Reproductive Toxicology 30 (2010) 558–565

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Tissue distribution of ^{35}S-labelled perfluorooctane sulfonate (PFOS) in C57Bl/6 mice following late gestational exposure

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ARTICLE INFO

Article history:
Received 22 January 2010
Received in revised form 31 May 2010
Accepted 12 July 2010
Available online 23 July 2010

Keywords:
Perfluorooctane sulfonate (PFOS)
Perfluoroalkylated compounds (PFCs)
Autoradiography
Liquid scintillation counting
Perinatal
Tissue distribution
C57Bl/6 mouse

ABSTRACT

Exposure of rodents *in utero* to perfluorooctane sulfonate (PFOS) impairs perinatal development and survival. Following intravenous or gavage exposure of C57Bl/6 mouse dams on gestational day (GD) 16 to ^{35}S-PFOS (12.5 mg/kg), we determined the distribution in dams, fetuses (GD18 and GD20) and pups (postnatal day 1, PND1) employing whole-body autoradiography and liquid scintillation counting. In dams, levels were highest in liver and lungs. After placental transfer, ^{35}S-PFOS was present on GD18 at 2–3 times higher levels in lungs, liver and kidneys than in maternal blood. In PND1 pups, levels in lungs were significantly higher than in GD18 fetuses. A heterogeneous distribution of ^{35}S-PFOS was observed in brains of fetuses and pups, with levels higher than in maternal brain. This first demonstration of substantial localization of PFOS to both perinatal and adult lungs is consistent with evidence describing the lung as a target for the toxicity of PFOS at these ages.

1. Introduction

Perfluoroalkylated compounds (PFCs) constitute a large family of fluorinated organic chemicals that have been produced for more than five decades. These compounds are used as components of and precursors for surfactants and surface protectors in industrial applications and consumer products, e.g., impregnating agents for fabrics, coatings for paper and packaging, waxes and cleaning agents, insecticides, fire-fighting foams and hydraulic fluids in airplanes [1,2]. The degradation of many PFCs derived from perfluorooctanesulfonyl fluoride (POSF) can potentially give rise to perfluorooctane sulfonate (PFOS) as a stable end-product [1,3].

During the past decade, PFOS and its precursors have been recognized as highly persistent environmental contaminants, displaying widespread global distribution in wildlife, humans and the environment [4–10]. These findings, together with the bioaccumulative and toxic properties of PFOS, have led to regulatory restrictions on its use both in the European Union [11] and the United States [12], as well as the inclusion of this substance in the Stockholm Convention on Persistent Organic Pollutants [13]. The major manufacturer discontinued its production of PFOS and its derivatives in 2002 [14], but this compound is still being produced elsewhere [15].

Exposure of rodents to PFOS *in utero* causes a dose-dependent reduction in birth weight and a marked increase in neonatal mortality, as well as developmental abnormalities such as cleft palate, delayed ossification and cardiac defects (reviewed by Lau et al. [16]). The mortality has been proposed to be due to impaired pulmonary function on the basis of observed labored breathing and cyanosis, but the exact cause has not yet been established. Morphological indications of delayed or impaired lung maturation are present [17,18] and direct interaction of PFOS with components of the pulmonary surfactant has also been proposed as a possible underlying mechanism [19,20]. There is a critical window of exposure in this context, with administration as late as gestational day (GD) 19 being sufficient to induce this toxicity [17].

Pharmacokinetic studies in adult rats have revealed that PFOS is absorbed rapidly following oral exposure [21] and is distributed thereafter primarily to the liver, but also to the serum, kidneys,

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0890-6238/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2010.07.004

heart and brain [22,23]. Knowledge concerning distribution to other organs and at other ages is, however, limited. PFOS does not appear to undergo any type of metabolism [24] and is eliminated primarily via the urine and feces in male rats (30% and 13%, respectively, within 89 days after exposure), with a half-life of approximately 100 days whereas the half-life in humans is approximately 5 years [23,25]. In rats as well as in humans, PFOS is transferred to the developing fetus via the placenta and to the neonate via the mother's milk [26–28].

The blood levels of PFOS in human populations living in different geographic regions and belonging to different demographic groups are similar, commonly in the range of 5–50 ng/ml serum (equivalent to 2.5–25 ng/ml blood [29]), with children exhibiting levels comparable to those in adults [9,28,30–34]. Although recent biomonitoring studies indicate that the levels of PFOS in human blood are declining [35–37], they remain higher than those of other perfluorochemicals [34,38,39]. Furthermore, in light of its pronounced persistence and continued production (see above), human and environmental exposure to PFOS will continue for a substantial period of time.

At present, only limited information is available concerning the overall tissue distribution of PFOS both in laboratory animals and in humans. Hence, important information concerning distribution and possible target tissues in fetuses, newborns and adults are missing. Therefore, the present investigation was designed to in detail characterize the tissue distribution of ^{35}S-labelled PFOS in C57Bl/6 mouse fetuses, newborns and dams following late gestational exposure, employing whole-body autoradiography in combination with liquid scintillation counting.

2. Materials and methods

2.1. Chemicals

All the solvents and other chemicals used in the synthesis and analysis of radioactive PFOS were of pro-analytical grade. ^{35}S-Sulfuric acid was purchased from the Institute of Isotopes Co. Ltd. (Budapest, Hungary); ethyl magnesium chloride solution (2 M in diethyl ether), hydrogen peroxide (30% in water) and dimethyl sulfoxide (DMSO, D5879) from Sigma–Aldrich Chemie (Steinheim, Germany); perfluorooctyl iodide (98%) from TCI Europe (Zwijndrecht, Belgium); and methanol from VWR (batch no. L743302, Leicester, UK).

