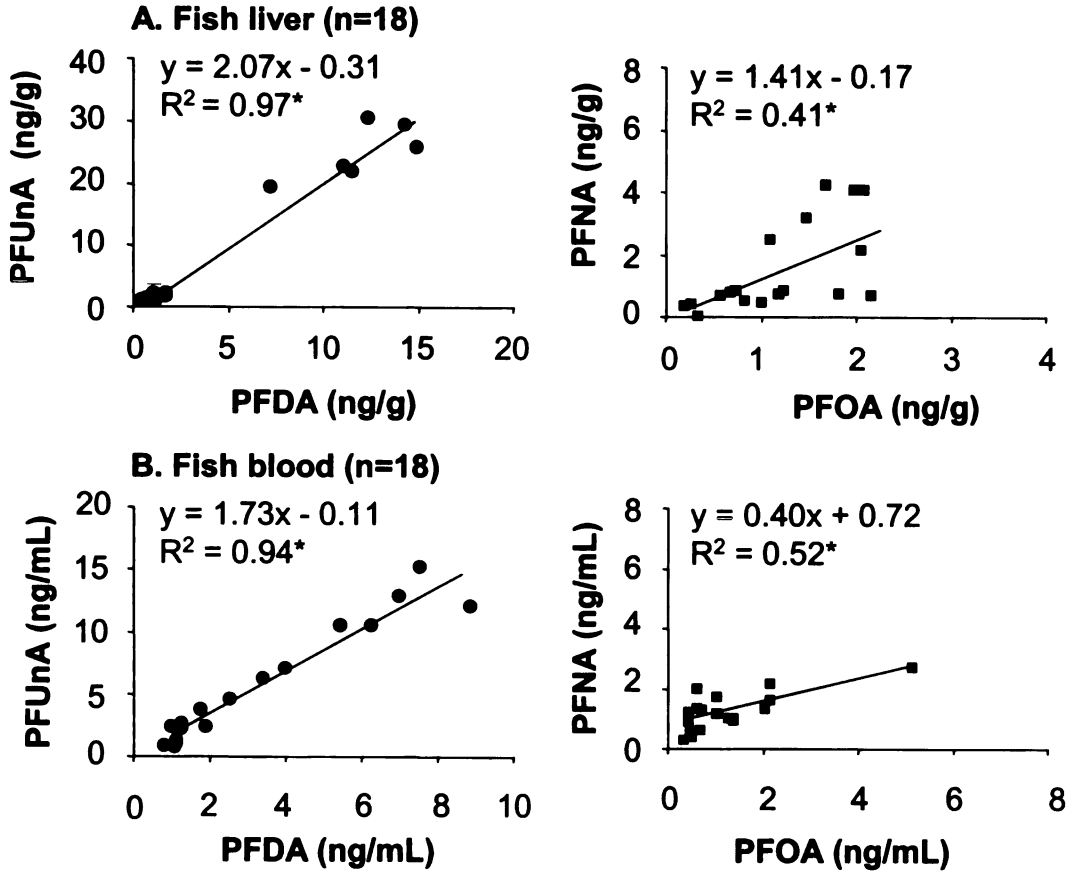

Figure 13. Plot of the odd-chain PFCA and the even-chain PFCA in liver (A) and blood (B) of fish collected from Lake Shihwa. Filled circle stands for the relationship between PFUnA and PFDA, and filled square represents for PFNA and PFOA (*: $p<0.05$).

The site-specific BCF values of PFCAs estimated for Lake Shihwa waters were greater than those estimated under laboratory conditions. For example, the BCF value for PFOA which is a ubiquitous PFA in water column was in the range of 5.8×10^{1} to 3.8×10^{2} in mullet samples, while laboratory-derived BCFs were only 8 to 2.7×10^{1} in rainbow trout. Field-determined BCF for PFDA (6.9×10^{3} in blood and 1.5×10^{4} in liver) in this study was also greater than those estimated in rainbow trout under laboratory condition (2.7×10^{3} and 1.1×10^{3} for blood and liver, respectively). Each fish species may have different toxicokinetics for PFCA accumulation, which results different BCF. However, this observation may suggest the existence of unidentified precursors of PFCAs in the region of Lake Shihwa, which makes significant contribution to the overall bioaccumulation of middle-length PFCAs (C8-C12) in marine organisms.

Hazard Assessment based on Concentrations of PFOS in Tissues

An earlier hazard assessment based on water-borne PFOS concentrations revealed that neither waters inland streams nor lake Lake Shihwa waters exceed water-quality (guideline) values that are protective of aquatic species (Rostkowski et al., 2006). Concentrations of PFOS in tissues determined in the present study were used to evaluate the ecological risk of PFOS exposure to fish in Lake Shihwa. The tissue concentration that would not be expected to cause acute effects in fish was determined to be 8.7×10^{1} mg PFOS/kg ww, which is less than the 95% confidence limit of the LD_{01} based on a study of the blue gill sunfish (Beach et al., 2005). Prior to hazard quotient (HQ) calculation, the hepatic PFOS concentrations were converted into whole carcass PFOS concentrations. To conduct the risk assessment based on whole body concentrations a

conversion ratio was developed by dividing the concentration in liver to that in whole body. The value of 4.9 was used to predict the whole body concentration and then a threshold water concentration was estimated by using the BCF value for PFOS that was determined from a water-only exposure of rainbow trout (Martin *et al.*, 2003a). By using this conversion, the whole body concentrations of PFOS in fish were approximated be in the range of 3.9 to $5.1x10^{1}$ ng/g, ww. The calculated HQs were less than 1.0 for all fishes. For example, an HQ calculated from the greatest PFOS in fish was estimated to be only 6×10^{-4}. These results, estimated from the measured concentrations in fish tissue were consistent with those predicted previously from measured water concentrations by use of a bioconcentration factor (So et al., 2004) that least current concentrations of PFOS in fish in Lake Shihwa are not expected to cause acute lethality of the fish. However, currently there is little information on the potential for chronic effects of PFOS on fish in Lake Shihwa or to assess the potential for effects of the mixture of PFAs.

