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Tissue distribution of ^{35}S-labelled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose

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ABSTRACT

The widespread environmental pollutant perfluorooctane sulfonate (PFOS), detected in most animal species including the general human population, exerts several effects on experimental animals, e.g., hepatotoxicity, immunotoxicity and developmental toxicity. However, detailed information on the tissue distribution of PFOS in mammals is scarce and, in particular, the lack of available information regarding environmentally relevant exposure levels limits our understanding of how mammals (including humans) may be affected. Accordingly, we characterized the tissue distribution of this compound in mice, an important experimental animal for studying PFOS toxicity. Following dietary exposure of adult male C57/BL6 mice for 1–5 days to an environmentally relevant (0.031 mg/kg/day) or a 750-fold higher experimentally relevant dose (23 mg/kg/day) of ^{35}S-PFOS, most of the radioactivity administered was recovered in liver, bone (bone marrow), blood, skin and muscle, with the highest levels detected in liver, lung, blood, kidney and bone (bone marrow). Following high daily dose exposure, PFOS exhibited a different distribution profile than with low daily dose exposure, which indicated a shift in distribution from the blood to the tissues with increasing dose. Both scintillation counting (with correction for the blood present in the tissues) and whole-body autoradiography revealed the presence of PFOS in all 19 tissues examined, with identification of thymus as a novel site for localization for PFOS and bone (bone marrow), skin and muscle as significant body compartments for PFOS. These findings demonstrate that PFOS leaves the bloodstream and enters most tissues in a dose-dependent manner.

1. Introduction

Due to their unique physicochemical properties, including potent surfactant activity and resistance to chemical hydrolysis and heat, perfluorooctane sulfonate (PFOS) and its congeners have been used for decades in numerous industrial and consumer applications (Lau et al., 2007). With its exceptional resistance to both biological and environmental degradation, PFOS, along with other perfluoroalkyls have emerged as global environmental contaminants, as exemplified by their detection in living organisms throughout North America, Europe, Antarctica, Asia and the Pacific Ocean (Giesy and Kannan, 2001; Houde et al., 2006; Lau et al., 2007). This world-wide occurrence also includes the serum and tissues of occupationally and non-occupationally exposed human populations (Calafat et al., 2007; Lau et al., 2007; Olsen et al., 2003a,b).

The long serum half-life of PFOS in humans (approximately 4–5 years; Olsen et al., 2007; Spliethoff et al., 2008) and its reported pathophysiological effects on experimental animals have raised concerns about potential adverse effects on human health. In contrast, the serum half-lives are considerably shorter in experimental animals, such as rats and mice (approximately 50 days, Butenhoff JL, personal communication) and monkeys (approximately 200 days, Seacat et al., 2002). One obvious problem in comparing serum half-lives and distribution of PFOS in humans to those of experi-

Abbreviations: PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; PPARα, peroxisome proliferator activated receptor α; OAT, organic anion transporter; OATP, organic anion transporting polypeptide.

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mental animals are the large differences in the serum levels and the length of exposure of this compound, which may profoundly influence both tissue distribution and biological half-life. To date, no detailed characterization has been reported concerning the tissue distribution in adult experimental animals following exposure to low, environmentally relevant doses of PFOS. In non-occupationally exposed humans, levels of PFOS have been examined in certain pooled post-mortem tissues (Maestri et al., 2006). Clearly, more toxicokinetic data of this nature is required in attempt to better understand the biological effects of PFOS.

The toxic effects observed after short-term, high-dose exposure to PFOS – including pronounced hepatomegaly and peroxisome proliferation, reduced appetite and loss of body weight and fat, developmental toxicity, and severe atrophy of the thymus and spleen – have been characterized most extensively in rats and mice (Andersen et al., 2008; Lau et al., 2007; Sohlenius et al., 1993; Qazi et al., 2009; Yang et al., 2000; Zheng et al., 2009). All toxicological data obtained in mice concerning PFOS, together with the mouse being one of the most extensively characterized experimental animals, makes this species a valuable experimental model for further toxicokinetic and mechanistic studies of PFOS. Because previous studies on the tissue distribution of this perfluorochemical in rodents have been limited in terms of tissues and doses examined (Cui et al., 2009; Johnson et al., 1979; Liu et al., 2009; Thibodeaux et al., 2003), the present investigation was designed to provide a detailed characterization of this distribution in one of the most widely studied murine strains, C57BL/6, following exposure for 1–5 days to two very different daily doses, i.e. a low, environmentally relevant and a high, experimentally relevant dose. For this purpose, we synthesized ^{35}S-PFOS to allow determination of tissue levels and distribution using liquid scintillation counting and whole-body autoradiography. The high daily dose employed here (156 μg/g in the diet, which corresponded to approximately 23 mg/kg/day) is a typical experimental daily dose that after 3–10 days of exposure causes, among other effects, extensive hypertrophy of the liver and atrophy of the thymus, spleen and fat depots in C57BL/6 mice (Sohlenius et al., 1993; Qazi et al., 2009; Xie et al., 2003). The 750-fold lower daily dose (1000-fold lower level in the diet; 0.156 μg/g, corresponding to approximately 0.031 mg/kg/day) was the lowest dose that would allow reliable analysis of the radioactivity and at the same time was anticipated to yield blood levels of PFOS similar to those found in general human populations (Calafat et al., 2007; Lau et al., 2007; Olsen et al., 2003b).

2. Materials and methods

2.1. Chemicals

All solvents and other chemicals used in synthetic and analytical procedures were of pro-analysis quality. ^{35}S-Sulfuric acid was purchased from the Institute of Isotopes Co. Ltd. (Budapest, Hungary); ethyl magnesium chloride (2 M dissolved in diethyl ether) and hydrogen peroxide (30% in water) from Sigma Aldrich Chemie (Steinheim, Germany); and perfluorooctyl iodide (98%) from TCI Europe (Zwijndrecht, Belgium). The solubilization reagents Solvable (Product No. 6NE9100), ULTIMA Gold Scintillation cocktail (Product No. 6013326) and Hionic Fluor Scintillation cocktail were purchased from Perkin Elmer (Perkin Elmer life and Analytical Sciences, Boston, USA). The hemoglobin Assay kit (DIHB-250) was obtained from BioAssay Systems (Hayward, CA, USA).

