

## Supporting Information

## Biomonitoring of Perfluoroalkyl Acids in Human Urine and Estimates of Biological

## Half-Life

Yifeng Zhang,<sup>†,‡</sup> Sanjay Beesoon,<sup>‡</sup> Lingyan Zhu,<sup>\*,†</sup> and Jonanthan W. Martin<sup>\*,‡</sup>

<sup>†</sup> Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, P.R. China

<sup>‡</sup> Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada, T6G 2G3

\*To whom correspondence may be addressed (zhuly@nankai.edu.cn and  
jon.martin@ualberta.ca)

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18    **Urinary creatinine analysis method**

19    Urinary creatinine was measured by the picric acid kinetic spectrophotometric method.<sup>1</sup>  
20    Working reagent was prepared by mixing equal volumes of picric acid and sodium hydroxide  
21    (RANDOX Laboratories Ltd. UK). The wavelength and temperature of spectrophotometer set  
22    as 492 nm and 25°C respectively. Method blank was prepared using 100 µL HPLC water and  
23    1000 µL working reagent. A three point calibration curve was prepared by mixing 100 µL of  
24    standard reagent (RANDOX Laboratories Ltd. UK) to 1000 µL working reagent. Test  
25    samples were prepared by mixing 100 µL urine and 1000 µL working reagent. Absorbances  
26    were read at 0.5 and 2.5 minutes and the difference calculated. Urinary creatinine  
27    concentrations were calculated based on the slope of the calibration curve.

28 **HPLC-MS/MS method for total and isomer-specific PFAAs and PFOSA**

29 An isomer-specific HPLC-MS/MS method developed by Benskin et al. was adapted<sup>2</sup>. Briefly,  
30 10 µL of the extracts was injected onto a FluoroSep RP Octyl column (ES Industries, West  
31 Berlin, NJ) at 38°C. The mobile phase started from 60 % A (100% water adjusted to pH 4.0  
32 with ammonium formate) and 40 % B (100% methanol) at a flow rate of 150 µl/min. The  
33 initial condition was held for 0.3 min and the gradient increased to 64 % B by 1.9 min,  
34 increased to 66 % B by 5.9 min, 70 % B by 7.9 min, 78 % B by 40 min, and finally to 100 %  
35 B by 42 min; held until 65 min; returned to initial conditions by 66 min; and the column  
36 equilibrated for a further 27 min. Mass spectral data were collected using an Applied  
37 Biosystems API 5000 (MDS Sciex, Concord, ON, Canada) equipped with an electrospray  
38 interface operating in negative ion mode. Chromatograms were recorded by multiple reaction  
39 monitoring (MRM) with 1 to 9 transitions per analyte.

40      **Table S1.** List of perfluorinated compounds monitored in the present study and their  
 41      acronyms, LC-MS/MS parent and product ions

