

Full Length Research Paper

Toxicokinetic behaviors and modes of perfluorooctane sulfonate (PFOS) and perfluorooctane acid (PFOA) on tilapia (*Oreochromis niloticus*)

Zhao-Xiang Han^{1*}, Meng Zhang¹ and Chun-Xia Lv²

¹School of Chemical Engineering, Huaihai Institute of Technology, Lianyungang, Jiangsu, 222005, China.

²Jiaonan Agricultural Bureau, Qingdao, Shandong, 266400, China.

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Perfluorooctane sulfonate (PFOS) and perfluorooctane acid (PFOA) are widely distributed environmentally persistent organic pollutants found at low levels in human and wildlife ecosystem. The objectives of the current study were to investigate toxicokinetic behaviors and modes of PFOS and PFOA on tilapia (*Oreochromis niloticus*). The toxicokinetic behaviors and modes of PFOS and PFOA are different in tilapia during experimental periods. Exposure to both PFOS and PFOA was approximately 5 to 6 times higher for male tilapia than for female tilapia. The terminal half-life of PFOA in serum was about 4 times longer for male tilapia than for female tilapia. The apparent volume of distribution for PFOS and PFOA in the serum was about 3 to 4 times higher for female tilapia than for male tilapia. The lymphocytes level decreased rapidly with the increased PFOA concentration. A similar pattern was observed with the PFOS exposure, and it is remarkable that PFOS and PFOA were significantly accumulated in both PFOS and PFOA exposure but PFOA showed a greater effect than PFOS. PFOA blood concentrations were lesser than the limit of quantification in non-exposure tilapia during an uptake period, while measured PFOS concentrations were at least six times lesser than those in PFOS exposure. Tilapia weight gain was also decreased with statistical significance in all PFOA-treated groups, and the effect of PFOA was higher than that of PFOS. The effects of PFOA in survival percent were more pronounced in this case than that of PFOS. Moreover, PFOA had similar mode, and PFOS and PFOA can inhibit the 17 β -HSD3 enzyme activity of tilapia.

Key words: Toxicokinetic, behaviors, perfluorooctane sulfonate (PFOS), perfluorooctane acid (PFOA), tilapia (*Oreochromis niloticus*).

INTRODUCTION

Many products of perfluorinated compounds (PFCs) are becoming ubiquitous in human and wildlife environment because of the continuous use of such products (Giesy and Kannan, 2002; Olsen et al., 2005) and the two PFCs that have received the greatest attention are perfluorooctane sulfonate (PFOS) and perfluorooctanoic

acid (PFOA). In a monitoring study of human serum PFOS was determined collected from the general public, to be the most abundant PFC followed by PFOA (Kannan et al., 2004). While exposure pathways of PFOA and PFOS to humans and aquaculture 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 PFOS and PFOA to exposed human populations.

A few pharmacokinetic studies with 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), but, the details of the toxicokinetic profile and modes of PFOS and PFOA have not been published

*Corresponding author. E-mail: hjyzx@sina.com. Tel: +86-518-85895409.

Abbreviations: PFOS, Perfluorooctane sulfonate; PFOA, perfluorooctane acid.

with aquatic animals. PFOS and PFOA are readily absorbed and are 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. Previously, it has been reported that repeated dose toxicity with PFOS and PFOA in mice, rats and monkeys (Seacat et al., 2002, 2003; Luebker et al., 2005; Thibodeaux et al., 2003; Lau et al., 2003) have shown that body burden is proportional to cumulative dose at lower and moderate experimental doses.

To control effectively the exposures to PFOS and PFOA, it is necessary to understand the kinetic mechanism and metabolism of PFOS and PFOA in the environment. Therefore, the objective of our study was to explore the toxicokinetic behaviors and modes of PFOS and PFOA in tilapia and evaluate if the pharmacokinetic profile and modes of PFOS and PFOA are similar to tilapia after oral administration.

MATERIALS AND METHODS

Experimental design

A total of 60 freshwater tilapia (*Oreochromis niloticus*) 20 to 30 g each (30 females and 30 males) were collected from a local fish farm at the industrial and agricultural areas. They were kept in a 300 L fiber-glass tank with recirculating water for 3 weeks ($22 \pm 0.5^\circ\text{C}$, 12 h light / 12 h dark cycles). Tilapia were randomly divided into three groups (sex for each group) in which they were treated with PFOS and PFOA in the form of pelleted dry feed at a dose of 0, 1.0, 5.0 and 10.0 mg/L, respectively for 24, 48, 72, 96, 120, and 144 h.

The fish were fed once every other day with commercial fish food. Doses applied in this study were designed based on the pharmacological and toxicological studies, and PFOS and PFOA were found to be effective at range of 1 to 20 mg/g. The fish were then killed immediately by a blow on the head and samples of the muscle, liver were taken, the samples were stored at -40°C until used.

Sample preparation and analysis

Blood samples were collected with an ion-pairing method with some modifications (Kannan et al., 2004). Measurement of PFOS and PFOA in blood or tissues was conducted using HPLC with high resolution and electro-spray tandem mass spectrometry (HPLC-MS/MS). Serum was prepared by standard methods. Serum samples were stored frozen (-20°C) until analysis using a validated HPLC-MS/MS method. Hemolymphs were instantly mixed with the same volume of ACD (1.5% citric acid, 2.5% trisodium citrate, 2% D-glucose) solution, and were kept frozen at -70°C . Hemolymph were thawed and vortexed to assure homogeneity before extraction.