2.2. Synthesis of ^{35}S-perfluorooctane-sulfonate

The synthesis of ^{35}S-labelled PFOS (hereafter referred to as ^{35}S-PFOS) was performed according to Sundström and colleagues (manuscript in preparation). In brief, ^{35}S-PFOS was synthesized employing a Grignard reaction, prepared by introducing perfluorooctyl iodide into dried diethyl ether under an inert atmosphere. This mixture was then cooled before drop-wise addition of a solution of ethyl magnesium chloride solution and subsequently stirred for 1 h prior to slow addition of ^{35}S-sulfur dioxide (formed from reduction of ^{35}S-sulfuric acid). After careful quenching 4 h later, the perfluorooctane-sulfinic acid formed was oxidized with excess hydrogen peroxide. Thereafter, the product (^{35}S-PFOS) was extracted and purified with hexane, yielding one batch of 1.13 mCi ^{35}S-PFOS (8% yield; 31.8 mCi/mmol), used for the high daily dose exposures, with a chemical purity of 97%, as determined by liquid chromatography–mass spectrometry (the major impurity being perfluorooctanoate (PFOA)). In a second synthesis designed to obtain higher specific radioactivity for low daily dose exposure, the ^{35}S-PFOS obtained had a specific radioactivity of 59 mCi/mmol and 90% chemical purity (the 10% contamination again being mainly PFOA). The radiochemical purity was determined by quantification of the mass (by liquid chromatography–mass spectrometry) and verification towards the specific activity. In the first batch of ^{35}S-PFOS the radiochemical purity was 97% and in the second 95%. The final products were dissolved in methanol.

2.3. Animals

The male C57BL/6 mice (6–8 weeks old and weighing 20–22 g) used for liquid scintillation counting were obtained from the Microbiology and Tumor Biology Center at Karolinska Institutet (Stockholm, Sweden) and divided randomly into 6 groups of 3 mice each. For whole body autoradiography male C57BL/6 mice (10–11 weeks old and weighing 27 g) were purchased from Scanbur B&K (Sollentuna, Sweden). All animals were housed individually in polycarbonate cages with bedding of heat-treated pine-shavings at the animal facilities of the Wenner-Gren Institute, Stockholm University. The mice were allowed to acclimatize to the conditions of the animal facilities, including a 12-h light/12-h dark cycle, relative humidity of 40–60% and temperature of 22 ± 2 °C, with access to standard RMI (E) laboratory chow (Rat and Mouse Standard Diet; B&K Universal AB, Sweden) and tap water *ad libitum* for at least one week prior to initiation of the experiments. All of the experiments performed on these animals were pre-approved by the Northern Stockholm Ethical Committee for Animal Experimentation (approval numbers N/150-07 and N405/08).

2.4. Preparation of the diet for dietary exposure

For low daily dose exposure, ^{35}S-PFOS (as the free acid with a specific radioactivity of 59 mCi/mmol and dissolved in methanol) was diluted 20-fold with double-distilled water, following which the appropriate amount of this stock solution (as determined by liquid scintillation counting) was mixed with powdered RMI (E) chow to obtain a concentration of 0.156 μg/g with 0.018 μCi/g food. With an average food intake of 4 g per day and a body weight of 20 g, this corresponds to an exposure of 0.031 mg/kg/day. In the case of high daily dose exposure, the ^{35}S-PFOS (as the free acid and with a specific radioactivity of 31.8 mCi/mmol) was supplemented with an appropriate amount of unlabelled perfluorooctane-1-octanesulfonic acid tetraethyl ammonium salt (98% purity; Aldrich) in methanol and thereafter diluted 20-fold with double-distilled water to obtain a final concentration of 156 μg/g with 0.81 μCi/g food. With an average food intake of 3 g per day (the mice ate less of this diet as an effect of PFOS in the diet) and a body weight of 20 g this corresponds to an exposure of 23 mg/kg/day. In both cases, the chow was subsequently dried in a ventilated hood and shaped into cookies to facilitate determination of food consumption (since mice tend to scatter powdered food in their cages; please see Xie et al., 2003 for further details).

2.5. Dietary exposure

Three groups of mice were allowed to consume the diet containing 0.156 μg/g PFOS for 1, 3 or 5 days; while the other three groups received the high daily dose diet containing 156 μg/g PFOS for these same periods of time. Body weight was measured at the beginning and end of each period of feeding and food consumption was estimated by subtracting the weight of the remaining food from the weight of the food initially supplied to each mouse.

2.6. Collection of blood and tissues after dietary exposure

At the end of the feeding period, each mouse was bled under iso-flurane anesthesia and thereafter sacrificed by cervical dislocation. The blood samples were collected in capillary collection tubes containing dipotassium EDTA as anticoagulant. The organs and tissues (liver, lungs, kidneys, heart, spleen, stomach, small and large intestine, epididymal fat, testes, inguinal fat pads, a muscle sample from *m. quadriceps femoris*, bone samples consisting of the whole femur and tibia, skin samples taken from the back between the two scapula, brain, thymus, thyroid gland and pancreas) were dissected out and washed in cold PBS. The stomach and intestines were emptied of their contents and then washed carefully again with cold PBS for cleaning. All tissues were then weighed (wet weight) and stored frozen at −20 °C prior to liquid scintillation counting and determination of hemoglobin content.

2.7. Determination of tissue PFOS contents

Tissue contents of ^{35}S-PFOS were determined by liquid scintillation counting in a Beckman LS 6000TA Liquid Scintillation Counter. Following low daily dose exposure, entire organs were subjected to liquid scintillation counting and determination of hemoglobin content, except for the skin, whole bone and the liver, from which portions were taken (for bone both femur and both tibia were used). In the high daily dose experiment portions of the tissues were used (for bone, only one femur or part of a femur was taken). Prior to liquid scintillation counting, the blood and

Table 1
The levels of hemoglobin in the blood and tissues of mice exposed to PFOS for 1, 3 or 5 days[a].

	Hemoglobin (mg/g)					
Period (days):	1	1	3	3	5	5
Dose:	Low	High	Low	High	Low	High
Blood	153 (18.5)	220 (28.3)*	157 (18.5)	220 (46.3)*	163 (20.2)	217 (29.6)*
Liver	9.1 (1.1)	9.8 (1.8)	8.1 (1.1)	13.8 (3.5)	9.4 (2.2)	15.0 (7.3)
Lung	16.5 (2.1)	30.8 (5.3)*	16.8 (2.1)	29.6 (6.5)*	21.2 (3.6)	32.4 (11.8)
Kidney	10.6 (3.6)	13.9 (1.9)	15.0 (3.6)	16.4 (3.5)	10.5 (2.0)	13.2 (0.9)
Skin	6.8 (1.8)	6.5 (1.8)	5.5 (1.8)	5.1 (1.5)	6.2 (1.7)	7.2 (3.0)
Whole bone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pancreas	2.4 (0.9)	2.4 (0.1)	3.1 (0.9)	2.1 (0.3)	1.8 (1.1)	3.1 (2.5)
Large intestines	2.5 (1.7)	4.1 (0.8)	2.8 (1.7)	3.5 (0.4)	2.4 (0.3)	3.9 (0.8)
Stomach	4.5 (1.9)	4.7 (1.4)	4.0 (1.9)	3.4 (0.4)	6.5 (2.8)	6.4 (2.7)
Spleen	27.6 (2.0)	40.8 (14.3)	28.0 (2.0)	56.0 (24.1)*	32.3 (5.1)	56.8 (16.0)*
Small intestines	2.3 (0.4)	2.7 (1.1)	2.9 (0.4)	3.3 (0.8)	3.0 (0.2)	2.3 (1.1)
Thymus	1.7 (0.5)	1.9 (0.4)	2.1 (0.5)	2.4 (0.3)	1.9 (2.6)	4.8 (2.8)
Heart	16.8 (2.5)	32.6 (11.1)*	16.2 (2.5)	21.7 (1.5)	20.0 (4.5)	17.8 (1.6)
Testis	0.8 (0.2)	1.3 (0.2)	1.2 (0.2)	1.7 (0.2)	4.6 (2.4)	1.5 (0.2)
Epididymal fat	2.7 (0.6)	1.0 (0.1)	1.9 (0.6)	1.7 (0.3)	2.6 (2.1)	1.6 (0.1)
Fat pads	2.6 (1.0)	2.6 (1.0)	3.0 (1.0)	5.1 (3.3)	3.7 (1.8)	9.9 (10.2)
Brain	2.2 (0.2)	3.6 (1.3)	2.0 (0.2)	2.5 (0.7)	2.2 (0.1)	3.7 (0.9)
Muscle	1.2 (0.3)	1.3 (0.1)	1.2 (0.3)	2.1 (1.4)	1.5 (0.2)	1.6 (0.3)
Thyroid gland	n.d.	3.1 (0.3)	n.d.	3.4 (0.3)	n.d.	4.3 (0.5)

