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Many per- and polyfluoroalkyl substances (PFAS) are a health risk because of their persistent, bioaccumulative and toxic properties. Determining how PFAS distribute throughout the body will improve our understanding of their impact on organ systems. In this study we used ^{19}F solid-state magic angle spinning nuclear magnetic resonance to detect and quantify PFAS in tissue samples from experimental mice that consumed perfluorooctanoic acid (PFOA). PFOA was detected in the liver and adipose tissue, challenging the established assumption that PFAS do not accumulate in lipids. PFOA was also detected in the placenta, with a greater detection rate in male compared to female placentas. This implies a sex-specific vulnerability to PFAS exposure and motivates future work to evaluate bioaccumulation of emerging PFAS.

Per- and polyfluoroalkyl compounds (PFAS) are a class of synthetic chemicals that have attracted attention due to their large-scale use in consumer products (*e.g.*, water-resistant clothing, cookware, cosmetics), their environmental persistence driven by the strength of the carbon–fluorine bond, and their negative health impacts. Restrictions imposed on the use of legacy PFAS, a term coined to refer to compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic

acid (PFOS), has resulted in a decline in their prevalence in wildlife and humans.¹ However, these legacy PFAS (sometimes referred to as “forever chemicals”) remain in the environment and continue to pose a global health concern.^{2–4} Exposure to legacy PFAS has been linked to thyroid disease, kidney and liver cancers, and immunotoxicity, as well as reproductive and developmental health complications.^{5–8}

Pregnancy and early life development are particularly vulnerable stages of life where exposure to environmental pollutants can have a long-lasting impact. The placenta plays a crucial role in fetal growth and development, thus the detection of PFAS in the human placenta and umbilical cord raises important questions about the effects of PFAS on pregnancy.^{9,10} PFOA and PFOS have been associated with pregnancy complications in humans including hypertension,¹¹ preterm birth,¹²

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and low birth weight infants.¹³ One way to establish causality between PFAS exposure and adverse health impacts is using experimental mice. From studies in mouse models, legacy PFAS exposure has been linked to wide-ranging effects including delayed fetal growth, neurological impairments, and increased risk for chronic diseases in adulthood.¹⁴ Sex differences arising from prenatal PFAS exposure have also been described in literature for both human and murine studies. For example, in humans, PFOS exposure has been linked to decreased weights in male infants in comparison to increased weights in females.¹⁵ In mice, prenatal exposure to PFOA resulted in a more significant decrease in fetal/birth weights in males compared to females¹⁶ and in accelerated sexual maturation in male offspring but not in females.¹⁷

The main routes of human exposure to PFAS are through food, drinking water, dust, and air.¹⁸ PFAS are able to enter the bloodstream and travel through the body by binding to serum albumin.^{19,20} Previous studies have used mass spectrometry to investigate PFAS accumulation in the human brain, liver, kidney, spleen, lung, pancreas, thyroid, gonads, bone, skeletal muscle as well as breast milk.^{21–25} ¹⁹F solution-state nuclear magnetic resonance (NMR) is also an effective technique for identifying PFAS, finding application in the study of environmental and wastewater samples as well as consumer products and biological liquid samples such as blood and urine.^{26–28} Mass spectrometry and ¹⁹F NMR can provide complementary information, illustrated by the comprehensive evaluation of PFAS contamination in surface water surrounding the Toronto Pearson International Airport following a spill of fluorinated fire retardant foam.²⁹ Solid-state magic angle spinning (MAS) NMR is a useful tool in studying biopsied tissue samples;^{30,31} however, to the best of our knowledge, its use as an analytical technique for studying the accumulation of PFAS in tissue samples has not been explored. Given the high sensitivity of the ¹⁹F nucleus (the ¹⁹F nucleus is 100% abundant and ~83% as NMR-sensitive as ¹H), the absence of endogenous fluorine in biological systems, and limited requirements for sample preparation (reducing sources of potential contamination), we used ¹⁹F solid-state MAS NMR to study the bioaccumulation of PFOA in tissue samples from throughout the body in PFOA-exposed pregnant and non-pregnant mice. In addition, we investigated bioaccumulation in the placenta and fetal brain during late gestation and determined whether the bioaccumulation depended on fetal sex.

Experimental

Chemicals

Perfluorooctanoic acid (PFOA) ammonium salt (≥98%, CAS number 3825-26-1) was purchased from Sigma-Aldrich (Darmstadt, Germany). Drinking water solutions were prepared at a concentration of 50 ppm (50 mg of PFOA salt dissolved in 1 L of filtered water (three-stage filtration system (5, 1 and 0.2 μm))). This concentration of PFOA was chosen to ensure the resonances are visible in the tissue samples and does not represent an environmentally relevant concentration of typical human exposure *via* drinking water.

Animal exposure and tissue collection

Nine healthy female adult CD-1 mice were individually housed in standard conditions following a 12 hour light/dark cycle and provided with *ad libitum* access to food and water. Control data (no exposure to PFOA, *n* = 4 dams) from a previously published study from our group was used for comparison of maternal, fetal and placental weights.³² Four mice were bred in-house and the detection of a vaginal plug the morning after mating was designated embryonic day 0.5 (E0.5). For comparison to tissue during pregnancy, a cohort of five non-pregnant mice were included. All mice were given 50 ppm PFOA drinking water for a period of 17 days (for E0.5 to E17.5 for pregnant mice), at which time they were euthanized by decapitation following an intraperitoneal injection of ketamine/xylazine (150 and 10 mg kg⁻¹, respectively). Adult brain, intestine, kidney, liver, uterus, and adipose tissues, as well as placentas and fetal brains from pregnant mice, were excised and flash-frozen in liquid nitrogen and stored at -80 °C for quantification of PFOA concentration. Fetal skin samples were collected for genotyping and stored at -18 °C. All animal experiments were conducted with the approval of the Institutional Care Committee at Memorial University of Newfoundland (23-03-LC) and in accordance with the Canadian Council of Animal Care's guidelines. Liver, kidney, and adipose samples were analyzed for all nine mice and two randomly selected samples of adult brain, intestine, liver, and uterus were analyzed. Three placentas per sex per dam (*n* = 24) and 8 fetal brains per sex (*n* = 16) were analyzed.

Special considerations to avoid sample contamination

Several adjustments needed to be made to the sample collection and preparation steps to avoid contamination in the ¹⁹F NMR spectra. First, the use of isoflurane as an anesthetic was avoided because the presence of isoflurane in the tissue can be detected using ¹⁹F NMR.³³ Additionally, use of compressed gas to dry the NMR rotors was found to produce a detectable ¹⁹F signal in the range -110 to -120 ppm (if the rotor is immediately closed after using the gas), likely from difluoroethane.