0.5 ml of hemolymph were placed into 10 ml polypropylene centrifuge, then 7.0 ml of the extraction solution (1 M HCl acetonitrile (4/500, v/v) was added. The tubes were vortexed for 2 min and centrifuged at 8000 rpm for 10 min and 4°C . The resulting clear supernatant was dried using a vortex evaporator and reconstituted in 1.0 ml of mobile phase. After filtration with 0.45 μm disposable syringe (filter units equipped with cellulose acetate membranes), the filtrate was injected into the HPLC system (Tang and Yang, 2006).

Preparation of microsomal protein and 17 β -HSD3 activity assay

Microsomal preparations of human and rat testes were done as described previously (Hu et al., 2009). Microsomal protein concentrations were adjusted to 4 mg/ml and used for measurement of 17 β -HSD3 activities. 17 β -HSD3 activity in spermary microsomes was recorded using the normal procedures (Hu et al., 2009).

Toxicokinetic analysis

All pharmacokinetic parameters were calculated using non-compartmental analysis from the individual concentrations of PFOS and PFOA in serum for tilapia and from the mean concentrations in serum, lymphocytes and blood. The data could not be consistently fit to a one-compartment model using WinNonlin version 5.2 (Pharsight Corporation, Mountain View, CA). The program used a compartmental open model based on non-linear regression analysis and a non-compartmental analysis based on statistical moment theory to analyze concentration-time data for PFOS and PFOA.

Statistical analysis

Assays were repeated three times. The IC_{50} was calculated using GraphPad version 4.0 using nonlinear regression of curve fit with one-site competition. Data were subjected to analysis by one-way ANOVA followed by Duncan multiple comparison test to identify significant differences between groups when three groups were calculated. All data were expressed as means \pm SEM. Differences were regarded as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Toxicokinetic behaviors of PFOS and PFOA in serum

The serum concentrations of PFOS and PFOA following oral administration dose of 5 mg/g are illustrated in Figure 1. Systemic exposures to PFOA were approximately 2 to 3 times higher than exposures to PFOS at equivalent dosages. This is partially due to a terminal half-life for PFOS that is shorter than that for PFOA. Exposure to both PFOS and PFOA was approximately 5 to 6 times higher for male tilapia than for female tilapia, this may be attributable to several related factors such as the shorter half-life, higher apparent clearance and higher distribution numbers of females than for males.

The terminal half-life of PFOS in serum was approximately 2-fold shorter for female tilapia (1.0 h) than for male tilapia (2.0 h). The terminal half-life of PFOA in serum was about 4-fold longer for male tilapia (1.4 h) than female tilapia (0.35 h). Apparent clearance of PFOS and PFOA from the serum was roughly 5 to 6 times higher for female tilapia than for male tilapia. The apparent volume of distribution for PFOS and PFOA in the serum was about 3 to 4 times higher for female tilapia than for male tilapia. Lower apparent volumes of distribution for both female and male may reflect rapid clearance, including protein binding, and elimination.

