Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information

Per- and polyfluoroalkyl substances (PFAS) in grocery store foods: method optimization, concentrations, and exposure assessment

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S1. MATERIALS AND METHODS

Materials and Reagents. All native and internal mass labeled PFAS standards were obtained from Wellington Laboratories (Canada) (**Table S1)**. Liquid chromatography-mass spectrometry (LC-MS)-grade Acetonitrile (≥ 99.9%, ACN) and methanol (MeOH; ≥ 99.9%) were obtained from Honeywell (United States). HPLC Methyl tert-butyl ether (MTBE) with a purity of ≥ 99% was obtained from J.T Baker (United States). Ammonium hydroxide (30%, Fisher Chemical), ammonium acetate (J.T Baker), and Optima™ LC/MS acetic acid (99.7%, Fisher Chemical), and H_2O_2 (30%, VWR Chemical) were purchased from VWR Chemicals BDH (United States). Envi-carb™ (Supelco). Tetrabutylammonium hydrogen sulfate (≥ 98%, TBAH) and LC-MS formic acid (≥ 98%) were obtained from TCI America (United States). ChloroFilter Tubes were obtained from UCT (United States). Sodium carbonate (99.95-100.05%, ACS, Na_2CO_3) was obtained from Alfa Aesar (Germany). Chromabond Diamino adsorbents were obtained from Thomas Scientific (United States).

Table S1. PFAS included in this study, mass labeled, extraction internal standards, and limits of quantitation (LOQs).

Compound	LOQ (ng/L)	Internal Standard
Perfluorobutanoic acid (PFBA)	5	$^{13}C_4$-PFBA
Perfluoropentanoic acid (PFPeA)	5	$^{13}C_5$-PFPeA
Perfluorohexanoic acid (PFHxA)	1	$^{13}C_5$-PFHxA
Perfluoroheptanoic acid (PFHpA)	1	$^{13}C_4$-PFHpA
Perfluorooctanoic acid (PFOA)	1	$^{13}C_8$-PFOA
Perfluorononanoic acid (PFNA)	1	$^{13}C_9$-PFNA
Perfluorodecanoic acid (PFDA)	1	$^{13}C_6$-PFDA
Perfluoroundecanoic acid (PFUnA)	1	$^{13}C_7$-PFUnA
Perfluorododecanoic acid (PFDoA)	5	$^{13}C_2$-PFDoA
Perfluorotridecanoic acid (PFTriA)	5	$^{13}C_2$-PFTeDA
Perflurotetradecanoic acid (PFTreA)	5	$^{13}C_2$-PFTeDA
Perfluorobutane sulfonate (PFBS)	2	$^{13}C_3$-PFBS
Perfluoropentane sulfonate (PFPeS)	5	$^{13}C_3$-PFHxS
Perfluorohexane sulfonate (PFHxS)	5	$^{13}C_3$-PFHxS
Perfluoroheptane sulfonate (PFHpS)	1	$^{13}C_3$-PFHxS
Perfluorooctane sulfonate (PFOS)	1	$^{13}C_8$-PFOS
Perfluorononane sulfonate (PFNS)	5	$^{13}C_8$-PFOS
Perfluorodecane sulfonate (PFDS)	5	$^{13}C_8$-PFOS
Perfluorooctanesulfonamide (FOSA)	2	$^{13}C_8$-FOSA
N-Methyl perfluorooctanesulfonamide acetic acid (MeFOSAA)	2	d_3-MeFOSAA
N-Ethyl perfluorooctanesulfonamide acetic acid (EtFOSAA)	2	d_3-EtFOSAA
4:2 Fluorotelomer sulfonate (4:2 FtS)	2	$^{13}C_2$-4:2 FtS
6:2 Fluorotelomer sulfonate (6:2 FtS)	2	$^{13}C_2$-6:2 FtS
8:2 Fluorotelomer sulfonate (8:2 FtS)	2	$^{13}C_2$-8:2 FtS

Sample Selection. When selecting matrices for sample screening, the project focused on sample types that were representative of produce, animal, and dairy tissues included in the food study. Dairy screening was limited to milk, so milk was the only sample type screened. Within produce and tissues, sample composition also demonstrates clear trends (**Table S2**). With the exception of corn, plant chemical composition is largely similar across multiple common produce types. Similar consideration applies to tissues which, as shown, are largely similar in composition (**Table 1**). Additionally, the protein content of earthworms and mammalian tissues is comprised primarily of structural proteins, and studies have shown that organic contaminants have a similar affinity (i.e., protein-water partitioning) for structural proteins across multiple organisms (e.g., pork, chicken, fish) [1]. Collectively this suggests that most mammalian tissues and earthworm tissues are representative of animal tissues consumed as food products. Although we view earthworm tissues as representative, in our study the results of earthworm screening were presented separately from screening of animal tissues. Although more sample types were screened for stronger vs. weaker performing extraction methods, plant extractions were screened on a minimum of radish shoots, radish roots, and lettuce, and tissue extractions were screened on a minimum of turkey liver in addition to earthworms (which, as noted, were considered separately).

Table S2. Chemical composition of some foods within matrices evaluated in this study [2–5].