Sry genotyping

In order to investigate sex differences in the PFOA accumulation in placentas and fetal brains, fetal skin samples were used to determine biological sex *via* Sry polymerase chain reaction (PCR). The Sry primers used were a forward primer (CTCATCGGAGGGCTAAAGTG) and a reverse primer (AAGCTTTGCTGGTTTTTGA), having a product size of 166 bp. Cyp24a1 primers were used as an extraction quality control with a forward primer (CCAAGTGTGCCATTCAAC) and a reverse primer (TCTCTCGCTGAACCTGGATT), having a product size of 557 bp.

Sample preparation

All tools (razor blade, tweezers, plastic Petri dish, NMR packing tools) were washed with methyl alcohol and dried using Kimwipes. Frozen tissue samples were thawed and a small piece (~0.5 cm³) was sectioned for analysis. Samples were packed into



a pre-weighed 3.2 mm zirconium NMR rotor and a sample's mass was determined through weighing by difference. The NMR rotor and packing tools were all cleaned in the same manner in between each sample.

¹⁹F solid-state nuclear magnetic resonance spectroscopy

¹H decoupled ¹⁹F solid-state MAS NMR experiments were performed on intact, unprocessed tissue samples using a Bruker NEO 600 MHz spectrometer (¹⁹F Larmor frequency of 564.80 MHz) and a solid-state NMR probe (3.2 mm triple-tuned MAS H-F-C). The experimental parameters were optimized to minimize the baseline distortion and by measuring the *T*₁ relaxation of a PFOA solution (¹⁹F *T*₁ values ranged from 0.3–1.0 seconds). The spectra were collected using a single pulse experiment (zg pulse sequence) with a 90° pulse length of 4 μs and a recycle delay of 2 s. Spectra were acquired with a spinning speed of 4 kHz at 298 K with 3072 scans (time = 1.75 hours). Clean, empty rotors were run repeatedly throughout the study to ensure there was no ¹⁹F contamination present within the rotor or rotor cap.

Spectral analysis and quantification of concentration

Spectral data was processed with MestReNova (version 15.1.0, Mestrelab, Research S.L., Norwich, CT). The spectra were referenced to Teflon tape (polytetrafluoroethylene) at −126 ppm. The data was processed with an exponential line

broadening of 100 Hz. Automatic phase correction and splines function for baseline correction were used on the spectra, with manual phasing being applied when necessary. A 5-point calibration curve was obtained using standard PFOA solutions with a concentration from 10 ppm to 50 ppm in order to quantify the concentration of PFOA within the tissue samples. The PFOA solutions were placed inside of a 3.2 mm solid-state NMR rotor and the spectra were acquired with the solid-state MAS NMR probe used for the tissue sample experiments (Fig. 1). The integration of each peak was manually obtained, and their absolute values were summed to obtain the entire peak area for each solution. The summed integrals were then plotted as a function of the mass of PFOA (SI Fig. 1) (*R*² = 0.9914). The limit of detection (LOD) was determined to be the lowest concentration for the alkyl CF₃ resonance with a signal-to-noise ratio of 3 (LOD = 10 μg g^{−1}). Several tissue samples showed a clear ¹⁹F signal above the baseline; however, they could not be accurately quantified (signal-to-noise < 3) and were not included in the reported detection frequency. If the PFOA concentration was found to be below the LOD, the concentration was designed as the LOD divided by √2 of the LOD.³⁴

Statistical analysis

Statistical analysis was conducted using R programming software (<https://www.r-project.org>). Data are presented as mean and 95% confidence intervals. Maternal weights were

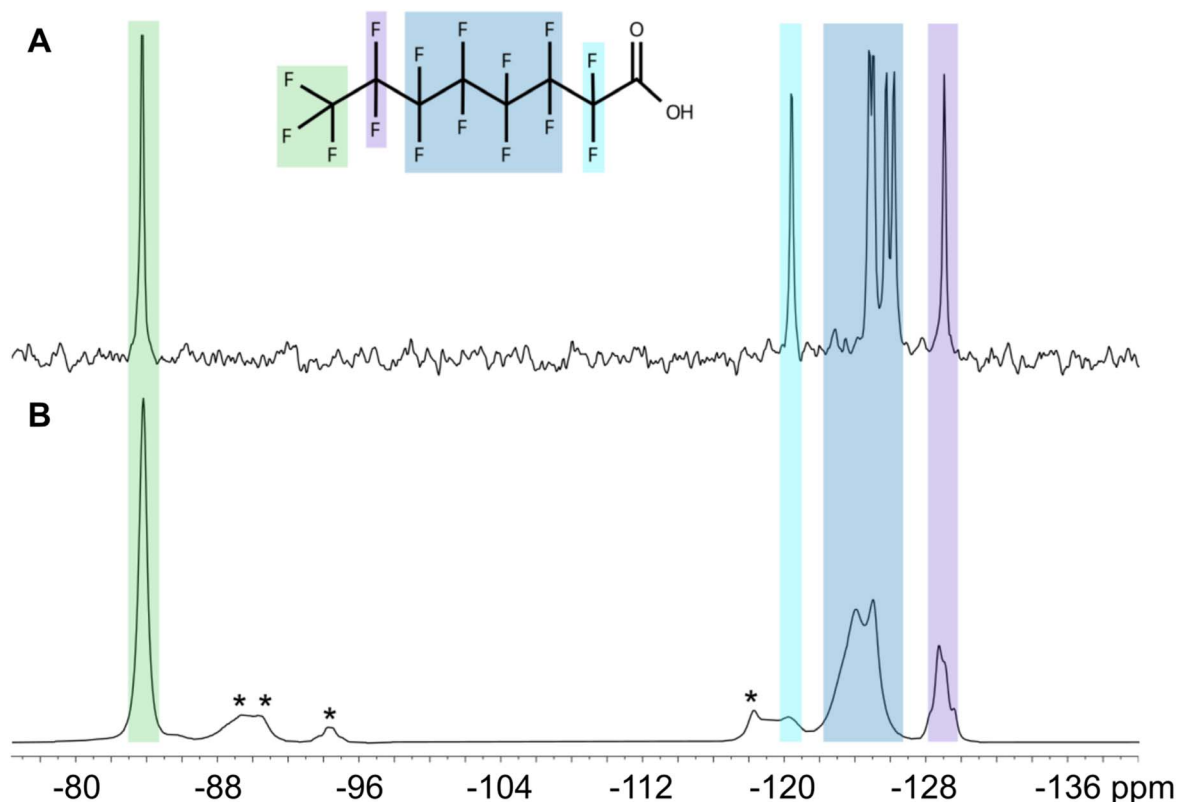


Fig. 1 Representative ¹⁹F spectra of (A) a 50 ppm solution of PFOA (MAS = 4 kHz) and (B) solid PFOA (MAS = 20 kHz). Both spectra were acquired using a 3.2 mm solid-state MAS NMR probe. Spinning sidebands are marked with asterisk. The resonances are colour-coded to match the chemical structure of PFOA (inset).



