



# Depuration kinetics and tissue disposition of PFOA and PFOS in white leghorn chickens (*Gallus gallus*) administered by subcutaneous implantation

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## Abstract

Elimination kinetics and tissue disposition of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in male chickens (*Gallus gallus*) was determined following exposure by subcutaneous implantation. Chickens were exposed to two levels of PFOA or PFOS for 4 wk and then allowed to depurate for an additional 4 wk. These exposures did not cause any statistically significant changes in body index, clinical biochemistry or histology among treatments relative to the controls ( $p > 0.05$ ), except that concentrations of total cholesterol and phospholipids were less in chickens exposed to PFOS. The elimination rate constant for PFOA ( $0.150 \pm 0.010 \text{ d}^{-1}$ ) was approximately six-fold greater than that of PFOS ( $0.023 \pm 0.004 \text{ d}^{-1}$ ). The greatest concentrations of PFOA and PFOS were found in kidney and liver, respectively. The organ to blood ratio of PFOS concentration was increased after the whole experiment, indicating the importance of organ partitioning of PFOS in elimination kinetics. The depuration half-life of PFOA ( $t_{1/2} = 4.6 \text{ d}$ ) and PFOS ( $t_{1/2} = 125 \text{ d}$ ) in chickens was calculated.

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## 1. Introduction

The use of perfluorinated compounds (PFCs) in a variety of products has resulted in them becoming ubiquitous in the environment, where they occur globally in humans and wildlife (Giesy and Kannan, 2002; Olsen et al., 2005). PFCs 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). Because they are the degradation products of a number of

chemicals used in industrial and commercial applications, the two PFCs 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 PFC followed by PFOA (Kannan et al., 2004). Similar profiles of PFCs have also been observed in tissues of terrestrial and marine wildlife with PFOS being the dominant PFC with concentrations in wildlife often exceeding those measured in human populations (Houde et al., 2006). Several food web studies have shown that some PFCs have the potential to bioaccumulate into lower trophic-level organisms and through trophic transfer and biomagnification, can accumulate into upper

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trophic level organisms (Van de Vijver et al., 2003; Martin et al., 2004). While exposure pathways of PFOA, PFOS and related PFCs 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 PFCs to exposed human populations. Nevertheless, efforts to reduce PFCs in the environment have been initiated.

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, PFCs do not behave in the same manner as the more studied organochlorine contaminants. Specifically, PFCs have fewer tendencies to partition into lipids, but rather are preferentially bound to proteins and retained in the blood and liver of wild animals (Kannan et al., 2001; Jones et al., 2003). A few pharmacokinetic studies with of PFOA or PFOS have been conducted with mammals such as rats, dogs, and monkeys (Seacat et al., 2002; Kudo et al., 2002; Lau et al., 2004). In these studies, administered PFOA has been shown to be readily absorbed but has a relatively short half-time for depuration with notable species- and gender-differences in rates of elimination when compared to PFOS. For example, in male rats the half-time for depuration of PFOA from blood is approximately a week while in female rats the half-time for depuration has been reported to be approximately 1 d (Kemper, 2003). In contrast, PFOS is also readily absorbed but is poorly eliminated from blood and other tissues with biological half-time for depuration that can range from several weeks to months, depending on the species and sex.

Concentrations of PFCs in wild birds have been studied globally and the resulting data suggest that birds from urban areas are more contaminated with PFCs than those from rural areas (Kannan et al., 2001; Verreault et al., 2005). Other studies have quantified PFOS in chickens and domestic livestock. In that study, concentrations of PFOS in blood plasma and liver were greater in chickens than the other farm animals that were evaluated (Guruge et al., 2005). While pharmacokinetic studies with PFOS have not been conducted with birds, 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 platyrhynchos*) (Newsted et al., 2005). The results of those studies indicated that the half-time for depuration of PFOS from blood and liver of juvenile birds ranged from approximately 7–18 d while in adult birds, the half-time for depuration from 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 those studies were greater than those 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, a study was conducted with lesser concentrations 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 an elimination period. In addition to pharmacokinetic evaluations, biochemical, and histological 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 PFCs exposure for avian species (Molina et al., 2006).

## 2. Materials and methods

### 2.1. 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  $\mu\text{m}$ , 13 mm i.d.) were purchased from Iwaki, Japan. Additional clean-up for tissue extracts was carried out using Oasis weak-anion exchange (WAX<sup>®</sup>) cartridge purchased from Waters Corp., Milford, MA, USA.

