Original Article

A comparative study on oxidative damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages

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ABSTRACT — Effects of perfluoroctane sulfonate (PFOS) on maleic dialdehyde (MDA) content, superoxide dismutase (SOD) activity and total antioxidation capability (T-AOC) were compared in mice at different postnatal developmental stages, and concentrations and distributions of PFOS in different tissues were measured simultaneously. The male and female mice at postnatal day (PD) 7, PD 14, PD 21, PD 28 and PD 35 were distributed randomly to dosage group (50 mg/kg body weight) and control group (0 mg/kg body weight). Mice were administered with PFOS by once subcutaneous injection. Subsequently, after 24 hr, MDA content, SOD activity and T-AOC in brain and liver were analyzed. The PFOS concentrations in blood, brain and liver were determined by high-performance liquid chromatography negative electrospray tandem mass spectrometry (LC-MS). PFOS induced depression of the body weights of mice evidently and increase of relative weights of liver. Meanwhile, it depressed the SOD activity and T-AOC in brain and liver. The concentrations and distribution percentages of PFOS in blood, brain and liver of mice were significantly different at various postnatal developmental stages. Achieved results in this study indicate that younger mice pups were more sensitive to PFOS exposure. In addition, significant distinctions in concentrations and distribution percentages of PFOS in various tissues were demonstrated in this study. The gender difference observed was greater in the older mice. Thus it is worth giving attention especially to adverse effects of PFOS on foetus and children.

Key words: Perfluoroctane sulfonate, Postnatal, Oxidative stress, Distribution percentages

INTRODUCTION

Perfluoroctane sulfonate (PFOS), as a representative substance of perfluoroochemicals (PFCs), has been utilized in a wide variety of products, such as detergent, fire-fighting foam, surfactant, pesticide and cosmetics. PFOS possesses strong physical, chemical and biological stability, because of its special chemical constitution. PFOS is ultimate products of decomposed PFCs in the organism and environment. However, no evidence has been found for metabolic or environmental degradation of PFOS. The properties of PFOS, involving good absorption, chemical stability toward degradation and poor elimination, may demonstrate its cumulative toxicity and produce long half-life in animals and humans (Seacat et al., 2002; Olsen et al., 2007). High concentrations of PFOS have been found in top predators (de Vos et al., 2008; Powley et al., 2008; Smithwick et al., 2005). Many stud-
ies have reported extensive distributions of PFOS in both occupational (Olsen et al., 2003a) and non-occupational exposed human (Jin et al., 2004; Kubwabo et al., 2004; Midasch et al., 2006), wildlife (Law et al., 2008; Sinclair et al., 2006; Lin et al., 2006; Bossi et al., 2005; Giesy and Kannan, 2001) and various environmental matrices (Jin et al., 2006; Senthilkumar et al., 2007; Kubwabo et al., 2005). It even has been detected in the Antarctica and the Arctic (Lin et al., 2006; Kannan et al., 2001). As a potential member of persistent organic pollutants (POPs), the environmental contamination status, human exposure levels and health effect evaluations of PFOS have been focused in the research fields of environmental science and preventative medicine.

The potential toxicities of PFOS have not been well known. However, the preceding reports have described that PFOS could induce the disturbances in lipometabolism and energy metabolism (Berthiaume and Wallace, 2002), degrade thyroxine level (Chang et al., 2008), interfere cellular signal transduction (Hu et al., 2002), lead to conspicuous hepatic toxicity (Sohlenius et al., 1993), cause developmental neurotoxic effects (Lau et al., 2003), and pose potential multiple organs toxicities (Austin et al., 2003; Luebker et al., 2005; Grasty et al., 2005). Moreover, the carcinogenicity of PFOS to occupationally exposed humans has been demonstrated (Alexander and Olsen, 2007).