Chapter 4

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

Abstract

Concentrations of perfluoroalkyl acids (PFAs) were measured in egg yolks of three species of birds collected in and around Lake Shihwa, Korea, which receives wastewaters from an adjacent industrial complex. Mean concentrations of perfluorooctanesulfonate (PFOS) ranged from 1.9×10^2 to 3.1×10^2 ng/g ww, with size-dependent accumulation among species. Measured concentrations of PFOS were similar to those reported for bird eggs from other urban areas. Concentrations of long-chain perfluorocarboxylic acids (PFCAs) were found in egg yolks. Mean concentrations of perfluoroundecanoic acid (PFUnA) in egg yolks of little egret *(Egretta garzetta)*, little ringed plover (*Charadrius dubius*), and parrot bill (*Paradoxornis webbiana)* were 9.5×10^1, 1.5×10^2, and 2.0×10^2 ng/g ww, respectively. Perfluorooctanoic acid (PFOA) was detected in a few samples, but concentrations were 100-fold less than those of PFOS. Relative concentrations of PFAs in all three species were similar, with the predominance of PFOS (45-50%) followed by PFUnA (25-30%). There was a statistically significant correlation between PFUnA and perfluorodecanoic acid (PFDA) in egg yolks ($r^2=0.54$). This suggests that fluorotelomer alcohols are important contributors to the occurrence of long chain PFCAs in bird eggs. Using measured egg concentrations as well as concentrations in the diet of the birds, ecological risk of PFOS and PFAs to birds in Lake Shihwa was evaluated using two different approaches. Estimated hazard quotients were similar between the two approaches. The concentration of PFOS associated with 90th centile in bird eggs was

100-fold less than the lowest observable adverse effect level (LOAEL) determined for birds and those concentrations were 4-fold less than the suggested toxicity reference values (TRV). Current concentrations of PFOS are less than what would be expected to have an adverse effect on birds in the Lake Shihwa region.

Introduction

Widespread occurrence of perfluoroalkyl acids (PFAs) in wildlife has spurred monitoring efforts and regulatory concerns regarding these emerging contaminants (Giesy and Kannan, 2001&2002; OECD, 2002; Environment Canada, 2006). The physico-chemical 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 fluorinated polymers (Kissa, 2001). Food web studies and examination of concentrations in biota suggest that perfluorooctanesulfonate (PFOS) and other related PFAs are bioaccumulative to some extent and are able to biomagnify to top-predators such as marine mammals and fish-eating birds (Kannan et al., 2001; Van de Vijver et al.; Martin et al., 2004; Houde et al., 2006). PFAs and in particular PFOS and perfluorooctanoate (PFOA), which were once considered to be biologically inert are relatively bioactive at the cellular level, causing diverse effects including blockage of cell-cell communication (Upham et al., 1998) and initiation of hepatic peroxisome proliferation (Intrasuksri et al., 1998). These two PFAs have also been shown to cause developmental toxicities in experimental animals including rodents, birds, and amphibians (Austin et al., 2003; Lau et al., 2004; Molina et al., 2006; Palmer and Krueger, 2000).

Birds from urbanized areas contain greater concentrations of PFAs in their tissues or egg than those from more remote areas (Kannan et al., 2001; Verreault et al., 2005; Sinclair et al., 2006). Concentrations of PFAs in osprey eggs were also correlated with a concentration gradient of other persistent contaminants in the environment suggesting local sources of exposures (Toschik et al., 2005). Furthermore, guillemot eggs collected from 1968 to 2003 showed increase in PFOS concentrations by 30-fold from 1968 (2.5×10^{1} ng/g ww) to 2003 (6.1×10^{2} ng/g ww), which corresponds to greater use of fluorochemicals (Holmström et al., 2005).

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 km^2) since its construction in 1994 (Figure 14). Investigation 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 (Khim et al., 1999; Li et al., 2004). 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 (Rostkowski et al., 2006; Yoo et al., *submitted*). These earlier studies found relatively great concentrations of PFOS in the water column and certain fishes and invertebrates. Concentrations of PFAs in livers of birds from other areas of Korea have been reported on limited sample sizes (Kannan et al., 2002). However, no attempt has been made to investigate the concentrations and effects of PFAs in the upper-tropic organisms, such as fish-eating birds in this region.

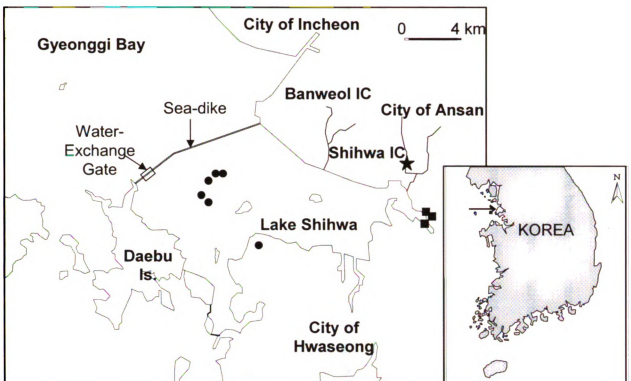

Figure 14. Map showing sampling locations for birds eggs in this study. Little egret eggs (star) were collected at one colony located at the hills in City of Ansan. Little ringed plover eggs (circles) were collected at various sites including islands and walkways in Lake Shihwa, while parrot bill eggs (squares) were sampled at locations in upstream wetlands of Lake Shihwa

The aim of this study was to measure concentrations of PFOS and other PFAs eggs of birds collected in the vicinity of Lake Shihwa. Egg yolks were selected for this study because the concentrations of PFAs in eggs are often used in exposure and risk assessments. Assessment of the potential risks of PFOS and a mixture of PFAs to birds in the Lake Shihwa area were made based on the currently available benchmark doses for toxicity in birds (Newsted et al., 2005). Concentrations of PFAs in water and fish-diet of birds were also incorporated to provide multiple lines of evidence for assessing risk. In biota, PFAs were present as a mixture of PFAs rather than a single congener. Thus, he gap junction intercellular communication (GJIC) cell-bioassay was used to obtain a relative toxic potency of individual PFAs to calculate PFOS-equivalent concentrations in egg samples for the risk assessment of PFA mixture (Villeneuve et al., 2000).