### 2.2. 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 three successive generations of specified-pathogen-free chickens maintained to insure the health of the birds used in the study. Eggs were hatched and male chickens were housed until 6 wk of age at a 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 ( $3.5 \times 10^2 \pm 1.6 \times 10^1$  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 or 0.5 mg/mL) or PFOS ( $2.0 \times 10^{-2}$  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<sup>®</sup> 2ML4), which has an average releasing rate of 2.5  $\mu$ L/h with over 95% of the filled volume will release end of 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 2–3 d, 1–2 mL of blood was drawn from the wing vein of chickens during experimental periods using a heparinized needle and stored in polypropylene (PP) centrifuge tube 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 wk 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 were released. All biological samples (blood and organs) were kept at negative 20 °C until instrumental analysis.

### 2.3. 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 0.5 g 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  $\mu$ m nylon filter. For the tissue analysis, solid-phase extraction (SPE) step was employed as an additional clean-up with some modifications (So et al., 2006). Briefly, a half-milliliter aliquot 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<sup>®</sup> cartridge (0.2 g, 6 cm<sup>3</sup>) at an elution rate of 1 drop/s. At the completion of sample loading, 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<sub>4</sub>OH dissolved in methanol. Teflon or glassware was avoided in extraction procedure to remove possible contamination of samples and sorption of analytes.

### 2.4. 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  $81 \pm 6.7\%$  and  $87 \pm 5.3\%$  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% to 88% ( $n = 4$ ) for PFOS-spiked tissues. Reported concentrations of PFOA and PFOS were not corrected for recoveries of matrix spikes. The limit of quantification (LOQ) was calculated for each analyte considering the maximum blank concentration, the concentration factors, the sample volume, and a signal-to-noise ratio of 3 (Taniyasu et al., 2005). The LOQ for both compounds varied from 1.0 to 5.0 ng/mL or ng/g wet wt., depending on the sample type.

### 2.5. Instrumental analysis and data analysis

Quantification of PFOA and PFOS in blood or tissues was conducted using HPLC with high resolution, electro-spray tandem mass spectrometry (HPLC-MS/MS). Separation of analytes was performed by an Agilent HP 1100 liquid chromatography (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro II mass spectrometer (Waters Corp., Milford, MA) operated in electro-spray negative mode. Ten microliter aliquot of extract was injected onto a Keystone Betasil C<sub>18</sub> column (2.1 mm i.d.  $\times$  50 mm length, 5  $\mu$ m) with 2 mM ammonium acetate and methanol as mobile phase, starting at 10% methanol. Analyte ions were monitored using multiple reaction monitoring (MRM) mode (Taniyasu et al., 2005). Concentration of PFOA or PFOS in extracts was quantified using calibration curves constructed by external standards ( $1.0 \times 10^{-2}$ ,  $5.0 \times 10^{-2}$ , 0.2, 1.0, 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.

## 2.6. Clinical chemistry and histopathology

Thirteen biochemical parameters were analyzed in plasma using a Hitachi model 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 phospholipids (PLs), free total cholesterol (F-Cho), total cholesterol (T-Cho), high-density lipoprotein (HDL), low-density lipoprotein (LDL), non-esterified fatty acids (NEFA), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), total protein (TP), and albumin (Alb). The following tissues were fixed in 10% phosphate-buffered formalin and processed for histological examination: liver, kidney, spleen, heart, lung, thymus, testis, bursa of fabricius, and brain.

## 2.7. 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. (1)).

$$C_t = C_0 \exp^{-kt}, \quad (1)$$

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)).

$$k = k' + k_g, \quad (2)$$

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

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

## 3. Results

### 3.1. Body index, clinical biochemistry, and histopathology

No statistically significant differences were observed for body-weight gains among doses (vehicle control, low-, 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 1). 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 from 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 brain to body ratio or the kidney to body ratios. In chickens collected at the termination of an exposure period, most of clinical chemistry parameters were not significantly different among treatments (Table 2) and no specific 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 PLs in

Table 1  
Mean body-weight gain (s.d.) and organ to body weight ratio at the end of (A) an exposure and (B) a depuration phase

	Body wt. (g)	Liver/body (%)	Kidney/body (%)	Testis/body (%)
<i>(A) Exposure</i>				
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)
<i>(B) Depuration</i>				
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 0.2 or 1.0 mg) or PFOS (total 0.04 or 0.2 mg) via subcutaneous implantation for 4 wk and were allowed to depurate for further 4 wk.