The geometric means of PFOS concentrations in the serum of 65 non-occupationally exposed male and female from Shenyang in China were 40.73 μg/l and 45.46 μg/l, respectively (Jin et al., 2004). They were markedly higher than the levels detected in the human serum of Canada, German and America. PFOS was detected in 56 blood specimens of Canadians at an average concentration of 28.8 μg/l (Kubwabo et al., 2004). The median of plasma concentrations of 105 non-smokers out of the German non-occupational exposed human was 22.3 μg/l for PFOS (Midasch et al., 2006). The geometric mean for the serum PFOS concentration was 14.7 μg/l for 24 donors of Americans (Olsen et al., 2003b). Contrary to reports that PFOS level in human serum has been described to reach a platform period or even decrease in some countries currently (Harada et al., 2007; Olsen et al., 2005; Olsen et al., 2007; Wiesmüller et al., 2007). Our group has found that the PFOS level in human serum in China increased during the last twenty years (Jin et al., 2007). Interestingly, the results of these reports were similar in the fact that no correlation was observed between the accumulation of PFOS in human body and age (Midasch et al., 2006; Ericson et al., 2007). It is suggested that the exposure levels of PFOS are identical in infant and adult, and leading higher risk to infants and children who were more susceptible to toxic stress caused by PFOS.

During the neonatal periods, the developing organs of mammals are inherently much more susceptible to injuries caused by environmental pollutants than mature ones, because of the unsoundness of organic physiologic functions in neonates. If a critical phase of their developmental processes is halted or inhibited, there is only a slight potential for the subsequent repairs, and the consequences can therefore be permanent and irreversible. Maternal PFOS could transfer into fetus via the placental barrier (Midasch et al., 2007; Inoue et al., 2004) or milk (Kärman et al., 2007; So et al., 2006). The correlation between concentrations of PFOS in neonatal mice tissues and the maternal exposure dosage has been observed (Lau et al., 2003).

In the present study, the disruption of PFOS on the oxidation-antioxidation system and its internal distributions were examined in mice at different postnatal stages. The purposes were to investigate the difference in the toxicities of PFOS to mice during the developmental processes and thereby support for the health risk assessments of this new persistent organic contaminant.

**MATERIALS AND METHODS**

**Chemicals**

Perfluorooctane sulfonate (PFOS, heptadecafluorooctanesulfonic acid solution, 40% in water, purity > 98%) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Tetrabutylammonium hydrogen sulfate (TBAHS) was provided by Acros Organics (Geel, Belgium). HPLC grade of methyl tert-butyl ether (MTBE) and methanol (MeOH) were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). Antioxidative enzyme detection kits (SOD, T-AOC), maleic dialdehyde (MDA) and protein detection kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Presep-C Agri Solid-phase Extraction (SPE) column was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Deionized water was passed through the Agri cartridge to remove any remaining contamination, called de-PFCs water. All solutions were prepared in de-PFCs water.

**Animal treatment**

Adult male and female KM mice were obtained from the Center of Laboratory Animals in Dalian Medical University. Animals were housed individually in cages with heat-treated pine shavings for bedding and were provided pellet chow and tap water ad libitum. Animal facili-
ties were controlled for temperature (18 to 29°C) and relative humidity (40 to 60%), and operated under a 12 hr light-dark cycle. Time of parturition for each animal was noted and the following day was designated as postnatal day (PD) 1. For this study, the pups were distributed randomly to nursing dams with a litter size of less than 10 to maintain a uniform nutritive status. All pups were weaned at PD 21 and separated by gender. Pups of both genders were randomly chosen from several litters, weighed, and divided into either treatment or control groups. PFOS was freshly prepared in 5 mg/ml and administered to pups once by subcutaneous injection at PD 7, PD 14, PD 21, PD 28 and PD 35. Controls received vehicle alone at an equivalent volume (10 ml/kg body weight). All pups from each group were sacrificed by decapitation after 24 hr after administration. Trunk blood was collected. Liver and brain were removed quickly on a cold box and weighed. All protocols involved in the use of animals were approved by Institutional Animal Care Committee of Dalian Medical University.

**Determination of PFOS concentrations**

The frozen tissue samples were thawed at room temperature before extraction. One hundred mg tissue was removed and pooled in a 15-ml polypropylene tube pre-cleaned with MeOH, into which 2 ml of de-PFCs water was added. The sample was then homogenized by a mechanical homogenizer. Blood sample (0.2 ml) was collected. All Teflon parts were removed from the homogenizer to avoid analytical interference. After samples of one type and one location were homogenized, the homogenizer probe and laboratory wares were thoroughly washed in the sequence of de-PFCs water and methanol in order to avoid cross-contamination.