compared using a one-way ANOVA. Fetal and placental weights were compared using a linear mixed effects model with group (control, PFOA-exposed) as the fixed effect and litter as the random effect. The PFOA concentrations between tissue types and between female and male placentas were compared using a one-way ANOVA. For the comparison of detection frequency between female and male placentas, a Fisher's exact test was used. Statistical significance was defined as $p < 0.05$ and statistical comparisons trending towards significant ($0.05 < p < 0.10$) are reported because of the small sample size and the potential clinical relevance.³⁵

Results and discussion

There were no visible signs of abnormal behaviour (e.g., grooming habits, activity levels, posture) in the PFOA-exposed mice and there were no differences in how much water the mice consumed per day (9 ± 2 mL d⁻¹). The maternal weights were significantly lower in the PFOA-exposed group compared to controls ($p = 0.046$); however, there were no differences in the size of the litters ($p = 0.6$). While there were no differences in fetal weights between PFOA-exposed and control groups ($p = 0.6$), the placentas from the PFOA-exposed group weighed 46% more than the controls ($p = 0.001$) (Fig. 2). This is consistent with previous work from our group and others on the impact of maternal PFAS exposure on pregnancy,^{32,36–38} suggesting an inefficient placenta that needs to increase in size to support the normal fetal growth trajectory.

To determine the bioaccumulation of PFOA throughout the body, ¹⁹F solid-state MAS NMR spectroscopy was conducted on biopsied tissue samples from pregnant and non-pregnant mice. Fig. 3 shows representative examples of ¹⁹F spectra for each of the tissue samples where PFOA was detected, as well as a fetal brain sample to illustrate a sample below the LOD. As summarized in Table 1, PFOA was detected above the LOD in a total of 11 of the 32 adult tissue samples. The liver had the highest detection frequency, with PFOA detected in all of the samples taken from pregnant and non-pregnant mice (9/9

samples). PFOA was detected in two of the five adipose tissue samples from non-pregnant mice and was not detected in adipose tissue from pregnant mice. The PFOA concentrations were highest in the liver (90 ± 10 µg g⁻¹) and were significantly lower in the adipose tissue (9 ± 3 µg g⁻¹) ($p < 0.0001$). One of the nine kidney samples showed a visible ¹⁹F signal that was below the LOD. Tissue from the adult brain, intestine, and uterus did not show any evidence of PFOA by ¹⁹F MAS NMR spectroscopy.

Detection of PFOA at high concentrations in the liver is consistent with biodistribution studies in animals^{39–41} and humans.^{21,23} The PFOA concentrations reported in healthy adult humans are lower (e.g. 3.24×10^{-4} µg g⁻¹ in the liver²¹), consistent with the high exposure used in our study. PFAS are known to interact and bind to liver fatty acid binding proteins, highly expressed in hepatocytes.⁴² Studies assessing the impact of PFOA exposure on liver metabolism in mice report metabolic signatures of oxidative stress,⁴³ as well as alterations to amino acid and lipid metabolism in the liver.⁴⁴ PFAS exposure has also been associated with liver disease such as non-alcoholic fatty acid liver disease.⁴⁵ In addition to the associations between PFAS and the liver, several human studies have reported bioaccumulation in the kidney and associations with kidney disease and kidney cancer.^{21,46,47} PFOA was detected in the kidney of adult rats following 28 days of exposure by daily gavage (average concentration = 220 µg g⁻¹).⁴¹ While PFOA was also detected in the kidney of adult mice following 1 to 5 days of dietary exposure in Bogdanska *et al.*, the methodology (administration of ¹⁴C-PFOA and liquid scintillation counting) has much higher sensitivity than NMR.⁴⁰ The primary route of excretion of PFAS from the body is through urine⁴⁸ and PFOA has been shown to have high renal resorption.⁴⁹ However, we did not detect quantifiable evidence of PFOA in the mouse kidney in this study. This result was unexpected and the reason for PFOA not accumulating in the kidney is not clear. Another unexpected result was the detection of PFOA in the adipose tissue. With their hydrophilic acid head group, PFAS compounds are rarely thought to concentrate in lipids^{21,24,25,50} and few studies include the analysis of adipose tissue. Consistent with our findings, Bogdanska *et al.* reported PFOA accumulation in the epididymal fat tissues of PFOA-exposed mice at concentrations similar to those detected in our study (10 µg g⁻¹).⁴⁰ Accumulation of environmental pollutants in adipose tissue (e.g., persistent organic pollutants such as polychlorinated biphenyls) is associated with obesity and related metabolic diseases.⁵¹ A recent review described a potential mechanism by which PFAS can dysregulate adipose tissue function, through targeting of peroxisome proliferator-activated receptors (a group of nuclear receptor proteins in adipose tissue).⁵² In support of the link between PFAS and adipose dysfunction, human exposure studies have reported associations between PFAS and increased risk of obesity in both adults and children.^{53–58} The detection of PFOA in adipose tissue challenges the accepted theory that PFAS do not accumulate in adipose tissue and should be the subject of future investigations.

We did not detect PFOA in the two adult brain tissue samples studied by ¹⁹F NMR spectroscopy. This is consistent with

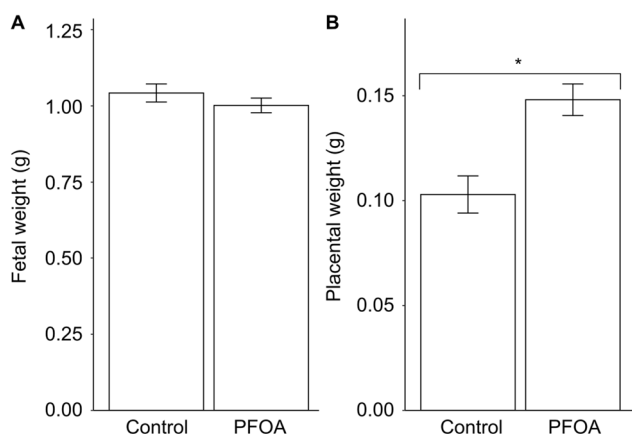


Fig. 2 Comparison of fetal weight (A) and placental weight (B) between control and PFOA-exposed pregnant mice. Data shown as means $\pm$ 95% confidence intervals. $n = 4$ dams per group. * $p = 0.001$.