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, Bergman 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 dropwise addition of ethylmagnesium chloride solution and subsequently stirred for 1 h prior to slow addition of ^{35}S-sulfur dioxide. 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 1.13 mCi ^{35}S-PFOS (8% yield; 31.8 mCi/mmol) with a chemical purity of 97%, as determined by liquid chromatography–mass spectrometry (the major impurity being PFOA). The final product was dissolved in methanol, which was evaporated off prior to treatment of the dams, and the ^{35}S-PFOS was re-dissolved in deionized water:tap water (50:50) for exposure via oral gavage and in DMSO for exposure via intravenous injection.

2.3. Animal maintenance

Female C57Bl/6 mice (age 10–11 weeks; Scanbur B&K, Sollentuna, Sweden) were mated and, based on the presence of vaginal plug, considered pregnant at gestation day (GD) 1 in accordance with the breeder's routines. These dams were allowed to acclimatize for 1 week prior to exposure to ^{35}S-PFOS, during which time they were housed in polycarbonate cages with heat-treated pine-shavings for bedding, with access to a standard pellet diet (Labfor R36, Lantmännen, Sweden) and tap water *ad libitum*. The animal house environment was controlled with respect to temperature (20° C), relative humidity (50–55%) and the light/dark cycle (12 h/12 h). At the start of the experiment, the dams were transferred to and housed individually in polycarbonate cages with a filter top and placed in a ventilated cupboard with filtered air outlets. The pups were designated as being at postnatal day (PND) 1 on the day of parturition. This study was approved by the Northern Stockholm Ethical Committee for Animal Research (approval number N405/08).

2.4. Animal exposure and sacrifice

On GD16, six dams (34 ± 3 g) received a single dose of S^{35}-PFOS (0.8 μCi/g, 12.5 mg PFOS/kg), five of these via oral gavage and the sixth by intravenous injection to address possible differences in bioavailability between these routes of administration. Thereafter, the animals were monitored visually each day for possible signs of toxicity until the time of sacrifice by exposure to gaseous CO_2 on GD18 (two dams), GD20 (one dam) or PND1 (three dams) for whole-body autoradiography and liquid scintillation counting of tissues. Mothers and pups (two per mother), were housed together until sacrifice within hours after birth (PND1) and then analyzed in the same manner. Following sacrifice, the animals were prepared for whole-body autoradiography by placing them in aqueous carboxymethyl cellulose (CMC), which was then frozen in a bath of hexane cooled with dry ice.

2.5. Whole-body autoradiography

The frozen tissues were processed for whole-body autoradiography as described by Ullberg and Larsson [40]. In brief, series of sagittal whole-body sections (20- and 40-μm thick; Jung Cryomacrocut, Leica), taken at 8–15 different levels, were collected onto tape (Scotch 6890, 3M Ltd., St. Paul, MN, USA) and freeze-dried. These sections were then air-dried and opposed to X-ray film (Structurix, Agfa, Mortsel, Belgium), at −20 °C, with subsequent development employing D19 (Kodak, Rochester, NY, UK). The exposure time for the autoradiograms presented in the figures was 9 days. Certain of the whole-body sections were also stained with hematoxylin Certistain® (Merck KGaA, Darmstadt, Germany) and eosin Y (BDH Ltd., UK). Samples of the liver, lungs, kidneys, brain and blood from dams, pups and all the remaining fetuses were dissected out of the remains of the CMC blocks for liquid scintillation counting as described below.

2.6. Liquid scintillation counting

To quantify radioactivity by liquid scintillation counting, tissue samples from the dams, fetuses and pups utilized for whole-body autoradiography (see Section 2.4) were weighed (range 0.5–103 mg wet weight), processed using the SOLVABLE™ kit (PerkinElmer, USA) in accordance with the manufacturer's protocol, and analyzed in a Beckman LS 6000 TA scintillation counter. The SOLVABLE™ solubilization reagent was not found to quench or affect the counting efficiency of ^{35}S-PFOS as compared to ^{35}S-PFOS dissolved in water. The process from chemical synthesis to completion of liquid scintillation results took up to 91 days and measured dpm levels were at least 6 times higher than background level. The radioactivity measured was subsequently converted into μg ^{35}S-PFOS/g tissue (wet weight) using the original specific radioactivity of this compound and compensation for radioactive decay.

2.7. Statistical analysis

The data sets were found to be normally distributed (as determined by the omnibus normality test of Kolmogorov–Smirnov or D'Agostino and Pearson, depending on group size) and demonstrated equal variances (as determined by Bartlett's test). For statistical comparisons, one-way analysis of variance (ANOVA) was performed using GraphPad Prism v5.02 (GraphPad Software, Inc., USA), followed by Bonferroni's Multiple Comparison Test.

3. Results

3.1. Observations of the animals

The pups were born on GD20, i.e. approximately 96 h after exposure of the pregnant dams to ^{35}S-PFOS and thus the PND1 pups had been exposed for essentially the same period of time as the fetuses collected on GD20. The pups were born alive, but some pups in the first litter displayed cyanosis within hours after delivery, and subsequent litters were therefore sacrificed during the first hour after birth. Only non-cyanotic pups were used in the study.

3.2. General observations concerning ^{35}S-PFOS levels in the tissues of dams and fetuses/pups

All radioactivity was assumed to reflect unaltered ^{35}S-PFOS, as this compound has been reported to be metabolically inert [24].