The samples were extracted using a method described before (Hansen et al., 2001). Briefly, TBAHS (0.5 M, 1 ml) and Na₂CO₃ buffer (0.25 M, 2 ml) were added into the polypropylene tube containing the water-tissue mixture of each sample. The extraction was conducted twice by addition of 5 ml of MTBE and 5 min of vortex. The organic and aqueous layers were separated by centrifugation, and the organic layer was removed to a new polypropylene tube. The organic layers were evaporated at room temperature under a nitrogen gas flow. The sample was reconstituted with 1.0 ml of methanol and analyzed by liquid chromatography negative electrospray tandem mass spectrometry (LC-MS).

**Chemical analysis**

Analyses were performed using LC-MS. Briefly, each methanol extract was chromatographed using HPLC (Shimadzu LC 2010A with Zorbax XDB C-18 (Agilent Brow-Broe 2.1 × 150 mm, 5 μm)) at a flow rate of 0.2 ml/min. Gradient conditions were used in the mobile phase. Initial mobile phase conditions were 35:65 CH₃CN/CH₃COONa buffer (10 mM), followed by a 5 min ramp to 45:55, hold for 13 min. The chromatographic column was kept at 40°C. Shimadzu 2010A mass spectrometer equipped with an electro-spray ionization source was used as detector, employing electrospray ionization in the negative mode (ESI). Atomization gas (N₂) flow rate was 1.5 l/min, and blowback gas (N₂) flow rate was 5 l/min. The ionization source voltage was set at −3.50 kV, and the capillary voltage was −25.0 V. The capillary temperature was 250°C, and the block temperature was 200°C. The detector voltage was −1.6 kV. The selected ion monitoring (SIM) was used for quantification of the analytes. The quantification ions were m/z 499 electronegativity molecular ion (CF₃OSO₂⁻) for PFOS. The injection volume was 1.0 μl.

**Activities of antioxidative enzymes**

Activities of superoxide dismutase (SOD), total antioxidation capability (T-AOC) and protein content were tested by xanthine oxidase technique, Fe³⁺ reduction method and Bradford's method, respectively. A portion of the liver or brain sample was prepared in 10% homogenate immediately. The homogenate was centrifuged at 2,500 rpm for 10 min at 4°C to precipitate insoluble material. The supernatant was removed and assayed for SOD activity, T-AOC and protein content using kits, following the manufacturer’s specifications.

**Lipid peroxidation**

MDA content was determined with thiobarbituric acid method. A portion of the liver or brain sample was prepared in 10% homogenate immediately. The homogenate was centrifuged at 2,500 rpm for 10 min at 4°C to precipitate insoluble material. The supernatant was removed and assayed for MDA level using kits, following the manufacturer’s specifications.

**Statistical analysis**

All data were presented as means ± S.D. Normality of data was verified through the Kolmogorov-Smirnov test, and homogeneity of variances was checked using Levene's test. Differences from control values were determined by t-test and one-way ANOVA, and later followed by a Fisher's LSD test with the level of statistical significance set at P < 0.05. Correlation coefficients were executed by Pearson test in quantitative data and Spearman rank test in enumeration data. Statistical analysis was per-
formed using SPSS 11.5 (SPSS, Chicago, IL, USA).

**RESULTS**

Effects of PFOS on body weights and relative liver weights in mice. After 24 hr of PFOS administration, body weights of male and female mice in control group at different postnatal developmental stages increased, but decreased slightly in the mice exposed to PFOS at PD 21, PD28 and PD35 (Table 1). The relative liver weights of mice in dosage group increased to some extent, which were significantly higher than control groups, except the male mice at PD 7 and PD 14.

Effects of PFOS on the oxidation and antioxidation in brain and liver in mice. Levels of MDA in brain and liver remained unchanged in PFOS exposure groups at all stages of postnatal development (Table 2). In brain, SOD activity in male mice was significantly depressed at PD 7 and PD 21, and a merely slight inhibition was observed at PD 14 in liver. There was a significant difference at PD 14 compared with control in SOD activity of female mice liver.