[a] Hemoglobin was measured in the same samples solubilized for liquid scintillation counting, as described in Section 2. Each value represents the mean (S.D.) for 3 mice. n.d. = not determined.

* Significantly different ($P < 0.05$) than the corresponding values for low daily dose treatment as evaluated by one-way ANOVA test followed by Duncan's test for multiple comparisons.

soft tissue samples were weighed (wet weight), solubilized in Solvable, and then divided into two parts: one for scintillation counting and the other for determination of hemoglobin (described in the next section). The samples were bleached with hydrogen peroxide, in accordance with the manufacturer's instructions. In this manner hemoglobin and PFOS could be determined in the same solubilized sample.

For solubilization of whole bone, a mixture of perchloric acid and hydrogen peroxide was utilized (according to Mahin and Lofberg, 1966), which did not allow determination of hemoglobin. In brief, ≤200 mg whole bone was chopped into small pieces and placed into 0.2 mL 60% perchloric acid in a glass vial before adding 0.4 mL 30% hydrogen peroxide. After screwing the vial caps on tightly, the samples were heated to 70 °C for 2–3 h with occasional agitation to ensure complete solubilization, and then allowed to cool to room temperature. Scintillation liquid cocktail (Hionic Fluor for bone samples and Ultima Gold for all other tissue samples) was added to all samples and before counting they were kept in ambient light and temperature for at least 1 h to minimize background counts derived from hydrogen peroxide-induced scintillation.

Neither the two solubilization reagents employed nor the solubilized tissues influenced the efficiency of liquid scintillation counting (as assessed by spiking with a known amount of ^{35}S-PFOS). Correction for the decay of sulfur-35 was made in all cases. The volumes of blood samples were measured and these volumes then converted to weights assuming a density of 1.06 g/mL (Wang et al., 2001). When only a portion of an organ/tissue was assayed, the total amount of PFOS present was obtained by adjusting for the total (wet) weight. However, in the case of the skin, muscle, whole bone and blood the total weights were not determined, and instead, literature values for the relative contributions of these tissues to total body weight were utilized in these calculations (16.5% for skin, 38.4% for muscle and 10.7% for whole bone, values for mice of similar weight to the animals used here, see Brown et al., 1997, and 8% for blood, as has been estimated in the almost identical C57BL/10 strain, Everds, 2007). Determination of the food consumption by each mouse (see above) provided the total amount of PFOS ingested.

2.8. Determination of the hemoglobin contents

For quantification of the hemoglobin content in each sample, the QuantiChrom Hemoglobin Assay Kit was applied to the unbleached solubilized samples (see above) in accordance with the manufacturerĩs instructions. In this assay hemoglobin is converted entirely into a colored end-product by utilization of the detergent Triton and NaOH, and the end-product is directly proportional to the hemoglobin concentration in the original sample. In brief, after dissolving the tissue samples in Solvable, 50 μL of the resulting mixture was added to each individual well on a 96-well plate, to which 200 μL of reagent was added. After 5 min incubation at room temperature, the optical density at 400 nm was determined and converted to mg/mL hemoglobin by comparison to a standard supplied by the manufacturer. The resulting contents are expressed as mg hemoglobin per g tissue or blood. To correct the PFOS concentrations in each tissue for PFOS derived from the blood present in the same tissue, the radioactivity per mg hemoglobin in blood was calculated; this value was multiplied by the mg hemoglobin in the specific tissue; and the value thus obtained subtracted from the total amount of PFOS present.

2.9. Whole-body autoradiography

An aliquot of ^{35}S-labelled PFOS (in acidic form with a specific activity of 59 mCi/mmol) was prepared for administration by evaporating the methanol and dissolving the substance in deionized water:tap water (1:1). Two male C57BL/6 mice then received a single oral dose (0.8 μCi/g, 12.5 mg PFOS/kg) via gavage. 48 h later these mice were sacrificed, mounted in aqueous carboxymethyl cellulose (CMC) and frozen in a bath of hexane cooled with dry ice. The frozen tissues were processed for whole-body autoradiography as described by Ullberg and Larsson (1981). In short, series of whole body sagittal sections (20 μm and 40 μm in thickness; Jung Cryomacrocut, Leica) taken at 10 different levels were collected onto tape (Scotch 6890, 3M Ltd., St. Paul, MN, USA) and freeze-dried. The sections were then apposed to X-ray film (Structurix, Agfa, Mortsel, Belgium) and stored at −20 °C during exposure, following which the film was developed using D19 (Kodak, Rochester, NY, USA). In addition, samples of liver, lungs, kidneys, brain and blood were dissected out of the remains of the CMC blocks for liquid scintillation counting as described above.

2.10. Histological examination

Sections used for the whole-body autoradiography were stained for calcified bone using von Kossa's staining (Presnell and Schreibman, 1997). In brief, the sections were placed in 5% silver nitrate in the dark for 30 min and then exposed to bright light (60 W bulb) for 30 min. The sections were then washed thoroughly in distilled water, counterstained in nuclear fast red for 5 min and rinsed in 70% alcohol before dehydration and mounting. A selection of the whole-body sections were also stained with hematoxylin Certistain® (Merck KGaA, Darmstadt, Germany) and eosin Y (BDH Ltd., UK) in the standard manner.

2.11. Statistical analyses

Statistical analyses were performed utilizing the WinStat software (R Finch, Germany). To ensure normal distribution, all data was first transformed by square root calculations (McDonald, 2009). This transformation revealed the data set to be normally distributed (evaluated by the Kolmogorov–Smirnov test) and with similar variance in all cases (evaluated by Bartlett's test). One-way ANOVA was performed to test for statistically significant differences between groups and an independent two-tailed *t*-test was utilized when only two groups were to be compared to one another.