The pattern of distribution and tissue levels of ^{35}S-PFOS were similar in all the dams, regardless of the time-point of sacrifice or route of exposure, and the concentrations in individual tissues all exhibited a Gaussian distribution (Figs. 1 and 2). Consequently, the values for all the dams have been pooled for comparisons to the corresponding values for fetuses and pups.

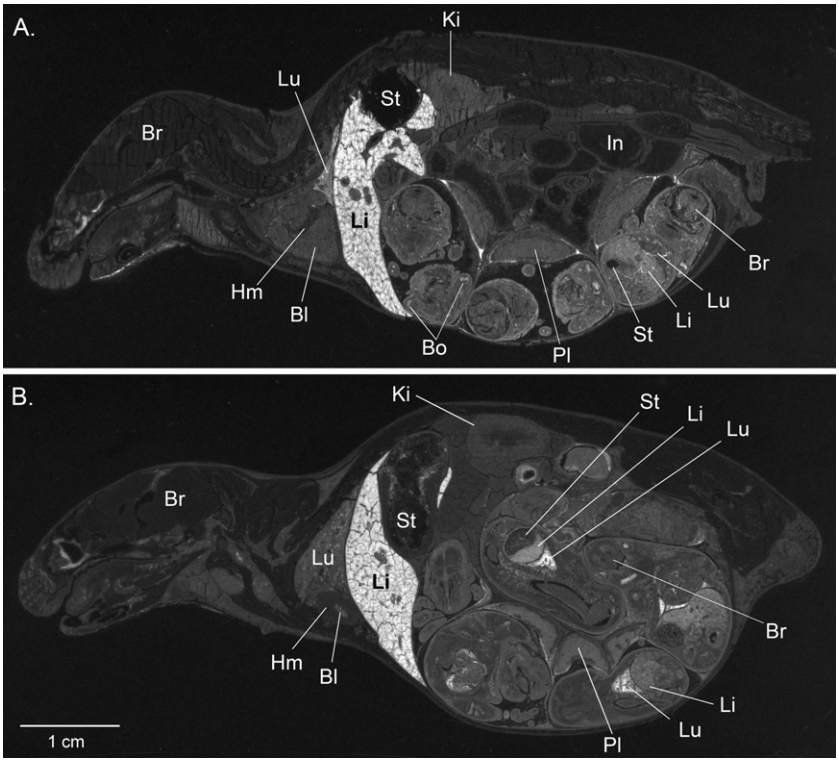

Fig. 1. Autoradiograms of 40-μm sections of pregnant mice on gestational days (GD) 18 and GD20, 48 h and 96 h, respectively, after administration of a single oral dose of ^{35}S-labelled PFOS (12.5 mg/kg) on GD16. These autoradiograms were treated identically with respect to exposure time and image processing. The brighter areas correspond to higher levels of radioactivity. ^{35}S-PFOS was readily transferred to the fetuses which on GD18 (A) and GD20 (B) generally demonstrated tissue levels similar to or higher than the level in the blood of the dams. In the dams, the liver and the lungs contained the highest levels of ^{35}S-PFOS, and in the fetuses the kidneys (not observable in this section) and the lungs contained the highest levels on GD18 and GD20, respectively. Bl = blood, Bo = bone, Br = brain, Hm = heart muscle, In = intestines, Ki = kidney, Li = liver, Lu = lung, Pl = placenta, St = stomach.

^{35}S-PFOS was readily transferred to the fetuses, which at all the time-points (GD18, GD20 and PND1) generally demonstrated tissue levels similar to or higher than the level in the blood of the dams (Figs. 1 and 2). The blood levels in the fetuses and pups were 1.1–2.3-fold that in the maternal blood (Table 1).

The distribution in the organs examined differed in certain respects between the dams and fetuses/pups. In the dams, the liver and lungs exhibited the highest concentrations of ^{35}S-PFOS at all the time-points (Fig. 2), whereas in the fetuses and pups, the kidneys and liver contained the highest concentrations on GD18 and the lungs and liver on GD20/PND1 (Fig. 2).

3.3. Distribution to the liver

In the dams, the hepatic level of ^{35}S-PFOS was approximately 4-fold higher than in the blood (Fig. 2(A)). The whole-body autoradiograms demonstrated a relatively homogenous distribution, although with a somewhat mottled pattern and a clearly lower level in blood vessels (Figs. 1(A,B) and 4(C)). Staining with hematoxylin/eosin did not reveal any apparent relationship between the distribution pattern and specific regions within hepatic lobuli. In the case of the fetuses and pups, the level of ^{35}S-PFOS in the liver was relatively constant and homogenously distributed at all the time-points examined (Figs. 2(A), 3(A–C), and 4(A)), being approximately 2.5-fold higher than in maternal blood and 1.7-fold lower than in the maternal liver (Table 1).

3.4. Distribution to the lungs

In the dams, the pulmonary levels of ^{35}S-PFOS were on average 2-fold higher than in the blood (Fig. 2(A)). In this case, whole-body autoradiography revealed a generally homogenous distribution, although with some regions showing higher levels of ^{35}S-PFOS (Figs. 1(A,B) and 4(C)).

In fetuses on GD18, the level of ^{35}S-PFOS in the lungs was similar to that in the maternal lungs (Fig. 2(B)) and approximately 2-fold higher than in maternal blood (Table 1). The whole-body autoradiography showed a relatively homogenous distribution within the lungs (Fig. 3(A)). On GD20, the level in the fetal lungs was slightly higher than at GD18, and lung and liver contained the highest levels of the fetal tissues examined (Fig. 2), approximately 2.5-fold higher than in maternal blood (Table 1). The distribution within the lungs was somewhat heterogeneous, with certain areas exhibiting higher levels.