| Nomenclature and acronyms   | Parent Ions ( <i>m/z</i> ) | Product Ions Monitored ( <i>m/z</i> )<br>quantitative ion (first one)   |
|---|----------------------------|---|
| <b>Perfluoroalkyl Acids (PFAAs)</b>   |                            |   |
| <b>Perfluoroalkyl carboxylates (PFCAs)</b>  |                            |   |
| Perfluoroheptanoate (PFHpA)   | 363                        | 319, 169  |
| Perfluorononanoate (PFNA)   | 463                        | 419, 169, 219   |
| Perfluorodecanoate (PFDA)   | 513                        | 469, 219, 269   |
| Perfluoroundecanoate (PFUnA)  | 563                        | 519, 219, 319   |
| Linear perfluoroctanoic acid ( <i>n</i> -PFOA)  |                            |   |
| Perfluoroisopropyl ( <i>iso</i> -PFOA)  |                            |   |
| 5-perfluoromethyl (5 <i>m</i> -PFOA)  | 413                        | 369 ( <i>n</i> , <i>iso</i> ), 169 (3 <i>m</i> ), 219 (5 <i>m</i> ), 119 (4 <i>m</i> ), 319, 269  |
| 4-perfluoromethyl (4 <i>m</i> -PFOA)  |                            |   |
| 3-perfluoromethyl (3 <i>m</i> -PFOA)  |                            |   |
| <b>Perfluoroalkane sulfonates (PFSAs)</b>   |                            |   |
| Perfluorohexane sulfonate (PFHxS)   | 399                        | 119, 80, 99, 169  |
| Linear perfluoroctane sulfonic acid ( <i>n</i> -PFOS)                                       |                            |   |
| Perfluoroisopropyl ( <i>iso</i> -PFOS)  |                            |   |
| 4-perfluoromethyl (4 <i>m</i> -PFOS)  | 499                        | 80 ( <i>n</i> , <i>iso</i> ), 130 (3+5 <i>m</i> , <i>m</i> <sub>2</sub> ), 330 (4 <i>m</i> ), 419 (1 <i>m</i> ), 99, 169, 230, 219, 119 |
| 3-perfluoromethyl+5-perfluoromethyl (3+5 <i>m</i> -PFOS) <sup>a</sup>                       |                            |   |
| 1-perfluoromethyl (1 <i>m</i> -PFOS)  |                            |   |
| Total dimethyls perfluoromethyl ( $\Sigma m_2$ -PFOS)                                       |                            |   |
| Perfluorooctane sulfonamide (PFOSA)   | 498                        | 78, 169   |
| <b>Internal Standards</b>   |                            |   |
| Perfluoro-1-hexane[ <sup>18</sup> O <sub>2</sub> ]sulfonate ( <sup>18</sup> O-PFHxS)        | 403                        | 103, 84   |
| Perfluoro-1-[1,2,3,4- <sup>13</sup> C <sub>4</sub> ]octanesulfonate ( <sup>13</sup> C-PFOS) | 503                        | 80, 99  |
| Perfluoro-n-[1,2- <sup>13</sup> C <sub>2</sub> ]hexanoic acid ( <sup>13</sup> C-PFHxA)      | 315                        | 270, 119  |
| Perfluoro-n-[1,2,3,4- <sup>13</sup> C <sub>4</sub> ]octanoic acid ( <sup>13</sup> C-PFOA)   | 417                        | 372   |
| Perfluoro-n-[1,2,3,4,5- <sup>13</sup> C <sub>5</sub> ]nonanoic acid ( <sup>13</sup> C-PFNA) | 468                        | 423   |
| Perfluoro-n-[1,2- <sup>13</sup> C <sub>2</sub> ]decanoic acid ( <sup>13</sup> C-PFDA)       | 515                        | 470   |
| Perfluoro-n-[1,2- <sup>13</sup> C <sub>2</sub> ]undecanoic acid ( <sup>13</sup> C-PFUnA)    | 565                        | 520   |
| Perfluoro-1-[1,2,3,4,5,6,7,8-C <sub>8</sub> ]octanesulfonamide ( <sup>13</sup> C-FOSA)      | 506                        | 78  |

42      <sup>a</sup> 3*m*- and 5*m*-PFOS isomers could not be baseline separated and thus are reported as the sum.

43 **Table S2.** Summary of the demographic information for participants

| Source of samples  | matrix      | Age group | Female            |                | Male              |                |
|--------------------|-------------|-----------|-------------------|----------------|-------------------|----------------|
|                    |             |           | Age: mean (range) | No. of samples | Age: mean (range) | No. of samples |
| Urban Shijiazhuang | Serum       | ≤50       | 47                | 1              | 33(25-42)         | 3              |
|                    |             | >50       | 66(55-78)         | 7              | 70(52-88)         | 8              |
|                    | Whole blood | ≤50       | 30(26-46)         | 7              | 26 (22-42)        | 20             |
|                    |             | >50       | 52                | 1              | 61                | 2              |
| Rural Shijiazhuang | Serum       | ≤50       | 31(22-41)         | 4              | 36(22-50)         | 4              |
|                    |             | >50       | 72(59-85)         | 7              | 61(54-68)         | 3              |
| Urban Handan       | Serum       | ≤50       | 29(21-37)         | 8              | 33(20-46)         | 5              |
|                    |             | >50       | 58(52-64)         | 4              | 62(60-64)         | 2              |
| Total              |             |           |                   | 39             |                   | 47             |

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**Table S3.** Recoveries for PFAAs and PFOSA at different concentrations with different extraction methods and LOD.