T-AOC in male brain declined at all stages of postnatal development, a significant difference appeared at PD 21. In liver, T-AOC in male mice was significantly depressed at PD 7 and PD 14. T-AOC in female mice was lower than control at PD 7, 14, 21 and 28, and there was a significant difference at PD 21.

PFOS concentrations and distribution percentages in blood, brain and liver of mice.

The volume of blood sample was calculated by weight according to the mouse blood density of 1.050 g/ml and whose whole blood volume of 77.8 ml/kg body weight (Shi and Mei, 2002). The levels of PFOS in blood, brain and liver of the mice from control groups were below the limit of detection (LOD) in the present study.

PFOS concentrations in the brain of both genders decreased with age at different postnatal developmental stages (Fig. 1), and appeared to increase gradually in liver. However, no significant difference was observed in blood. Furthermore, significant correlation of PFOS levels in brain and liver with postnatal days was determined (Male: brain, r = -0.676; liver, r = 0.844; Female: brain, r = -0.726; liver, r = 0.850; Table 3). Statistically, there was no correlation between blood PFOS concentration and postnatal days (Male: r = 0.007; Female: r = 0.101; Table 3). PFOS concentrations in the brain of male and female mice were correlated with that in liver (Male: r = -0.614; Female: r = -0.762; Table 3). However, there was no correlation between PFOS concentration in blood and that in brain or liver.

Although the variance of relative organ weight existed at different developmental stages, it was found that the tendency in the change of PFOS distribution percentages was accordant to its concentrations, which suggested that the variance in relative organ weight had not effect on the organic distribution of PFOS. In brain, the distribution percentages of male mice were 5.04 ± 1.49%, 1.02 ± 0.28% at PD 7 and PD 35, respectively (Table 4). Accord-

| Table 1. Effects of PFOS on body weights and relative liver weights in mice at different postnatal |
|-----------------|-----------------|-----------------|-----------------|
| Subjects PD     | Control         | Exposure        | Control         | Exposure        |
|                 | Male            | Female          | Male            | Female          |
|                 | n Before After  | n Before After  | n Before After  | n Before After  |
|                 |                 |                 |                 |                 |
| BW (g)          |                 |                 |                 |                 |
| 7 6             | 4.3 ± 1.0 4.9 ± 1.2 | 6 4.1 ± 1.0 4.3 ± 1.2 | 6 3.9 ± 1.2 4.5 ± 1.4 | 6 4.2 ± 1.1 4.4 ± 1.2 |
| 14 4            | 7.6 ± 0.7 7.7 ± 0.7 | 6 7.4 ± 1.3 7.5 ± 1.5 | 6 8.1 ± 1.3 8.3 ± 1.9 | 6 7.5 ± 1.0 7.4 ± 1.1 |
| 21 6            | 11.8 ± 2.8 13.1 ± 2.5 | 6 11.5 ± 2.0 11.0 ± 2.4 | 3 11.1 ± 1.3 12.0 ± 1.5 | 6 11.6 ± 1.9 10.9 ± 1.9 |
| 28 6            | 16.5 ± 4.5 17.6 ± 5.0 | 6 17.6 ± 3.5 17.2 ± 3.8 | 6 16.7 ± 5.4 17.7 ± 6.2 | 6 16.2 ± 4.6 14.9 ± 4.4 |
| 35 5            | 19.0 ± 5.8 19.7 ± 6.4 | 6 18.6 ± 6.4 17.6 ± 6.8 | 5 18.3 ± 1.9 18.8 ± 2.3 | 6 21.0 ± 3.1 19.3 ± 3.0 |
|                 |                 |                 |                 |                 |
| RLW (%)         |                 |                 |                 |                 |
| 7 6             | 3.3 ± 0.3 4.2 ± 0.4 | 6 3.6 ± 0.3 4.5 ± 0.4 | 6 3.1 ± 0.4 3.9 ± 0.3 | 6 3.8 ± 0.2** 4.7 ± 0.5** |
| 14 4            | 5.0 ± 0.2 5.4 ± 0.6 | 6 6.4 ± 0.2** 6.4 ± 0.5** | 3 5.0 ± 0.2 5.5 ± 0.6 | 6 6.1 ± 0.2** 6.3 ± 0.6* |
| 28 6            | 5.3 ± 0.4      | 6 6.9 ± 0.7**   | 5 5.4 ± 0.9     | 6 6.9 ± 1.1*   |