In pups on PND1, the pulmonary levels of ^{35}S-PFOS increased further, being 3-fold higher than in maternal blood (Table 1), and lung and liver remained the highest among the tissues analyzed in pups (Fig. 2). Moreover, this level was significantly higher than in maternal lungs and in fetal lungs on GD18 (Fig. 2(B)). In this case, whole-body autoradiography revealed a more heterogeneous distribution (Figs. 3(C) and 4(A)), with certain areas exhibiting higher levels of radioactivity. Staining with hematoxylin/eosin demonstrated that the fetal lung tissue was intact and homogenous in appearance, ruling out an artifact of processing as the explanation for this heterogeneity in distribution (Fig. 4(A) and (B)). Only in some cases could areas with lower levels be identified as blood vessels.

3.5. Distribution to the kidneys

The average level of ^{35}S-PFOS in maternal kidneys was similar to that in maternal blood (Fig. 2(C)), and this radioactivity

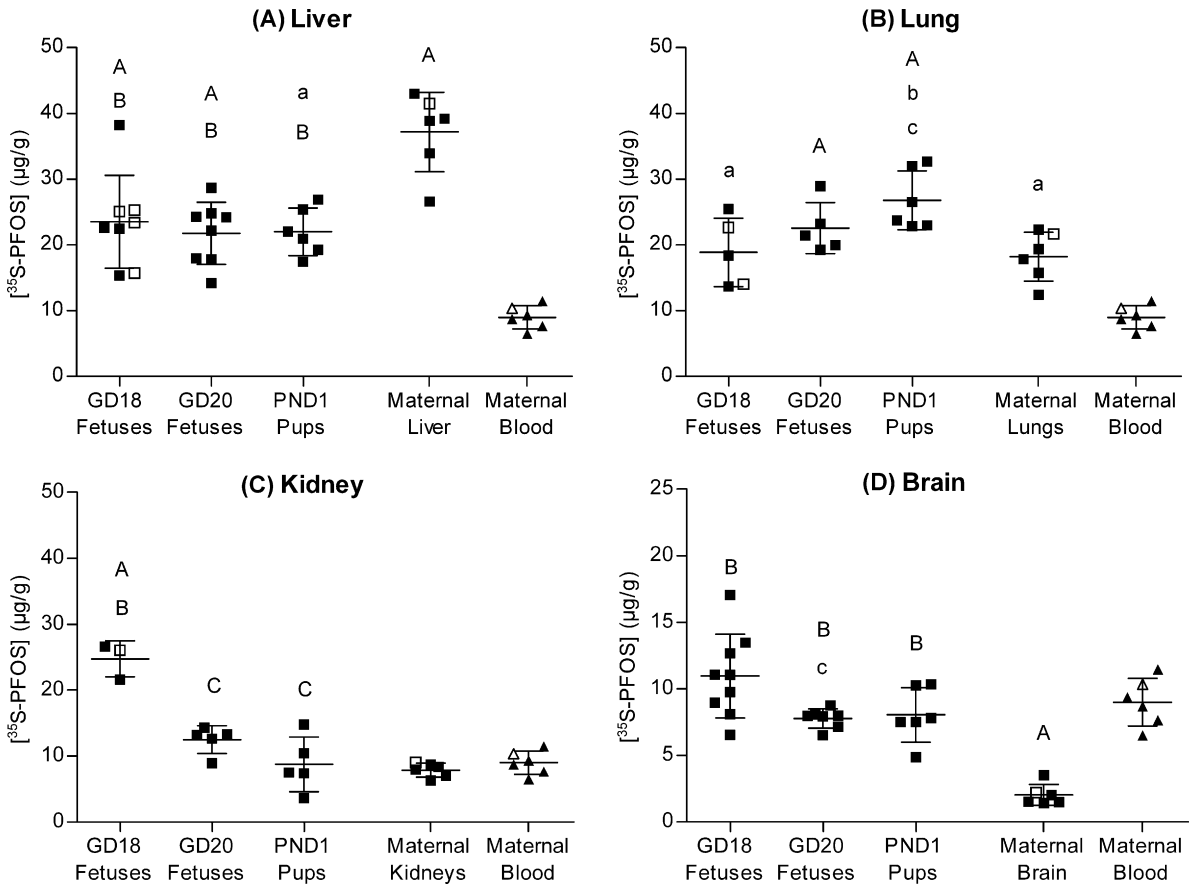

Fig. 2. Liquid scintillation counting of ^{35}S-labelled PFOS in organs. Livers (A), lungs (B), kidneys (C) and brains (D) of individual fetuses at GD18, GD20 or pups at PND1 are displayed in relationship to the level in maternal tissues and blood, following exposure of the pregnant dams to a single oral ($n = 5$) or intravenous ($n = 1$) dose (12.5 mg/kg) on GD16. Filled symbols represent individuals after oral exposure and open symbols after intravenous exposure, respectively. Data from GD18 and GD20 fetuses and PND1 pups originate from 2, 1 and 3 dams respectively. The longer horizontal bars depict arithmetic means and the vertical bars standard deviations. Since the dams demonstrated essentially identical levels in all the tissues and blood at all the time-points, all of their values have been combined in this analysis. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test. a = $P \leq 0.01$, A = $P \leq 0.001$ in comparison to the maternal blood; b = $P \leq 0.05$, B = $P \leq 0.001$ in comparison to maternal tissue; c = $P \leq 0.05$, C = $P \leq 0.001$ in comparison between fetuses/pups on GD20/PND1 with the corresponding value on GD18.

was present primarily in the renal cortex (Fig. 1(B)). In fetuses on GD18, the renal level of ^{35}S-PFOS was similar to that in fetal liver (Fig. 2) and approximately 3-fold higher than in the maternal blood and kidneys (Table 1). By GD20 and PND1, this level had decreased significantly, becoming similar to that in the maternal kidneys and blood (Fig. 2(C)). On PND1, the level of radioactivity appeared to be slightly higher in the cortex than in the medulla (Fig. 3(C)).