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 14). In this area, one colony of little egret (*Egretta garzetta*) was found in a section of the city of Ansan, while eggs of little ringed plover (*Charadrius dubius*) were sampled at various locations including the islands. Nests of parrot bill (*Paradoxornis webbiana)* occurred in wetlands located upstream of Lake Shihwa. One or two eggs per nest were collected and a total of ten, seven, and four nests were surveyed for little egret, little ringed plover, and parrot bill, respectively. Mean shell length (mm) and egg weight (g) of each species are summarized (Table 12). To avoid potential contamination during sampling, eggs 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 (Kannan et al., 2004). Briefly, an aliquant of homogenized egg yolk (1 g ww) was diluted 4 fold (w/v) with Milli-Q water before extraction. Five nano-grams of [^{13}C]PFOS and [^{13}C]PFOA were spiked as internal standards. Tetrabutyl ammonium hydrogensulfate (TBA) and methyl *tert*-butyl ether (MTBE) were used as an ion-pairing agent and extraction solvent, respectively. The resulting extracts were concentrated under a gentle stream of nitrogen and reconstituted in 1.0 mL methanol. Teflon or glassware was avoided in whole extraction procedure to remove possible contamination of samples and sorption of analytes.

TABLE 12. Bird Egg Sample Descriptions

Species	No. of Nest	No. of Egg[d]	Shell length (mm)		Egg weight (g)	
			Mean	s.d	Mean	s.d.
Little Egret[a]	10	20	$4.4x10^1$	3.5	$2.7x10^1$	5.8
Ringed plover[b]	9	17	$3.1x10^1$	1.7	8.3	0.7
Parrot bill[c]	4	7	$1.3x10^1$	0.7	1.2	0.2

[a]*Egretta garzetta*. [b]*Charadrius dubius*. [c]*Paradoxornis webbiana*. [d]One or two eggs per nest were collected

Instrumental Analysis and QA/QC

Target analytes of perfluoroalkyl acids in extracts were quantified using an Agilent 1100 series high-performance liquid chromatograph (HPLC) coupled with an Applied Biosystems API 2000 tandem mass spectrometer (MS/MS). The extract was injected separately for sulfonate and carboxylic acids. For sulfonate acids, 10 % methanol at a flow rate of 300 µl/min was increased to 100 % methanol at 10 min and was held for 5 min and then was back to 10 % methanol. For carboxyl acids, 100 % methanol was used at 7 min and was held for 3 min. The MS/MS was operated in electrospray negative ion mode. Analyte ions were monitored in multiple-reaction monitoring mode. Parent and daughter ion transitions used for identification and quantification were 399>80 (perfluorohexane sulfonate, PFHS), 499>99 (perfluorooctane sulfonate, PFOS), 498>78 (perfluorooctane sulfonamide, PFOSA) for sulfonate acids and 413>369 (perfluorooctanoic acid, PFOA), 463>419 (perfluorononanoic acid, PFNA), 513>469 (perfluorodecanoic acid, PFDA), 563>519 (perfluoroundecanoic acid, PFUnDA), 613>569 (perfluorododecanoic acid, PFDoDA) for carboxylic acids. Two internal standards of 503>99 (^{13}C-PFOS) and 417>372 (^{13}C-PFOA) were also monitored for analytical recovery of sulfonate and carboxylic acids, respectively. Concentrations of PFAs in extracts were quantified using an extracted calibration curve containing target analytes spiked into a chicken egg matrix (0.1, 0.2, 0.5, 1.0, 5.0, and 20.0 mg/L). The chicken egg was found not to contain measurable concentrations of target compounds. The coefficient of determination (r^2) for each constructed curve was greater than 0.99. Acquired data were deemed acceptable if QC standard measured after every 10 injections fell within 30% of the long-term average, otherwise analysis was stopped and samples

were reanalyzed with a new calibration curve. The limit of quantification (LOQ) was estimated at the lowest concentration point on the calibration curve within ±30% of its theoretical value. The LOQ in this study was determined to be 0.8 ng/g ww for all analytes. Procedural blanks and matrix-spiked recovery tests were used to ensure the quality of the analytical procedure. Concentrations of any target PFAs in the procedural blank (n=6) were below the LOQ. Mean recoveries (n=3) from egg yolk samples spiked with 5.0 ng PFAs were 83.3% for PFOS, 90.0% for [^{13}C]PFOS, 68.0% for PFOSA 63.6% for PFHS, 151.2% for PFDoA, 157.1 for PFUnA, 128.2% for PFDA, 128.6% for PFNA, 68.4% for PFOA, and 79.5% for [^{13}C]PFOA. For calculation of mean concentrations, values below LOQ were arbitrarily assigned as half of LOQ.

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 University of North Carolina. The cells were cultured as described previously (Upham et al., 1998). GJIC was measured using the scrape loading dye transfer technique (Hu et al., 2002). Briefly, confluent cells were trypsinized with 1× trypsin-EDTA and cell solution was harvested. Two milliliters of the diluted cell solution were seeded to 35 mm tissue culture plates, and cells were allowed 48 h for attachment before the PFA dosing. Total twelve PFAs (PFBA, PFBS, PFHA, PFHS, 6:2 FTOH, PFOS, PFOA, 8:2 FTOH, PFDA, 10:2 FTOH, 10:1 FTOH, and PFTetraDA) 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 μg/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 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 epifluorescence 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 gap junction. The migrated area was calculated using the Gel Expert program (Nucleotech, San Mateo, CA). Each PFA concentration was tested in triplicate.