Table 2

Mean serum lipids and biochemistry measurements for experimental chickens determined at the end of (A) an exposure and (B) a depuration period ( $n = 3$ )

Treatment	T-Cho <sup>a</sup> (mg/dL)	F-Cho <sup>b</sup> (mg/dL)	HDL <sup>c</sup> (mg/dL)	LDL <sup>d</sup> (mg/dL)	TP <sup>e</sup> (g/dL)	Alb <sup>f</sup> (g/dL)	BUN <sup>g</sup> (mg/dL)	NEFA <sup>h</sup> (mEq/L)	PL <sup>i</sup> (mg/dL)	AST <sup>j</sup> (IU/L)	ALT <sup>k</sup> (IU/L)	ALP <sup>l</sup> (IU/L)	LDH <sup>m</sup> (IU/L)
<i>(A) Exposure</i>													
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
<i>(B) Elimination</i>													
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

\* $p < 0.05$ ; <sup>a</sup>Total cholesterol; <sup>b</sup>Free cholesterol; <sup>c</sup>High-density lipoprotein; <sup>d</sup>Low-density lipoprotein; <sup>e</sup>Total protein; <sup>f</sup>Albumin; <sup>g</sup>Blood urea nitrogen; <sup>h</sup>Non-esterified fatty acid; <sup>i</sup>Phospholipid; <sup>j</sup>Aspartate aminotransferase; <sup>k</sup>Alanine aminotransferase; <sup>l</sup>Alkaline phosphatase; <sup>m</sup>Lactate dehydrogenase.

chickens exposed to both low- (2.0 mg PFOS/mL) and high-dosed ( $1 \times 10^{-1}$  mg PFOS/mL) treatments.

### 3.2. Uptake and elimination profile 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 an increase ( $t_{\max} = 7$  d) followed by sustained blood levels in later periods, while blood-borne PFOS fluctuated over a 4 wk-uptake period (Figs. 1 and 2). Concentrations of PFOA and PFOS in blood reflected the dose from capsules containing target PFCs. For example, the maximum PFOA concentrations in blood taken from low- and high-dosed treatment were  $1.0 \times 10^2$  and  $3.3 \times 10^2$  ng/mL, respectively. In non-exposed chickens during an uptake period, PFOA blood concentrations were less than the LOQ, while measured PFOS concentrations ( $3.5 \pm 0.7$  ng/mL) were at least eight times less than those in any given PFOS treatment.

Rates of elimination of PFOA and PFOS from blood of chickens were significantly different (Figs. 1 and 2). Concentrations of PFOA in blood decreased rapidly during the depuration phase, with PFOA concentrations at the termination of depuration being only 2–3% compared to those at the beginning of depuration. In contrast, only 48–52% reduction of PFOS was eliminated from the blood during the same period (Figs. 1 and 2). The first-order elimination rate constant was similar between treatments administered with the same compound (PFOA:  $1.50 \times 10^{-1} \pm 1.0 \times 10^{-2} \text{ d}^{-1}$  and PFOS:  $2.3 \times 10^{-2} \pm 4.0 \times 10^{-3} \text{ d}^{-1}$ ). The overall elimination half-time ( $t_{1/2}$ ) of treatment-averaged PFOA (4.6 d) was almost six-fold less than that of treatment-averaged PFOS (31.1 d). Growth dilutions that occurred during depuration did not influence

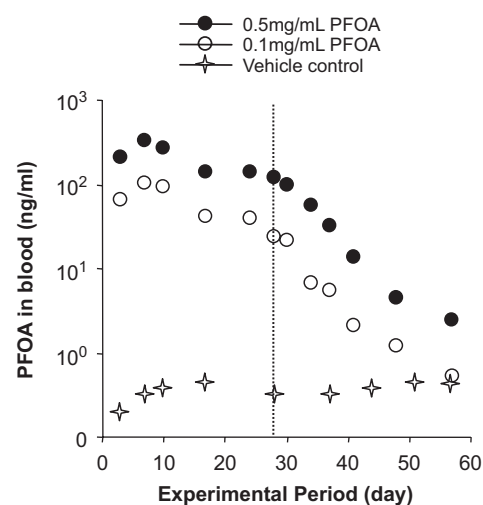


Fig. 1. Changes in the blood concentrations of PFOA (ng/mL) introduced into male chickens using an implantation. Chickens were exposed for 28-d (dashed line) and allowed for 28-d depuration. Closed circles and open circles symbolize high-dosed (0.5 mg PFOA/mL) and low dosed-treatment (0.1 mg PFOA/mL). Blood samples were pooled and analyzed by duplication. All PFOA measurements from vehicle control were less than the LOQ (1 ng/mL).