3.6. Distribution to the brain

In the adult female brain, ^{35}S-PFOS was distributed homogeneously at a level considerably lower than that in the blood (Figs. 1 and 2(D)). In fetuses and pups, the level in brain was similar at all the time-points (Fig. 2(D)) corresponding to the level in maternal blood (Table 1) but 3.8–5.4-fold higher than in the maternal brain (Fig. 2(D)). Regional differences in the distribution within the

Table 1
Ratios between the average concentrations of ^{35}S-labelled PFOS ([^{35}S-PFOS]) in the liver, lungs, kidneys, brain and blood of C57BL/6 mouse dams, fetuses and pups versus the average concentration in maternal blood following exposure of the dams to a single oral ($n = 5$) or intravenous ($n = 1$) dose (12.5 mg/kg) on gestational day (GD) 16. Values are presented as means ± standard deviation followed by the number of samples in brackets.

	$[^{35}S\text{-PFOS}]_{organ}/[^{35}S\text{-PFOS}]_{maternal\ blood}$				
	Liver	Lungs	Kidneys	Brain	Blood
Dams	4.2 ± 0.7*** (6)	2.0 ± 0.4** (6)	0.9 ± 0.1 (6)	0.2 ± 0.05*** (6)	1.0 ref (6)
Fetus on GD18[a]	2.6 ± 0.8*** (8)	2.1 ± 0.6** (5)	2.8 ± 0.3*** (3)	1.2 ± 0.3 (9)	2.3 (1)
Fetus on GD20[b]	2.4 ± 0.5*** (8)	2.5 ± 0.4*** (5)	1.4 ± 0.2 (5)	0.9 ± 0.1 (7)	1.1 ± 0.04 (3)
Pups on PND1[c]	2.4 ± 0.4** (6)	3.0 ± 0.5*** (6)	1.0 ± 0.5 (5)	0.9 ± 0.2 (6)	1.7 ± 0.3*** (5)

Since the dams demonstrated essentially identical levels in all the tissues and blood at all the time-points, all of their values have been combined in this analysis. Also, the levels following oral and intravenous administration were essentially identical, so that these values have also been combined. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test. *$P \leq 0.05$ in comparison to the maternal blood. **$P \leq 0.01$ in comparison to the maternal blood. ***$P \leq 0.001$ in comparison to the maternal blood.

[a] Data from 2 dams.
[b] Data from 1 dam.
[c] Data from 3 dams.