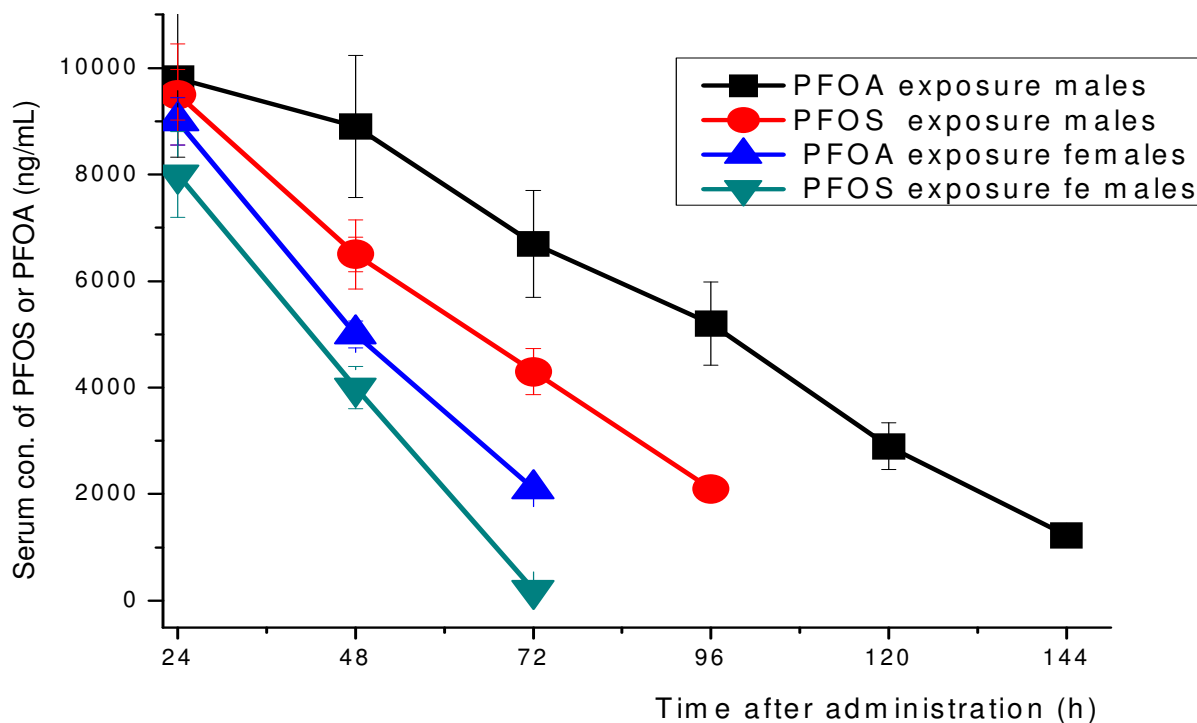


Figure 1. Mean concentrations (\pm S.E.) of PFOS and PFOA in serum of male and female tilapia following oral administration of 5.0 mg/g. Bars indicate SD.

The serum concentrations of PFOS and PFOA followed repeated oral doses of 10 mg/g, respectively for male and female tilapia. Oral administration of PFOS and PFOA resulted in systemic exposure, which was proportional to the dosage level. Exposure tended to decrease upon repeated dosing. The terminal half-life of PFOS and PFOA in serum, however, was about 1 to 2 h regardless of dosage level, gender or number of doses. Studies with PFOS and PFOA in rats have demonstrated a sex-specific difference in elimination, with females more actively excreting these materials via the kidney than males (USEPA OPPTS, 2002; Vanden Heuval et al., 1991). This difference contributes to large differences in toxicokinetic behavior. For example, Vanden Heuval et al. (1991) reported that the half-life for ^{14}C -PFOA was 9 days for male rats and 4 h for female rats. Ylinen et al. (1990) reported a half-life of 24 h in female and 105 h in males Wistar rats. In the study, there was also appreciable gender difference in the half-life of PFOS and PFOA in serum of tilapia following oral doses.

Toxicokinetic behaviors of PFOS and PFOA in peripheral lymphocytes

The PFOS and PFOA concentration-time curves for the tests showed instant absorption followed by distribution phase as shown in Figure 2. After an oral administration of 5.0 mg/g body weight of tilapia, the peripheral

lymphocytes PFOA concentration peaked at 600.09 ± 15.8 ng/ml at the first sampling time of 24 h and then the lymphocytes PFOA concentration decreased rapidly from 600.09 ± 15.8 to 468.32 ± 12.6 ng/ml post-treatment. A second absorption peak (480.65 ± 9.5 ng/ml) was observed at 96 h post administration, after which the PFOA concentration declined slowly and continuously until the end of the experiment. A similar pattern and dosage was observed with the PFOS exposure, including a peak lymphocytes PFOS concentration of 453.0 ± 8.6 ng/ml at the first sampling time of 24 h and a second absorption peak (385.78 ± 11.4 ng/ml) at 72 h post-treatment.

It is possible that there is recirculation of PFOS and PFOA, resulting in second absorption peaks. The lymphocytes PFOS and PFOA concentration at 5.0 mg/g dosage body weight were analyzed with pharmacokinetics software (DAS ver 1.0), and both could be described by a two compartment open model with first-order absorption. Both the absorption half-time ($T_{1/2Ka}$) and the lag time before initiation of absorption (T_{lag}) were approximately zero. With respect to apparent volume of distribution and total body clearance, no significant differences were observed between PFOS and PFOA. The distribution rate constant (α) of PFOS in lymphocytes was approximately 1.5-fold lesser than for PFOA, and the elimination rate constant (β) of PFOS in lymphocytes was about 2-fold higher than for PFOA. After non-compartment model analysis, the value of area under