the final  $t_{1/2}$  of PFOA (5.2 d). However, there was an effect of growth dilution on the elimination half-life of PFOS. The elimination half-time for depuration was less for the greater dose ( $t_{1/2} = 7.2 \times 10^1$  d) than that observed for lesser dosed chickens ( $t_{1/2} = 1.8 \times 10^2$  d). In a vehicle control group, concentrations of PFOA and PFOS were similar during the depuration period to those measured during the exposure period.

### 3.3. Organ concentrations

Distinctive accumulation features of these two perfluorinated compounds were observed among organs (brain,

kidney, and liver) of chickens (Fig. 3). After a 4 wk dosing period, the greatest concentration of PFOA occurred in kidney, followed by liver and brain (Figs. 3A–C). In chickens exposed to a total of 1.0 mg PFOA dosed, the concentration of PFOA in kidney was as great as  $1.9 \times 10^2 \pm 40$  ng/g ww. A measurable concentration of PFOA was also detected in brain homogenate of PFOA-treated chickens. Concentrations of PFOA in all tissues decreased at a rate that fit a first-order model. By the completion of a

deuration 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 over the entire experimental period. Tissue disposition and elimination patterns of PFOS were significantly different than those observed for PFOA. Following cessation of exposure, the greatest concentration of PFOS occurred in the liver, with lesser concentrations in kidney and brain (Figs. 3D–F). Concentrations of PFOS in liver of chickens exposed to a total of 0.2 mg PFOS were as great as  $7.7 \times 10^2$  ng/g ww, which was five- and 55-fold greater than those in kidney and brain, respectively. Even after a 4 wk deuration period, concentrations of PFOS in organs had not significantly changed, while PFOS concentrations in brain tissue of the least dosed chickens was greater after 4 wk deuration ( $p < 0.05$ ). Chickens exposed to only saline buffer also contained detectable amounts of PFOS in liver tissues at all time periods, but in brain and kidney only after the deuration phase.

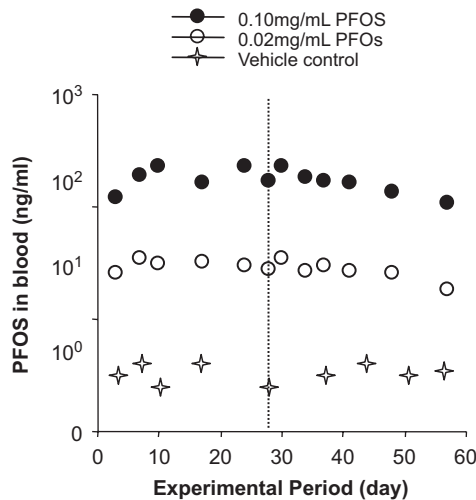


Fig. 2. Changes in the blood concentrations of PFOS (ng/mL) introduced into male chickens using an implantation. Chickens were exposed for 28-d (dashed line) and allowed for 28-d deuration. Closed circles and open circles symbolize high-dosed (0.10 mg PFOS/mL) and low dosed-treatment (0.02 mg PFOS/mL). Blood samples were pooled and analyzed by duplication.

## 4. Discussion

### 4.1. PFOA vs. PFOS elimination kinetics

The observation that introduced PFOS was retained longer in *G. gallus* than PFOA is consistent with the empirical results from studies of other species (Butenhoff, et al., 2004; Lau et al., 2004). Different elimination kinetics were observed even though PFOS and PFOA are structurally similar anionic surfactants that possess some similar physico-chemical properties such as  $pK_a$  (presumably  $< 3$ ), Henry's Law constant, and similar hydrophobicity based on critical micelle concentration (CMC for