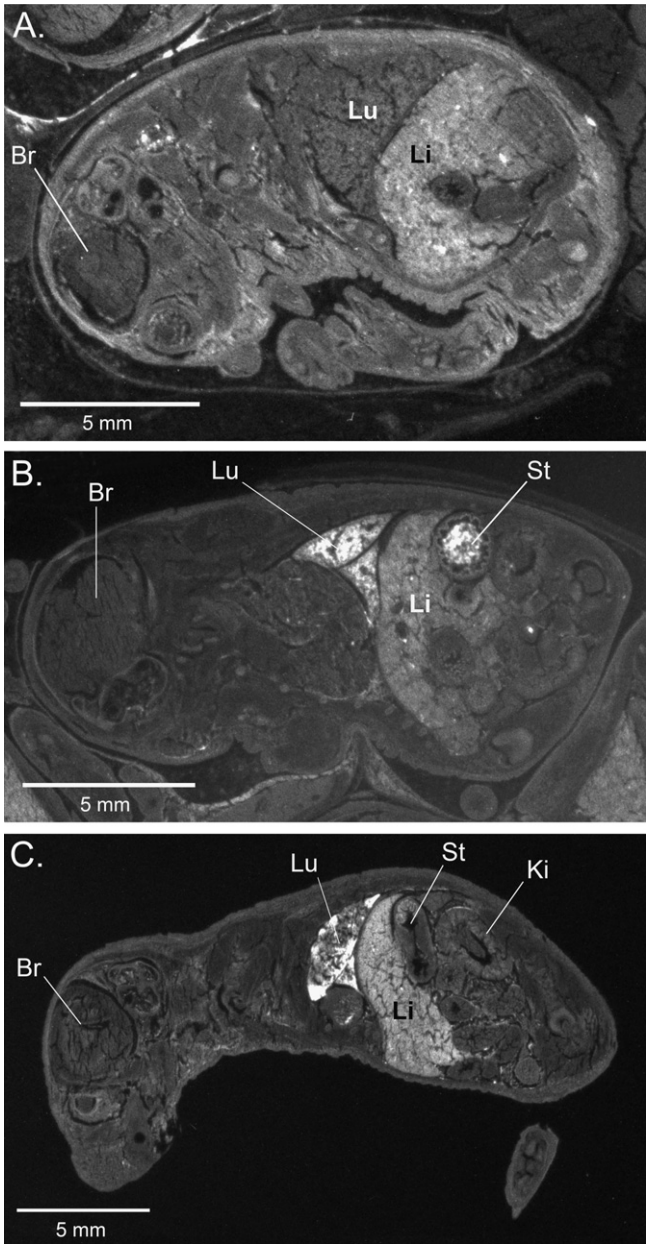

Fig. 3. Autoradiograms of 40-μm thick sections of mouse fetuses on GD18 and GD20 and of pups on PND1 following exposure of the pregnant dams to a single dose of ^{35}S-labelled PFOS (12.5 mg/kg) orally or intravenously on GD16. The fetus on GD18 (A) originate from a intravenously exposed dam and the fetus on GD20 (B) and pup on PND1 (C) from an orally exposed dam, respectively. These autoradiograms were treated identically with respect to exposure time and image processing. The brighter areas correspond to higher local levels of radioactivity. On GD18 (A), the level of ^{35}S-PFOS was lower in fetal lungs than in the liver and with a relatively homogenous distribution. On GD20 (B), local levels in the lungs were higher than in the liver and the lungs demonstrated a somewhat heterogeneous distribution. On PND1 (C), local levels in the lungs was further increased displaying a more heterogeneous distribution. Br = brain, Ki = kidney, Li = liver, Lu = lung, St = stomach.

brain were apparent, with a somewhat lower level in the perinatal cortex (Figs. 1(B), 3(C), and 4(D)).

3.7. Other organs and tissues

In the dams, ^{35}S-PFOS was also detected in the yolk sac epithelium and in the placenta (Fig. 1(A) and (B)). ^{35}S-PFOS was also present in the stomach and intestines, the harderian gland (not shown) and nasal tissues (though not in the olfactory turbinates) (Fig. 1(A) and (B)). In fetuses and pups, ^{35}S-PFOS was observed in developing bone on GD18, and somewhat less apparently, on GD20 and PND1, and in the glandular portion of the stomach, the stomach content, and in the intestines (Figs. 1(A and B), 3(A–C), and 4(A)).

4. Discussion

After administration of a single oral or intravenous dose to pregnant mouse dams on GD16, ^{35}S-PFOS was rapidly transferred to their fetuses, where the levels in certain tissues were higher than in maternal blood. This difference was most notable for the lungs, where the average level of ^{35}S-PFOS in the lungs rose from 2-fold higher than in maternal blood at GD18 to 3-fold higher on PND1. Indeed, the lungs contained higher levels of ^{35}S-PFOS than any of the other examined organs of fetuses and pups on PND1. The distribution within the lungs changed, from homogenous on GD18 to heterogeneous on PND1. Certain areas with lower levels could be identified as blood vessels, but the limited histological analysis possible on tape-sections could not reveal any other explanation for this heterogeneity.

Rodents exposed to PFOS *in utero* during late stages of gestation exhibit dose-dependent cyanosis and reduced survival after birth [26,41]. The marked elevation in neonatal mortality is observed within a remarkably small dose-interval [26,41,42] and occurs within minutes to days after birth, depending on the dose administered. Although the underlying mechanism(s) has not yet been established, impaired pulmonary function has been proposed to be the direct cause (reviewed by Lau et al. [43]).

Grasty et al. [17,18] found indications of altered lung maturation in newborn rats following exposure to PFOS *in utero* and proposed that this fluorochemical interferes with late developmental stages, involving cellular differentiation as also observed by Luebker et al. [26] in newborn rats. An alternative and/or complementary mechanism could be direct interaction of PFOS with components of the pulmonary surfactant [19,20,44], resulting in elevated surface tension and atelectasis, i.e. incomplete expansion of the lung [45]. Atelectasis has been observed in neonatal mice following exposure to PFOS *in utero* [46] and, moreover, PFOS has been shown to interact directly with and thereby disturb the function of components of the pulmonary surfactant *in vitro* [20]. Our findings that ^{35}S-PFOS is present in particularly high levels in perinatal lungs is consistent with the hypothesis that PFOS directly impairs pulmonary function, possibly by interacting with the pulmonary surfactant in the alveoli, although the exact localization of PFOS in the lungs remains to be determined.

Moreover, the 2-fold higher level of ^{35}S-PFOS in maternal lungs than in maternal blood observed here is also consistent with a recent observation that the lung is a target for PFOS in adult animals as well. Cui et al. [47] observed dose-related pulmonary congestion and thickened epithelial walls in the lungs of adult rats following 28 days of exposure to PFOS. The heterogeneous pulmonary distribution of PFOS observed in the present investigation, primarily in dams and pups and to some extent in fetuses on GD20, could be an indication of pulmonary congestion or atelectasis.

In fetal liver, the level of ^{35}S-PFOS observed here was approximately 2.5-fold higher than in maternal blood, although significantly lower (approximately 40%) than in maternal liver. These findings are similar to previous reports [41,42,48,49], when the serum/whole blood ratio for PFOS of 2:1 described by Ehresman et al. [29] is taken into account. PFOS has been reported to cause centrilobular to midzonal hepatocellular hypertrophy in adult rats [3]. Unfortunately, the limited histological analysis that can be performed on tape-sections could not reveal any morphological basis for the heterogeneous distribution of ^{35}S-PFOS in this organ.