In the pregnant mice, PFOA was detected in the placentas of 3 of the 4 PFOA-exposed dams. ^{19}F signal was visible in 11 of the 24 placentas (8 male and 3 female) and was above LOD for 6 placentas (4 male and 2 female). The average PFOA concentration of the placentas was $30 \pm 40 \mu\text{g g}^{-1}$ for the male fetuses and $8 \pm 2 \mu\text{g g}^{-1}$ the female fetuses. While the concentration of

PFOA was not statistically different between males and females ($p = 0.3$), the detection frequency showed a trend towards significantly higher detection of PFOA in the male compared to female placentas ($p = 0.099$). The detection frequency (including below LOD) had a high odds ratio of 5.5 (95% confidence interval 0.96–39.0), suggesting a strong association between fetal sex and PFOA accumulation. This is consistent with a study of PFAS in human placentas that reported PFOA in the placenta and in fetal liver, lung, heart, brain, and adipose tissue.⁶⁵ As with our results, the accumulation of PFAS in human male placentas was significantly higher than in the female placentas. There is also a sexual dimorphism in adult PFAS concentrations, with adult males having higher serum concentrations than females,⁶⁶ and in elimination of PFAS from the body, with PFOA reported to have a shorter half-life and faster clearance in female mice compared to males.⁵ The differences in bioaccumulation between male and female placentas may be related to the known differences in placental cellular and vascular composition.^{67,68} The increased accumulation of PFAS and known sex differences in placental blood flow^{69,70} and placental metabolism⁷¹ may partially explain the sex-specific vulnerability to PFAS.^{15–17} PFAS are known to be transported from the mother, across the placenta to the fetus^{72,73} and therefore we investigated the concentration of PFOA in fetal brain tissue. While below the LOD, PFOA was

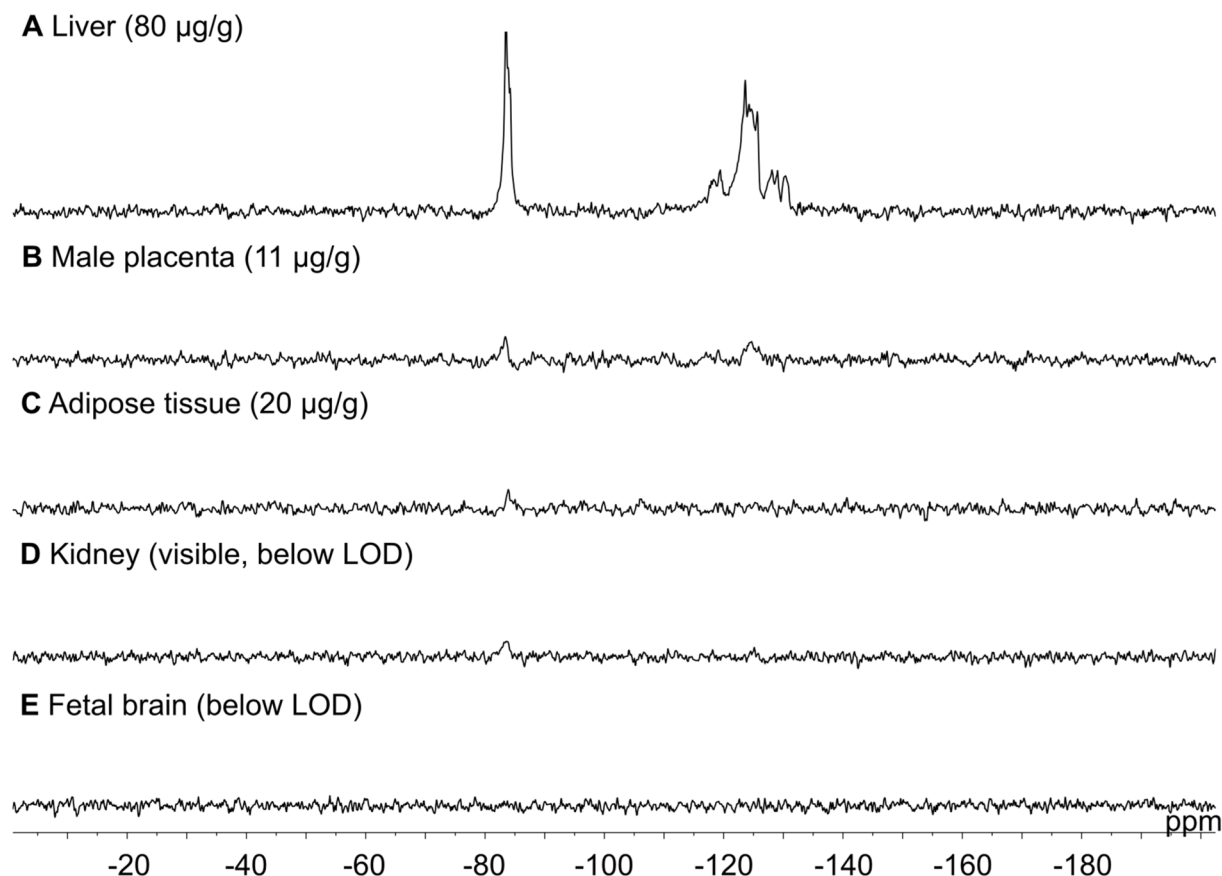


Fig. 3 Representative ^{19}F spectra of (A) liver, (B) placenta (male), (C) adipose tissue, (D) kidney, and (E) fetal brain tissue samples from mice exposed to PFOA for 17 days. Tissue concentration determined using calibration curve and tissue sample weight.



Table 1 Summary of quantification and detection frequency of all tissue samples^a

Tissue type	Average [PFOA] ($\mu\text{g g}^{-1}$)	Detection frequency above LOD (%)	Detection frequency including below LOD (%)
Adult brain ($n = 2$)	Below LOD	0 (0/2)	0 (0/2)
Intestine ($n = 2$)	Below LOD	0 (0/2)	0 (0/2)
Kidney ($n = 9$)	Below LOD	0 (0/9)	11.1 (1/9)
Liver ($n = 9$)	90 ± 10^b	100 (9/9)	100 (9/9)
Uterus ($n = 2$)	Below LOD	0 (0/2)	0 (0/2)
Adipose tissue ($n = 8$) ^c	9 ± 3	25 (2/8)	25 (2/8)
Placenta ($n = 24$)	Male: 30 ± 40	Male: 33.3 (4/12)	Male: 66.7 (8/12)
	Female: 8 ± 2	Female: 16.7 (2/12)	Female: 25 (3/12)
Fetal brain ($n = 16$)	Male: Below LOD	Male: 0 (0/8)	Male: 11.1 (1/8)
	Female: Below LOD	Female: 0 (0/8)	Female: 11.1 (1/8)

^a Concentrations are shown as means $\pm$ 95% confidence intervals. ^b Excluded one sample from quantification due to significant line broadening.