Developmental stages after exposure to PFOS for 24 hr.
* Significant differences (P < 0.05) from control values by t-test.
** Significant differences (P < 0.01) from control values by t-test.
Table 2. Effects of PFOS on oxidation and antioxidation in brain and liver at different postnatal developmental stages after exposure to PFOS for 24 hr

<table>
<thead>
<tr>
<th>PD</th>
<th>Gender</th>
<th></th>
<th>Brain</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Liver</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MDA</td>
<td>SOD</td>
<td>T-AOC</td>
<td>MDA</td>
<td>SOD</td>
<td>T-AOC</td>
<td></td>
<td>MDA</td>
<td>SOD</td>
<td>T-AOC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Exposure</td>
<td>Control</td>
<td>Exposure</td>
<td></td>
<td></td>
<td>Control</td>
<td>Exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>3.38 ± 0.69</td>
<td>2.91 ± 0.84</td>
<td>115.5 ± 17.0</td>
<td>91.0 ± 13.2*</td>
<td>4.87 ± 1.65</td>
<td>4.49 ± 1.93</td>
<td>1.52 ± 0.36</td>
<td>1.59 ± 0.68</td>
<td>351.8 ± 75.4</td>
<td>339.8 ± 78.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.56 ± 1.02</td>
<td>2.15 ± 0.66</td>
<td>92.3 ± 23.3</td>
<td>99.4 ± 37.0</td>
<td>4.73 ± 1.89</td>
<td>4.72 ± 1.23</td>
<td>1.95 ± 0.58</td>
<td>1.88 ± 0.81</td>
<td>392.5 ± 66.4</td>
<td>412.8 ± 87.9</td>
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<tr>
<td>14</td>
<td>Male</td>
<td>1.27 ± 0.36</td>
<td>1.74 ± 0.66</td>
<td>181.3 ± 77.3</td>
<td>140.7 ± 44.0</td>
<td>6.11 ± 2.09</td>
<td>4.82 ± 1.68</td>
<td>0.92 ± 0.16</td>
<td>1.22 ± 0.46</td>
<td>513.5 ± 75.3</td>
<td>468.0 ± 48.7</td>
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<tr>
<td></td>
<td>Female</td>
<td>1.77 ± 0.52</td>
<td>1.35 ± 0.77</td>
<td>152.1 ± 60.6</td>
<td>124.9 ± 19.2</td>
<td>6.34 ± 2.62</td>
<td>4.61 ± 1.31</td>
<td>1.25 ± 0.37</td>
<td>1.11 ± 0.21</td>
<td>488.5 ± 61.1</td>
<td>395.9 ± 79.9*</td>
</tr>
<tr>
<td>21</td>
<td>Male</td>
<td>1.29 ± 0.37</td>
<td>1.49 ± 0.64</td>
<td>177.6 ± 8.9</td>
<td>154.3 ± 11.8*</td>
<td>4.80 ± 0.53</td>
<td>4.09 ± 0.42*</td>
<td>1.68 ± 0.50</td>
<td>1.52 ± 0.43</td>
<td>523.1 ± 48.2</td>
<td>510.4 ± 102.0</td>
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<tr>
<td></td>
<td>Female</td>
<td>1.29 ± 0.31</td>
<td>1.10 ± 0.37</td>
<td>151.8 ± 28.3</td>
<td>151.1 ± 18.3</td>
<td>4.02 ± 0.74</td>
<td>4.18 ± 0.60</td>
<td>0.99 ± 0.34</td>
<td>1.23 ± 0.56</td>
<td>466.7 ± 123.2</td>
<td>461.8 ± 104.9</td>
</tr>
<tr>
<td>28</td>
<td>Male</td>
<td>1.32 ± 0.44</td>
<td>1.36 ± 0.37</td>
<td>174.8 ± 65.3</td>
<td>179.6 ± 56.0</td>
<td>3.41 ± 1.72</td>
<td>3.03 ± 1.51</td>
<td>1.28 ± 0.39</td>
<td>1.58 ± 0.79</td>
<td>557.9 ± 60.0</td>
<td>611.6 ± 124.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.25 ± 0.27</td>
<td>1.19 ± 0.53</td>
<td>177.8 ± 83.8</td>
<td>135.0 ± 44.8</td>
<td>3.20 ± 1.37</td>
<td>2.95 ± 0.79</td>
<td>1.71 ± 0.65</td>
<td>1.25 ± 0.21</td>
<td>579.2 ± 98.4</td>
<td>562.7 ± 97.4</td>
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<tr>
<td>35</td>
<td>Male</td>
<td>1.93 ± 0.64</td>
<td>2.28 ± 0.77</td>
<td>128.0 ± 10.3</td>
<td>125.1 ± 12.8</td>
<td>3.06 ± 0.55</td>
<td>3.04 ± 0.68</td>
<td>1.49 ± 0.35</td>
<td>1.70 ± 0.52</td>
<td>494.7 ± 106.1</td>
<td>513.3 ± 97.6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.01 ± 0.80</td>
<td>2.12 ± 0.75</td>
<td>117.6 ± 12.6</td>
<td>108.5 ± 10.0</td>
<td>2.80 ± 0.39</td>
<td>2.54 ± 0.55</td>
<td>1.28 ± 0.38</td>
<td>2.03 ± 0.88</td>
<td>483.7 ± 89.6</td>
<td>546.8 ± 78.7</td>
</tr>
</tbody>
</table>