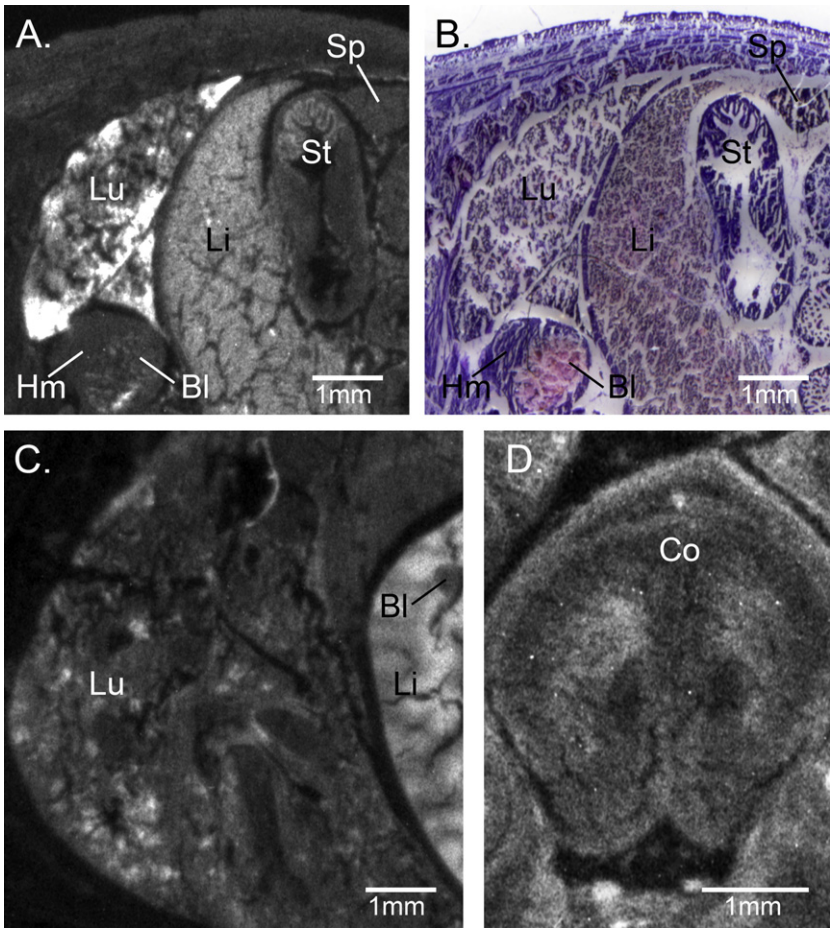

Fig. 4. Detailed images of autoradiograms. Pulmonary region of the mouse pup on PND 1 shown in Fig. 3(C) (A) with corresponding haematoxylin/eosin staining (B). Pulmonary region from a dam at PND1 (C) and, frontal section of the fetal brain on GD20 (D), both shown in Fig. 1(B). The pregnant dams were exposed to a single dose of ^{35}S-labelled PFOS (12.5 mg/kg) orally on GD16. These autoradiograms of 40-μm thick sections were treated identically with respect to exposure time and image processing. The brighter areas correspond to higher levels of radioactivity. A heterogeneous distribution of ^{35}S-PFOS was observed in the lungs (A) and the corresponding hematoxylin/eosin staining (B) revealed that the fetal lung tissue was intact and homogenous in appearance. In the dams, the distribution in the lungs of ^{35}S-PFOS was generally homogenous although some regions showed higher levels, and in the liver the distribution of ^{35}S-PFOS was somewhat mottled (C). A regional difference in the distribution within the fetal brain was apparent, with a lower level in the perinatal cortex (D). Bl = blood, Co = cerebral cortex, Hm = heart muscle, Li = liver, Lu = lung, Sp = spleen, St = stomach.

Our demonstration of ^{35}S-PFOS in the perinatal brain at a level of approximately 90% of that in maternal blood is similar to the corresponding ratios reported earlier (ranging from 1.0 to 1.4 in GD20 fetuses exposed to PFOS *in utero*) [48]. It should be noted, however, that the distribution within the perinatal brain observed here was not homogenous, with the cortex containing lower levels. Furthermore, the level of PFOS in the perinatal brain was significantly higher than in the maternal brain, in agreement with previous reports showing that rats exposed to PFOS *in utero* have higher levels in the brain than do their dams, both before [48] and after birth [48,50]. This difference is presumably due to the incomplete development of the fetal blood–brain barrier [48,50] and provides support to the conclusion that PFOS can affect the central nervous system and thereby cause behavioral defects in both mice [51] and rats [52].

Another observation made here that requires further investigation is the high level of PFOS in fetal kidneys on GD18, which declined significantly on GD20 and PND1 to the same level as in maternal blood. To our knowledge, this is the first report on the accumulation of PFOS in fetal kidneys. The average renal levels in the dams were similar to those in their blood, in agreement with a previous report on adult rats [23]. In the kidneys of both dams and fetuses, higher levels of ^{35}S-PFOS were detected in the cortex and outer medulla than in the inner medulla.

^{35}S-PFOS was also detected in the stomach and intestines of dams and their fetuses and pups. In the case of the dams, this may reflect to the oral route of exposure, but possibly also enterohepatic circulation, which has been reported previously [24]. The latter possibility is supported by our detection of radioactivity in the gastrointestinal tract of the dam exposed intravenously as well (not shown). ^{35}S-PFOS in the stomach content of fetuses could reflect the ingestion of amniotic fluid, in which PFOS was found in the present study as well as in other studies following exposure of pregnant dams [19,53]. The occurrence of ^{35}S-PFOS in the stomach of pups might originate from the milk of their dams.

^{35}S-PFOS was also present in the Harderian gland of the dams. More than 20% of the wet weight of this gland consists in rodents of lipids [54] and it also contains proteins, such as the fatty-acid binding protein, to which PFOS has shown affinity *in vitro* [55]. The observation that PFOS delays ossification and causes cleft palate in mice following exposure *in utero* [49,53] may be related to the present detection of PFOS in the bones of perinatal mice.