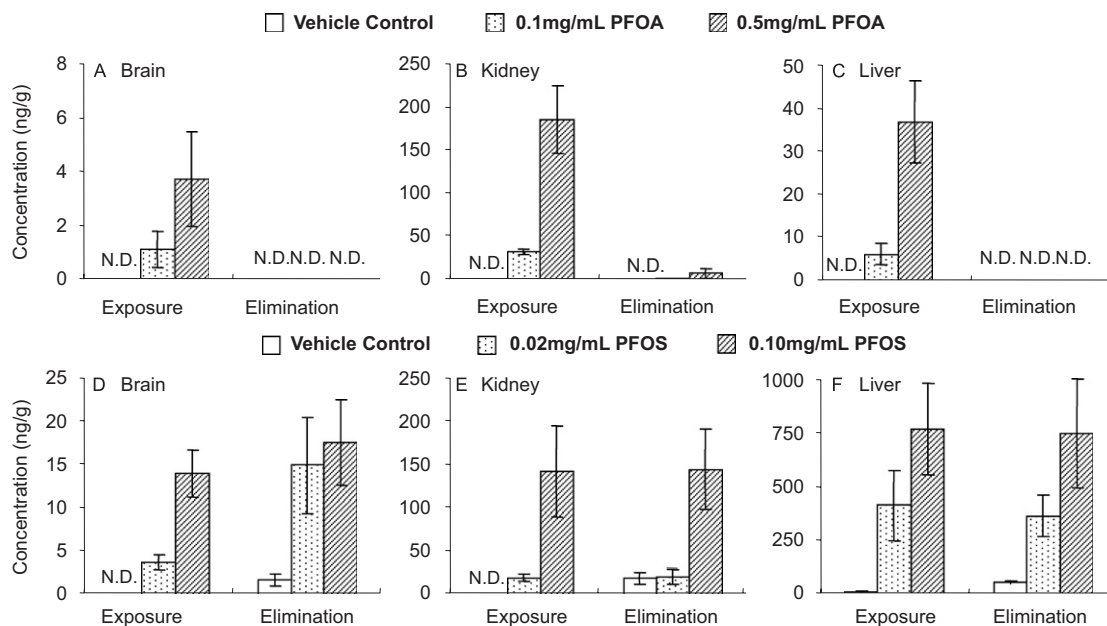


Fig. 3. Concentrations (ng/g ww) of PFOA (A–C) or PFOS (D–F) in organs retrieved from chickens at the completion of the exposure and the elimination period ( $n = 3$ ). Tissue homogenates (brain, kidney, and liver) were extracted using the combination of ion-pairing extraction and subsequent solid-phase extraction. Concentrations that were less than the LOQ were marked with “N.D.”

$C_7F_{15}COOH = 8.7\text{--}10.5$  mmol/L; CMC for  $C_8F_{17}SO_3K = 8$  mmol/L) (Giesy and Kannan, 2002; Kissa, 2001). In contrast, they can be distinguished by 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 PFCs 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 rats 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). In castrated male rats, expression of OAT2 mRNA was greater than in the controls and they were still able to eliminate PFOA at a rate similar to that of female rats. This result is consistent with an important role of these transporter proteins for PFOA removal via the kidney and also influences of sex-hormones (Kudo et al., 2001, 2002). For PFOS, clearance mechanisms, such as active transport via OATs have not been well examined. Rather than facilitating PFOS removal, retardation processes of PFOS removal could be hypothesized such as enterohepatic-recycling. In addition, greater binding strength of PFOS for serum proteins may also contribute the increased retention of PFOS compared to 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.

#### 4.2. Organ distribution

The disposition of these persistent and anionic PFCs 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 PFCs are blood, liver, and kidney rather than lipids. One of the reasons for the favored accumulation in those sites is the binding of PFCs to protein albumin, which is abundant in blood and liver (Jones et al., 2003). Though the octanol-water partitioning coefficients ( $K_{ow}$ ) are incorporated in a bioaccumulation modeling for hydrophobic compounds, the amphiphilic nature of PFCs makes impossible for calculation of  $K_{ow}$ . Even though a