3. Results

3.1. General observations on tissue levels of PFOS

In line with earlier investigations (Sohlenius et al., 1993; Qazi et al., 2009; Xia et al., unpublished results) mice exposed to the high daily dose for 5 days exhibited hypertrophy of the liver (a dou-

Table 2
Levels of PFOS in the blood and tissues of mice following low daily dose exposure for 1, 3 or 5 days[a].

Compartment	PFOS (pmol/g)		
	1 day	3 days	5 days
Blood	61 (6)	129 (41)[#]	99 (21)
Liver	114 (13)[**]	343 (24)[**,#]	578 (39)[**,#]
Lung	39 (29)	88 (6)[#]	141 (10)[*,#]
Kidney	38 (19)	65 (13)	93 (11)[#]
Skin	17 (3)[**]	36 (1)[*,#]	49 (7)[*,#]
Whole bone	113 (15)[**]	98 (24)	109 (6)
Pancreas	23 (15)[*]	46 (4)[#]	65 (6)[*,#]
Large intestines	49 (43)	37 (12)[*]	62 (2)
Stomach	36 (14)	36 (6)[**]	59 (4)[*,#]
Spleen	11 (7)[*]	21 (17)[*]	46 (2)[*,#]
Small intestines	32 (7)[*]	49 (5)	79 (24)[#]
Thymus	21 (13)[*]	26 (5)[**]	40 (1)[*,#]
Heart	12 (8)[**]	20 (10)[**]	28 (3)[**,#]
Testis	12 (7)[**]	21 (4)[*]	31 (2)[*,#]
Epididymal fat	5 (1)[**]	10 (2)[*,#]	11 (1)[**,#]
Fat pads	10 (3)[**]	12 (2)[*]	13 (1)[**]
Brain	9 (1)[**]	12 (1)[*,#]	17 (1)[**,#]
Muscle	8 (2)[**]	12 (1)[*,#]	22 (4)[**,#]

[a] PFOS levels, determined by liquid scintillation counting as described in Section 2, are presented as pmol per g (wet weight) tissue or blood. Each tissue level has been corrected for the contribution by the blood present (with the exception of whole bone samples) and represents the mean (S.D.) for 3 mice. n.d. = not determined. The tissues (except for blood) are ordered from the highest to the lowest level of PFOS detected after 5 days of high daily dose exposure (see Table 3).
[*] Significantly different ($P < 0.05$) than the blood at the same time-point as evaluated by an independent *t*-test (two-tailed).
[**] Significantly different ($P < 0.01$) than the blood at the same time-point as evaluated by an independent *t*-test (two-tailed).
[#] Significantly different ($P < 0.05$) from the value for the same tissue at day 1 as determined by one-way ANOVA followed by Duncan's test for multiple comparisons.

Table 3
Levels of PFOS in the blood and tissues of mice following high daily dose exposure for 1, 3 or 5 days[a].

Compartment	PFOS (nmol/g)		
	1 day	3 days	5 days
Blood	67 (4)	171 (21)[#]	287 (9)[#]
Liver	246 (31)[**]	698 (71)[**,#]	1044 (114)[**,#]
Lung	135 (18)[**]	336 (69)[*,#]	445 (42)[**,#]
Kidney	62 (3)	166 (8)[#]	233 (12)[**,#]
Skin	54 (3)[*]	141 (22)[#]	222 (14)[**,#]
Whole bone	55 (6)[*]	155 (17)[#]	207 (8)[**,#]
Pancreas	48 (2)[**]	129 (11)[*,#]	155 (32)[**,#]
Large intestines	35 (6)[**]	82 (3)[**,#]	125 (16)[**,#]
Stomach	35 (2)[**]	81 (4)[**,#]	123 (7)[**,#]
Spleen	29 (8)[**]	102 (36)[*,#]	113 (10)[**,#]
Small intestines	34 (2)[**]	80 (5)[**,#]	110 (48)[*,#]
Thymus	23 (2)[**]	63 (5)[**,#]	104 (4)[**,#]
Heart	22 (2)[**]	65 (3)[**,#]	94 (8)[**,#]
Testis	16 (0)[**]	51 (1)[**,#]	86 (3)[**,#]
Epididymal fat	12 (4)[**]	30 (10)[**,#]	53 (7)[**,#]
Fat pads	12 (1)[**]	44 (8)[**,#]	49 (19)[**,#]
Brain	9 (1)[**]	29 (1)[**,#]	44 (1)[**,#]
Muscle	9 (0)[**]	28 (4)[**,#]	37 (4)[**,#]
Thyroid gland	31 (3)[**]	81 (35)[*,#]	134 (12)[**,#]

[a] PFOS levels, determined by liquid scintillation counting as described in Section 2, are presented as nmol per g (wet weight) tissue or blood. Each tissue level has been corrected for the contribution by the blood present (with the exception of whole bone samples) and represents the mean (S.D.) for 3 mice. The tissues (except for blood and thyroid gland) are ordered from the highest to the lowest level of PFOS detected after 5 days.
[*] Significantly different ($P < 0.05$) than the blood at the same time-point as evaluated by an independent *t*-test (two-tailed).
[**] Significantly different ($P < 0.01$) than the blood at the same time-point as evaluated by an independent *t*-test (two-tailed).
[#] Significantly different ($P < 0.05$) from the value for the same tissue at day 1 as determined by one-way ANOVA followed by Duncan's test for multiple comparisons.

bling of liver:body weight ratio), atrophy of fat pads (approximately 50% of fat pads:body weight ratio) and epididymal fat (approximately 75% of epididymal fat:body weight ratio) when compared to the corresponding group of mice exposed to the low daily dose.

The utilization of ^{35}S-labelled PFOS in this study allowed a simple and reliable determination of its distribution *in vivo*, since no metabolism of this perfluorochemical is known to occur (reviewed in Lau et al., 2007). In order to calculate the amount of radioactivity recovered that was due to blood present in the tissues, the hemoglobin contents of all samples were determined (Table 1). By then correcting for PFOS present in the blood, we could determine the actual tissue levels following low or high daily dose exposure (Tables 2 and 3). These corrections were small for most tissues, being no more than 5% following low daily dose exposure and 10% following high daily dose exposure. However, for some tissues with high levels of blood the corrections were considerably larger; values for PFOS levels in the spleen had corrections of 20–50% and 20–30% following low and high daily dose exposure respectively, and values for levels in the heart between 25–40% and 15–25% following low and high daily dose exposure respectively.

The level of PFOS in all tissues examined increased significantly with time at low daily dose exposure, with the exceptions of whole bone samples and fat pads (Table 2). This increase over time was even more pronounced in the case of high daily dose exposure (Table 3), in which case PFOS levels in all of the tissues examined were statistically higher after both 3 and 5 days compared to 1 day of exposure. Thus, there was no indication of saturation of PFOS binding or uptake for most of the tissues, with the possible exceptions of the spleen and fat pads after 5 days of high daily dose treatment (Table 3).