^c Excluded one sample due to contamination during sample preparation.

visible in one female and one male fetal brain. Despite the lack of quantifiable evidence of PFOA in the fetal brain, the sex-specific impact on the placenta is likely to result in abnormal fetal neurodevelopment that extends into postnatal life. For example, the Maternal-Infant Research on Environmental Chemicals (MIREC) study showed that higher prenatal PFAS exposure was associated with lower performance IQ in preschool males.⁷⁴

The use of ¹⁹F NMR has been shown to be effective for analyzing PFAS in various media, including solid biological tissue samples. Unlike static (non-spinning) NMR, solid-state MAS NMR results in decreased line broadening and improved resolution, allowing for structural determination and accurate quantification of PFAS concentrations. Compared to techniques such as gas-chromatography or liquid-chromatography mass spectrometry, NMR does not face the same complications of reduced quantification of analytes as a result of matrix effects or a time-consuming sample preparation method.^{26,75,76} One drawback of NMR is the inherent insensitivity, resulting in higher LODs and requiring long scan times. Recent advances in NMR hardware and software has led to improvements in ¹⁹F NMR for quantification of PFAS in biological and environmental samples, detecting higher concentrations in total and class-specific PFAS in comparison to mass spectrometry.^{26,28} For future applications, solid-state MAS NMR could be used as a screening tool to identify affected tissues, followed by more sensitive mass spectrometry methods to detect novel PFAS or the biotransformation products formed from the analyte.

There are several limitations to this study. The study population was only female mice and we only used a small portion of each tissue type. There are known sex differences in PFAS concentrations in tissue (e.g., PFOA levels in male livers are higher than in females for humans²³ and rats³⁹) and therefore future studies will include both male and female subjects. While we tried to collect tissue from the same part of the organ, there may be variability and repeated measures from each organ would determine tissue heterogeneity in PFOA accumulation. Human studies have reported high accumulation of PFAS in lung tissue^{21,24,25} and future studies should include lung samples in the study design. Finally, our exposure period was

only 17 days, which does not accurately represent human lifetime exposure.

Conclusions

In summary, ¹⁹F solid-state MAS NMR spectroscopy was a useful tool to investigate bioaccumulation of PFOA in mouse tissue samples. PFOA was detected in all of the liver tissue samples, consistent with the well-known toxic effects of PFAS in the liver. The observed detection of PFOA in adipose tissue suggests PFOA accumulation is more pervasive in the body than previously believed. PFOA was more frequently detected in male placentas compared to female placentas, consistent with male fetuses being at increased risk of complications following exposure to environmental pollutants. These findings support the use of ¹⁹F solid-state MAS NMR to study bioaccumulation of emerging PFAS, particularly those shown to be persistent in the environment and toxic (e.g., fluorotelomer ethoxylates^{32,77}).

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the SI. See DOI: <https://doi.org/10.1039/d5va00220f>.

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References

- 1 M. Land, C. A. de Wit, A. Bignert, I. T. Cousins, D. Herzke, J. H. Johansson and J. W. Martin, What is the effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl acids and their