Organism	% Lipid	% Protein	% Carbohydrate
Lettuce	<1	1	5
Corn (U.S.)	4	9	72
Strawberries	2	3	6
Radish shoots	<1	3	4
Radish root	1	7	4
Earthworms	5-15	60-70	8-20
Deer meat	10	90	<1
Beef (roast)	20	80	<3
Chicken liver	7	18	2

Sample Preservation. Upon receipt or purchase, aliquots of fresh and canned plants were dried (70°C), and the dried materials refrigerated until extraction. Surplus fresh and canned vegetables were archived in the freezer (-20 °C). A subset of lettuce and radish shoots were dried, homogenized, and spiked with a mix of the 24 PFAS to achieve low (10 ng/L) and high (200 ng/L) extract concentrations. After spiking, the samples were vortexed and dried overnight in a fume hood to allow evaporation of solvent. Spiked samples were aged in the cool room (4 °C) for a period of 90 days prior to extraction to evaluate the impacts of aging on extraction efficiency. The 90-day aging period was based on a 90-day holding time in some standard methods such as EPA Method 1633.[6] All dairy and animal tissues were preserved in freezer until extraction. Prior to extraction milk powder was prepared using the instructions on the label.

Table S3. Sample media and extraction method summary

Media	Method Selected	Mass or Volume extracted	Sample Prep	Extraction Solvent	Rounds Extraction	Sample Cleanup	Reconstitution solvent	Matrices applied to[1]
Plants	MTBE	0.2-0.5 g_{dw}	Homogenized, dried	MTBE+ TBA, Na2CO3	3	ENVI-carb	MeOH	Lettuce, organic lettuce, radish root, radish shoot, carrot shoot, carrot root, tomato, strawberry, canned green beans, green beans, canned corn, corn, celery, baby food (3/5 carrot; 1/4 veggie, 2 tsp rice, 1.75 tsp beef; 22 green beans; 1/2 apple, 1 strawberry, 1/10 banana; 3/5 sweet potato, 1/5 corn; carrots)
Milk	ACN	10 mL	Reconstitute (powdered only), add 0.5 g/mL QuEChERs (all types)	100% ACN @ 1:1 ACN:milk	1	ENVI-carb	MeOH	Organic whole milk, Goat milk powder
Muscle Tissue	MeOH	0.2-0.5 g_{dw}	Homogenized, dried	100% MeOH	1	ENVI-carb	MeOH	Rabbit tissue, beef baby food
Liver	MeOH	0.2-0.5 g_{dw}	Homogenized, dried	100% MeOH	1	ENVI-carb	MeOH	Turkey liver, guinea pig liver
Earthworms	ACN	0.2-0.5 g_{dw}	Homogenized, dried	100% ACN	1	ENVI-carb	MeOH + 10% formic acid	Earthworms

1. Unless otherwise stated, all plants were not organic and fresh.

ACN Extraction. This method was used in previous studies for the extraction fish fillet,[7] earthworms,[8] and with the addition of QuECHERs salts, for milk and plant samples.[9–22] It was screened in the current study for use on plants, milk, and earthworm samples. Ultimately, the method was selected for use on milk (with QuECHERs) and earthworm (no QuECHERs) extractions (**Table S3**). Briefly, 0.2-1 g of dried homogenized plant tissues or 10 mL of milk (fresh or reconstituted powder; see **Table S1**) were measured into a 50 mL polypropylene (PP) centrifuge tubes. For earthworms, an aliquot of 0.25 g of homogenized, dried tissue was added to a 15 mL polypropylene (PP) tube. IS (2ng; **Table S1**) were spiked into all matrix types prior to extraction, and 5 mL (earthworms) to 10 mL (all other matrices) of ACN and 5g of QuECHERs anhydrous salt were added to each tube, vortexed (30s), and placed on a shaker table for 20min (earthworms) to two hours (all other matrices). Earthworm extracts were put in a freezer (-20 °C) for at least 1 hour to facilitate protein precipitation. All extracts were separated by centrifuge (5000 rpm, 20min) and transferred to a clean 20 mL glass scintillation vial, evaporated to dryness under nitrogen (Organomation Associates Inc. N24EVAP, Berlin, MA), and reconstituted with 1000 µL LC-MS grade MeOH or MeOH with 10% formic acid (earthworms only). Prior to evaporation, all needles for the evaporation unit were washed, submerged in a container of PFAS-free methanol, and sonicated for 30 minutes, and oven dried prior to use. Reconstituted extracts were transferred to a 2mL microcentrifuge tube containing 20-40 mg Envi-carb, vortexed, and centrifuged (15000 rpm; Beckman Coulter Microfuge® 20 Centrifuge) for 30 minutes. For targeted analysis, 340 µL of extract was transferred to an autosampler vial and amended with 170 µL of LC-MS grade methanol (MeOH) and 1190 µL ultrapure water to achieve a final vial composition of 70% water: 30% methanol containing 200 ng/L IS.