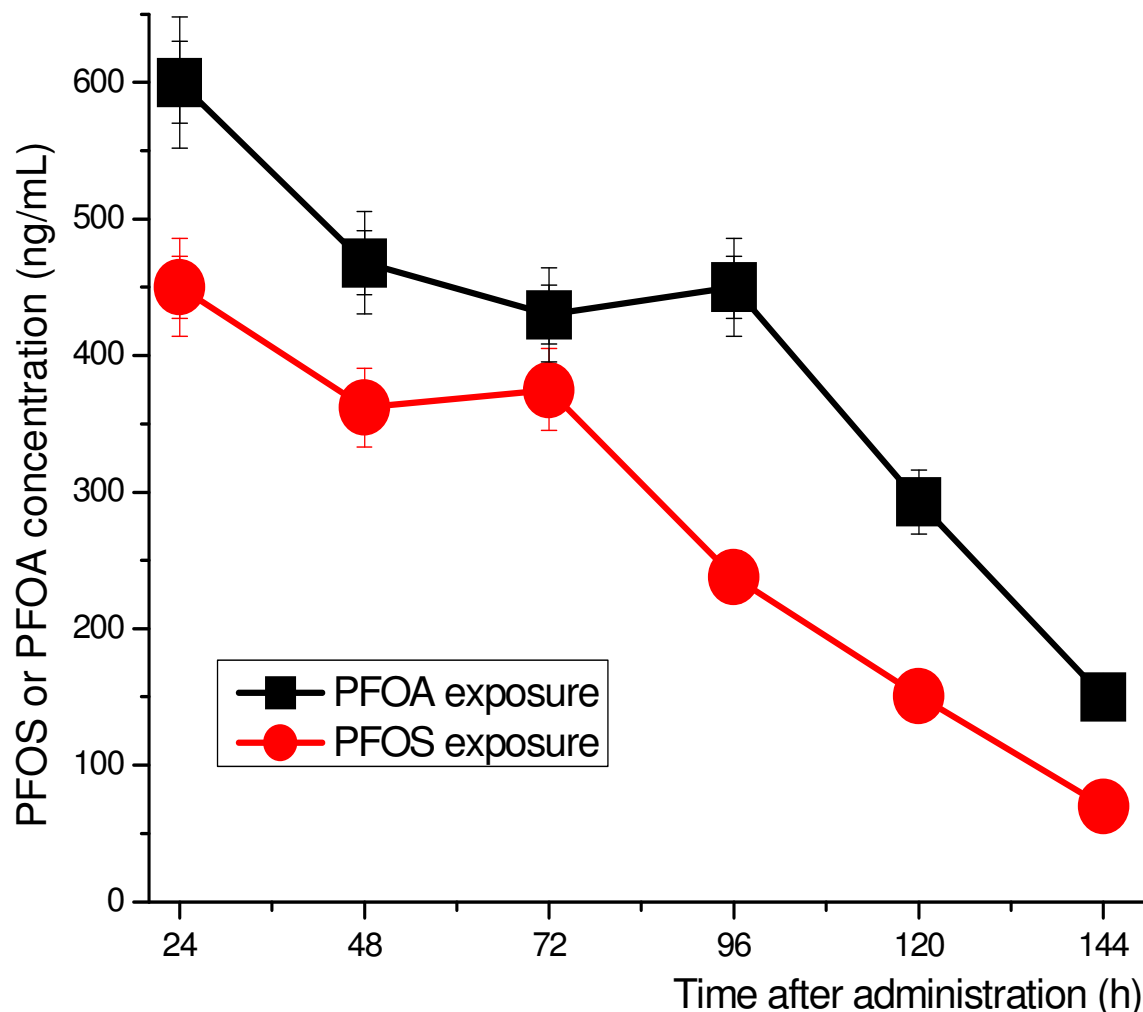


Figure 2. Peripheral lymphocytes concentrations of PFOS and PFOA in tilapia following oral administration at 5.0 mg/g dosage, respectively. Symbols indicate the means and standard deviations.

the peripheral lymphocytes concentration-time curve ($AUC_{0-\infty}$) for PFOA was 2-fold that of the lymphocytes PFOS concentration. It is remarkable that PFOS and PFOA were significantly accumulated in our study in both PFOS and PFOA exposure, with PFOA showing a greater effect than PFOS. Similar results have been observed in few fishes, such as the zebrafish (Huang et al., 2010) and common carp (*Cyprinus carpio*) (Hoff et al., 2003a).

Uptake and elimination behaviors of PFOS and PFOA in blood

A one-compartment model was used to describe the elimination behavior of PFOS or PFOA from blood in tilapia according to the equation: $\ln(C_t/C_0) = -kt$, where C_t is the concentration of PFOS or PFOA at the time (t) in an elimination phase, C_0 is the concentration at the onset of depuration (ng/mL) and k is the elimination rate constant.

The time-course concentrations of PFOS and PFOA released from oral administration of different uptake profiles in tilapia blood over an exposure period was recorded. The maximum PFOS and PFOA concentrations in blood taken from same dosage treatment were 7.3×10^3 and 9.6×10^3 ng/ml at primary exposure time (24 h), respectively (Figure 3).

Subsequently, introduced PFOS fluctuated over 72 h uptake period, while PFOA in 5.0 mg/ml treatment had an increase at 24 h followed by decreased blood levels in later exposure periods. PFOA blood concentrations were lesser than the limit of quantification in non-exposure tilapia during an uptake period, while measured PFOS concentrations were at least six times lesser than those in PFOA exposure. Concentrations of PFOS and PFOA in blood reflected the dose from oral administration target PFCs. Nevertheless, elimination rates of PFOS and PFOA from blood of tilapia were significantly different (Figure 3). Concentrations of PFOS in blood decreased