MDA content was expressed as nmol/mg prot, and SOD, T-AOC activities expressed as U/mg prot. Data are expressed as means ± S.D. of 3-6 determinations.
* Significant differences (P < 0.05) from control values by t-test.
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PFOS distribution percentages in the brain of male and female mice were correlated with that in liver (Male: $r = -0.896$; liver, $r = 0.865$; Table 3). PFOS distribution percentages in the brain of male and female mice were correlated with that in liver (Male: $r = -0.699$; Female: $r = -0.850$; Table 3). Similarly, there was no correlation between PFOS distribution percentages in blood and that in brain or liver.

The total percentages of PFOS including blood, brain and liver in male and female mice increased with age after exposure to PFOS for 24 hr (Fig. 2). There was a positive correlation between postnatal days and PFOS total percentage (Male: $r = 0.919$; Female: $r = 0.858$). The total percentages of male mice were 29.13%, 85.26% at PD 7 and PD 35, respectively. Comparatively, the total percentages of female mice were 32.45% and 76.41% at PD 7 and PD 35, respectively.

**DISCUSSION**

In the present study, the body weights of male and female mice increased at earlier postnatal period (PD 7 and PD 14) after exposure to 50 mg/kg BW of PFOS for 24 hr, but the increase was smaller comparing with control. In addition, loss in body weights of male mice was observed after PD14, and the loss was greater in that of female mice. The general variation of experimental animals exposed to exogenous compounds can be reflected by the change in body weight, and thus being an objective, convenient and comprehensive index. The results in this study showed that PFOS might impact fat and energy metabolisms severely through the acute high-dose exposure. The relative liver weights of PFOS exposed male mice increased at later developmental stages with respect to control, while higher than control in female mice at all stages. Therefore, it is suggested that PFOS possibly poses higher hepatotoxicity to female mice.

Many exogenous chemical compounds could induce oxidative stress injuries by producing a great quantity of reactive oxygen species (ROS) which are naturally generated in small amounts during the body’s metabolic oxidation-reduction reactions, as well as reacting with complex cellular molecules such as fat, protein, or DNA (Kelly et al., 1998). The adverse effects caused by ROS involve damage to membrane structure and electrophysiologic activity and disruption in receptor function, interference to calcium homeostasis (Kimura et al., 2005). SOD is a member of important antioxidants which construct the antioxidant defense system that could eliminate ROS with antioxidant, and T-AOC could represent the total activity of the antioxidant system in organism. MDA reflects the degree of lipid peroxidation. In the primary cultured hepatocytes of freshwater tilapia (Oreochromis niloti-
Oxidative damage and distributions of PFOS in postnatal mice

Table 3. Correlation of PFOS concentrations, distribution percentages and developmental stages in mice