DCM extraction. In prior studies, this method was used for the extraction of plants,[23–25] so it was included in methods screened for plant extraction in the current study. Briefly, 0.5-2.0 g (fresh weight) or 0.06-0.24 g (dried weight) of homogenized plant tissue was added to pre-labeled 50 mL PP tubes containing 2ng IS (**Table S1**). A 7 mL aliquot of extraction solvent was added to each tube. The extraction solvent was 50% by volume dichloromethane (DCM) and 50% 99:1 (v/v) MeOH and ammonium hydroxide (i.e., basic MeOH). Samples were put in a heated sonication bath (60 °C, VWR, 97044-006) for 30 minutes and then placed on a shaker table for 1 hour. Samples were then centrifuged for 20 minutes (2700 rpm), and the extract was decanted into a clean scintillation vial. The extraction was repeated two additional rounds for a total of three rounds. Combined extracts were evaporated to dryness under nitrogen. A 1 mL aliquot of 30% H_2O_2 was added to the 50 mL tube containing the plant material, tubes were vortexed (30s), and placed in a heated sonication bath (30 °C) for 2 hours. Another three rounds of extraction using DCM and basic MeOH were conducted, combined into the scintillation of dried extract, and evaporated to dryness under nitrogen. Cleaning protocol for the nitrogen evaporation unit is described in **ACN Extraction**. Extracts were reconstituted using 1 mL LC-MS MeOH. Reconstituted extracts were transferred to 2mL microcentrifuge tubes containing 100 mg of diamino and 100 mg of ENVI-carb for cleanup. The microcentrifuge tube was vortexed (30 sec) and centrifuged (15000 rpm for 30 minutes). Finally, 170 µL of the supernatant was transferred to an autosampler vial and amended with 340 µL of LC-MS grade methanol and 1190 µL ultrapure water to achieve a final vial composition of 70% water: 30% MeOH, with 200 ng/L IS.

Methyl tert-butyl ether (MTBE) + tetrabutylammonium hydrogen sulphate (TBA) and Na2CO3 extraction. This method has been used in previous studies for extraction of animal

tissues[12–22] and plants.[26–33] So, it was screened in the current study for use with animal tissues (liver, earthworm) and plants in the current study, but was ultimately selected for use on plants only. Briefly, 0.5-2.0 g (fresh weight) or 0.06-0.24 g (dried weight) homogenized tissues were added to pre-labeled 50 mL PP tubes containing 2ng IS (**Table S1**). Aliquots of 1 mL 170g/L tetrabutyl ammonium hydrogen sulfate solution and 2 mL of 26.5 g/L sodium carbonate buffer were added to each tube and vortexed (30s). Next, 5 mL of methyl tert butyl ether (MTBE) was added and tubes were placed on a shaker table for 1 hour, and then the organic and aqueous phases were separated by centrifuge (5000 rpm, 20 min). MTBE extracts were transferred by pipet to a 20 mL scintillation vial. This extraction was repeated 2 additional times for a total of 3 rounds. The combined extracts were evaporated to dryness under nitrogen and reconstituted in 1 mL LC-MS methanol. Cleaning protocol for the nitrogen evaporation unit is described in **ACN Extraction**. Reconstituted extracts were transferred to 2mL microcentrifuge tubes containing 20-40 mg Envi Carb, vortexed (30 sec), and centrifuged (15000 rpm) for 30 min. A 170 µL aliquot of the final extract was transferred to an autosampler vial and amended with 340 µL of LC-MS grade methanol and 1190 µL ultrapure water to achieve a final vial composition of 70% water: 30% MeOH containing 200 ng/L IS.

MeOH extraction. MeOH extraction using basic MeOH has been used extensively in previous studies for soils, sediments, and biosolids.[34,35] It was trialed here for use with earthworms. An alternate version of the MeOH extraction using unmodified (i.e., not basic) MeOH has been used in prior studies for animal tissues, [36–38] so it was screened for use on liver and earthworm tissues in the current study and ultimately selected for use on muscle tissues. Briefly, 0.5 g of homogenized earthworm (wet weight) or tissue was weighed into 50 mL PP centrifuge tubes containing 2-4 ng of IS (**Table S1**). A 4mL aliquot MeOH or 7 mL aliquot of basic MeOH was added to each tube, vortexed (30s), placed in heated sonication bath (60 °C, VWR, 97044-006) for one hour, and the on a shaker table for two hours. Samples were then centrifuged (5000 rpm, 20 min), and the supernatant was transferred into a clean, 20 mL glass scintillation vial. Extractions using basic MeOH were repeated twice for a total of three rounds. For both MeOH (single round extraction) and basic MeOH extractions (triplicate) the supernatant was evaporated under nitrogen and reconstituted in 1000 µL acidic methanol (1% [V/V] acetic acid in LC-MS grade methanol). Cleaning protocol for the nitrogen evaporation unit is described in **ACN Extraction**. The extract was transferred to a microcentrifuge tube containing 20 - 40 mg ENVI carb (Millipore Sigma, USA) for clean-up. The microcentrifuge tube was vortexed (30 sec) and centrifuged (15000 rpm for 30 minutes). For targeted analysis, 170 µL of the supernatant was transferred to an autosampler vial and amended with 340 µL of LC-MS grade MeOH and 1190 µL ultrapure water to achieve a final vial composition of 70% water, 30% methanol, containing 200 ng/L of each IS.