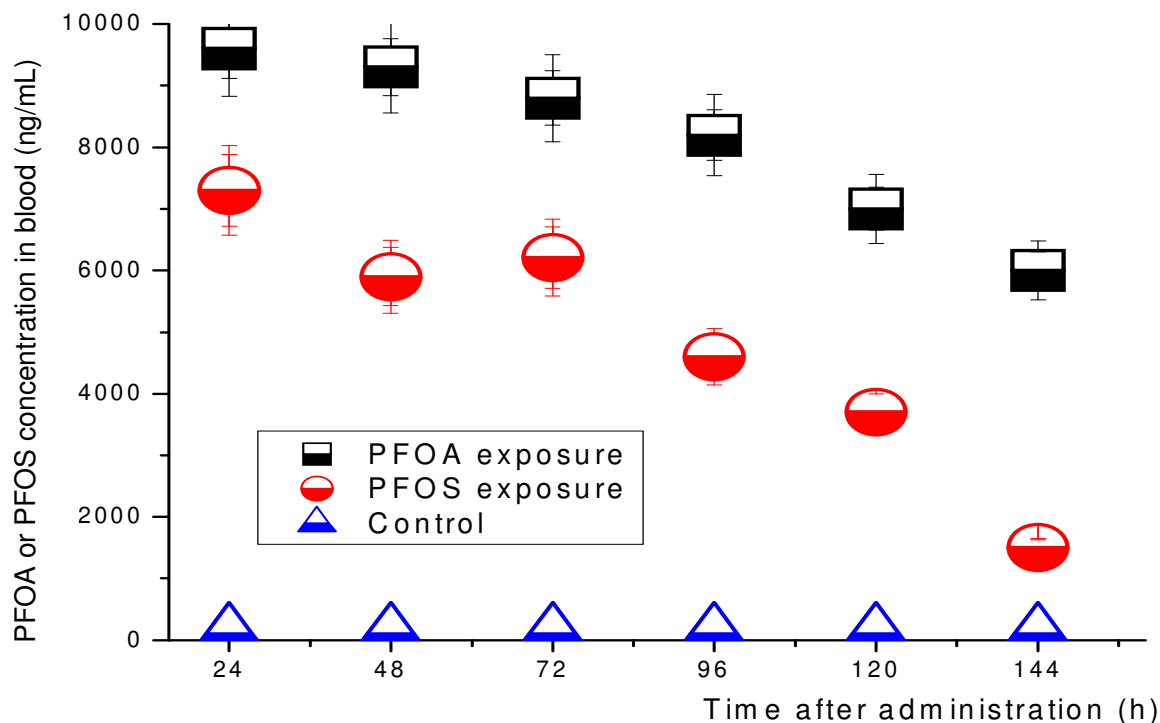


Figure 3. Changes in the blood concentrations of PFOA or PFOS in tilapia following oral administration at 5.0 mg/g dosage, respectively. Blood samples were pooled and analyzed by duplication.

rapidly during the depuration phase, with PFOS concentrations at the termination of depuration being 56 to 59% as compared to those at the beginning of depuration. However, only 18 to 23% reduction of PFOA was eliminated from the blood during the same exposure period. The elimination half-time ($t_{1/2}$) of treatment-averaged PFOS (10.6 h) was almost four-fold more than that of treatment-averaged PFOA (42.5 h). The elimination rate constant of PFOS and PFOA was 0.12 ± 0.01 and $0.03 \pm 0.002 \text{ h}^{-1}$ with the same dosage, respectively. Concentrations of PFOS and PFOA were similar during the depuration period to those measured during the exposure period in control group. 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 , Henry's Law constant, and similar hydrophobicity based on critical micelle concentration (Giesy and Kannan, 2002; Kissa, 2001).

Dose-response modes of PFOS and PFOA on weight and percent survival

We chose 144 h as the standard incubation time for the experiments according to the primary experiment. Tilapia weights, as an average of each experiment group, are presented in Figure 4A and were decreased with

statistical significance in all PFOS-treated groups. Tilapia weight gains were also decreased with statistical significance in all PFOA-treated groups, and the effect of PFOA was higher than that of PFOS. It appears that there is positive dependence between PFOS and PFOA concentration and tilapia weight to PFOS and PFOA exposure environment, represented with a high coefficient of correlation ($p = 0.021$, $R = 0.9316$ for PFOS and $R = 0.9258$ for PFOA). Figure 4B presents data for the mean percent of tilapia that survived in each experiment group. The mean percent survival of live born tilapia was decreased in a dose-dependent manner and became statistically significant in the 5.0 and 10.0 mg/l oral administration dose groups for PFOS-treated groups. The mean percent survival of live tilapia decreased from 88 to 26% with 0 and 10.0 mg/L PFOA exposure, respectively. Furthermore, the effects of PFOA in percent survival were more pronounced in this case than that of PFOS. Multiple linear regression analysis revealed a significant correlation between PFOS and PFOA concentration and survival ($p = 0.011$, $R = 0.9056$ for PFOS and $R = 0.9403$ for PFOA).

Inhibition modes of PFOS and PFOA on 17 β -HSD3 activity

17 β -HSD3 levels were determined only in male tilapia. Activity of the 17 β -HSD3 enzyme, which catalyzes the