Data and Statistical Analysis

From a concentration-response relationship, EC50 of each PFA was calculated. Toxic equivalent factor (TEF) was obtained by normalizing the EC50 of individual PFA congener to that of the PFOS. PFOS equivalent concentrations (PEQ) in samples were calculated by multiplying a corresponding TEF with its concentration in the sample. Assuming additivity of toxic effects, a summed concentration of PEQs was used to characterize the risks associated with a mixture of PFAs in bird eggs.

To evaluate the differences in accumulation of PFOS and total PFCAs in egg yolk among the bird species, one-way analysis of variance (ANOVA) was conducted with the Bonferroni post-hoc criterion. Prior to analysis, values for concentrations of PFOS and total PFCAs were log-transformed to attain the normality (one-sample Kolmogorov-Smirnov test) and equal variances (Levene's test). Probable associations among

perfluorinated carboxylic acids were tested using a Pearson correlation analysis. A probit analysis was used to derive EC_{50} of inhibition of cellular communication (GJIC) using Excel® program. Statistical significance was set at the level of $p \leq 0.05$, unless otherwise noted. All statistical analyses other than EC_{50} determinations were performed with the SYSTAT® 11 statistical package (SYSTAT Software Inc., Richmond, CA).

Results and Discussion

Concentrations in Birds

Mean concentrations of PFHS, PFOS, PFOSA, PFOA, PFNA, PFDA, PFUnA, and PFDoA in egg yolks are summarized in Table 13. Significant amounts of PFOS were detected in all the egg yolk samples. PFOA was measurable in a few samples at concentrations two orders of magnitude less than that of PFOS. The long chain PFCA, PFUnA was the second most prevalent perfluorinated compound in the egg samples. Mean concentration of PFOS in egg yolk of little egret, little ringed plover, and parrot bill was 1.9×10^2, 2.2×10^2, and 3.1×10^2 ng/g ww, respectively (Figure 15). The greatest concentration of PFAs in this study was 1.2×10^3 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 (5.6×10^2 - 8.7×10^2 ng PFOS/g ww during 2000-2003 sampling campaigns) (Kannan et al., 2001; Verreault et al., 2005; Holmström et al., 2005; Toschik et al., 2005). PFHS was also measured in some egg samples, at concentrations as great as 9.5 ng/g ww. PFOSA was frequently found in the diet of birds (fish samples), but were not quantifiable in any yolk samples (<LOQ).

Table 13. Concentrations of Perfluoroalkyl Acids in the Egg Yolks of Birds Collected around Lake Shihwa (ng/g ww)

Species		PFHS	PFOS	PFOSA	PFOA	PFNA	PFDA	PFUnA	PFDoA
Little	Max	9.5	$1.2x10^3$	1.4	4.0	$6.9x10^1$	$9.0x10^1$	$2.6x10^2$	$3.2x10^1$
Egret	Min	<LOQ	$3.0x10^1$	<LOQ	<LOQ	3.9	$1.5x10^1$	$2.6x10^1$	6.2
(n=20)	Mean	2.3	$1.9x10^2$	0.7	1.7	$2.5x10^1$	$4.4x10^1$	$9.5x10^1$	$2.0x10^1$
	% detected [a]	85%	100%	40%	60%	100%	100%	100%	100%
Ringed	Max	9.5	$8.9x10^2$	NA	$2.5x10^1$	$1.1x10^2$	$1.1x10^2$	$3.1x10^2$	$4.7x10^1$
plover	Min	<LOQ	$6.0x10^1$	NA	0.9	6.4	$1.1x10^1$	$2.3x10^1$	6.1
(n=17)	Mean	2.3	$2.2x10^2$	NA	8.4	$5.1x10^1$	52.7	$1.5x10^2$	$2.1x10^1$
	% detected	82%	100%	0%	100%	100%	100%	100%	100%
Parrot bill	Max	5.3	$7.6x10^2$	NA	1.4	$9.7x10^1$	$2.9x10^2$	$6.8x10^2$	$5.4x10^1$
(n=7)	Min	<LOQ	$3.5x10^1$	NA	<LOQ	5.7	$1.1x10^1$	$2.6x10^1$	$1.5x10^1$
	Mean	1.3	$3.1x10^2$	NA	0.8	$4.0x10^1$	$1.1x10^2$	$2.0x10^2$	$2.6x10^1$
	% detected	57%	100%	0%	43%	100%	100%	100%	100%
	LOQ	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8

[a] % 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.