Chromatographic Separation. The chromatographic separation was performed on a C18 analytical column (Gemini®, 3 uM, 100 X 3 mm ID, Phenomenex, CA, USA) coupled with a guard column (Gemini®, C18 4 x 2.0mm ID, Phenomenex, CA, USA) with a Sciex Exion high pressure liquid chromatography (HPLC) system. A delay column (Luna®, 5 µm, C18, 30 x 3mm, Phenomenex, CA, USA) was installed between mobile phase mixer and sample injector to minimize background contamination that may come from solvent reservoir tubing and pump parts. The C18, delay, and guard columns were maintained at 40°C throughout the run. The aqueous phase consisted of 20 mM ammonium acetate solution (A), and the organic phase was 100% MeOH (B). Sample injections of 500 µL were used for the analysis. Mobile phase flow rate was

maintained at 600 µL/min throughout the run, and the composition was ramped from 95% A to 35% A over the first minute, and further ramped to 5% A at 8 minutes, 1% to in next 0.1 minutes, held constant until 12.5 minutes, and at the end ramped to 95% A at 13.00 minutes and equilibrated the column for 3.5 minutes. The first 3.5 minutes of eluent was diverted to waste.

QTOF-MS analysis. All analyses (targeted and suspect screening) were performed on a quadrupole time of flight-mass spectrometry (QTOF-MS) system (X500R, SCIEX, Framingham, MA, USA). Turbo ion spray was used as ion source and maintained at 500° C during the sample acquisition with following conditions: n spray voltage -4500 (v); curtain gas 30 (PSI); ion source gas 140 (PSI), ion source gas 260 (PSI). Collision activated dissociation (CAD) gas was maintained at 10 PSI. Ultra-pure nitrogen was used as the source, collision, and exhaust gas. For the targeted analysis, 24 PFAS were monitored and analyzed using a MRMHR acquisition method. The QToF-MS was operated in a multiple reaction monitoring high resolution (MRMHR) mode that acquired two transitions (quantifier and qualifer) for each PFAS, where possible (**Table S4**). Data were acquired and processed using SCIEX OS (versions 1.5 and 2.2). PFAS were quantified using isotope dilution over a calibration range of 0.5-5000 ng/L (R^2>0.99).

Table S4. MRM transitions and retention time of PFAS monitored during this study

	Precursor (Q1)		Quantifier (Q3)		Qualifier (Q3)		
Analyte	m/z (Da)	DP[2] (V)	m/z (Da)	CE[3] (V)	m/z(Da)	CE (V)	RT (min)
PFBA[1]	212.9	-25	168.9894	-12			5.26
13C4_PFBA	217	-25	171.99944	-12			5.24
PFPeA[1]	262.9	-50	218.9862	-12			5.84
13C5_PFPeA	267.9	-50	222.9996	-12			5.84
PFBS	298.9	-55	79.9574	-58	98.9558	-40	5.87
13C3_PFBS	302	-55	79.9574	-55			5.88
PFHxA	313	-25	268.983	-12	118.9926	-28	6.35
13C5_PFHxA	318	-25	272.9964	-12			6.35
4:2 FTS	327	-95	306.9681	-25	80.9652	-45	6.29
13C2-4:2 FTS	329	-95	80.9652	-66			6.29
PFPeS	349	-60	79.9574	-66	98.9558	-45	6.35
PFHpA	363	-25	318.9798	-12	168.9894	-20	6.92
13C4_PFHpA	367	-25	321.98985	-12			6.92
PFHxS	399	-60	79.9574	-74	98.9558	-50	6.86
13C3_PFHxS	402	-60	79.9574	-50			6.87
PFOA	413	-25	368.9766	-14	168.9894	-22	7.49
13C8_PFOA	421	-25	376.00008	-14			7.49
6:2 FTS	427	-45	406.9617	-30	80.9652	-45	7.49
13C2-6:2FTS	429	-45	80.9652	-45			7.49
PFHpS	449	-65	79.9574	-88	98.9558	-50	7.49
PFNA	463	-25	418.9734	-14	168.9894	-24	8.12
13C9_PFNA	472	-25	427.00024	-14			8.12
FOSA[1]	498	-60	77.9655	-85			8.67

PFOS	499	-165	79.9574	-70	98.9558	-50	8.06
13C8_FOSA	506	-60	77.9655	-85			8.68
13C8_PFOS	507	-165	79.9574	-108			8.07
PFDA	513	-25	468.9702	-16	168.9894	-26	8.74
13C6_PFDA	519	-25	473.98698	-16			8.75
8:2 FTS	527	-50	506.9553	-35	80.9652	-60	8.81
13C2-8:2FTS	529	-50	80.9652	-40			8.81
PFNS	549	-70	79.9574	-110	98.9558	-70	8.69
PFUdA	563	-25	518.967	-18	168.9894	-28	9.35
13C7_PFUdA	570	-25	524.98714	-18			9.35
N-MeFOSAA	570	-75	418.9734	-28	482.9353	-22	9.09
d3-MeFOSAA	573	-75	418.9734	-28			9.09
N-EtFOSAA	584	-90	418.9734	-28	525.9775	-28	9.41
d5-EtFOSAA	589	-90	418.9734	-28			9.41
PFDS	599	-85	79.9574	-118	98.9558	-84	9.31
PFDoA	613	-25	568.9638	-18	168.9894	-30	9.95
13C2_PFDoA	615	-25	569.96718	-18			9.95
PFTrDA	663	-25	618.9606	-20	168.9894	-36	10.45
PFTeDA	713	-25	668.9574	-22	168.9894	-38	10.9
13C2_PFTeDA	715	-25	669.96079	-22			10.92