Following low-dose exposure the ratio of tissue:blood levels of PFOS for most tissues (again with the exception of whole bone samples) increased with time (Fig. 1A). In contrast, the ratios were virtually constant over time in the high daily dose animals (Fig. 1B). Moreover, the ratios were in general higher after high daily dose treatment (Fig. 1A and B). Taken together, these findings indicate a shift in distribution from the blood to the tissues with increasing dose.

To obtain complementary information concerning the distribution of PFOS, mice exposed to a single oral dose of 12.5 mg/kg were subjected to whole-body autoradiography (Fig. 2). The overall distribution and relative levels of PFOS indicated by this experiment correlated well with the values in Tables 2 and 3, i.e., liver exhibited the strongest signal, followed by lungs, blood and kidney, and all tissues appeared to contain PFOS. The whole-body autoradiography was combined with quantitative determination of PFOS levels in certain tissues of these same mice using liquid scintillation counting, which yielded levels of 19 nmol/g in blood, 129 nmol/g in liver, 47 nmol/g in lung, 18 nmol/g in kidney and 5 nmol/g in brain. Although these values could not be corrected for PFOS derived from blood in the tissues, they are generally in good agreement with the findings following one day of high daily dose exposure presented in Table 3.

3.2. PFOS levels in liver

With both daily dose regimes, liver contained the highest levels of PFOS at all time-points (Tables 2 and 3 and Fig. 2) except for after one day of low daily dose exposure, when the level in whole bone samples was equally high (Table 2). In mice exposed to the low daily dose the level in liver relative to blood increased with time (Fig. 1A), whereas with high daily dose exposure this ratio remained relatively constant (Fig. 1B). This reflects that a plateau in the blood levels was reached after 3 days of low daily dose exposure (Table 2), rather than any difference in the almost

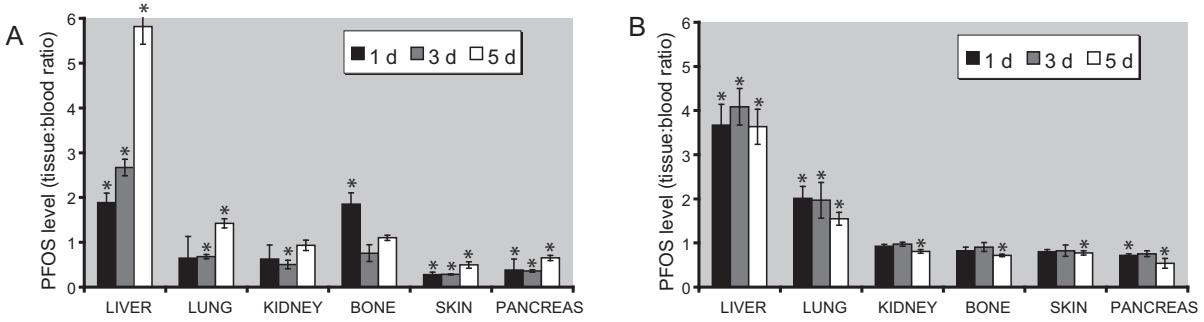

Fig. 1. The ratios of tissue-to-blood levels of PFOS for some tissues containing high levels of this compound following (A) low- and (B) high daily dose exposure. The mice were exposed for 1 (black), 3 (grey) or 5 (white) days. The data presented are the means for 3 mice per group, with the error bars depicting S.D. *Significantly different ($P < 0.05$) from the corresponding blood levels (i.e., from a ratio of 1) as determined by one-way ANOVA followed by Duncan's test. Whole bone including the marrow was analyzed.

linear relationship between the period of exposure and hepatic levels (Tables 2 and 3). Whole-body autoradiography and staining with hematoxylin/eosin demonstrated a heterogeneous, somewhat mottled distribution of PFOS within the liver, with no obvious relationship to any specific regions within hepatic lobules apart from the clearly lower level in the blood vessels (Fig. 2A and B).

3.3. PFOS levels in lungs

Pulmonary levels of PFOS were higher than blood levels following 5 days of low daily dose exposure (Table 2 and Fig. 1A) and higher than in blood at all time-points in the high daily dose situation (Table 3 and Fig. 1B). The distribution within the lungs was