As also observed by others in rodents [17–19,26,41,42,46], cyanosis occurred here in some of the pups within hours after birth, which was somewhat surprising since only a single oral dose of 12.5 mg PFOS (acid form)/kg was administered on GD16. The cyanosis in newborn rodents has been proposed to be correlated to their body burden of PFOS [43]. Previously, approximately

50% neonatal mortality has been observed following gestational exposure of CD-1 or ICR mice to a cumulative dose of 170 or 180 mg PFOS/kg, respectively [41,46,49], whereas in the case of 129S1/Svlmj mice a cumulative dose of 18 or 34 mg PFOS/kg gives the same effect, indicating 5–10-fold higher sensitivity in the latter strain [19]. No studies on the developmental toxicity of PFOS in C57Bl/6 mice have yet been reported and the sensitivity of this strain may be similar to that of 129S1/Svlmj mice. An alternative and/or complementary explanation for the cyanosis observed here could be the higher solubility of the acid form of PFOS in aqueous solutions in comparison to the potassium salt (Sundström et al., manuscript in preparation) employed in previous toxicity studies, which might have resulted in more rapid uptake and, subsequently, a higher peak level in the blood in the present case. The blood level of ^{35}S-PFOS in dams in the current study was in the μg/ml range i.e. similar to many experimental studies and approximately a 1000-fold higher than blood levels in non-occupationally exposed human populations. A study on the effect of dose on tissue distribution in male mice demonstrated that the pattern of distribution of PFOS was similar with the two oral doses used (resulting either in blood levels similar to those in the present study or to those detected in human populations (Bogdanska et al., manuscript in preparation)). This finding supports that the tissue distribution observed here may also be similar at blood levels of humans relevance.

PFOS is transferred from mother to fetus also in humans, with the level in human cord serum at the time of delivery being approximately 2–3-fold lower than in maternal serum [27,56,57]. Here, blood levels of ^{35}S-PFOS in the fetuses and pups were 1.1–2.3-fold higher than in the dams, in line with previous findings [41,42,48,49].

Biomonitoring has revealed that serum levels of PFOS in children are similar to those in adults, despite the long half-life of approximately 4.5 years in humans. Different patterns of exposure have been proposed as an explanation [33], however, the present investigation indicates that children may even at birth carry a burden of PFOS with a different pattern of tissue distribution than in adults. Although measurements of blood or serum levels of PFOS are in general the only feasible approach for human biomonitoring, the findings documented here suggest that blood levels may not reflect the burden of PFOS in individual organs, and moreover, that the tissue-to-blood ratios may vary with age.

Little is presently known concerning the overall tissue distribution of PFOS in humans. Olsen et al. demonstrated that PFOS levels in liver samples from human donors were on average 2.6-fold higher than the corresponding blood levels [58], which is comparable to the 2.7-fold higher level shown by Maestri et al. in pooled liver samples obtained in connection with human post-mortem examinations [59]. These results are relatively similar to the results of the present and other studies on rodents [41,42,48,49], in which hepatic levels were approximately 4-fold higher than those in blood. To our knowledge, the only current information about levels of PFOS in human lungs was reported by Maestri et al., who found a 1.5-fold higher level in pooled human lung samples than in the corresponding blood samples [59].

Exposure of experimental animals to certain chemicals at specific stages of development can cause damage to the lung, leading to malformations or improper function that may persist into adult life [60–62]. Examples of such chemicals include the organophosphorus compound O,O,S-trimethyl phosphorothioate [61] and the herbicide nitrofen, to which the effects of PFOS have been compared [41]. Furthermore, experimental and epidemiological evidence concerning humans has revealed that the susceptibility of the lung to damage by environmental toxicants is particularly high during the early postnatal period of development [63]. There is a well documented association between exposure to environmental pollutants and, e.g., reduced pulmonary function in infants, children and adults [64], and it cannot be excluded that an accumulation of PFOS in human lungs could represent an additional contributing risk factor. Furthermore, our observation that ^{35}S-PFOS is present in the perinatal as well as the adult mouse lung indicates the possibility of long-term local pulmonary exposure.

In conclusion, this investigation demonstrates that PFOS is readily transferred to the mouse fetus following oral or intravenous exposure of the pregnant dam, resulting in fetal tissue levels similar to or higher than in maternal blood. Distribution of PFOS to the lungs was substantial and highest among the tissues analyzed on GD20 and PND1, and may explain, at least in part, the respiratory distress seen at birth. The finding that PFOS also distributes to the adult lung is consistent with evidence that the lung may be a target for the toxicity of PFOS at all ages.

Conflict of interest statement

None of the authors had any conflicts of interest in connection with the design and performance of these experiments or preparation and submission of this manuscript.

Acknowledgements

This work was supported financially by the Swedish Council for Environment, Agricultural Sciences and Spatial Planning – FORMAS (to UB, and KH), by the Magnus Bergvall Foundation (to UB), by unrestricted research grants from the 3M Company (St. Paul, MN, USA; to ÅB and JdP), and by scholarships from the Swedish Institute (JB) and Sture Eriksson's Fund at Stockholm University (SN). We are grateful to Margareta Mattsson (Department of Environmental Toxicology, Uppsala University) and Associate Professor Manuchehr Adebi-Valugerdi (Department of Biochemistry and Biophysics, Stockholm University, Sweden) for excellent technical assistance. We would also like to thank Professor Nils-Gunnar Lindqvist (Swedish Chemicals Agency, Stockholm, Sweden) and Buck Nelson (Department of Biochemistry and Biophysics, Stockholm University, Sweden) for valuable comments during the preparation of this manuscript.

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