[1]Only one transition available for monitoring; [2]Declustering potential (DP); [3]Collision energy (CE)

Quality Control. Method blanks, solvent (i.e., analytical) blanks, instrument sensitivity checks, and calibration verifications were included as quality control samples. IS and matrix spike (MS) recoveries were used to account for matrix effects and analyte losses during extraction. IS recoveries in unknown samples were calculated according to **Equation S1 (Eq S1).**

$$IS\ recovery\ (\%) = \left(\frac{Peak\ area\ of\ IS\ in\ unknown\ samples}{Average\ peak\ area\ of\ IS\ in\ calibration\ standards}\right) \times 100 \qquad \text{(Eq S1)}$$

If IS recoveries were outside the acceptable range of (50-150%), target analyte concentrations were flagged. Peaks of internal standards and calibrants (target analytes) in unknown samples were only considered for further analysis if retention times were ±30 s of calibration standards, signal to noise ratios were greater than 10 and also at least 3X higher than the response in instrument blanks.

Matrix spike (MS) recoveries were used a second line of evidence for evaluation of extraction efficiency and matrix effects during analysis. Matrix spike recoveries in unknown samples were calculated according to **Eq S2**.

$$MS\ recovery\ (\%) = \left(\frac{[PFAS]\ MS - [PFAS]\ Background}{Spiked\ Concentration}\right) \times 100 \qquad \text{(Eq S2)}$$

where [PFAS]MS is the concentration of each PFAS in the matrix spike, [PFAS] background is the background concentration (if any) detected in the unspiked sample, and the spiked

concentration is the concentration of each PFAS spiked into the sample (200 ng/L). If MS were recoveries were outside of the acceptable range of 70-130%, MS results were flagged.

Each analytical run consisted of 14 calibration standards (0.5-5000 ng/L), method blanks, instrument blanks, instrument sensitivity checks (ISCs, 0.5-10 ng/L), low concentration continuing calibration verification (CCV, 10 ng/L), and mid-point CCV (200 ng/L). All quality control samples except instrument blanks contained 200 ng/L of each IS. Vial composition of all quality control samples was the same as unknown samples (30% methanol/70% water).

ISCs were performed by running 0.5-10 ng/L standards immediately prior to unknown samples. The limit of quantitation (LOQ) of an analyte was the lowest ISC where the calculated concentration was ±30% of true concentration or the concentration detected in the method blank, whichever was higher. CCV was performed by injecting a standard after every 10 unknown samples (alternating between 10 and 200 ng/L) and sample data were accepted only if CCVs were ±30% of true value. Calibration curves were fit with regression equations (R^2>0.99) and used to quantify analytes in unknown samples. Every sample was quantified using an isotope dilution method, and concentrations of samples are reported as average of triplicates. Relative standard deviation (RSD) of replicates was calculated and presented as a measure of variability during the analysis.

Exposure Assessment Model. Exposure intakes (EIs) applicable to various age groups were estimated using **Eq S3**, which utilizes exposure frequency (EF, days/year), age-specific exposure duration (ED, days), averaging time (AT; time period over averaged exposure, days), body weight (BW, kg), and ingestion rates (IR, $g_{ww}/kg_{bw\text{-}day}$) as seen in a recently published study.[39] BW and IR were estimated using a Monte Carlo simulation and published resources from USEPA Exposure Factors Handbook.[40] BW and IR are multiplied to yield the daily consumption of vegetables per age group (kg_{wet}/day). These were combined with PFAS concentrations (C_{PFAS}) measured in the food survey (**Table S11**) to generate the daily EI values (ng/kg_{bw}-day). The Monte Carlo simulations generated ranges of BW and IR based on age, and these were used in Eq 3 to yield ranges of EI values for children 1-2 years of age and adults over 20 years. Within each age range, the EI values from the 5th, 50th, and 95th percentile were compared to reference doses summarized in **Table 1** of the manuscript.

$$EI = \left(\frac{C_{PFAS}(plant\ i) * \left(BW * \frac{IR}{1000} \right) * EF * ED}{(BW * AT)} \right) * 10^6 \qquad \text{(Eq S3)}$$

Where:

- EI is daily dietary exposure intake, ng/kg_{bw}-day.
- $C_{PFAS(plant\ i)}$ is detected concentration in our grocery food survey from each plant, mg/kg_{ww}.
- BW is age-specific body weight generated from distribution, kg.
- IR is age-specific intake rate of edible vegetables (uncooked), g/kg_{bw}-day
- EF is Exposure frequency of vegetable consumption, which is averaged growing season of fresh vegetables (4 months), days/year.
- ED is exposure duration based on maximum length of time for age-specific age range, years.
- AT is averaging time, which is equal to ED for non-cancer risks, days.

S2. RESULTS

The optimal extraction methods selected for each media are summarized in **Table S3** and in the main manuscript. The following results describe the outcomes of QA/QC samples, earthworm method screening, and extraction methods that were screened for plants, animal tissues, and milk and deemed unacceptable for use on that media.

