

## PAPER

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Cite this: *Environ. Sci.: Adv.*, 2025, 4, 1612

# <sup>19</sup>F solid-state nuclear magnetic resonance as a tool to study the bioaccumulation of per- and polyfluoroalkyl substances in murine tissue samples

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Many per- and polyfluoroalkyl substances (PFAS) are known to be persistent in the environment and are associated with adverse health effects including kidney and liver disease and developmental toxicity. While PFAS are also known to have high bioaccumulation potential, whether these compounds can be detected in biological tissue using nuclear magnetic resonance (NMR) has not been established. In this study, we used <sup>19</sup>F solid-state magic angle spinning (MAS) NMR to investigate the accumulation of a legacy PFAS, perfluorooctanoic acid (PFOA), in murine tissue samples including the adult brain, intestine, kidney, liver, uterus, adipose tissue, placenta and fetal brain. Healthy pregnant ( $n = 4$ ) and non-pregnant ( $n = 5$ ) female CD-1 mice were exposed to 50 ppm of PFOA through their drinking water for 17 days. PFOA was detected above the limit of detection ( $10 \mu\text{g g}^{-1}$ ) in all of the liver samples ( $n = 9/9$ ), 25% ( $n = 2/8$ ) of the adipose tissue samples, 33.3% ( $n = 4/12$ ) of the male placenta samples, and 16.7% ( $n = 2/12$ ) of the female placenta samples. The detection of PFOA in adipose tissue challenges the current understanding about the behaviour of PFAS in the human body. These results demonstrate that <sup>19</sup>F solid-state MAS NMR is a promising tool for detection and quantification of PFAS in tissue samples and motivate further work to evaluate accumulation of unregulated, emerging PFAS that have different chain lengths and head groups.

Received 18th July 2025  
Accepted 22nd August 2025

DOI: 10.1039/d5va00220f

rsc.li/esadvances

## Environmental significance

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 <sup>19</sup>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.

## Introduction

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.<sup>1</sup> However, these legacy PFAS (sometimes referred to as “forever chemicals”) remain in the environment and continue to pose a global health concern.<sup>2–4</sup> Exposure to legacy PFAS has been linked to thyroid disease, kidney and liver cancers, and immunotoxicity, as well as reproductive and developmental health complications.<sup>5–8</sup>

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.<sup>9,10</sup> PFOA and PFOS have been associated with pregnancy complications in humans including hypertension,<sup>11</sup> preterm birth,<sup>12</sup>

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and low birth weight infants.<sup>13</sup> 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.<sup>14</sup> 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.<sup>15</sup> In mice, prenatal exposure to PFOA resulted in a more significant decrease in fetal/birth weights in males compared to females<sup>16</sup> and in accelerated sexual maturation in male offspring but not in females.<sup>17</sup>

The main routes of human exposure to PFAS are through food, drinking water, dust, and air.<sup>18</sup> PFAS are able to enter the bloodstream and travel through the body by binding to serum albumin.<sup>19,20</sup> 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.<sup>21–25</sup> <sup>19</sup>F 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.<sup>26–28</sup> Mass spectrometry and <sup>19</sup>F 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.<sup>29</sup> Solid-state magic angle spinning (MAS) NMR is a useful tool in studying biopsied tissue samples;<sup>30,31</sup> 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 <sup>19</sup>F nucleus (the <sup>19</sup>F nucleus is 100% abundant and ~83% as NMR-sensitive as <sup>1</sup>H), the absence of endogenous fluorine in biological systems, and limited requirements for sample preparation (reducing sources of potential contamination), we used <sup>19</sup>F 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.<sup>32</sup> 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<sup>-1</sup>, 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 <sup>19</sup>F NMR spectra. First, the use of isoflurane as an anesthetic was avoided because the presence of isoflurane in the tissue can be detected using <sup>19</sup>F NMR.<sup>33</sup> Additionally, use of compressed gas to dry the NMR rotors was found to produce a detectable <sup>19</sup>F 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 cm<sup>3</sup>) 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.

### <sup>19</sup>F solid-state nuclear magnetic resonance spectroscopy

<sup>1</sup>H decoupled <sup>19</sup>F solid-state MAS NMR experiments were performed on intact, unprocessed tissue samples using a Bruker NEO 600 MHz spectrometer (<sup>19</sup>F 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*<sub>1</sub> relaxation of a PFOA solution (<sup>19</sup>F *T*<sub>1</sub> 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 <sup>19</sup>F 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*<sup>2</sup> = 0.9914). The limit of detection (LOD) was determined to be the lowest concentration for the alkyl CF<sub>3</sub> resonance with a signal-to-noise ratio of 3 (LOD = 10 μg g<sup>−1</sup>). Several tissue samples showed a clear <sup>19</sup>F 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.<sup>34</sup>