| PFAAs and<br>PFOSA            | Calf serum spiked at<br>5 ng/0.5mL |             |               | Calf whole blood spiked at<br>5 ng/0.5mL |             |               | Whole<br>blood | Synthetic urine spiked at  |       |            | Urine      |
|-------------------------------|------------------------------------|-------------|---------------|--|-------------|---------------|----------------|----------------------------|-------|------------|------------|
|                               | Mean %<br>Recovery<br>±S.D         | Range       | LOD<br>(ng/L) | Mean %<br>Recovery<br>±S.D               | Range       | LOD<br>(ng/L) |                | Mean %<br>Recovery<br>±S.D | Range | % Recovery | % Recovery |
| PFHxS                         | 119.5±19.5                         | 97.1-132.4  | 9             | 105.1±7.3                                | 96.6-109.4  | 38            | 94.3±6.7       | 88.0-101.3                 | 111.3 | 136.8      | 45         |
| <i>n</i> -PFOS                | 92.3±7.8                           | 83.7-99.0   | 111           | 127.0±13.9                               | 118.7-143.0 | 159           | 118.2±7.1      | 113.2-126.3                | 109.2 | 98.4       | 567        |
| <i>iso</i> -PFOS              | 88.7±14.2                          | 77.1-104.5  | 13            | 85.8±7.5                                 | 79.6-94.1   | 217           | 111.5±10.9     | 99.5-120.6                 | 117.0 | 86.3       | 205        |
| 1 <i>m</i> -PFOS              | 60.9±6.2                           | 53.9-65.8   | 4             | 58.9±17.6                                | 46.3-79.3   | 4             | 90.7±7.5       | 83.1-98.2                  | 89.7  | 102.0      | 36         |
| 4 <i>m</i> -PFOS              | 114.4±26.3                         | 87.5-140.0  | 1             | 94.1±14.4                                | 77.6-104.2  | 77            | 148.7±10.5     | 136.6-154.3                | 124.8 | 85.7       | 105        |
| 3+5 <i>m</i> -PFOS            | 71.4±19.4                          | 53.9-92.2   | 1             | 86.2±17.6                                | 70.1-105.0  | 154           | 113.9±13.7     | 100.7-128.0                | 117.4 | 84.8       | 110        |
| Σ <i>m</i> <sub>2</sub> -PFOS | 74.2±5.5                           | 68.3-79.3   | 1             | 77.6±15.1                                | 67.7-94.9   | 20            | 97.8±17.1      | 78.5-111.5                 | 105.9 | 83.2       | 117        |
| PFHpA                         | 99.2±28.1                          | 73.6-129.2  | 30            | 92.2±12.1                                | 80.2-104.4  | 11            | 91.6±17.5      | 80.4-111.7                 | 101.0 | 120.4      | 173        |
| PFNA                          | 103.4±10.1                         | 94.1-114.2  | 51            | 111.1±12.3                               | 98.5-123.2  | 108           | 111.4±8.0      | 102.6-118.2                | 113.6 | 119.9      | 133        |
| PFDA                          | 110.3±20.4                         | 89.7-130.5  | 8             | 108.2±3.8                                | 107.0-112.4 | 5             | 99.8±0.5       | 99.4-100.4                 | 114.1 | 135.7      | 77         |
| PFUnA                         | 144.4±23.3                         | 117.9-161.4 | 27            | 102.8±5.7                                | 96.2-106.7  | 18            | 90.4±4.9       | 84.8-94.0                  | 113.9 | 134.2      | 32         |
| <i>n</i> -PFOA                | 117.8±10.1                         | 107.3-127.5 | 34            | 114.3±3.1                                | 111.2-117.3 | 30            | 108.8±10.3     | 99.8-120.1                 | 135.3 | 114.9      | 515        |
| <i>iso</i> -PFOA              | 89.1±4.6                           | 85.7-94.4   | 6             | 105.7±13.5                               | 97.5-121.2  | 6             | 96.9±11.8      | 87.9-110.3                 | 119.4 | 93.8       | 58         |
| 3 <i>m</i> -PFOA              | 83.5±14.5                          | 69.4-98.3   | 20            | 97.8±10.6                                | 87.1-108.2  | 20            | 115.8±10.1     | 100.3-122.9                | 75.8  | 91.8       | 178        |
| 4 <i>m</i> -PFOA              | 117.3±9.2                          | 109.1-127.3 | 1             | 92.9±6.8                                 | 87.8-100.7  | 1             | 86.1±11.0      | 78.3-98.7                  | 118.4 | 96.4       | 14         |
| 5 <i>m</i> -PFOA              | 78.6±6.7                           | 72.2-85.5   | 2             | 111.7±13.2                               | 97.9-124.3  | 2             | 113.8±19.7     | 98.3-127.7                 | 126.4 | 96.5       | 18         |
| <i>n</i> -PFOSA               | 107.4±11.3                         | 95.3-117.8  | 30            | 97.1±2.4                                 | 95.0-99.6   | 3             | 112.2±1.6      | 110.4-113.5                | 112.4 | 90.2       | 3          |