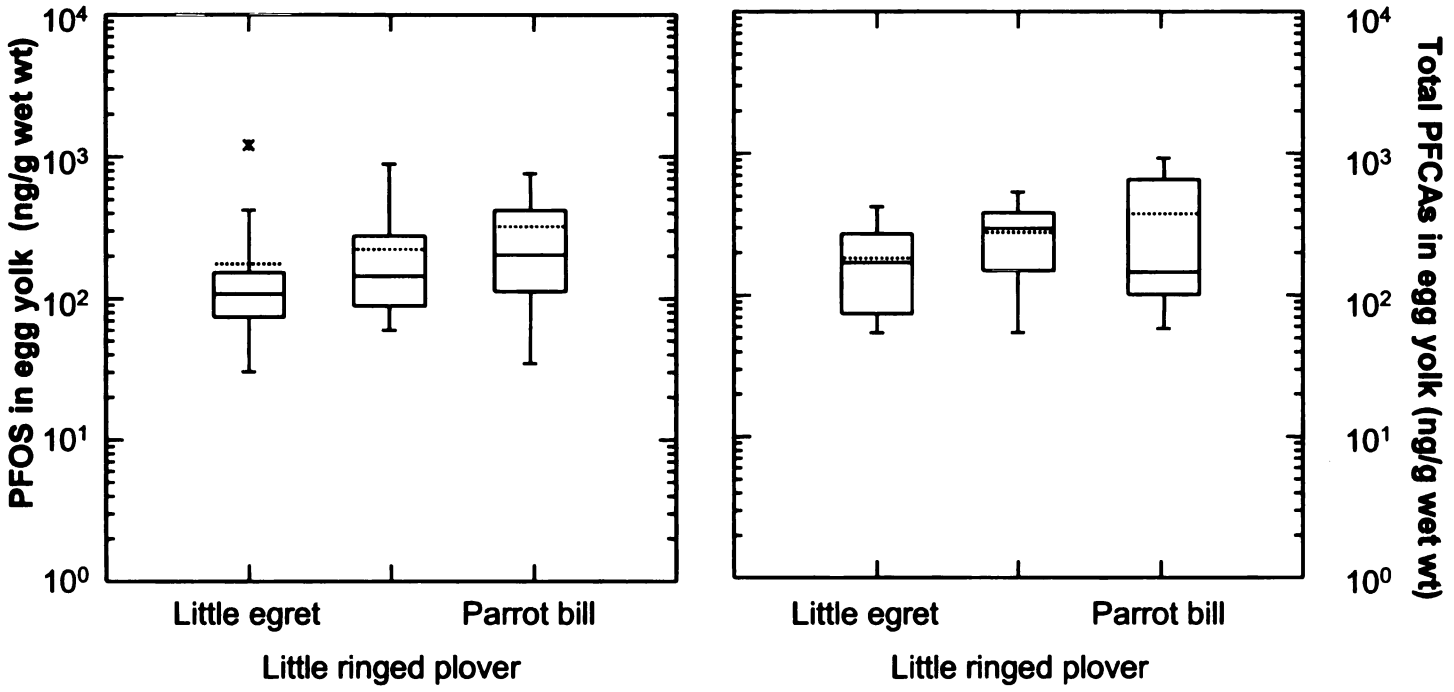

Figure 15. Species comparison of PFOS and total PFCAs concentrations (ng/g wet wt) in little egret, little ringed plover, and parrot bill from the Lake Shihwa area. The straight line is the median, and the dotted line represents the mean. The 25th and 75th percentiles define the box (asterisks are outliers). One-way ANOVA resulted in no statistical difference in accumulation among species (p<0.05).

The concentrations of PFCAs in egg yolks of the birds analyzed were some of the greatest ever found in wildlife species. In particular, the concentrations of long chain PFCAs were elevated in all three species (Table 13). Among the PFCAs 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 little egret, little ringed plover, and parrot bill were 9.5×10^{1}, 1.5×10^{2}, and 2.0×10^{2} ng/g ww, respectively. The predominance of PFUnA was also observed in osprey eggs from the Delaware River and Bay. PFOA was detected in 60%, 100%, and 43% of little egret, plover, and red-billed tit eggs, respectively. In plover, the concentration of PFOA was as great as 2.5×10^{1} ng/g ww. In other studies, PFOA was not detectable in the eggs of guillemot and glaucous gulls from northern Europe. Concentrations of PFCAs in eggs from our study were 10-fold greater than those in glaucous gulls from the Norwegian Arctic (total PFCAs was 4.2×10^{1} ng/g ww with the dominance of PFUnA).

Mean concentrations of PFOS in birds were inversely proportional to 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 yolks. However, this difference was not statistically significant (Figure 15) ($p>0.05$). The occurrence of great mean concentrations of PFOS and total PFCAs in parrot bill is interesting because these eggs were sampled at upper freshwater wetlands away from known sources such as industrial complexes and populated areas, whereas little ringed plover eggs were collected on islands and walkways in the vicinity of PFA contaminated Lake Shihwa. Although the foraging ranges of investigated species were not studied, high

concentrations found in samples from wetland waters of Lake Shihwa need to be investigated.

Contamination Sources to Birds and Relationship among PFCAs

Little egret and plover prey on aquatic organisms from Lake Shihwa 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 pf PFAs in Lake Shihwa has been suggested previously (Rostkowski et al., 2006). Concentrations of PFA in water were elevated in drains and streams receiving effluents from the nearby Shihwa Industrial Complex, and gradually decrease 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 PFCAs in fish and blue crabs also decreased as a function of distance from wastewater discharges to the water exchange gate in Lake Shihwa (Yoo et al., *submitted*). Information on neither the amounts of PFAs used in the industrial complexes nor their emissions from these industries bordering Lake Shihwa were not available, but the results of previous studies have indicated that there are local sources in this area.

Profiles of relative concentrations of PFAs in the three species of birds examined were similar to each other (Figure 16). The relative contribution of PFOS to the total PFAs was less than what has been reported for other species, such as fish, reptiles, and polar bears. Although PFOA concentration ($1.7\text{-}1.1\times10^{1}$ ng/L) in waters of Lake Shihwa was comparable to that of PFOS ($7.3\text{-}1.8\times10^{1}$ ng/L), the relative concentration of PFOA was less than 2% in egg yolks. PFUnA accounted for 25-30% of the total mass of PFAs. In contrast, PFNA which is often a dominant PFCA in arctic marine mammals accounted

for only 10-20% of total PFCA concentration. 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.