### 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

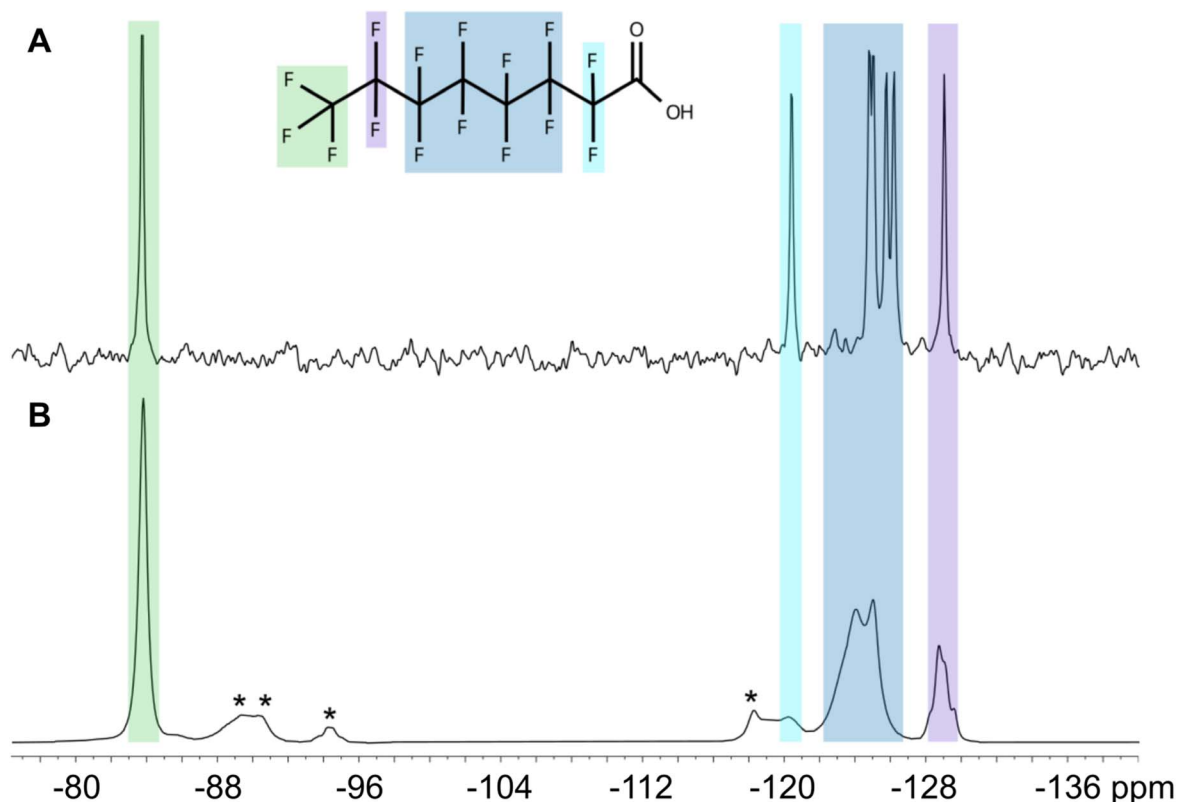


Fig. 1 Representative <sup>19</sup>F spectra of (A) a 50 ppm solution of PFOA (MAS = 4 kHz) and (B) solid PFOA (MAS = 20 kHz). Both spectra were acquired using a 3.2 mm solid-state MAS NMR probe. Spinning sidebands are marked with asterisk. The resonances are colour-coded to match the chemical structure 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.<sup>35</sup>

## 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 ( $9 \pm 2$  mL d<sup>-1</sup>). 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,<sup>32,36–38</sup> 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, <sup>19</sup>F solid-state MAS NMR spectroscopy was conducted on biopsied tissue samples from pregnant and non-pregnant mice. Fig. 3 shows representative examples of <sup>19</sup>F 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 ( $90 \pm 10$  µg g<sup>-1</sup>) and were significantly lower in the adipose tissue ( $9 \pm 3$  µg g<sup>-1</sup>) ( $p < 0.0001$ ). One of the nine kidney samples showed a visible <sup>19</sup>F signal that was below the LOD. Tissue from the adult brain, intestine, and uterus did not show any evidence of PFOA by <sup>19</sup>F MAS NMR spectroscopy.

