STUDIES ON THE TOXIC EFFECTS OF PFOA AND PFOS IN MALE RATS DURING A SUBCHRONIC EXPOSURE: HISTOPATHOLOGY, DISTRIBUTION, AND EXCRETION

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Abstract
In the present study, the toxic effects of PFOA and PFOS, including the histological change, distribution, and excretion in male Sprague-Dawley (SD) rats were examined after 28-day subchronic exposure. Histopathological observation showed that relatively serious damage occurred in the liver and lung, and higher toxicity of PFOS than PFOA. The distribution of two PFCs in the rats indicated that liver, lung and kidney might serve as the main target organs, and PFOS had relatively higher accumulation ability than PFOA. Urinary excretion was regarded as the principal clearance route for either PFOA or PFOS, according to faster excretion rates in urine than those in feces. Compared to PFOA, the higher levels in the target organs and lower excretion rates of PFOS, may explain the relatively higher toxicity of PFOS than PFOA.

Introduction
As an emerging class of synthetic surfactants that are widely used, perfluorinated compounds (PFCs), especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have become globally environment contamination. Their high persistence, bioaccumulation and potential toxicity have resulted in withdrawing of PFOS from the marketplace. Considerable public attention has been paid to their toxicities because the available toxicological datum still greatly limited the risk assessment of the environmental pollution caused by PFCs. In addition, an increasing number of papers have described the pharmacokinetics of these compounds following in-vivo exposure, however, few of them focused on the excretion of PFCs during consecutive dosing. This current study was undertaken to investigate the histopathological alterations in various organs in the SD rats exposed to PFOA and PFOS at 5 and 20 mg/kg/day. The distribution and excretion of the two perfluorochemicals in the rats during a 28-day subchronic exposure were compared for the evaluation on the toxic effects induced by them.

Materials and Methods
Fifty male SD rats (SPF grade, two months old, 190-210 g) were randomized into five groups (10/group) and administrated with PFC solutions (0.5, 2 mg/mL) by gavage once a day for 28 days. The first group (named by G0) receiving Milli-Q water only, was used as the blank for the control. The other four groups were administered to 5 mg/kg/day PFOA (G1), 20 mg/kg/day PFOA (G2), 5 mg/kg/day PFOS (G3), and 20 mg/kg/day PFOS (G4), respectively. Urine and feces samples were collected after daily gavage with PFCs by moving rats to standard metabolism cages respectively overnight for 24-h intervals on Days 1, 3, 5, 7, 10, 14, 18, 21, 24, and 28. All the
rats were sacrificed after the exposure for collecting the whole blood and tissue samples including liver, kidney, lung, heart, spleen, testis and brain. A cubical sample (about 0.5 cm³) was removed from each tissue and prepared specimen for histopathological observation. The remaining tissues, blood samples and excretion samples were stored at -40°C until chemical analysis.

The analytical procedure for PFOA and PFOS in all the samples was in accordance to a ion-pair method by Hansen et al. with some minor modifications, and the details had been described in our previous work. Separation and quantitation of PFOA and PFOS in the concentrated extract were performed by a high performance liquid chromatography-electrospray tandem mass spectrometry system (HPLC/ESI-MS/MS).

Results and Discussion

Except G4, no significant ethological abnormality was observed in the rats of G1, G2 and G3 from the third week. Death occurred in G4 after 11 days’ exposure and all the animals died within 26 days. General increase trends of the average body weight in G0, G1, G2 and G3 were observed over the whole study period (Figure 1); however, the body weights in G4 decreased sharply from the second week, attributing to the significant decrease of food consumption. Above evidence showed the high toxicity of PFOS at a relatively high exposure level. All the values of viscera-somatic indices in exposure groups significantly higher than those of the control (P<0.05), indicated obvious hepatomegaly, renal hypertrophy and orchioncus could be caused by PFOA and PFOS.

Based on the microscope observation, different extents of the histopathological changes were observed in the prepared tissue specimens. The liver and the lung were regarded as the most primary target organs. Comparatively, more serious histopathological lesion was found in PFOS dose groups, denoting higher toxicity of PFOS than PFOA.

Through determination of PFOA and PFOS in exposed rats, the liver, kidney and lung as the predominant deposit sites for PFOA and PFOS, might contribute to the corresponding histological changes. Compared with PFOA, PFOS was easier to accumulate in rats, relating to some specific proteins in the tissues binding with PFOS therein.

For both two test compounds’ exposure, there were upward trends during the whole test period for urinary, fecal and total excretion under the conditions of administration with 5 and 20 mg/kg/day. In general, the values in urinary excretion were more or less greater than those in fecal excretion, particularly for PFOA, indicating faster excretion rates by urine. More mass contents of PFOA than PFOS were found in the excretion of the rats gavaged with the same doses, especially at a relatively high dose (20 mg/kg). By this token, PFOA has a faster elimination rate, lower bioaccumulation, thereby leading to lower toxicities than PFOS as observed above.

In conclusion, the combined properties of rapid absorption and poor elimination could contribute to high bioaccumulation of PFOS and PFOA during repeated exposures, thus leading to observable toxicities. The toxic effects induced by PFOA and PFOS on the exposed male rats might be helpful in the assessment of environmental risk and the threat to human health posed by universal PFCs pollution.

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References


Figure 1. The changes in the rat body weights during 28 days’ exposure. Data are expressed as mean ± SD (n=10). * P < 0.05, ** P < 0.01, *** P<0.001, as compared with control group or compared between two groups indicated, one-way ANOVA.

Figure 2. Concentrations of PFOA and PFOS in rat whole blood (µg/mL) and various tissues (µg/g) after 28 days’ exposure. Data are expressed as mean ± SD (n≥5). No data of the whole blood samples were available because all ten rats died during the experiment.