<table>
<thead>
<tr>
<th>Gender</th>
<th>Involved items</th>
<th>Concentrations</th>
<th>Percentages</th>
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<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>Brain</td>
</tr>
<tr>
<td>Male</td>
<td>PD</td>
<td>0.007</td>
<td>-0.676**</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>0.101</td>
<td>-0.726**</td>
</tr>
<tr>
<td>Male</td>
<td>Blood</td>
<td>1</td>
<td>0.160</td>
</tr>
<tr>
<td>Female</td>
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<td>1</td>
<td>0.154</td>
</tr>
<tr>
<td>Male</td>
<td>Brain</td>
<td>1</td>
<td>-0.614**</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>1</td>
<td>-0.762**</td>
</tr>
</tbody>
</table>

The correlation coefficients of PFOS concentrations, distribution percentages in male and female mice blood, brain and liver were executed by Pearson test, and the correlation coefficients were determined between PFOS concentrations, distribution percentages and postnatal days by Spearman rank test. ** Significant correlation (P < 0.01).

Table 4. Distribution percentages of PFOS at different developmental stages in mice (%)

<table>
<thead>
<tr>
<th>PD</th>
<th>Male</th>
<th>Blood</th>
<th>Brain</th>
<th>Liver</th>
<th>Female</th>
<th>Blood</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>11.78 ± 2.88</td>
<td>5.04 ± 1.49</td>
<td>14.84 ± 4.01</td>
<td>10.77 ± 1.16</td>
<td>4.17 ± 1.17</td>
<td>16.23 ± 4.84</td>
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<tr>
<td>14</td>
<td>13.78 ± 1.52</td>
<td>1.61 ± 0.80**</td>
<td>26.50 ± 7.36</td>
<td>12.31 ± 2.24</td>
<td>3.26 ± 0.58</td>
<td>26.30 ± 4.54</td>
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<tr>
<td>21</td>
<td>9.85 ± 2.74</td>
<td>2.40 ± 0.60**</td>
<td>51.35 ± 11.06**</td>
<td>12.37 ± 3.80</td>
<td>2.14 ± 0.38**</td>
<td>51.48 ± 3.44**</td>
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<tr>
<td>28</td>
<td>9.89 ± 2.94</td>
<td>0.85 ± 0.19**</td>
<td>63.39 ± 19.78**</td>
<td>12.16 ± 2.32</td>
<td>2.10 ± 0.73**</td>
<td>51.05 ± 10.59**</td>
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<tr>
<td>35</td>
<td>13.33 ± 0.89</td>
<td>1.02 ± 0.28**</td>
<td>73.68 ± 6.86**</td>
<td>11.54 ± 1.28</td>
<td>0.90 ± 0.23**</td>
<td>69.92 ± 18.52**</td>
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** Significant differences (P < 0.01) from PD 7 values by Fisher’s LSD test.

Percus) that were exposed to PFOS at concentrations of 0, 1, 5, 15 and 30 mg/l for 24 hr, induction of ROS accompanied by increases in activities of SOD was observed, while no effect occurred on MDA level (Liu et al., 2007). Although the MDA content of male and female mice at different developmental stages exhibited no marked difference, the SOD activity and T-AOC decreased. In addition, the degree of injury was reduced with the mice growing up. As above, it is manifest that there was a distinction in the degree of oxidative damage that originated by PFOS at different developmental stages. The defense capability of organisms plays an important role in this process. Meanwhile, the oxidative damage in male mice was more severe than that in female mice. Conspicuous behavior disorders were found at PD 7 in experimental session, such as erethism, ataxia and generalized convulsive seizures. The phenomenon was seldom observed in the other developmental periods.

The highest concentration of PFOS was found in liver, followed by serum and brain. The liver PFOS level was positively correlated with postnatal age in both male and female mice. However, a visible negative correlation was observed between brain PFOS level and age. In contrast, no correlation was found between blood PFOS concentration and age. These results suggested that the major cumulative organs of PFOS in mice varied at different postnatal developmental stages. The variation in the distribution of PFOS in the mice observed may be formed accompanying the development process which mainly includes the establishment and ripeness of blood-brain barrier function (Watson et al., 2006), change in liver metabolic capability and piecemeal completion of heptaoenteral circulation and tubular reabsorption.