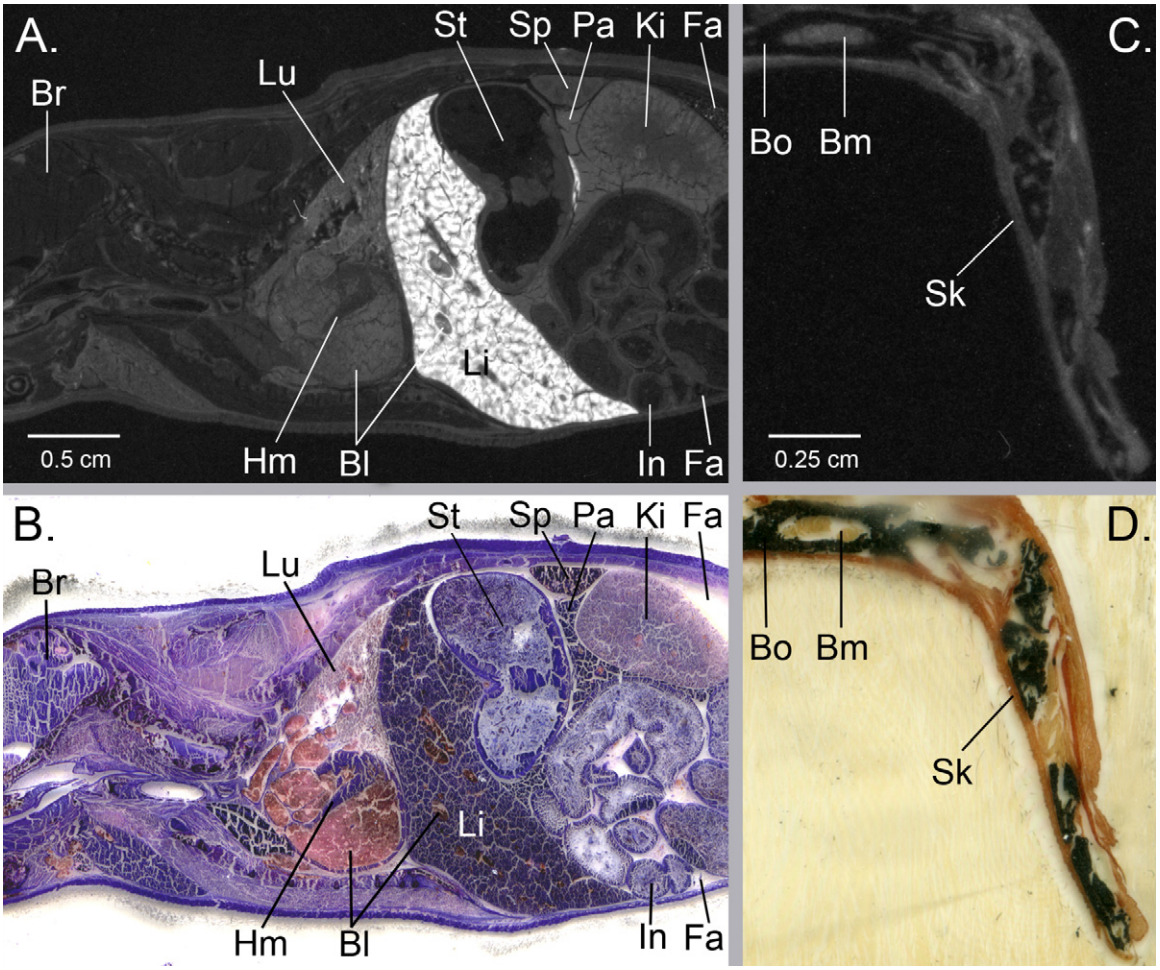

Fig. 2. (A) Whole body autoradiogram of a 40-μm section of a mouse 48 h after administration of a single oral dose of ^{35}S-PFOS (12.5 mg/kg), and (B) the same section with hematoxylin/eosin staining. (C) Autoradiogram of a 40-μm section of a mouse femur 48 h after administration of a single oral dose of ^{35}S-PFOS (12.5 mg/kg) and (D) the same section with von Kossa's staining for calcified bone. In (A) and (C) the brighter areas correspond to higher levels of radioactivity and in (D) dark areas correspond to calcified bone. In (B) unstained areas correspond to fat (as caudal of kidney, surrounding the intestines and sub dermal), body cavities and freezing artifacts (cracks). Bl = blood, Bm = bone marrow, Bo = bone, Br = brain, Fa = fat, Hm = heart muscle, In = intestine, Ki = kidney, Li = liver, Lu = lung, Pa = pancreas, Sk = skin, Sp = spleen, and St = stomach. Von Kossa's staining revealed that the ^{35}S-PFOS was present only in the bone cavities, i.e. bone marrow, and not in the calcified bone. Section (A) and (C) were exposed for 9 and 14 days, respectively.

generally homogenous, although certain surface areas exhibited higher levels (Fig. 2A).

3.4. PFOS levels in whole bone samples

Although the PFOS levels in whole bone samples could not be corrected for PFOS derived from the blood present (see Section 2), the error due to this is expected to be minor, since bone contains relatively little blood (approximately 10% of the total bone volume; see Brown et al., 1997, and even less in terms of weight). In the low daily dose situation, whole bone samples, together with liver, displayed the highest levels of PFOS after one day of exposure (almost double the PFOS level in blood, see Table 2 and Fig. 1A); but in contrast to liver, this level remained more or less constant thereafter with PFOS levels similar to those in blood. However, higher levels did occur in association with the high daily dose exposure, with an increase over time yielding final levels similar to those in blood (Table 3 and Fig. 1B). As depicted in the autoradiogram and the von Kossa staining of a femur (Fig. 2C and D), PFOS was detected in the cavities of the bone, containing the bone marrow, and not in the calcified bone. It should be noted that the quantification of PFOS contents in Tables 2 and 3 were made on whole bone samples (femur and tibia), including bone marrow.

3.5. PFOS levels in skin

PFOS was also detected in skin samples taken from the upper back of the mice, at levels half to a third of those in blood during low daily dose exposure (Table 2 and Fig. 1A) and close to those in blood in the high daily dose situation (Table 3 and Fig. 1B). Whole-body autoradiography indicated that distribution within the skin was relatively homogeneous (Fig. 2).

3.6. PFOS levels in other organs

As shown in Tables 2 and 3 and Fig. 2, PFOS was present to a varying extent in all murine tissues examined. One of the lowest levels of PFOS was found in fat depots and brain, which may to some extent reflect the relatively low partition coefficient (Kow) of PFOS (Sundström et al., manuscript in preparation). Notably, low levels similar to those in fat were also detected in skeletal muscle. Renal levels of PFOS reached blood levels after 5 days of low daily dose exposure and were on average the same as blood levels at all time-points in the high daily dose situation (Fig. 1). The distribution within the kidneys was heterogeneous, with higher levels in the cortex (Fig. 2A). PFOS was homogeneously distributed in the pancreas (Fig. 2A).

3.7. Comparison of body compartments for PFOS

Fig. 3 depicts the estimated body compartments in which most of the PFOS were recovered. With both doses, the liver was clearly the major compartment, with the striking exception that after one day of low-dose exposure, whole bone (including bone marrow) contained almost twice as much PFOS as the liver, a situation that was reversed at later time-points (Fig. 3A; see Fig. 2C for localization in bone marrow). At these later time-points, blood and whole bone contained, on the average, equal amounts of PFOS, followed by skin and skeletal muscle. High-dose exposure resulted in a different profile, with the liver containing even more (40–50%) of all PFOS recovered and the skin being the second largest compartment with approximately 20%. In this case, blood and whole bone contained similar amounts of PFOS (10–12%), followed closely by muscle.

3.8. Increased hemoglobin levels in blood following high daily dose exposure

A noteworthy observation was that at all time-points the levels of hemoglobin in the blood of mice exposed to a high daily dose of PFOS was significantly higher (133–143%) than the corresponding values associated with low daily dose exposure. In agreement with this finding, hemoglobin levels in blood-rich organs such as liver, lungs, heart and spleen were also higher after high daily dose exposure, with statistically significant differences after 1 and 3 days of exposure for the lungs, after 3 and 5 days in the case of spleen and after 1 day for heart (Table 1). This pronounced elevation of hemoglobin levels could either be due to increased production of erythrocytes (increased synthesis of hemoglobin) and/or a decreased blood volume (dehydration).

4. Discussion

4.1. The low daily dose exposure was environmentally relevant

Following the low daily dose exposure employed here, the blood levels of PFOS in our mice were at the high end of the range of those detected in serum or whole blood of general human populations at different locations in the United States and in several countries in Europe and Asia (Lau et al., 2007; Olsen et al., 2003b) taking into account a serum:whole blood ratio of 2:1 (Ehresman et al., 2007). Thus, after one day of low-dose exposure here, the mean murine blood level of PFOS was 3-fold higher than the corresponding mean blood levels of PFOS reported by the NHANES 2003–2004 study on general US populations (Calafat et al., 2007). Furthermore, both the blood and hepatic levels of PFOS detected here after low daily dose exposure were within the range of those detected in different forms of wild-life, including seals, birds and polar bears (Giesy and Kannan, 2001). Accordingly, our low daily dose exposure resulted in environmentally relevant blood levels of PFOS.