$K_{ow}$  for PFOA or PFOS could be driven using its solubility in water and octanol determined separately, an estimate of  $K_{ow}$  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 (Fig. 4). As already discussed, renal uptake processes by the OAT family for removal of PFOA may also contribute accumulation of PFOA in kidney tissues as if an intermediate step before urinary excretion. Efficient removal of PFOA organ residue once an exposure source removed may also imply the dynamic redistribution of organ-accumulated PFOA into the blood compartment where subsequent active elimination process follows. Liver is recognized as a central site for PFOS storage, thus this tissue has been frequently analyzed in monitoring studies of PFCs (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 did not decrease during depuration. Rather a statistically significant increase in the concentration of PFOS in brain was observed. The ratio of PFOS concentration in liver to that in blood from the lesser treatment ( $PFOS_{liver: blood} = 15$ ) was three times greater than that observed for the greater of the two doses ( $PFOS_{liver: blood} = 5$ ) following an exposure period, but was similar at the end of a depuration phase with increased ratios ( $PFOS_{liver: blood} = 33$  and 29 for the lesser and greater exposures, respectively). This result is consistent with the hypotheses that either the mechanism of elimination of PFOS from liver is a slow process compared to that from blood, or that blood-borne PFOS may be redistributed to liver. These ratios could be different in wildlife, where precursors of PFOS, either identified or not, also contribute to the overall bioaccumulation of PFOS which is the ultimate degradation product of perfluorooctane sulfonyl fluoride based products (Giesy and Kannan, 2002).

Analysis of the relative mass of PFOA or PFOS in each organ following exposure and depuration phases demonstrated that blood and liver are the largest reservoirs controlling PFOA and PFOS body-burden in exposed chickens, respectively (Fig. 5). 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

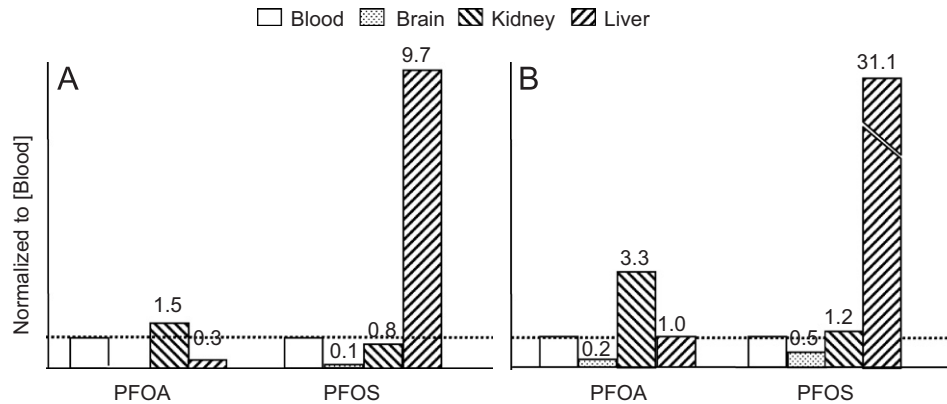


Fig. 4. Normalization of individual organ concentrations to concentrations in blood after exposure (A) and depuration (B). Number on top of the bar represents fold difference of concentration on average of two exposures.

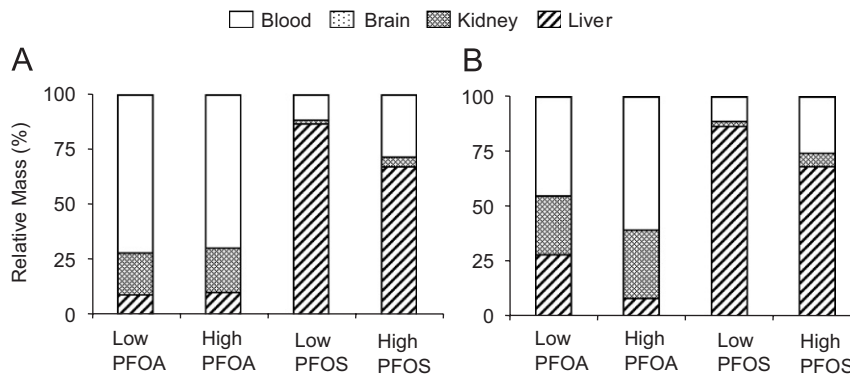


Fig. 5. Relative mass (%) of PFOA or PFOS in body reservoirs (blood, brain, kidney, and liver) after exposure (A) or depuration (B), respectively. Lesser and greater doses of PFOA had a total of 0.2 mg and 1.0 mg of PFOA for 4 wk of exposure, respectively, while one-fifth of amounts of PFOS were administered into lesser and greater doses. A 5% of body weight was assumed to be total volume of blood in the experimental chickens.