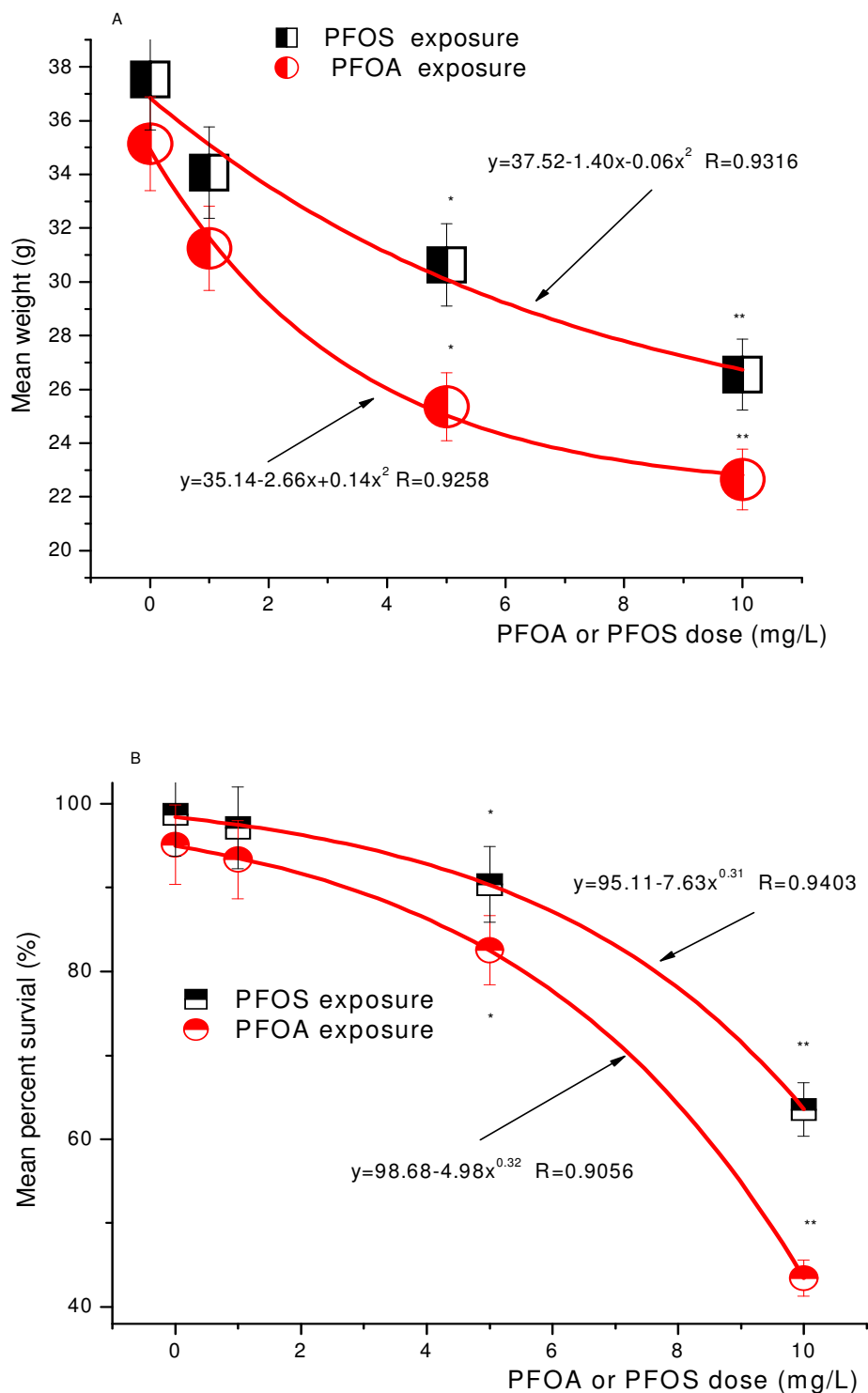


Figure 4. Mean weight (A) and mean percent survival (B) of tilapia treated with PFOS or PFOA. Error bars represent 95% confidence limits. Modeled dose–response curves are drawn based on the equation shown, and means that are statistically different from control means are noted by either a single asterisk (* $p<0.05$) or a double asterisk (** $p<0.01$).

conversion of androstenedione to testosterone, was measured in male tilapia spermary microsomes in the presence of PFOS and PFOA exposure. Figure 5 shows

the effect of PFOS and PFOA on 17 β -HSD3 activities when tested at different concentrations, respectively. 17 β -HSD3 activity was strongly reduced by PFOS, which is