Method and solvent blanks. As noted in Section S1 (Quality Control), this study employed both extraction (i.e., method) and solvent (i.e., analytical) blanks (Table S5). Through the duration of the study both method and solvent blanks using MeOH were below detection for all target analytes. DCM or reagents used in the DCM (e.g., H2O2, diamino adsorbents) led to background levels of PFAS in extraction blanks (see Table S5, DCM extraction blank, 11/9/19). No attempt was made to modify the source of these extraction reagents because this method was not selected for application to any matrix based on results of method screening described below. Background PFAS were also routinely detected in ACN extraction and solvent blanks (e.g., Table S5, ACN extraction blank 5/21/20 and solvent blank 5/21/20). The source of PFAS was isolated to the ACN (Fisher Brand, Optima LC-MS grade), so the source of the ACN was changed (Honeywell, LC-MS grade). The latter was free of background (e.g., Table S5, extraction blank, 7/26/22). The PFAS-impacted ACN was used in method screening, but only to evaluate IS recovery (i.e., Table S6, dried radish roots and shoots and dried lettuce). MS recoveries were not evaluated due to poor method performance, so no background subtraction was required. The remaining ACN extractions (e.g., milk and earthworm results, Table S6) used the Honeywell ACN and no background was detected in blanks.

Method screening: Plants. IS recoveries in plant extractions using the ACN method were outside of the target range (50-150%) for most PFAS in radish roots, shoots, and lettuce expect for $^{13}C_3$-PFBS (48.5-53.7%), $^{13}C_3$-PFHxS (71.5-82.0%), and $^{13}C_8$-PFOS (70.3-80.7%; **Table S6**). MS recoveries were not evaluated because of low IS recoveries. IS and MS recoveries using the DCM extraction varied by matrix, but $13C_4$-PFBA (16.5-36.9%), $13C_7$-PFUnA (39.5-49.9%), $13C_2$-PFDoA (2.2-10.6%), $13C_2$-PFTeDA(4.4-15.0%), $13C_8$-FOSA (0.0-4.9%), $13C_5$-PFPeA (38.4-42.6%), d_3-EtFOSAA (41.6-43.6%), $^{13}C_8$-PFOS(36.1-49.0%) and $13C_2$-8:2 FtS (29.0-41.8%) were outside of the 50-150% range for at least 2 of 3 matrices (**Table S7**). $^{13}C_5$-PFHxA (47.3±2.1%), $^{13}C_9$-PFNA (34.3±23.8%), d_5-MeFOSAA (48.0±1.0%), and $^{13}C_2$-6:2 FtS (38.6±25.6%) failed to meet IS recovery criteria in 1 of 3 tested matrices. In MS samples, PFBA (148.6-165.6%), PFTrDA (0-168%), PFBS (53.6-64.3%), PFPeS (0%), PFHxS (0-1.6%), PFDS (32.8-63.5%), FOSA (0%), MeFOSAA (0-46.0%), 6:2 FtS (0-19%), and 8:2 FtS (0-65.0%) failed to meet the target range of 70-130% in at least 2 of 3 matrices. PFHpA (133.2%), PFNA (171.8%), PFTeDA (64%, and PFHpS (65.9%) were outside of the target range for 1 of 3 tested matrices.

Method screening: Animal Tissue. Using the MTBE extraction method (**Table S8b**), IS recoveries of 8 of 19 PFAS in turkey liver were within 50-150% range, including $13C_9$-PFNA (58.0±11.0%), $13C_8$-PFOS (51.6±8.9%), $13C_8$-FOSA (56.7±8.5%), $^{d}_3$-EtFOSAA (64.1±10.8%), $^{d}_5$-MeFOSAA (75.4±12.1%), $13C_2$-4:2 FtS (69.8±12.0%), $13C_2$-6:2 FtS (117.6±17.3%), and $13C_2$-

8:2 FtS (119.3±20.5%). MS recoveries of 3 of 24 PFAS failed 70-130% range, including PFTrDA (161.4±21.2%), PFPeS (150.8±12.6%), and 6:2 FtS (200.7±24.6%).

Method screening: Earthworms. Using the MTBE method (**Table S8b**), IS recoveries for 8 of 19 PFAS in earthworm extracts were outside of the 50-150% range including $13C_5$-PFPeA (10.1±0.3%), $13C_5$-PFHxA (29.7±3.0%), $13C_4$-PFHpA (3.1±0.3%), $13C_3$-PFBS (10.9±0.8%), $13C_3$-PFHxS (19.7±1.6%), $13C_2$-4:2 FtS (7.6±2.1%), $13C_2$-6:2 FtS (156.5±35.5%), and $13C_2$-8:2 FtS (225.3±14.6%). Only 9 of 24 PFAS met the MS recovery criteria (70-130%), including PFHxA (77.0±4.1%), PFHpA (88.5±5.0%), PFOA (78.0±1.6%), PFNA (70.6±1.6%), PFDA (70.9±3.6%), PFUdA (71.7±2.5%), PFDoA (77.7±1.2%), and PFHxS (73.7±8.6%). Using the MeOH extraction method (with basic MeOH; **Table 9b**), IS recoveries of 7 of 19 PFAS in earthworm extracts passed 50-150% range, including $^{13}C_9$-PFNA (50.5±3.4%), $^{13}C_6$-PFDA (50.0±3.5%), $^{13}C_2$-PFTeDA (67.7±9.9%), $^{13}C_8$-PFOS (79.0±9.5%), d_5-MeFOSAA (93.8±12.5%), $^{13}C_2$-6:2 FtS (67.8±3.9%), and $^{13}C_2$-8:2 FtS (126.2±13.7%). MS recoveries of 9 of 24 PFAS passed 70-130% range, including PFHxA (85.9±3.7%), PFOA (78.9±6.6%), PFNA (70.2±2.8%), PFDA (73.5±3.8%), PFTrDA (70.5±11.1%), PFPeS (99.0±6.3%), PFHxS (70.0±0.0%), PFHpS (70.0±0.0%), and FOSA (74.0±8.5%).