4.2. High PFOS levels in blood

Blood levels of PFOS in the high daily dose groups in our study are in good agreement with earlier studies in mice. For instance, the blood levels were similar to those of KM mouse pups (Liu et al., 2009), as well as to levels in pregnant mice following 17 days of administration of 10 mg PFOS/kg/day (Thibodeaux et al., 2003) which is also consistent with our earlier finding that blood levels in pups and adult mice were in the same range (Borg et al., 2010). The occurrence of PFOS at relatively high levels in blood seems to be a general phenomena for most species investigated to date (reviewed in Lau et al., 2007) and probably involves binding to serum proteins such as albumin (Zhang et al., 2009).

4.3. Highest levels of PFOS in liver

The high hepatic levels found here following high daily dose exposure were similar to other values reported for mice (Liu et al., 2009; Thibodeaux et al., 2003). High levels of PFOS in liver also appears to be a general phenomenon in all species investigated to date (reviewed in Lau et al., 2007) and thus probably reflects general features of this compartment such as high levels of xenobiotic- and/or lipid-binding proteins. For example, hepatic fatty acid-binding protein is known to bind PFOS (Luebker et al., 2002). Furthermore, transport systems expressed in the liver may contribute to the high uptake into this organ, similar to the manner in which renal organic anion transporters appear to transport the congener PFOA in the kidney (Katakura et al., 2007; Kudo et al., 2002). Indeed, uptake of PFOA in isolated hepatocytes has

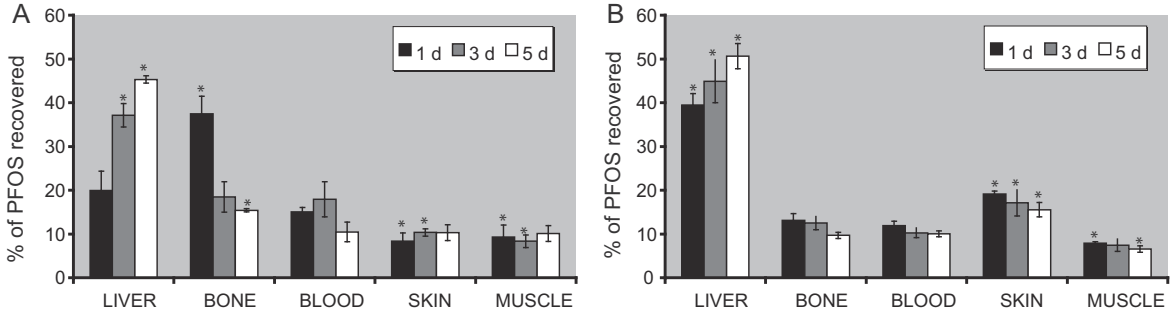

Fig. 3. The murine tissues containing the highest amounts of PFOS (expressed as percentages of the total tissue recovery) following (A) low- or (B) high daily dose exposure for 1 (black), 3 (grey) and 5 (white) days. Except for liver, the total amounts of PFOS in the tissues were estimated using literature values for their total weights, expressed as proportions of body weight; bone (10.7%), blood (8%), skin (16.5%) and muscle (38.4%). *Significantly different ($P < 0.05$) than blood at the same time-point as evaluated by one-way ANOVA followed by Duncan's test. The values shown are means, with error bars depicting S.D. ($n = 3$ for each group). Whole bone including the marrow was analyzed.

been indicated to be largely dependent on either organic anion-transporting polypeptides (OATPs) or organic anion transporters (OATs) (Han et al., 2008). The high levels of PFOS in liver of mice are most likely responsible for the local effects on this organ, leading to rapid enlargement at intermediate-to-high doses, as well as activation of hepatic nuclear receptors, including PPARα (Sohlenius et al., 1993).

4.4. Lungs demonstrates the second highest level of PFOS

The considerable levels of PFOS detected in lungs (second only to the liver with the high daily dose) agrees well with our previous findings on pregnant mice and their offspring (Borg et al., 2010), and with its acute toxic effects on this organ in adult rats (Cui et al., 2009; Dean et al., 1978). In addition, in post-mortem human lungs, PFOS levels were 1.6 times higher than those in blood (Maestri et al., 2006). The mechanism of PFOS retention in lungs is not yet known, but may involve interactions with components of the pulmonary surfactant, such as phosphatidylcholine (Lehmler et al., 2006).

4.5. PFOS levels in whole bone and skin were similar to those in blood

Both skin and whole bone samples exhibited PFOS levels similar to those in blood (Tables 2 and 3). As our whole-body autoradiography study indicates, PFOS appears to be localized to the bone marrow and not to the calcified bone (Fig. 2). Accordingly, the PFOS levels for whole bone given in Tables 2 and 3 are most likely underestimating PFOS levels in the bone marrow. Since the bone marrow has been reported to constitute approximately 50% of the skeleton weight (Brown et al., 1997) and assuming that this proportion is similar for all skeletal bones, PFOS levels in the bone marrow would be approximately two times higher than the levels given for whole bone in Tables 2 and 3, i.e. around 200 pmol/g in the low daily dose groups and 100, 300, 400 nmol/g after 1, 3, and 5 days, respectively, of high daily dose exposure. Based on these calculations bone marrow would demonstrate the second highest levels of PFOS in the low daily dose exposure and the third highest level of PFOS in the high daily dose exposure, close to the levels in lungs. Our finding of PFOS in the bone marrow and skin is consistent with a previous report on rats (Johnson et al., 1979), however, the tissue:blood ratios that can be calculated from their values, 0.13 for skin and 0.17 for bone marrow, are significantly lower than those observed here (see Fig. 1). The differences probably reflect the different experimental procedures as their data was based on measurements 89 days after a single intravenous dose of 4.2 mg/kg.

These observations motivate examination of the potential effects of PFOS on bone marrow and skin. Of interest in this connection is the recent report that exposure of primary cultures of dolphin skin cells to 13 μg/g PFOS (or 26 nmol/mL, which is in the same range as the blood level after 1 day of our high daily dose exposure) alters the transcription of certain genes, such as those encoding collagen and actin (Mollenhauer et al., 2009). Some of the well documented immunotoxicological effects of PFOS (reviewed in Andersen et al., 2008 and Lau et al., 2007), as well as our present finding of enhanced hemoglobin levels in blood following high daily dose exposure might reflect effects of this compound on the production of immune cells and erythrocytes, respectively, by the bone marrow.

4.6. Kidney and pancreas demonstrate levels of PFOS close to those in blood

After our high daily dose exposure, the kidney:blood level ratios were on average close to 1 (Fig. 1B) which is a little lower than reported for rats (kidney:blood level ratio of 1.3) exposed to a similar total dose of PFOS (28 days exposure to 5 mg/kg/day, Cui et al., 2009) and for human post-mortem renal samples (kidney:blood level ratio 1.2, Maestri et al., 2006). Effects of PFOS on the kidney are not well studied, however, Cui and colleagues reported on mild symptoms in the rat such as congestion in renal cortex and medulla as well as cytoplasmic acidosis. Renal hypertrophy was also observed in the rat (Cui et al., 2009) which correlate with our findings of a slight increase in renal wet weight after 5 days of high daily dose exposure (approximately 125% increase compared to corresponding low dose group).