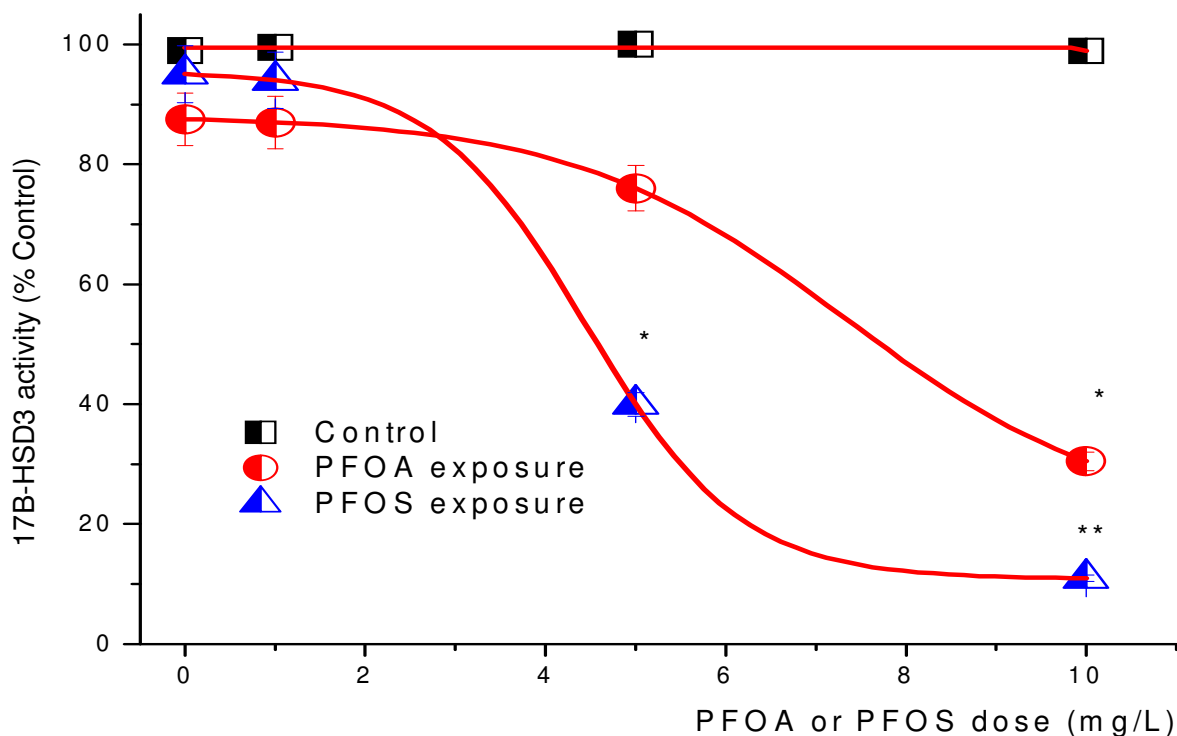


Figure 5. The inhibition of PFOS or PFOA on male tilapia 17 β -HSD3 activities in testicular microsomes (mean \pm SEM). Statistical difference of control are noted by either a single asterisk (* p <0.05) or a double asterisk (** p <0.01).

also in a dose-dependent manner. The activities of the tilapia 17 β -HSD3 enzymes in the absence of PFOS were determined to be 5.36 ± 0.06 nmol/mg protein/min. PFOS inhibited the tilapia 17 β -HSD3 enzyme by 15, 60 and 88% as compared to control at the oral dose of 1.0, 5.0, and 10.0 mg/L, respectively. Moreover, PFOA had similar mode, PFOA inhibited the tilapia 17 β -HSD3 enzyme by 23, 75 and 93% as compared to control at the oral dose of 1.0, 5.0, and 10.0 mg/L, respectively. The IC_{50} for PFOS and PFOA in the inhibition of tilapia 17 β -HSD3 activity were determined to be 4.18 ± 0.05 and 5.33 ± 0.02 , whereas the IC_{50} of PFOS and PFOA in the inhibition of tilapia 17 β -HSD3 activity were 102 and 106 nmol/mg protein min, respectively as previously reported (Chu et al., 2009). The mode of PFOS and PFOA inhibition of tilapia 17 β -HSD3 activity differs from that previously reported for the rat enzyme in and PFOS and PFOA inhibited rat 17 β -HSD3 activity with competitive inhibition on substrate DIONE binding site and noncompetitive inhibition on cofactor NADPH binding site of the enzyme (Zhao et al., 2010; Chu et al., 2009). The biological roles of 11 β -HSD2 in fish are complex. 11 β -HSD2 is expressed in fish liver, kidney, brain and reproductive organs (Michael and Baker, 2004; Zhao et al., 2010). 11 β -HSD2 and 17 β -HSD3 catalyzes the conversion of 11 β -hydroxytestosterone and androstenedione to 11-keto-testosterone, a fish androgen, consistent with the presence of 11 β -HSD2 and 17 β -HSD3 in

gonads. Fish 11 β -HSD2 and 17 β -HSD3 are expressed in testis (Michael and Baker, 2004; Zhao et al., 2010), where they can both inactivate cortisol and synthesize 11-keto-testosterone.

Conclusion

The results present in this study showed that though they are structurally similar, the toxicokinetic behaviors and modes of PFOS and PFOA are different in tilapia during experimental periods. The toxicokinetics behaviors and modes of PFOS and PFOA were estimated after oral administration to the tilapia. Systemic exposures to PFOA were approximately 2 to 3 times higher than exposures to PFOS at equivalent dosages; this is partially due to a terminal half-life for PFOS that is shorter than that for PFOA. Exposure to both PFOS and PFOA was approximately 5 to 6 times higher for male tilapia than for female tilapia. The terminal half-life of PFOA in serum was about 4 times longer for male tilapia than female tilapia. The apparent volume of distribution for PFOS and PFOA in the serum was about 3 to 4 times higher for female tilapia than for male tilapia. Lower apparent volumes of distribution for both female and male may reflect rapid clearance, including protein binding and elimination. After an oral administration of 5.0 mg/g body weight of tilapia, the peripheral lymphocytes PFOA

