BIOCHEMICAL RESPONSES AND BIOACCUMULATION PATTERNS IN DAY-1 CHICKENS UPON EXPOSURE TO MIXTURES OF PFOS/PFDA/PFOA

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Introduction

The widespread occurrence of perfluorinated compounds (PFCs) in humans, wildlife, and the environment has prompted studies on the potential toxicity of these compounds in test animals[1-3]. Pharmacokinetic studies on perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been conducted with mammals such as rats, dogs, and monkeys[3-5]. The results from these studies showed that PFOA was readily absorbed but had a relatively short half-life with notable species- and gender-differences in rates of elimination when compared to PFOS. The half-life for PFOA in the blood of male rats is approximately one week, whereas in females the half-life is approximately one day[6]. In contrast, PFOS was readily absorbed but it was poorly eliminated from blood and other tissues, with biological half-lives ranging from several weeks to several months, depending on species and sex[3-5]. However, although the effects of PFCs have been investigated in mammals, few similar investigations have been conducted on avian species.

Avian kinetic data are available from acute and chronic dietary studies that have been conducted with two species, the northern bobwhite quail (*Colinus virginianus*) and mallard (*Anas platyrhynchos*)[7]. The results of these studies indicated that the half-life of PFOS in the blood and liver of juvenile birds ranged from approximately 7 to 18 d, while PFOS half-lives in blood ranged from 14 to 21 d in adult birds. A recent study investigated elimination kinetics in male chickens exposed to either PFOS or PFOA via subcutaneous implants[8]. Calculated half-lives for PFOS and PFOA were 125 and 5.2 days, respectively. Previous toxicological studies have investigated the effects of PFCs via egg injection with PFOS before hatching[9], or by exposing six-week-old chickens to PFOS/PFOA[8], but toxicological data and pharmacokinetic data for newly hatched chickens are lacking. In view of this deficit, one of the objectives of the present study was to fill this data gap by exposing 1-day-old chickens to PFCs. In addition, several food web studies have shown that some PFCs have the potential to bioaccumulate in organisms at lower trophic levels and then accumulate at upper trophic levels through trophic transfer and biomagnification[10-11]. Biomonitoring data also suggest that PFCs with longer carbon-fluorine chain length are more bioaccumulative[12]. Therefore, the second objective of the present study was to understand the pharmacokinetic properties of long chain PFCs (PFOS, perfluorodecanoic acid (PFDA) and PFOA) in chickens exposed to mixtures of these compounds.

Material and Methods

Day-1 male PDL-1 strain white leghorn chickens (*Gallus gallus)* were housed in humidity-controlled facilities in accordance with the guidelines of the National Institute of Animal Health, Japan. Three groups of 12 randomly selected day-1 male chickens were exposed via oral gavage to equal-concentration mixtures of PFOS, PFOA, and PFDA at either a low dose (0.1 mg/kg body weight) or a high dose (1 mg/kg body weight), or a saline vehicle control. Chicks were dosed three times per week for three weeks. Three individuals from each group were sacrificed at the end of exposure. The remaining animals were allowed to depurate for three weeks before being sacrificed. Blood/plasma samples were collected for chemical, biochemical and pathological analysis, and the following tissues were fixed in 10% phosphate–buffered formalin and routinely processed for histopathological examination: liver, kidney, spleen, heart, lung, thymus, testis, bursa of Fabricius, and brain.

Fourteen biochemical parameters were analyzed in plasma in 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. The parameters measured were as follows: 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), lactate dehydrogenase (LDH), triglyceride (TG), and uric acid (UA).

Individual PFCs in blood samples were extracted using an ion-pairing method and were reduced to a final volume of 1 mL. In addition, 0.5 mL of the sample extract was subjected to solid phase extraction (SPE) cleanup. The concentrations of PFOS, PFDA, and PFOA were determined by using HPLC-MS/MS[12,13]. Non-parametric Kruskal-Wallis tests were used to assess significant differences among treatments. The significance level for the tests was α=0.05.