- precursors in the environment? A systematic review, *Environ. Evid.*, 2018, **7**, 4.
- 2 M. Houde, A. O. De Silva, D. C. G. Muir and R. J. Letcher, Monitoring of perfluorinated compounds in aquatic biota: an updated review, *Environ. Sci. Technol.*, 2011, **45**, 7962.
 - 3 B. Khan, R. M. Burgess and M. G. Cantwell, Occurrence and bioaccumulation patterns of per- and polyfluoroalkyl substances (PFAS) in the marine environment, *ACS ES&T Water*, 2023, **3**, 1243.
 - 4 X. Wang, N. Yu, Y. Qian, W. Shi, X. Zhang, J. Geng, H. Yu and S. Wei, Non-target and suspect screening of per- and polyfluoroalkyl substances in Chinese municipal wastewater treatment plants, *Water Res.*, 2020, **15**, 115989.
 - 5 S. E. Fenton, A. Ducatman, A. Boobis, J. C. DeWitt, C. Lau, C. Ng, J. S. Smith and S. M. Roberts, Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research, *Environ. Toxicol. Chem.*, 2021, **40**, 606.
 - 6 D. Melzer, N. Rice, M. H. Depledge, W. E. Henley and T. S. Galloway, Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition examination survey, *Environ. Health Perspect.*, 2010, **118**, 686.
 - 7 L. Zhang, A. Louie, G. Rigutto, H. Guo, Y. Zhao, S. Ahn, S. Dahlberg, M. Sholinbeck and M. T. Smith, A systematic evidence map of chronic inflammation and immunosuppression related to per- and polyfluoroalkyl substance (PFAS) exposure, *Environ. Res.*, 2023, **220**, 115188.
 - 8 B. E. Blake and S. E. Fenton, Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: a review including the placenta as a target tissue and possible driver of peri- and postnatal effects, *Toxicology*, 2020, **443**, 152565.
 - 9 Z. Xu, B. Du, H. Wang, Z. Li, Y. Wu, Q. Wang, Y. Niu, Q. Zhang, K. Sun, J. Wang and S. Chen, Perfluoroalkyl substances in umbilical cord blood and blood pressure in offspring: a prospective cohort study, *Environ. Health*, 2023, **22**, 72.
 - 10 Y. Lu, L. Meng, D. Ma, H. Cao, Y. Liang, H. Liu, Y. Wang and G. Jiang, The occurrence of PFAS in human placenta and their binding abilities to human serum albumin and organic anion transporter 4, *Environ. Pollut.*, 2021, **273**, 116460.
 - 11 C. R. Stein, D. A. Savitz and M. Dougan, Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome, *Am. J. Epidemiol.*, 2009, **170**, 837.
 - 12 Q. Meng, K. Inoue, B. Ritz, J. Olsen and Z. Liew, Prenatal exposure to perfluoroalkyl substances and birth outcomes: an updated analysis from the Danish National Birth Cohort, *Int. J. Environ. Res. Publ. Health*, 2018, **15**, 1832.
 - 13 P. I. Johnson, P. Sutton, D. S. Atchley, E. Koustas, J. Lam, S. Sen, K. A. Robinson, D. A. Axelrad and T. J. Woodruff, The Navigation Guide—evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth, *Environ. Health Perspect.*, 2014, **122**, 1028.
 - 14 Z. Aghaei, K. L. Steeves, K. J. Jobst and L. S. Cahill, The impact of perfluoroalkyl substances on pregnancy, birth outcomes, and offspring development: a review of data from mouse models, *Biol. Reprod.*, 2022, **106**, 397.
 - 15 S. M. Hall, S. Zhang, K. Hoffman, M. L. Miranda and H. M. Stapleton, Concentrations of per- and polyfluoroalkyl substances (PFAS) in human placental tissues and associations with birth outcomes, *Chemosphere*, 2022, **295**, 133873.
 - 16 E. Negri, F. Metruccio, V. Guercio, L. Tosti, E. Benfenati, R. Bonzi, C. La Vecchia and A. Moretto, Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data, *Crit. Rev. Toxicol.*, 2017, **47**, 482.
 - 17 C. Lau, J. R. Thibodeaux, R. G. Hanson, M. G. Narotsky, J. M. Rogers, A. B. Lindstrom and M. J. Strynar, Effects of perfluorooctanoic acid exposure during pregnancy in the mouse, *Toxicol. Sci.*, 2006, **90**, 510.
 - 18 H. Fromme, S. A. Tittlemier, W. Völkel, W. Wilhelm and D. Twardella, Perfluorinated compounds – exposure assessment for the general population in western countries, *Int. J. Hyg Environ. Health*, 2009, **212**, 239.
 - 19 T. W. Jackson, C. M. Scheibly, M. E. Polera and S. M. Belcher, Rapid characterization of human serum albumin binding for per- and polyfluoroalkyl substances using differential scanning fluorimetry, *Environ. Sci. Technol.*, 2021, **55**, 12291.
 - 20 L. Zhao, M. Teng, X. Zhao, Y. Li, J. Sun, W. Zhao, Y. Ruan, K. M. Y. Leung and F. Wu, Insight into the binding model of per- and polyfluoroalkyl substances to proteins and membranes, *Environ. Int.*, 2023, **175**, 107951.
 - 21 F. Nielsen, F. C. Fischer, P. M. Leth and P. Grandjean, Occurrence of major perfluorinated alkylate substances in human blood and target organs, *Environ. Sci. Technol.*, 2024, **58**, 143.
 - 22 W. Völkel, O. Genzel-Boroviczeny, H. Demmelmair, C. Gebauer, B. Koletzko, D. Twardella, U. Raab and H. Fromme, Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk: results of a pilot study, *Int. J. Hyg Environ. Health*, 2008, **211**, 440.
 - 23 A. Kärman, J. L. Domingo, X. Llebaria, M. Nadal, E. Bigas, B. van Bavel and G. Lindström, Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples, *Environ. Sci. Pollut. Res. Int.*, 2010, **17**, 750.
 - 24 L. Maestri, S. Negri, M. Ferrari, S. Ghittori, F. Fabris, P. Danesino and M. Imbriani, Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2728.
 - 25 F. Pérez, M. Nadal, A. Navarro-Ortega, F. Fàbrega, J. L. Domingo, D. Barceló and M. Farré, Accumulation of perfluoroalkyl substances in human tissues, *Environ. Int.*, 2013, **59**, 354.
 - 26 D. Camdzic, R. A. Dickman, A. S. Joyce, J. S. Wallace, P. L. Ferguson and D. S. Aga, Quantitation of total PFAS