Detection of PFOA at high concentrations in the liver is consistent with biodistribution studies in animals<sup>39–41</sup> and humans.<sup>21,23</sup> The PFOA concentrations reported in healthy adult humans are lower (e.g.  $3.24 \times 10^{-4}$  µg g<sup>-1</sup> in the liver<sup>21</sup>), 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.<sup>42</sup> Studies assessing the impact of PFOA exposure on liver metabolism in mice report metabolic signatures of oxidative stress,<sup>43</sup> as well as alterations to amino acid and lipid metabolism in the liver.<sup>44</sup> PFAS exposure has also been associated with liver disease such as non-alcoholic fatty acid liver disease.<sup>45</sup> 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.<sup>21,46,47</sup> PFOA was detected in the kidney of adult rats following 28 days of exposure by daily gavage (average concentration = 220 µg g<sup>-1</sup>).<sup>41</sup> 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 <sup>14</sup>C-PFOA and liquid scintillation counting) has much higher sensitivity than NMR.<sup>40</sup> The primary route of excretion of PFAS from the body is through urine<sup>48</sup> and PFOA has been shown to have high renal resorption.<sup>49</sup> 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<sup>21,24,25,50</sup> 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<sup>-1</sup>).<sup>40</sup> Accumulation of environmental pollutants in adipose tissue (e.g., persistent organic pollutants such as polychlorinated biphenyls) is associated with obesity and related metabolic diseases.<sup>51</sup> 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).<sup>52</sup> 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.<sup>53–58</sup> 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 <sup>19</sup>F NMR spectroscopy. This is consistent with

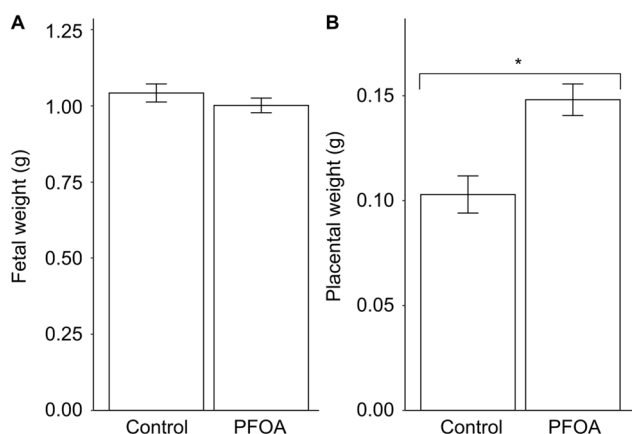


Fig. 2 Comparison of fetal weight (A) and placental weight (B) between control and PFOA-exposed pregnant mice. Data shown as means  $\pm$  95% confidence intervals.  $n = 4$  dams per group. \* $p = 0.001$ .





reports in humans and mice where the concentration of PFOA detected in the brain is low but quantifiable by sensitive analytical techniques such as mass spectrometry.<sup>21,24,40,50,59</sup> Whether the blood–brain barrier protects the brain from PFAS is debated and it may be that PFAS is able to disrupt the integrity of the blood–brain barrier.<sup>60</sup> Regardless of the affinity for bioaccumulation of PFOA in the brain, exposure to legacy PFAS has been associated with neurotoxicity in mice<sup>44,61</sup> and humans.<sup>62</sup> Similar to the brain, we also did not detect any <sup>19</sup>F signal in the tissue samples from the intestine and uterus. PFOA was detected in the intestine of adult mice in Bogdanska *et al.* and has been associated with inflammatory bowel disease and gut inflammation in humans in a community with high exposure to PFAS through drinking water.<sup>40,63</sup> While PFAS exposure is known to impact pregnancy and fetal development and has been associated with endometriosis,<sup>64</sup> detection of PFAS in the uterus has not been reported previously. The absence of detection of PFOA by <sup>19</sup>F NMR in the brain, intestine, and uterus must be treated with caution given the small sample size.

In the pregnant mice, PFOA was detected in the placentas of 3 of the 4 PFOA-exposed dams. <sup>19</sup>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.<sup>65</sup> 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,<sup>66</sup> 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.<sup>5</sup> The differences in bioaccumulation between male and female placentas may be related to the known differences in placental cellular and vascular composition.<sup>67,68</sup> The increased accumulation of PFAS and known sex differences in placental blood flow<sup>69,70</sup> and placental metabolism<sup>71</sup> may partially explain the sex-specific vulnerability to PFAS.<sup>15–17</sup> PFAS are known to be transported from the mother, across the placenta to the fetus<sup>72,73</sup> and therefore we investigated the concentration of PFOA in fetal brain tissue. While below the LOD, PFOA was