Results and Discussion

No statistically significant differences in body weight were observed among doses (vehicle control, low (a mixture of 0.1 mg PFOS/kg + 0.1 mg PFDA/kg + 0.1mg PFOA/kg), and high (a mixture of 1.0 mg PFOS/kg + 1.0 mg PFDA/kg + 1.0 mg PFOA/kg)) doses after three weeks of exposure (body weight range: 201-218 g) or after three weeks of depuration (range: 463-486 g). There were no significant differences in liver/kidney weight-to-body-weight ratios among doses after three weeks of exposure (liver: 2.59-3.04; kidney: 1.22-1.28) or after three weeks of depuration (liver: 2.48-2.98; kidney: 1.07-1.18). No specific lesions were observed in tissues from the exposed groups relative to those from vehicle controls. None of the 13 blood clinical chemistry parameters analyzed in plasma samples were significantly different from vehicle controls in any of the PFC-exposed chickens (Table 1).

Table 1. Mean values of the clinical chemistry measurements in experimental chickens determined after PFC exposure and depuration at different doses (Control: saline/ethanol; low dose 0.1 mg of each PFOS/PFDA/PFOA /kg b.w.; high dose 1.0 mg of each PFOS/PFDA/PFOA /kg b.w.).

Treatment	T-Cho mg/dL	F-Cho mg/dL	HDL mg/dL	LDL mg/dL	TP g/dL	Alb g/dL	BUN mg/dL	NEFA mEq/L	PL mg/dL	AST IU/L	ALT IU/L	LDH IU/L	TG mg/dL	UA mg/dL
Day 21 (End of expoure)														
Control *n=3*	141.3	34.7	86.2	48.3	3.0	1.7	1.7	2.3	263.0	196.3	1.7	747.7	103.3	5.3
S.E.	7.8	1.8	4.4	6.1	0.1	0.0	0.0	0.2	13.7	10.7	0.7	40.0	12.6	0.4
Low dose *n=3*	143.3	35.3	87.9	45.3	3.0	1.7	1.7	1.6	258.3	205.3	1.7	688.3	105.0	6.5
S.E.	2.9	2.0	3.5	3.3	0.1	0.0	0.4	0.3	5.2	10.7	0.3	95.0	9.6	1.3
High dose *n=3*	141.0	34.0	89.6	42.3	3.0	1.6	1.2	1.5	247.3	189.0	1.0	639.7	92.7	5.4
S.E.	9.5	4.9	2.5	6.6	0.2	0.1	0.1	0.4	19.8	17.3	0.0	42.7	26.4	0.3
Day 42 (End of depuration)														
Control *n=3*	130.7	27.0	89.2	36.0	3.1	1.7	1.4	1.2	225.0	187.3	1.0	481.7	82.3	4.0
S.E.	1.3	1.0	1.9	1.5	0.1	0.1	0.2	0.2	3.2	14.4	0.0	41.6	3.2	0.5
Low dose n=3	142.0	29.0	99.6	40.0	3.0	1.7	1.3	1.4	245.0	178.3	1.0	501.3	83.0	5.1
S.E.	5.0	0.0	6.0	1.0	0.0	0.1	0.1	0.1	10.6	3.3	0.0	21.3	7.0	0.4
High dose n=3	146.0	31.3	95.9	47.3	3.2	1.8	2.0	1.4	251.3	217.3	1.3	726.3	110.0	5.5
S.E.	7.0	0.7	5.1	4.1	0.0	0.0	0.6	0.3	4.8	25.7	0.3	147.8	8.5	2.4

T-Cho: Total cholesterol; F-Cho: Free cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; TP: Total protein; Alb: albumin; BUN: Blood urea nitrogen; NEFA: Non-esterified fatty acid; PL: Phospholipid; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; TG: triglyceride; UA: uric acid; S.E.: Standard error

These results were similar to our previous exposure experiment with chickens in which no effects on body index, clinical biochemistry and histopathology were observed in male chickens after subcutaneous implant exposure to 0.02/0.1 mg PFOS/kg, and 0.1/0.5 mg PFOA/kg with the exception of a decrease in T-cho/PL[8]. The different outcomes of these two studies might be attributed to their differing experimental designs (mode of exposure, and the age of the chickens). In the present study, no signs of toxicity were observed, suggesting that these doses did not have any adverse health effects on male chickens.