- including trifluoroacetic acid with fluorine Nuclear Magnetic Resonance spectroscopy, *Anal. Chem.*, 2023, **95**, 5484.
- 27 M. Thijs, E. Laletas, C. M. Quinn, S. V. Raguraman, B. Carr and P. Bierganns, Total and class-specific determination of fluorinated compounds in consumer and food packaging samples using fluorine-19 solid-state nuclear magnetic resonance spectroscopy, *Anal. Chem.*, 2024, **96**, 8282.
 - 28 J. R. Gauthier and S. A. Mabury, Noise-reduced quantitative fluorine NMR spectroscopy reveals the presence of additional per- and polyfluorinated alkyl substances in environmental and biological samples when compared with routine mass spectrometry methods, *Anal. Chem.*, 2022, **94**, 3278.
 - 29 C. A. Moody, W. C. Kwan, J. W. Martin, D. C. G. Muir and S. A. Mabury, Determination of perfluorinated surfactants in surface water samples by two independent analytical techniques: liquid chromatography/tandem mass spectrometry and ¹⁹F NMR, *Anal. Chem.*, 2001, **73**, 2200.
 - 30 E. Kaebisch, T. L. Fuss, L. A. Vandergrift, K. Toews, P. Habbel and L. L. Cheng, Applications of high-resolution magic angle spinning MRS in biomedical studies I-cell line and animal models, *NMR Biomed.*, 2017, **30**, e3700.
 - 31 C. Dietz, F. Ehret, F. Palmas, L. A. Vandergrift, Y. Jiang, V. Schmitt, V. Dufner, P. Habbel, J. Nowak and L. L. Cheng, Applications of high-resolution magic angle spinning MRS in biomedical studies II-Human diseases, *NMR Biomed.*, 2017, **30**, e3784.
 - 32 K. L. Steeves, J. Hanrahan, N. E. Harvey, K. J. Jobst and L. S. Cahill, Fluorotelomer ethoxylates cause developmental toxicity in mice, *Environ. Sci.: Adv.*, 2024, **3**, 603.
 - 33 A. M. Wyrwicz, C. B. Conboy, K. R. Ryback, B. G. Nichols and P. Eisele, In vivo ¹⁹F-NMR study of isoflurane elimination from brain, *Biochim. Biophys. Acta*, 1987, **927**, 86.
 - 34 R. W. Hornung and L. D. Reed, Estimation of Average Concentration in the Presence of Nondetectable Values, *Appl. Occup. Environ. Hyg.*, 1990, **5**, 46.
 - 35 M. S. Thiese, B. Ronna and U. Ott, P value interpretations and considerations, *J. Thorac. Dis.*, 2016, **8**, E928.
 - 36 H. Adams, J. Hanrahan, S. Kieft, T. O'Brien, G. V. Mercer, K. L. Steeves, C. M. Schneider, K. J. Jobst and L. S. Cahill, Differential impact of perfluorooctanoic acid and fluorotelomer ethoxylates on placental metabolism in mice, *Chemosphere*, 2024, **356**, 141923.
 - 37 B. Zhu, N. Sheng and J. Dai, Adverse effects of gestational exposure to hexafluoropropylene oxide trimer acid (HFPO-TA) homologs on maternal, fetal, and placental health in mice, *Sci. Total Environ.*, 2024, **912**, 169151.
 - 38 B. E. Blake, H. A. Cope, S. M. Hall, R. D. Keys, B. W. Mahler, J. McCord, B. Scott, H. M. Stapleton, M. J. Strynar, S. A. Elmore and S. E. Fenton, Evaluation of maternal, embryo, and placental effects in CD-1 mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene oxide dimer acid (HFPO-DA or GenX), *Environ. Health Perspect.*, 2020, **128**, 027006.
 - 39 S. J. Kim, S. H. Heo, D. S. Lee, I. G. Hwang, Y. B. Lee and H. Y. Cho, Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats, *Food Chem. Toxicol.*, 2016, **97**, 243.
 - 40 J. Bogdanska, D. Borg, U. Bergström, M. Mellring, Å. Bergman, J. DePierre and S. Nobel, Tissue distribution of ¹⁴C-labelled perfluorooctanoic acid in adult mice after 1-5 days of dietary exposure to an experimental dose or a lower dose that resulted in blood levels similar to those detected in exposed humans, *Chemosphere*, 2020, **239**, 124755.
 - 41 L. Cui, Q.-f. Zhou, C.-y. Liao, J.-j. Fu and G.-b. Jiang, Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis, *Arch. Environ. Contam. Toxicol.*, 2009, **56**, 338.
 - 42 D. Yang, J. Han, D. R. Hall, J. Sun, J. Fu, S. Kutarna, K. A. Houck, C. A. LaLone, J. A. Doering, C. A. Ng and H. Peng, Nontarget screening of per- and polyfluoroalkyl substances binding to human liver fatty acid binding protein, *Environ. Sci. Technol.*, 2020, **54**, 5676.
 - 43 B. Ü. Endirlik, A. Eken, H. Canpınar, F. Öztürk and A. Gürbay, Perfluorooctanoic acid affects mouse brain and liver tissue through oxidative stress, *Arh. Hig. Rada Toksikol.*, 2022, **73**, 148.
 - 44 N. Yu, S. Wei, M. Li, J. Yang, K. Li, L. Jin, Y. Xie, J. P. Giesy, X. Zhang and H. Yu, Effects of perfluorooctanoic acid on metabolic profiles in brain and liver of mouse revealed by a high-throughput targeted metabolomics approach, *Sci. Rep.*, 2016, **6**, 23963.
 - 45 E. Costello, S. Rock, N. Stratakis, S. P. Eckel, D. I. Walker, D. Valvi, D. Cserbik, T. Jenkins, S. A. Xanthakos, R. Kohli, S. Sisley, V. Vasiliou, M. A. La Merrill, H. Rosen, D. V. Conti, R. McConnell and L. Chatzi, Exposure to per- and polyfluoroalkyl substances and markers of liver injury: a systematic review and meta-analysis, *Environ. Health Perspect.*, 2022, **130**, 46001.
 - 46 J. J. Shearer, C. L. Callahan, A. M. Calafat, W. Y. Huang, R. R. Jones, V. S. Sabbisetti, N. D. Freedman, J. N. Sampson, D. T. Silverman, M. P. Purdue and J. N. Hofmann, Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma, *J. Natl. Cancer Inst.*, 2021, **113**, 580.
 - 47 B. E. Blake, S. M. Pinney, E. P. Hines, S. E. Fenton and K. K. Ferguson, Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort, *Environ. Pollut.*, 2018, **242**, 894.
 - 48 L. Cui, C.-y. Liao, Q.-f. Zhou, T.-m. Xia, Z.-j. Yun and G.-b. Jiang, Excretion of PFOA and PFOS in male rats during a subchronic exposure, *Arch. Environ. Contam. Toxicol.*, 2010, **58**, 205.
 - 49 M. E. Andersen, H. J. Clewell, Y. M. Tan, J. L. Butenhoff and G. W. Olsen, Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys—probing the determinants of long plasma half-lives, *Toxicology*, 2006, **227**, 156.