Pancreas contained significant levels of PFOS, as much as two-thirds of the corresponding blood levels (Fig. 1), similar to post-mortem human pancreatic samples (Maestri et al., 2006). This finding is of interest since mice treated with high doses of PFOS experience a loss of appetite (Lau et al., 2007), which could conceivably be related to the appetite-regulating hormone ghrelin, produced, among other sites, in the stomach, intestines and pancreas, and which also influences insulin secretion by the pancreas (DeVriese and Delporte, 2008). Although the incidence of pancreatic cancer in laboratory animals following exposure to PFOS has not been reported, exposure of rats to other PPARα agonists, such as PFOA, has been observed to cause pancreatic acinar cell adenoma (reviewed in Klaunig et al., 2003).

4.7. Dose-dependent tissue distribution of PFOS

The ratios of tissue:blood levels for PFOS (Fig. 1) indicate a time- and/or dose-dependency of the tissue distribution of PFOS, i.e. in most tissues this ratio rose at longer time-points and/or higher daily dose, a phenomenon that has not been reported previously. In con-

trast, ratios of tissue:blood levels of PFOA at two different doses (0.041 and 16.56 mg/kg intravenously) were reported to be almost identical for most tissues, with the exception that the liver:blood ratio decreased from 4 with the lower dose to 1.5 with the higher dose (calculated from Kudo et al., 2007). These observations may indicate that PFOS is retained longer in tissues, especially in the liver, than PFOA, as proposed previously by Tan et al. (2008). In the case of rats, Johnson et al. (1979) reported lower tissue:blood ratios than those observed here, which is probably due to the fact that these investigators performed their analyses 89 days after exposure. The exception to these general differences is their higher liver:blood ratio of 8 (calculated from their data), indicating that not only does the liver accumulate most PFOS, but this tissue also appears to retain it the longest.

Interestingly, the tissue distribution we observed in connection with our low daily dose exposure (especially after 5 days of exposure) is similar to that found in post-mortem human material (Maestri et al., 2006), where the tissue:blood ratios calculated from their data were similar to those reported here for most of the tissues examined. More detailed analysis of the pharmacokinetics of PFOS in mice is required, including comparison of the pharmacokinetics to the situation in humans.

4.8. Estimations of body compartments for PFOS and total tissue recovery

In the present study estimations of the major body compartments for PFOS were made (Fig. 3). It should be emphasized that these calculations are all based on estimations of the total tissue recovery of PFOS in the mice where the tissue compartments with the largest masses such as whole bone, skin, muscle and blood were calculated using literature values of their total mass (see Section 2). Nevertheless, our estimations of liver and blood as compartments for PFOS are in line with earlier published data such as our estimation of 40–50% recovery in liver in the high daily-dose treatment (Fig. 3) which agrees well with other studies in rodents (Austin et al., 2003; Cui et al., 2009; Liu et al., 2009) and our estimation of blood to contain 10–12% of the total PFOS recovered in tissues following high daily-dose exposure which is similar to the earlier report of 10–13% recovery in the blood of KM mouse pups (Liu et al., 2009).

In the case of whole bone and skin nothing has been reported in terms of body compartments for PFOS. Following our low or high daily-dose exposures, whole bone and skin, respectively, were estimated to be the second major compartments for PFOS (Fig. 3). In the case of bone samples, this rests on the assumption that the whole bone used here (femur and tibia) are representative. If our finding that PFOS in femur (Fig. 2) is localized to the bone marrow is also the case for all other bones, the value we present for the amount of PFOS in this particular compartment might be an overestimate, since each femur contains approximately 6% of all the red bone marrow (Chervenick et al., 1968) but accounts for approximately 2.3% of the total bone dry weight (including bone marrow, DiMasso et al., 1998). Taken together, although only estimations are given for the body compartments, the liver, bone marrow, skin, blood and muscle are likely significant compartments for PFOS and should all be taken into account as body compartments when constructing pharmacokinetic models for PFOS.

As described, the estimation of total tissue recovery of PFOS in the present study was based on an approximation of whole body mass using both measured values and literature values of the mass of the examined tissues. The total tissue recovery of ^{35}S-PFOS thus calculated varied between the doses, where approximately 100% (95–105%) of ingested PFOS was recovered in the tissues following the high daily dose exposures, but only 30–60% of ingested PFOS was recovered in the tissues following the low daily dose exposures. The dose-dependent recovery of PFOS in tissues could indicate that upon low daily dose exposure, there was a reduced uptake of PFOS and/or a more rapid excretion as compared to the case for the high daily dose groups. A potential dose-dependent effect on total tissue recovery and thus on uptake and/or excretion is interesting and further studies are required to determine if this is the case.

4.9. Hemoglobin levels

The hemoglobin content of the blood of mice receiving a low daily dose of PFOS in this study lies within the normal range reported for various laboratory strains of mice (130–180 mg/mL, Everds, 2004). Information on tissue levels of hemoglobin in rodents is sparse, but one report that hepatic hemoglobin levels in the rat are 7–8 mg/g (Walker et al., 1985) which is consistent with our present findings (see Table 1). Our surprising observation here that blood levels of hemoglobin are elevated by as much as 40% at all time-points during high daily dose exposure clearly requires further investigation. One explanation could be dehydration, however, our observation that PFOS localizes to the bone marrow raises the possibility that this compound may affect hematopoiesis. In contrast, others have reported slightly reduced blood levels of hemoglobin in monkeys treated with PFOS, although that study involved long-term exposure with lower doses (Seacat et al., 2002).

5. Conclusions

The present investigation documents the distribution of PFOS to most organs and tissues of mice following 1–5 days of exposure to two very different daily doses, an experimentally relevant high daily dose and an environmentally relevant daily dose that was 750-fold lower. Our dietary administration mimics what is considered to be the major route via which humans and most other animals are exposed to this compound (Vestergren et al., 2008). After correcting for PFOS in the blood present in the tissues, we could demonstrate that this perfluorochemical enters all of the tissues examined, including the thymus which has not been evaluated previously in this context. The body compartments containing the largest amounts of PFOS were the liver, bone (likely bone marrow), blood, skin and muscle, with the highest levels being found in liver, lung, blood, kidney, and whole bone samples (including bone marrow). Dose-dependent effects on the distribution between the major body compartments were observed, with a lower proportion in the blood and a higher proportion in the tissues, especially the liver, at the higher dose. Moreover, we found that the level of PFOS in most of the tissues examined increased with the length of exposure (1–5 days). In fact, even higher levels would be predicted for longer periods of exposure, since no saturation was evident for most of the tissues, even with the higher dose. The ratios of tissue:blood levels after our low daily dose exposure were similar to those found earlier in post-mortem human material (Maestri et al., 2006).

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Conflict of interest statements

The authors declare that there are no conflicts of interest.

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