concentration peaked at 600.09 ± 15.8 ng/ml at the first sampling time of 24 h and then the lymphocytes PFOA concentration decreased rapidly from 600.09 ± 15.8 to 468.32 ± 12.6 ng/ml post-treatment. A similar pattern was observed with the PFOS exposure. With respect to apparent volume of distribution and total body clearance, no significant differences were observed between PFOS and PFOA. The distribution rate constant (α) of PFOS in lymphocytes was approximately 1.5 times lesser than for PFOA, and the elimination rate constant (β) of PFOS in lymphocytes was about 2 times higher than for PFOA. It is remarkable that PFOS and PFOA were significantly accumulated in both PFOS and PFOA exposure, with PFOA showing a greater effect than PFOS. PFOA blood concentrations were lesser than the limit of quantification in non-exposure tilapia during an uptake period, while measured PFOS concentrations were at least six times lesser than those in PFOS exposure. Tilapia weight gains were also decreased with statistical significance in all PFOA-treated groups, and the effect of PFOA was higher than that of PFOS. The effects of PFOA on percent survival were more pronounced than that of PFOS. Moreover, PFOS and PFOA can inhibit the 17 β -HSD3 enzyme activity of tilapia.

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REFERENCES

- Chu Y, Zhao B, Huang Y (2009). Inhibition of 3 β - and 17 β -hydroxysteroid dehydrogenase activities in rat Leydig cells by PFOA. *Asian J. Androl.* 142: p. 11.
- Falandysz J, Taniyasu S, Gulkowska A, Yamashita N, Schulte-Oehlmann U (2006). Is fish a major source of fluorinated surfactants and repellents in human living on the Baltic coast? *Environ. Sci. Technol.* 40: 748–751.
- Giesy JP, Kannan K (2002). Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* 36: 147–152.
- Guruge KS, Manage MP, Miyazaki S, Yamanaka N, Taniyasu S, Hanari N, Yamashita N (2005). Species-specific accumulation of perfluorinated compounds in farm and pet animals from Japan. *Organohalogen. Compd.* 67: 823–826.
- Hoff PT, Van Dongen W, Esmans EL, Blust R, De Coen WM (2003a). Evaluation of the toxicological effects of perfluorooctane sulfonic acid in the common carp (*Cyprinus carpio*). *Aquat. Toxicol.* 62: 349–359.
- Hu GX, Zhou HY, Zh XW (2009). The (+)- and (-)-gossypols potentially inhibit both 3 β -hydroxysteroid dehydrogenase and 17 β -hydroxysteroid dehydrogenase3 in human and rat testes. *J. Steroid Biochem. Mol. Biol.* 115(12): 14–19.
- Huang HH, Hua ChJ (2010). Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonic acid (PFOS). *Aquat. Toxicol.* 98(2): 139–147.
- Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, Mohd MA, Olivero J, Wouwe NV, Yang JH, Aldous KM (2004). Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* 38: 4489–4495.
- Kissa E (2001). *Fluorinated Surfactants and Repellents*, second ed. Marcel Dekker, New York.
- Kudo N, Katakura M, Sato Y, Kawashima Y (2002). Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem. Biol. Interact.* 139: 301–316.
- Lau C, Butenhoff JL, Rogers JM (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol. Appl. Pharmacol.* 198: 231–241.
- Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II. Postnatal evaluation. *Toxicol. Sci.* 74: 382–392.
- Luebker DJ, Case MT, York RG, Moore JA, Hansen KJ, Butenhoff JL (2005). Two-generation reproduction and crossfoster studies of perfluorooctanesulfonate (PFOS) in rats. *Toxicology*, 215: 126–148.
- Michael E Baker (2004). Evolutionary analysis of 11 β -hydroxysteroid dehydrogenase-type 1,-type 2, -type 3 and 17 β -hydroxysteroid dehydrogenase-type 2 in fish. *FEBS Lett.* 574: 167–170.
- Olsen GW, Huang HY, Helzlsouer KJ, Hansen KJ, Butenhoff JL, Mandel JH (2005). Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ. Health Perspect.* 113: 539–545.
- Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, Butenhoff JL (2003). Subchronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology*, 183: 117–131.
- Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL (2002). Subchronic toxicity studies on perfluorooctane-sulfonate potassium salt in cynomolgus monkeys. *Toxicol. Sci.* 68: 249–264.
- Tang J, Yang XL (2006). Pharmacokinetics and the active metabolite of enrofloxacin in Chinese mitten-handed crab (*Eriocheir sinensis*). *Aquaculture*, 260: 69–76.
- Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, Butenhoff JL, Stevenson LA, Lau C (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I. Maternal and prenatal evaluations. *Toxicol. Sci.* 74: 369–381.
- USEPA OPPTS(2002). Risk Assessment Division. Revised draft hazard assessment of perfluorooctanoic acid and its salts. AR226-1136. Washington, DC.
- Vanden Heuvel J, Kuslikis B, Van Rafelghem MJ, Peterson R (1991). Tissue distribution, metabolism, and elimination of perfluoroactanoic acid in male and female rats. *J Biochem. Toxicol.* 6(2): 83–92.
- Ylisen M, Kojo A, Hanhijdrvi H, Peura P (1990). Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull. Environ. Contam. Toxicol.* 44: 46–53.
- Zhao BH, Hu GX, Hua GX (2010). Inhibition of human and rat 3 β -hydroxysteroid dehydrogenase and 17 β -hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances. *Chem. Biol. Interac.* 188: 38–43.