- 50 P. M. Lind, L. Lind, S. Salihovic, H. Ahlström, K. Michaelsson, J. Kullberg and R. Strand, Serum levels of perfluoroalkyl substances (PFAS) and body composition - a cross-sectional study in a middle-aged population, *Environ. Res.*, 2022, **209**, 112677.
- 51 E. Jackson, R. Shoemaker, N. Larian and L. Cassis, Adipose tissue as a site of toxin accumulation, *Compr. Physiol.*, 2017, **7**, 1085.
- 52 A. B. Kirk, S. Michelsen-Correa, C. Rosen, C. F. Martin and B. Blumberg, PFAS and potential adverse effects on bone and adipose tissue through interactions with PPAR γ , *Endocrinology*, 2021, **162**, bqab194.
- 53 H. B. Lauritzen, T. L. Larose, T. Øien, T. M. Sandanger, J. Ø. Odland, M. van de Bor and G. W. Jacobsen, Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study, *Environ. Health*, 2018, **17**, 9.
- 54 J. M. Braun, M. Eliot, G. D. Papandonatos, J. P. Buckley, K. M. Cecil, H. J. Kalkwarf, A. Chen, C. B. Eaton, K. Kelsey, B. P. Lanphear and K. Yolton, Gestational perfluoroalkyl substance exposure and body mass index trajectories over the first 12 years of life, *Int. J. Obes.*, 2021, **45**, 25.
- 55 S. D. Geiger, P. Yao, M. G. Vaughn and Z. Qian, PFAS exposure and overweight/obesity among children in a nationally representative sample, *Chemosphere*, 2021, **268**, 128852.
- 56 Y. Liu, N. Li, G. D. Papandonatos, A. M. Calafat, C. B. Eaton, K. T. Kelsey, A. Chen, B. P. Lanphear, K. M. Cecil, H. J. Kalkwarf, K. Yolton and J. M. Braun, Exposure to per- and polyfluoroalkyl substances and adiposity at age 12 years: evaluating periods of susceptibility, *Environ. Sci. Technol.*, 2020, **54**, 16039.
- 57 M. S. Bloom, S. Commodore, P. L. Ferguson, B. Neelon, J. L. Pearce, A. Baumer, R. B. Newman, W. Grobman, A. Tita, J. Roberts, D. Skupski, K. Palmares, M. Nageotte, K. Kannan, C. Zhang, R. Wapner, J. E. Vena and K. J. Hunt, Association between gestational PFAS exposure and Children's adiposity in a diverse population, *Environ. Res.*, 2022, **203**, 111820.
- 58 W. Qi, J. M. Clark, A. R. Timme-Laragy and Y. Park, Per- and polyfluoroalkyl substances and obesity, type 2 diabetes and non-alcoholic fatty liver disease: a review of epidemiologic findings, *Toxicol. Environ. Chem.*, 2020, **102**, 1.
- 59 M. Y. Xie, Z. Y. Lin, X. F. Sun, J. J. Feng, L. Mai, C. C. Wu, G.-L. Huang, P. Wang, Y.-W. Liu and E. Y. Zeng, Per- and polyfluoroalkyl substances (PFAS) exposure in plasma and their blood-brain barrier transmission efficiency-a pilot study, *Environ. Int.*, 2024, **187**, 108719.
- 60 J. Wang, Y. Pan, Q. Cui, B. Yao, J. Wang and J. Dai, Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans, *Environ. Sci. Technol.*, 2018, **52**, 13553.
- 61 R. Neita, K. L. R. Hynes, G. V. Mercer, C. Schneider and L. S. Cahill, Metabolomics using high-resolution magic angle spinning nuclear magnetic resonance: neurotoxicological applications, *J. Pharmacol. Toxicol. Methods*, 2025, **135**, 108382.
- 62 M. Y. Xie, X. F. Sun, C. C. Wu, G. L. Huang, P. Wang, Z. Y. Lin, Y. W. Liu, L. Y. Liu and E. Y. Zeng, Glioma is associated with exposure to legacy and alternative per- and polyfluoroalkyl substances, *J. Hazard. Mater.*, 2023, **441**, 129819.
- 63 Y. Xu, Y. Li, K. Scott, C. H. Lindh, K. Jakobsson, T. Fletcher, B. Ohlsson and E. M. Andersson, Inflammatory bowel disease and biomarkers of gut inflammation and permeability in a community with high exposure to perfluoroalkyl substances through drinking water, *Environ. Res.*, 2020, **181**, 108923.
- 64 S. Campbell, M. Raza and A. Z. Pollack, Perfluoroalkyl substances and endometriosis in US women in NHANES 2003-2006, *Reprod. Toxicol.*, 2016, **65**, 230.
- 65 L. S. Mamsen, B. D. Björvang, D. Mucs, M. T. Vinnars, N. Papadogiannakis, C. H. Lindh, C. Y. Andersen and P. Damdimopoulou, Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies, *Environ. Int.*, 2019, **124**, 482.
- 66 K. Góralczyk, K. A. Pachocki, A. Hernik, P. Struciński, K. Czaja, C. H. Lindh, B. A. G. Jönsson, V. Lelters, W. Korcz, M. Minorczyk, M. Matuszak and J. K. Ludwicki, Perfluorinated chemicals in blood serum of inhabitants in central Poland in relation to gender and age, *Sci. Total Environ.*, 2015, **532**, 548.
- 67 S. Cvitic, M. S. Longtine, H. Hackl, K. Wagner, M. D. Nelson, G. Desoye and U. Hiden, The human placental sexome differs between trophoblast epithelium and villous vessel endothelium, *PLoS One*, 2013, **8**, e79233.
- 68 A. E. Braun, O. R. Mitchel, T. L. Gonzalez, T. Sun, A. E. Flowers, M. D. Pisarska and V. D. Winn, Sex at the interface: the origin and impact of sex differences in the developing human placenta, *Biol. Sex Differ.*, 2022, **13**, 50.
- 69 C. Widnes, K. Flo, T. Wilsgaard, T. Kiserud and G. Acharya, Sex differences in umbilical artery Doppler indices: a longitudinal study, *Biol. Sex Differ.*, 2018, **9**, 16.
- 70 D. Jagota, H. George, M. Walker, A. Ravi Chandran, N. Milligan, S. Shinar, C. L. Whitehead, S. R. Hobson, L. Serghides, W. T. Parks, A. A. Baschat, C. K. Macgowan, J. G. Sled, J. C. Kingdom and L. S. Cahill, Sex differences in fetal Doppler parameters during gestation, *Biol. Sex Differ.*, 2021, **12**, 26.
- 71 J. Hanrahan, K. L. Steeves, G. V. Mercer, T. M. O'Brien, D. P. Locke, N. E. Harvey, A. S. Maekawa, A. N. Stephenson, C. M. Schneider and L. S. Cahill, Sex differences in mouse placental metabolite profiles: an NMR metabolomics study, *Can. J. Chem.*, 2024, **102**, 108.
- 72 F. Chen, S. Yin, B. C. Kelly and W. Liu, Isomer-Specific Transplacental Transfer of Perfluoroalkyl Acids: Results from a Survey of Paired Maternal, Cord Sera, and Placentas, *Environ. Sci. Technol.*, 2017, **51**, 5756.
- 73 S. E. Fenton, J. L. Reiner, S. F. Nakayama, A. D. Delinsky, J. P. Stanko, E. P. Hines, S. S. White, A. B. Lindstrom, M. J. Strynar and S. S. E. Petropoulou, Analysis of PFOA in dosed CD-1 mice. Part 2. Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups, *Reprod. Toxicol.*, 2009, **27**, 365.



- 74 C. V. Goodman, C. Till, R. Green, J. El-Sabbagh, T. E. Arbuckle, R. Hornung, B. Lanphear, J. R. Seguin, L. Booij, M. Fisher, G. Muckle, M. F. Bouchard and J. Ashley-Martin, Prenatal exposure to legacy PFAS and neurodevelopment in preschool-aged Canadian children: the MIREC cohort, *Neurotoxicol. Teratol.*, 2023, **98**, 107181.
- 75 H. Chen, S. Viel, F. Ziarelli and L. Peng, 19F NMR: a valuable tool for studying biological events, *Chem. Soc. Rev.*, 2013, **42**, 7971.
- 76 D. Camdzic, R. A. Dickman and D. S. Aga, Total and class-specific analysis of per- and polyfluoroalkyl substances in environmental samples using nuclear magnetic resonance spectroscopy, *J. Hazard. Mater. Lett.*, 2021, **2**, 100023.
- 77 K. L. Steeves, M. J. Bissram, S. Kleywegt, D. Stevens, F. L. Dorman, A. J. Simpson, M. J. Simpson, L. S. Cahill and K. J. Jobst, Nontargeted screening reveals fluorotelomer ethoxylates in indoor dust and industrial wastewater, *Environ. Int.*, 2023, **171**, 107634.