IS recoveries for all mass labeled PFAS in the ACN method (dried extraction) were within 50-150% range except for $^{13}C_2$-4:2 FtS (15.3%) (**Table S6, Figure S1**). MS recoveries (dried extraction) were within 70-130% range except for PFTrDA (55.8%) and 4:2 FTS (39.3%) (**Table S6, Figure S1**). As a result, the ACN method was carried forward to additional optimization.

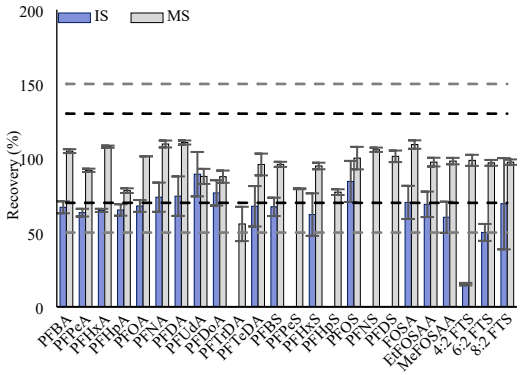

Figure S1. IS and MS recoveries in earthworm using the ACN method. Target IS recoveries were 50-150% (dashed gray lines) and target MS recoveries were 70-130% (dashed black lines). Where IS recoveries are not shown, this indicates that a mass labelled analog was not available for that PFAS.

Method screening: Milk. Using ACN+QuECHERs with the 1:9 ratio, IS recoveries of 15 of 19 PFAS passed 50-150% range except for $^{13}C_2$-PFDoA (39.7±8.1%), $^{13}C_2$-PFTeDA (219.0±40.4%), d_3-EtFOSAA (164.4±20.7%), and $^{13}C_2$-8:2 FtS (205.9±45.5%; **Table S6**). However, only 4 of 24 PFAS met the target MS recovery range of 70-130%: PFOA (85.1±2.0%), PFHxS (72.3±5.2%), PFHpS (71.6±1.2%), and EtFOSAA (70.5±2.0%; **Table S6**).

Table S6. IS and MS recoveries (%) for matrices extractions using the ACN extraction method.

See Excel file.

Table S7. IS and MS recoveries (%) for plant extractions using the DCM extraction method.
See Excel file

Table S8. IS and MS recoveries (%) for plant extractions (8a) and earthworms (8b) using the MTBE method. Unless otherwise noted, all foods were extracted at fresh weight.
See Excel file

Table S9. IS and MS recoveries (%) for animal tissues (9a) and earthworms (9b) using the MeOH method.
See Excel file

Table S10. Comparison of matrix spike recoveries in aged vs. non-aged samples using the MTBE extraction method
See Excel file

Table S11. Impacts of 0.2 μm filter, ChlorofilterTM tubes, and QuECHERs salts in IS recovery in extraction blanks and lettuce using the ACN extraction method
See Excel file

Table S12. IS recovery (200 ng/L; 12a) and MS recovery (12b) with and without Envi-carb cleanup for extraction blanks and samples prepared with the MTBE and MeOH methods
See Excel file

Table S13. Concentrations of PFAS detected in grocery store foods included in the food survey. Reported as the average and standard deviation of triplicate extractions.
See Excel file

Aged vs. Non-aged Matrix Spikes. MS recoveries were also evaluated using both aged and newly spiked materials before applying the MTBE plant extraction method for the food survey. Lettuce and radish shoots were spiked to achieve a concentration of 200 ng/L in the final, prepared sample, aged for 90 days, and extracted. High background concentrations of 8 out of 24 PFAS spiked in both radish and lettuce used for aged samples led to over recovery, even after background subtraction (**Table S10**). So, these PFAS were excluded when evaluating impacts of aging. MS recoveries of the remaining 16 PFAS at 200 ng/L in aged lettuce were 48% (PFTrDA) - 138% (PFHxA) and 10 of the 16 PFAS had recoveries within 70-130% (**Table S10**). Alternate radish and lettuce samples were acquired for the non-aged samples. The radish was free of background PFAS, but PFHxS was eliminated from consideration in non-aged lettuce due to background. MS recoveries in freshly spiked lettuce samples were 63% (PFDS) - 139% (PFTrDA) with the exception of 4:2 FtS (0%) (**Table S10**). 4:2 FtS recovery was attributable to the loss of the $^{13}C_2$-4:2 IS due to an isolated instrument issue that was not seen in other plant extractions. Of the 16 PFAS that could be compared across aged and freshly spiked lettuce samples, a similar number of PFAS had recoveries between 70 and 130% in aged (10 of 16) and non-aged (12 of 16) lettuce. In aged radish shoots, MS recoveries of 16 PFAS were 30% (PFTrDA) - 134% (PFHxA) and 4 of 16 PFAS had recoveries within 70-130% (**Table S10**). MS recoveries in non-aged radish shoots were

79% (PFTrDA) – 124% (8:2 FTS) with the exception of PFHpS (131%). Based on these results, 200 ng/L non-aged MS were used as QA/QC samples in each analytical batch for the remainder of the food survey.