There was a statistically significant positive correlation between PFUnA and PFDA (r^2=0.54), but not between PFNA and PFOA (r^2=0.17) (Figure 17). There is evidence that fluorotelomer alcohols (FTOHs) could generate PFCAs via atmospheric oxidation, aqueous photolysis, and biodegradation (Ellis et al., 2004; Wang et al., 2005). Studies have shown that 8:2 FTOH could degrade into PFNA and PFOA with even number PFCA being a major product. Degradation of 10:2 FTOH has not been tested, but presumably its degradation pathways may lead to PFUnA and PFDA. In most samples of egg yolk concentrations of PFCA with an odd number carbon were greater than the adjacent even number of carbon. For example, the slopes of correlation were 1.7 for PFUnA and PFDA, and 2.1 for PFNA and PFOA. This observation is explained by differences in exposures and bioaccumulation potential among the PFCAs. Fish and marine invertebrates from Lake Shihwa contained moderate to large concentrations of PFCAs as well as PFOS (Yoo et al., *submitted*). In marine animals, concentrations of PFCAs with an odd number of carbon atoms were greater than PFCA with an even number of carbon atoms. For example, the concentrations of PFUnA in liver (mean, 9.2; range, 0.9-3.1$\times10^1$ ng/g ww) were almost twice the hepatic concentrations of fish (mean, 4.6; range 0.5-1.5$\times10^1$ ng/g ww) collected from Lake Shihwa. Therefore, accumulation pattern of PFCAs in birds from Lake Shihwa is influenced by PFCA concentrations in prey items and BAF of individual PFCAs.

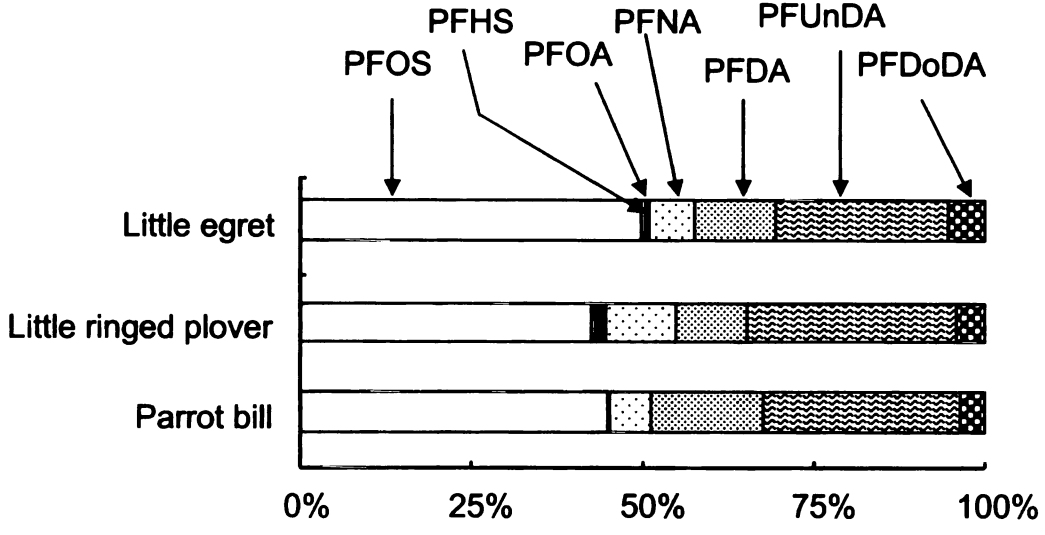

Figure 16. Composition profiles of PFAs in the egg yolks of little egret, little ringed plover, and parrot bill collected from Lake Shihwa and its vicinity.

Based on the current understanding of the degradation of FTOHs, a strong relationship between PFUnA and PFDA in egg yolks suggests that 10:2 FTOH and/or its unidentified precursors are likely contributors to the overall concentrations of long chain PFCAs in the Lake Shihwa area. At present, however, we do not have atmospheric concentrations of fluorinated contaminants in this region.

PFOS-Equivalent Concentration

Among twelve PFAs tested, eight PFAs elicited a concentration-dependent inhibition of cellular communication in rat liver epithelial cells (Figure 18 and Table 14). As consistent to previous results, inhibition potential of gap junction cellular communication was dependent on the chain length of PFAs irrespective of its head group (Upham et al., 1988). Only PFAs with a carbon-length of 5 to 9 in their fluorinated backbone block the cellular communication. Among those potent PFAs, four PFAs (PFOS, PFHS, PFOA, and PFDA) were detected in egg yolks. PFOS (EC_{50} = 1.3×10^{1} mg/L) was the most potent among the PFAs investigated except 6:2 FTOH which was not present in the sample and the potency decreased in the order of PFHS (1.7×10^{1} mg/L), PFOA (2.2×10^{1} mg/L), and PFDA (2.3×10^{1} mg/L). Previous studies have reported an EC50 for PFOS of 1.5×10^{1} mg/L (Hu et al., 2002). This observation demonstrates both the robust nature of the assay and the consistency of results.