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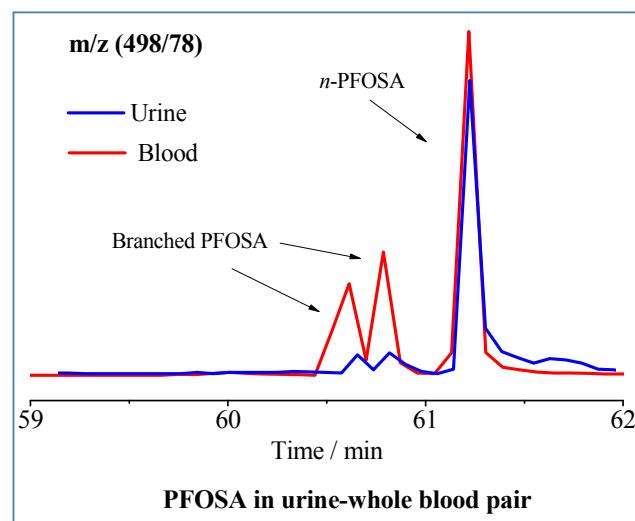
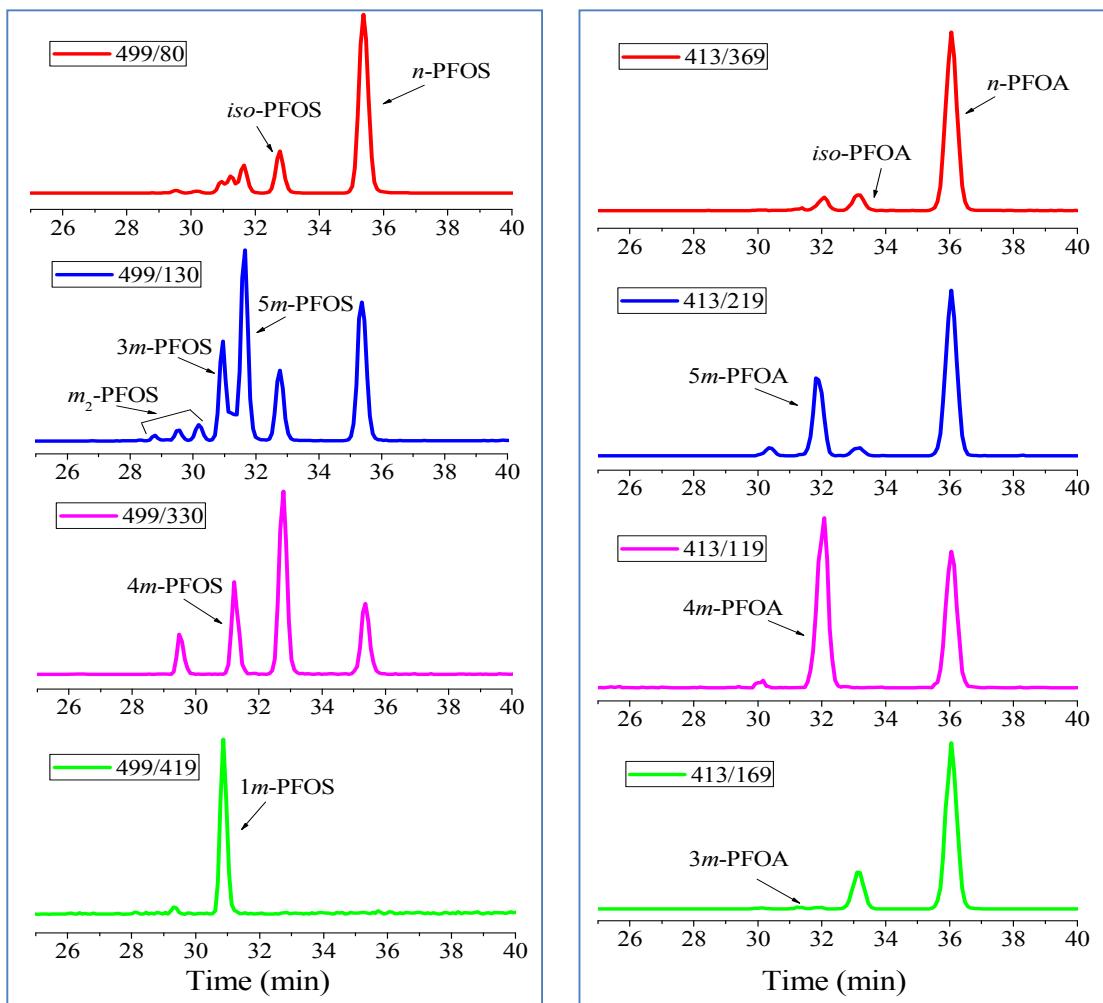
47 **Table S4.** Probability of Significant Correlations (Spearman Rank Correlation Coefficient,  
 48 and *p*-Value) between PFAA and PFOSA concentrations in urine normalized by urine volume  
 49 and urinary creatinine (R1); between PFAA and PFOSA concentrations in serum/blood and  
 50 urine normalized by urine volume (R2) and urinary creatinine (R3), respectively. The number  
 51 of blood-urine pairs that were available for analysis of Correlation Coefficient (for R2 and R3)  
 52 is shown as N.