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 PFCs 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 also observed in non-exposed chickens (Figs. 3D–F). Until the first 4-wk exposure phase, concentrations of PFOS in all of the organs were less than the LOQ or fewer. However, following a 4-wk of period measurable concentrations of PFOS were observed in brain, kidney, and liver of unexposed chickens. Effects of age on PFOS accumulation have been 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 PFCs to human population. Detection of PFOA and PFOS in brain of the exposed chickens

suggests, if birds are exposed to PFCs, 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., 2005). 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, 2001).

#### 4.3. Sublethal effects

The observation of statistically significant lesser total cholesterol and PL concentrations 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). The significant decrease in total cholesterol and PLs at the end of depuration rather than at the end of exposure suggests the current exposure levels and/or 4-wk exposure duration were not sufficient to cause altered lipid metabolism in chickens, but prolonged PFOS exposure until the depuration phase pronounced the reduction of those lipids levels. However, there were no alterations observed in tissues when they were examined histologically. It was 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 discussed in this experiment.

#### 4.4. Comparison with other studies

The biological half-lives of PFOA and PFOS in *G. gallus* were compared with males of other species and summarized in Table 3. The half-time for elimination 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 approximately four-fold faster than that of the dog ( $t_{1/2} = 20$ – $23$  d) or monkey ( $t_{1/2} = 21$  d). Thus, the half-time for elimination seems to be a function of body size and/or physiological parameters, such that fish ( $t_{1/2} = 4.5$  d) exhibit the fastest clearance of PFOA, followed by the chicken = rat > dog = monkey > human (Harada et al., 2005). In contrast, the biological half-time for elimination of PFOS in our treated chicken ( $t_{1/2} = 125$  d) was similar to that of mammals including the rat ( $t_{1/2} > 90$  d) and the monkey ( $t_{1/2} = 100$ – $200$  d). This suggests that, unlike PFOA body behavior, that a number of vertebrates share common accumulation mechanism for PFOS, for example, through binding to plasma proteins as a governing process except invertebrates (e.g., fish PFOS  $t_{1/2} = 12$  d). However, most of kinetic studies have not considered the effects of growth dilution on the half-life of PFCs, in part they employed the adult animals in their studies. Thus, the calculated half-life of PFOA and PFOS

in this study may have another importance in understanding of the elimination kinetics where animals are in a growing phase.

The only available pharmacokinetic studies for other species of birds reported shorter biological half-times for PFOS than those obtained in our experiment. Several factors can influence on the pharmacokinetics in experimental animals. 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 on pharmacokinetic behavior in animals. However, PFOA or PFOS has virtually non-metabolized property, thus different dosing scheme may not result in observed different pharmacokinetics in two studies. Second, exposure levels were far different in two studies, which could modify removal kinetics. At the highest treatment in the current study, blood PFOS concentrations were realized at most tens of ppb level that were approximately 1000-fold less than those measured when animals were exposed to greater levels of PFOS-spiked diets in previous studies. That high exposure scheme was attributed in fact that they designed 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 d after cessation appeared to be faster than that in toward the end of a 1-yr depuration period. Similarly, chickens exposed to the greater concentration of PFOS eliminated blood-borne PFOS almost twice as fast as those exposed to the lesser of the two concentrations of PFOS. These observations may allude that the biological mechanism of PFOS elimination is acting differently at the lower- and higher-body burden.

## 5. Conclusions

The results presented in this report show that though they are structurally similar, the pharmacokinetic behaviors of PFOA and PFOS are different in chicken *G. gallus* during 8 wk experimental periods. Of the various blood chemistry measurements, only decreases of total cholesterol and PLs were observed in PFOS-dosed treatment. As observed in other animals, significant portion of PFOA could be removed from the blood and organs as fast as a few days. In contrast, blood-borne PFOS and tissue-accumulated PFOS were eliminated more slowly with a half-time for depuration of 125 d. Major accumulation sites of PFOA and PFOS were found to be kidney and liver, respectively. This result implies that those depository organs may be more susceptible to toxicological insults from PFOA and PFOS exposure which are ubiquitous in the environment. Age-dependent accumulation of PFOS in chickens also requires studies for exposure pathways to domestic farm animals in light of human exposure concerns.

Table 3  
Elimination half-life (days) of PFOA and PFOS in blood or serum from other studies

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

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