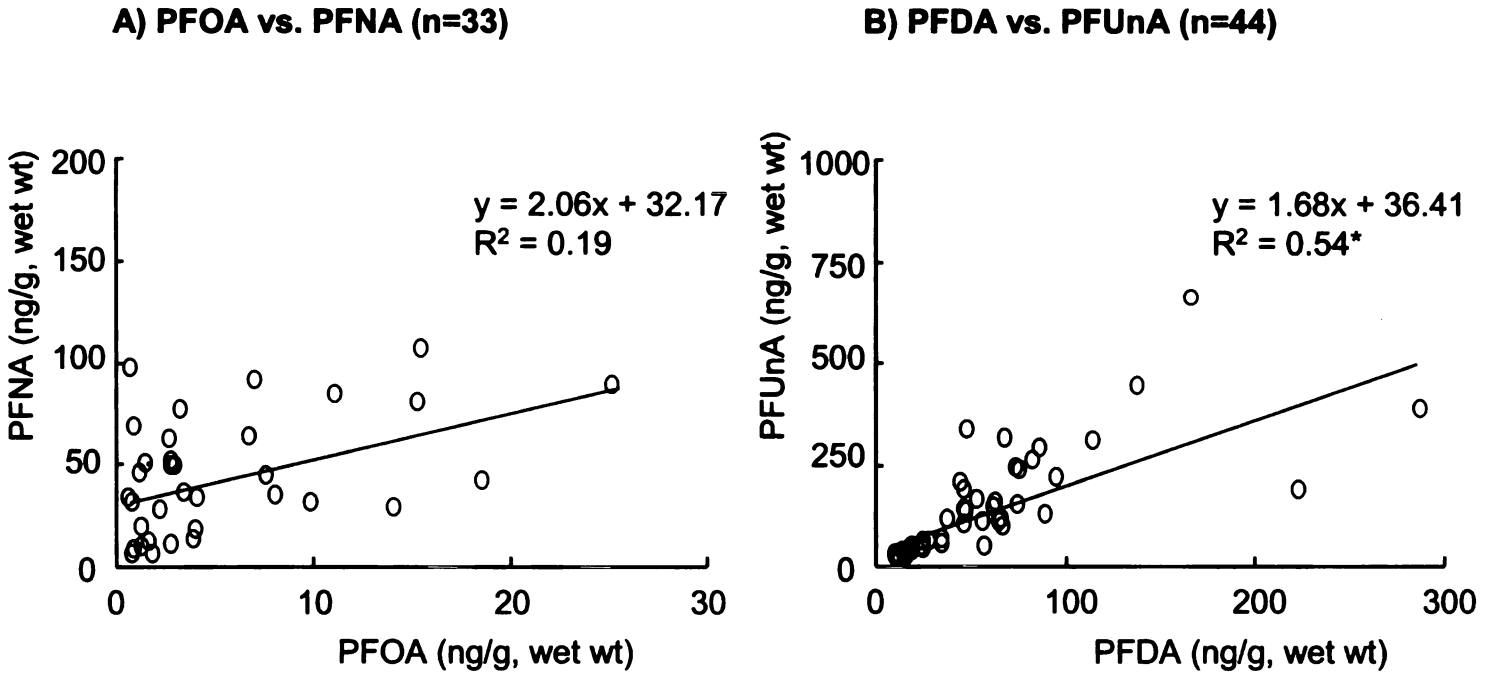

Figure 17. Plot of the odd-chain PFCA and the even-chain PFCA in the egg yolks of birds from Lake Shihwa and its vicinity (*: $p<0.05$)

A TEF value for each PFA was obtained by normalizing the EC_{50} concentrations to PFOS EC_{50} (TEF_{PFA} = $EC50_{PFOS}$ / $EC50_{PFA}$) (Table 14), which showed TEF values of 7.5×10^{-1}, 5.6×10^{-1}, and 4.7×10^{-1} for PFHS, PFOA, and PFDA, respectively. A PFOS-equivalent concentration (PEQ) was calculated for each mixture of PFAs by multiplying TEF_{PFA} with corresponding concentration in egg samples and summing (Villeneuve et al., 2000). 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. 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 endpoints (Trosko and Ruch, 1998; Trosko et al., 1998). Therefore, the results of this epigenetic test can be considered as being predictive of potential toxicity of PFAs in mixture.

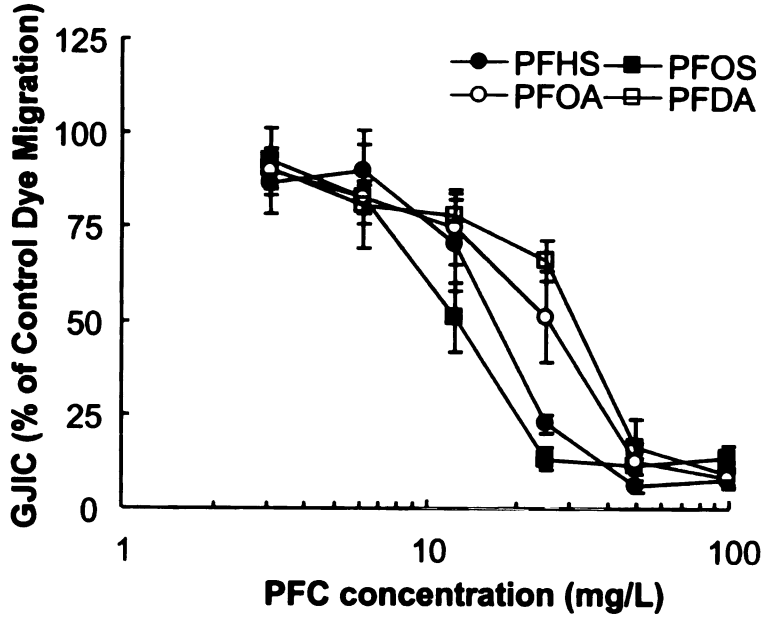

Figure 18. Concentration-response relationship between inhibition of cellular communication and a gradient of selected PFAs (PFHS, PFOS, PFOA, and PFDA) on rat liver epithelial cells.

Table 14. EC50 of PFAs and toxicity equivalent factor (TEF). PFOS equivalent concentrations were calculated by multiplying TEF and individual PFA concentration in a sample.

PFA	C-lengths	EC50	TEF
PFBA	3 (4)	N.A.	N.A.
PFBS	4 (4)	53.38	0.23
PFHA	5 (6)	31.15	0.40
PFHS	6 (6)	16.56	0.75
6:2 FTOH	6(8)	11.29	1.11
PFOA	7 (8)	22.31	0.56
PFOS	8 (8)	12.48	1.00
8:2 FTOH	8 (10)	11.00	1.13
PFDA	9 (10)	26.30	0.47
10:2 FTOH	10 (12)	N.A.	N.A.
10:1 FTOH	10 (11)	N.A.	N.A.
PFTeDA	13 (14)	N.A.	N.A.