Provided that the PFOS concentration in blood is one unit, and the concentration ratios in brain, blood and liver of male and female mice were 0.40 (0.29 to 0.58): 1:5.07 (2.72 to 6.99) and 0.41 (0.27 to 0.62): 1:5.00 (3.11 to 7.38), respectively. PFOS concentration in male brain at
PD 7 was 2.02 times of that at PD 35. However, PFOS concentration in male liver at PD 35 was 2.21 times of that at PD 7. The ratios in brain and liver of female mice correspondingly were 2.28 and 2.38, respectively. The PFOS concentration ratio of humans in serum and liver is 1:1.4 (Olsen et al., 2003c), which is similar to the experimental result (1:0.9 to 1:2.2) performing on cynomolgus (Seacat et al., 2002), and 1:3 to 1:12 in rat (Seacat et al., 2003), regardless of the administered dosages. Considering the variance of PFOS concentration in serum, plasma and whole blood, the ratio is near to 2:2:1 (Ehresman et al., 2007). PFOS concentration ratio in this study is slightly higher than that reported in the literatures mentioned above. The lower accumulation of PFOS in liver of young mice is possibly attributed to inferior capability of absorption and accumulation to chemical materials and incomplete hepatointerual circulation at early developmental stages. Similarly, provided that the PFOS distribution percentage in blood is one unit, PFOS distribution percentage in male brain at PD 7 was 5.60 times of that at PD 35. In contrast, PFOS distribution percentage in male liver at PD 35 was 4.39 times of that at PD7. Comparatively, the ratios in brain and liver of female mice were 4.96 and 4.02, respectively. There were significant negative correlations in both PFOS concentrations and distribution percentages between brain and liver. However, the mutual correlation was not established in blood. It might be explained by the relative stabilization of plasma protein level at different developmental stages and binding of PFOS to plasma proteins.

PFOS distribution percentage corrects the variance caused by the relative weight of various organs at different developmental stages, and reflects information on the distributions of PFOS in tissues precisely. The PFOS concentration and distribution percentage in blood are independent of age, suggesting that there could be higher PFOS concentrations and distribution percentages in brains of infant, children and imuberism than that of adults when the PFOS level is identical in blood. For humans, the development of the central nervous system (CNS) begins during the first two months of gestation and continues developing throughout the following two years. Postnatal development spans the first three to four weeks of neonatal life and peaks around PD 10 in mice and rats (Johansson et al., 2008). Due to the longer developmental process of humans, toxicants would pose higher risk to the CNS. The CNS develops rapidly at these stages, which are more sensitive to chemical materials exposures (Rodier, 1994). The PFOS-induced phenomenon should be paid much attention under the health risk assessments of PFOS. Although the gender difference in PFOS concentrations and distribution percentages in blood, brain and liver could be ignored in early postnatal developmental periods, it was obvious in older mice after PD 14. It is probably concerned with the diversity of behavior and endocrinum in development.

There was no obvious gender difference in total PFOS percentages at earlier postnatal stages. However, the PFOS accumulation of male mice was higher than that of the female in the later developmental periods. The results indicated that the elimination ability of female mice might be higher than that of the male mice. In addition, the accumulation of PFOS in mice increased with age. It exhibited relatively stronger ability to tolerate and accumulate PFOS in older mice. Many factors could affect the accumulation of PFOS in mice at different developmental stages including the body characters (such as moisture content, permeability and developmental degree of organs), ingestion and excretion pathways.

But it should indicate the limitation of the design for this study. Usual route of exposure to PFOS may be oral route. However, subcutaneous injection was used in the experiment. When the route of exposure is different, absorption, distribution and excretion of PFOS may be different. PFOS is absorbed by the gastrointestinal mucosa and experiences the first-pass effect in oral route which has a slow rate of absorption. PFOS is absorbed by capillaries in subcutaneous injection with a quick and complete absorption. The difference in absorption of PFOS will induce the differences in distribution and excretion of PFOS.

In conclusion, the present study demonstrated that there were visible distinctions in the toxic effects of PFOS on
mice at different developmental stages, and the juvenile mice were more sensitive to PFOS exposure. Moreover, the accumulation and distributions of PFOS in different tissues showed significant differences in mice at different stages and slight gender difference was also observed in older mice. The neurotoxicities induced by PFOS at earlier developmental stages are warranted in future research.

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