Dried vs. Fresh Extractions. Evaluation of dried vs. fresh produce is described in the main manuscript, but dried vs. fresh extractions were also evaluated for earthworms and rabbit muscle tissue (**Table S6, Table S9a**). In earthworm tissues, recovery of $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, increased from less than 50% to within the target range of 50-150%. Recoveries of $^{d}_5$-MeFOSAA, $^{13}C_2$-8:2 FtS decreased from greater than 150% to within the target range. All other IS were within the target range in both wet and dry extractions with the exception of $^{13}C_2$-4:2 FtS, which was low (~15%) in both extractions. MS recoveries in earthworms were comparable in both the extraction of dry and wet earthworm tissues; however, because IS recoveries were more favorable in dried tissues and because drying tissues facilitated evaluation of the water content, the dried extractions were selected. For rabbit tissues, MS recovery was comparable in dried vs. wet samples (**Table S9a**). Of 5 IS that were outside of the range of 50-150% in wet extractions, 4 of those were within the target range in dried extractions (**Table S9a**). Recovery of one IS ($^{13}C_2$-6:2 FtS) decreased to below the target range in wet extractions, and one IS ($^{13}C_2$-8:2 FtS) was enhanced in both extracts (**Table S9a**). Overall, extraction of dried samples was favorable for rabbit muscle tissue.

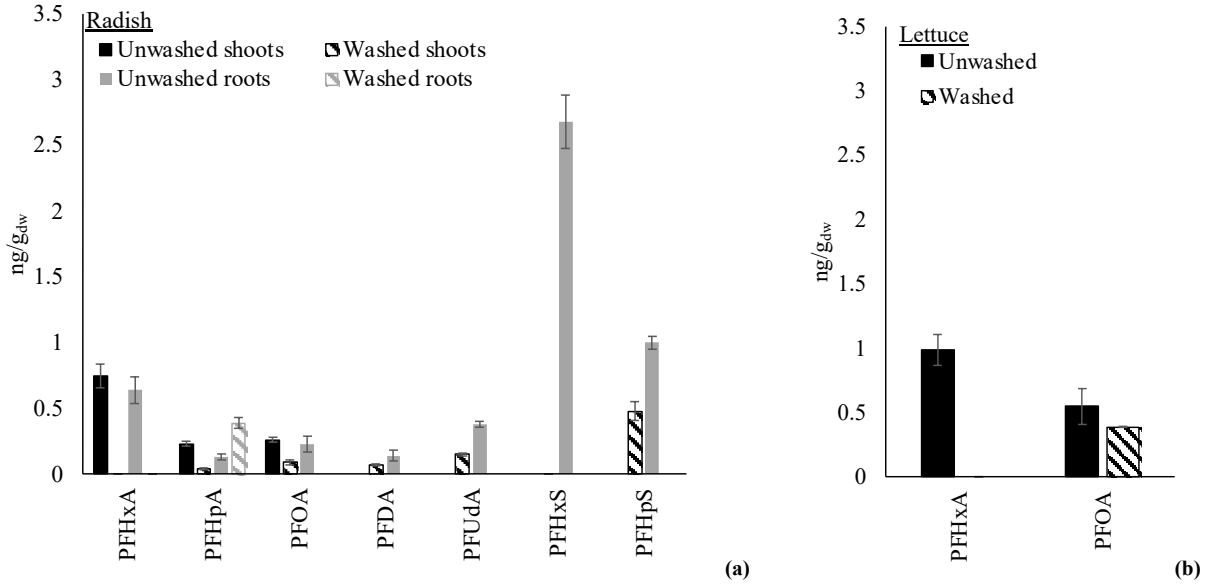

Figure S2. Concentrations (ng/g_{dw}) of PFAS in radish shoots and roots (a) and lettuce shoots (b) before and after washing.

Exposure Assessment. EI calculations required IR and BW values determined for each age group using Monte Carlo simulations, completed using the same approach as Brown et al. 2020.[39] Because the Monte Carlo simulations rely on a random number generator, the resulting 5th, 50th, and 95th percentiles of IR and BW within each age group are not identical to Brown et al. 2020, but values herein are similar (**Table S14**).

Table S14. Results of age-specific BW and IR values generated via Monte Carlo Simulation.

Distribution Parameters	BW (kg)		IR (g_{ww}/kg_{bw}-day) [ab]	
	1-2 yrs	>20 yrs	1-2 yrs	>20 yrs
5%	10	53	0	0
50%	13	77	5	3
95%	16	113	105	34
Average (This Study)	13	80	5	3
Average (Brown, 2020)	12.6	79.1	5.2	3.3
STD (This Study)	2	19	6	4
STD (Brown, 2020)	NA	NA	6.19	4.05

[a]Estimates are statistically generated based on exponential format of LN (IR).
[b]Log normal distribution is generated to avoid any negative values because of high standard deviation. Used LN($IR_{average}$), LN(IR_{STD}) to define the distribution for each population group.

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