Uptake and elimination profiles for PFCs in the blood of exposed chickens were compared (Figures 1a-c). PFC concentrations in the control group were much lower than those of the exposed groups and there were no significant changes in PFC concentrations throughout the experimental period. PFOS/PFDA/PFOA bioaccumulated to much higher levels in the low dose groups (0.1 mg/kg of each PFC) than in the vehicle control group after 1 week of exposure. PFOA concentrations in the low-dose group were steady throughout the exposure period (days 7-28), whereas PFOS and PFDA levels increased from days 7-21 and accumulated at higher levels on day 28. In contrast, greater accumulation of all three PFCs in the high dose group (1.0 mg/kg of each PFC) occurred after the first week of exposure compared to the vehicle control and low dose groups. PFOS and PFDA levels increased throughout the exposure period, whereas the PFOA level decreased over time.

A one-compartment model was constructed to understand the elimination of PFOS/PFDA/PFOA from blood in male chickens (Figures 2a,b). Two elimination rate constants were obtained for each PFC for the different dosing regimes. Two sets of half-lives for each PFC were calculated using different elimination constants. The half-lives for PFOS were around 12.0 days and 7.5 days for the 0.1 mg/kg and 1.0 mg/kg doses, respectively; 10.3 days and 7.0 days for PFDA; and 3.2 days and 0.8 days for PFOA, correspondingly. It was clear that the half-lives of PFDA and PFOS were similar to one another, whereas that of PFOA was quite different.

Similar studies have been conducted on PFCs in test animals[4,7,14,15]. Most of these studies investigated single PFCs, while a mixture of PFOS, PFDA, and PFOA was given to male chickens in the present study in order to evaluate the pharmacokinetics of these PFCs. Different half-lives for different PFCs among studies have been observed. For example, the half-lives of PFOS ranged from 7.5 days (present study) to 125 days in male chickens[8]. These differences might be due to experimental design (oral gavage vs subcutaneous implanted pump; a dose of a PFC mixture vs a single PFC dose). Nonetheless, it is clear from several studies that among PFOS, PFDA, and PFOA, PFOA is eliminated by test animals more rapidly than either PFOS or PFDA[4,8,14,16]. The validity of these data for female chickens needs further investigation. However, these results further confirm that longer carbon-fluorine chain PFCs are more likely to bioaccumulate in male chickens—findings which were similar to those in rats[18]—and may help account for the higher concentrations of PFDA than PFOA measured in water bird eggs[12]. In conclusion, the present study demonstrated different half-lives/elimination rates for PFOA, PFDA, and PFOS in male chickens after exposure to a mixture of these PFCs. PFOS and PFDA had similar half-lives, whereas PFOA had a shorter half-life in male chickens.

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Figure 1 a)PFOS/ b)PFDA/, and c)PFOA concentrations in chicken blood during the exposure and depuration periods

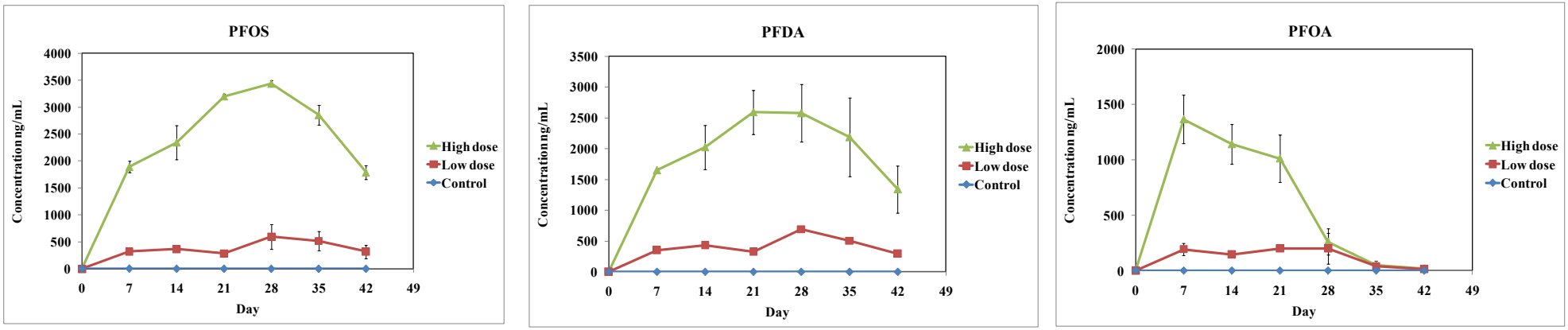

Figure 2 PFC concentrations during depuration periods in blood samples at (a: left) low dose, and (b: right) high dose.