Risk Characterization of PFOS and other PFAs

Ecological risks of PFOS and PFAs to birds in the Lake Shihwa region were evaluated by using two approaches. First, concentrations 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 endpoints with uncertainty factors assigned so that they are protective (Beach et al., 2006). Second, an average daily intake (ADI) value was determined for PFOS based on the concentrations of PFAs in the diet of birds. The ADI benchmark dose was then compared with the calculated dietary dose. To estimate the risk associated with the protection of 90% of birds in a population inhabiting

the region surrounding Lake Shihwa, a cumulative probability function was developed for PFOS concentrations in egg yolks of the three species examined (Figure 19).

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 liver, serum, and egg yolk were reported from dietary exposure studies with mallard and bobwhite quail (Newsted et al., 2005). The lowest observable adverse effect level (LOAEL) and toxicity reference value (TRV) for birds were determined to be 6.2×10^{4} ng PFOS/mL and 1.7×10^{3} ng PFOS/mL in egg yolk, respectively. In the present study, the 90th centile of PFOS concentrations in the yolk of bird eggs from the Lake Shihwa area was 4.8×10^{2} ng/g ww and the corresponding HQ was 8.0×10^{-3} based on the LOAEL and 2.8×10^{-1} based on the TRV. As discussed earlier, concentrations of PFCAs 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 PFAs mixture in egg yolk. In this estimation, a slightly greater 90th centile (5.4×10^{2} ng PFOS-EQ/g ww) resulted in greater HQs (LOAEL = 9.0×10^{-3} and TRV = 3.2×10^{-1}) but none exceeded a value of 1.0.

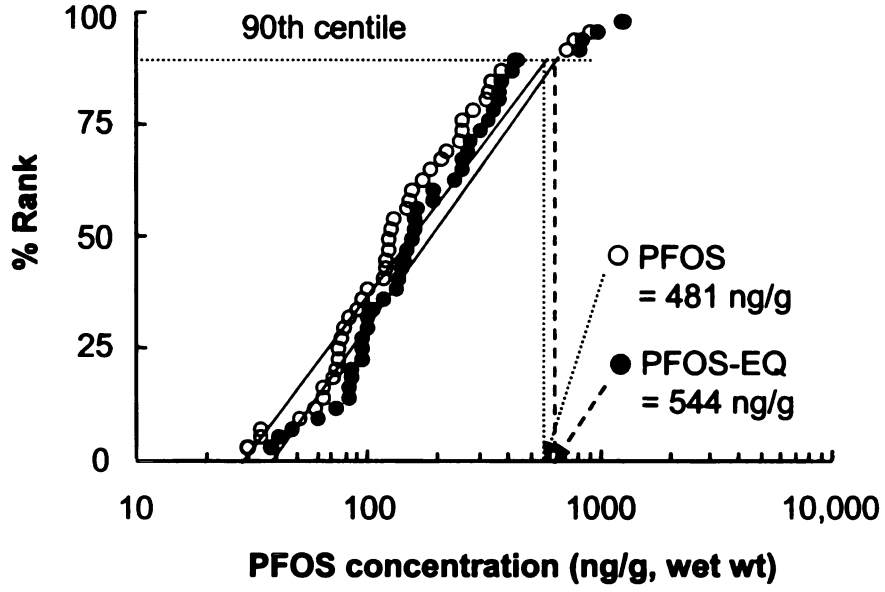

Figure 19. Cumulative percent rank against concentrations of PFOS and PFOS-EQ in the egg yolk of birds from Lake Shihwa and its vicinity. Regression lines were constructed to derive the 90th centile of PFOS concentration in egg yolk.

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 carcass were observed around the little egret colony. Thus, in this diet-based assessment, mullet caught in 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 = 7.7×10^{-1} mg PFOS/kg/d and TRV = 2.2×10^{-2} mg PFOS/kg/d) (Newsted et al., 2005). The amount of food ingested (FI) per day to body weight for wading birds was calculated for the little egret (5×10^{2} - 5×10^{3}g ww) (FI = 9.7×10^{-1} log (BW) -6.4×10^{-1}, BW= body weight of bird) (Kushlan et al., 1978). The mean concentrations of PFOS in carcass of mullet were 3.9×10^{1} ± 6.6 ng/g ww (Yoo et al., submitted). The ADI determined for little egret was 7.1×10^{-3} mg PFOS/kg/d. Based on this ADI value, HQs (=ADI/benchmark dose) were calculated to range from 8×10^{-3} - 9×10^{-3} based on the LOAEL and 3.1×10^{-1} – 3.3×10^{-1} based on the TRV.

Estimated risks of PFAs in avian species based on residue concentration in egg yolk (upper 10% of bird population) and based on dietary exposure approaches were quite similar. Although some assumptions have been made with limited toxicological data, the comparable HQs derived allow us to evaluate the risks associated with current exposures from PFOS and PFAs mixtures in Lake Shihwa at the screening level. Approximately hundred-fold difference between PFAs exposure level and the threshold values suggests that immediate threats such as reproductive failure are unlikely to occur due to PFAs in birds in the Lake Shihwa region. In addition, considering the conservative nature of TRV values (Beach et al., 2006), current concentrations of PFOS

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 the new data pertaining to toxicities of PFAs become available.

CONCLUSION

As a first risk assessment for PFAs in wildlife to date in Korea, overall results suggest a low ecological risk of PFAs exposure to marine wildlife in the Lake Shihwa area. In general, contamination status of PFAs in biota is comparable to other urban areas. However, as mentioned earlier, most of toxicological benchmarks for wildlife have been derived using a limited number of data generated with few endpoints to evaluate the health of aquatic wildlife. Toxic effects from chronic PFA exposure are not well studied at present. Therefore, more ecologically meaningful benchmark values are needed to draw conclusions about the status of environmental health. Finally, regional monitoring programs should be established in this area until we are confident that on-going world-wide efforts to reduce the emission of PFA into the environment are working as well as intended.

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