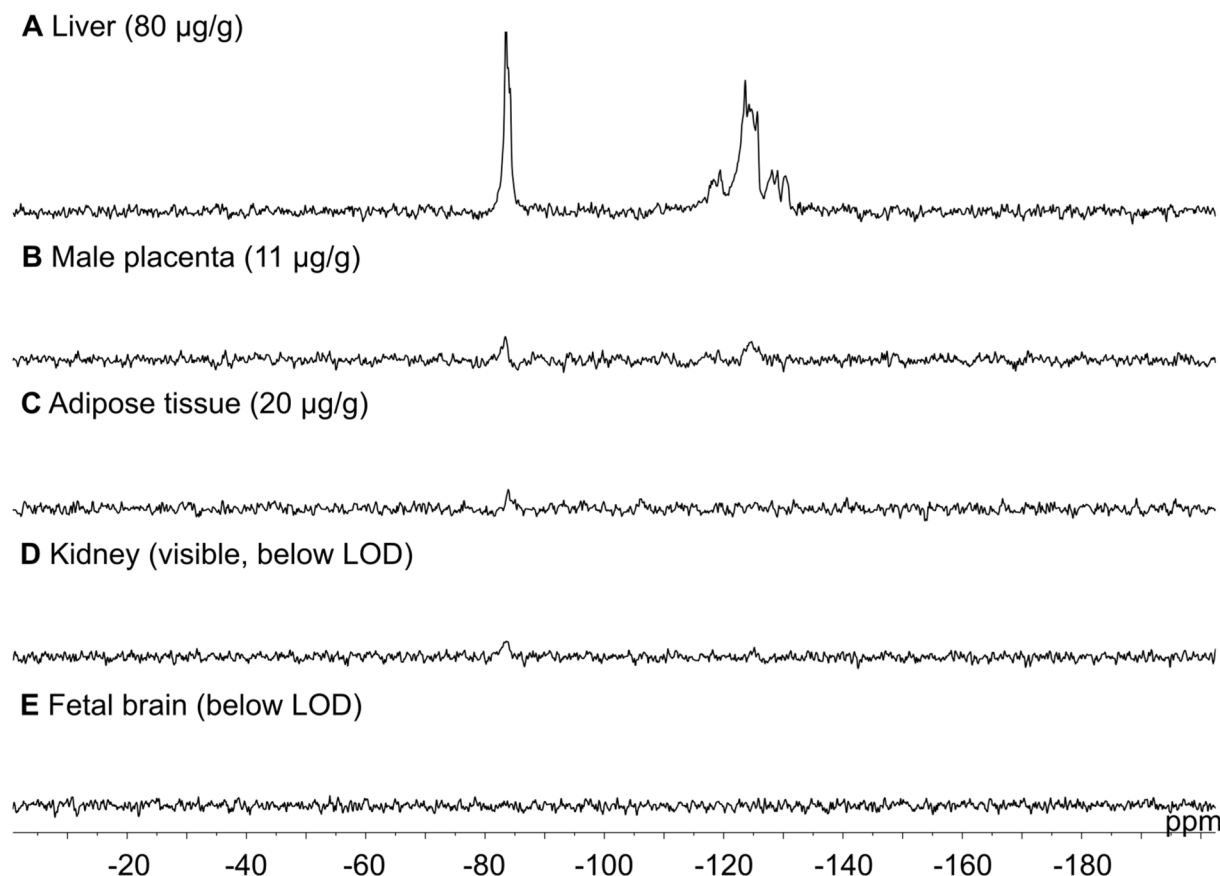


Fig. 3 Representative <sup>19</sup>F spectra of (A) liver, (B) placenta (male), (C) adipose tissue, (D) kidney, and (E) fetal brain tissue samples from mice exposed to PFOA for 17 days. Tissue concentration determined using calibration curve and tissue sample weight.



Table 1 Summary of quantification and detection frequency of all tissue samples<sup>a</sup>

| 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 ( $n = 9$ )                       | $90 \pm 10^b$                           | 100 (9/9)                         | 100 (9/9)                                   |
| Uterus ( $n = 2$ )                      | Below LOD                               | 0 (0/2)                           | 0 (0/2)                                     |
| Adipose tissue ( $n = 8$ ) <sup>c</sup> | $9 \pm 3$                               | 25 (2/8)                          | 25 (2/8)                                    |
| Placenta ( $n = 24$ )                   | Male: $30 \pm 40$                       | Male: 33.3 (4/12)                 | Male: 66.7 (8/12)                           |
|                                         | Female: $8 \pm 2$                       | 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)                          |

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

<sup>c</sup> 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.<sup>74</sup>

The use of  $^{19}\text{F}$  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.<sup>26,75,76</sup> 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  $^{19}\text{F}$  NMR for quantification of PFAS in biological and environmental samples, detecting higher concentrations in total and class-specific PFAS in comparison to mass spectrometry.<sup>26,28</sup> 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 humans<sup>23</sup> and rats<sup>39</sup>) 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<sup>21,24,25</sup> 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,  $^{19}\text{F}$  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  $^{19}\text{F}$  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<sup>32,77</sup>).

## 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>.

## Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council (NSERC) Discovery Grant.

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