| PFAAs and<br>PFOSA | R1          |                 | R2          |                 | R3          |                 | N  |
|--------------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|----|
|                    | Coefficient | <i>p</i> -Value | Coefficient | <i>p</i> -Value | Coefficient | <i>p</i> -Value |    |
| PFHxS              | 0.86        | < 0.001         | 0.74        | < 0.001         | 0.74        | < 0.001         | 83 |
| <i>n</i> -PFOS     | 0.71        | < 0.001         | 0.38        | < 0.001         | 0.24        | 0.025           | 86 |
| <i>iso</i> -PFOS   | 0.77        | < 0.001         | 0.46        | < 0.001         | 0.40        | < 0.001         | 86 |
| 1 <i>m</i> -PFOS   | 0.90        | < 0.001         | 0.39        | 0.001           | 0.44        | < 0.001         | 66 |
| 4 <i>m</i> -PFOS   | 0.80        | < 0.001         | 0.55        | < 0.001         | 0.54        | < 0.001         | 84 |
| 3+5 <i>m</i> -PFOS | 0.74        | < 0.001         | 0.53        | < 0.001         | 0.50        | < 0.001         | 86 |
| $\sum m_2$ -PFOS   | 0.78        | < 0.001         | 0.56        | < 0.001         | 0.40        | < 0.001         | 78 |
| $\sum$ PFOS        | 0.73        | < 0.001         | 0.43        | < 0.001         | 0.34        | 0.001           | 86 |
| PFHpA              | 0.93        | < 0.001         | 0.43        | 0.005           | 0.32        | 0.034           | 43 |
| PFNA               | 0.88        | < 0.001         | 0.24        | 0.052           | 0.21        | 0.098           | 66 |
| PFDA               | 0.86        | < 0.001         | 0.29        | 0.010           | 0.25        | 0.026           | 79 |
| PFUnA              | 0.74        | < 0.001         | 0.19        | 0.089           | 0.05        | 0.631           | 82 |
| <i>n</i> -PFOA     | 0.91        | < 0.001         | 0.32        | 0.003           | 0.18        | 0.090           | 86 |
| <i>iso</i> -PFOA   | 0.91        | < 0.001         | 0.32        | 0.010           | 0.25        | 0.048           | 62 |
| 3 <i>m</i> - PFOA  | 0.52        | < 0.001         | 0.26        | 0.279           | 0.09        | 0.706           | 19 |
| 4 <i>m</i> -PFOA   | 0.75        | < 0.001         | 0.37        | 0.003           | 0.21        | 0.098           | 61 |
| 5 <i>m</i> -PFOA   | 0.80        | < 0.001         | 0.38        | 0.002           | 0.26        | 0.039           | 63 |
| $\sum$ PFOA        | 0.90        | < 0.001         | 0.34        | 0.002           | 0.19        | 0.074           | 86 |
| <i>n</i> -PFOSA    | 0.85        | < 0.001         | 0.39        | 0.033           | 0.45        | 0.012           | 30 |

53 R1-Spearman Correlation Coefficient (urine normalized by urine volume and urinary creatinine);

54 R2-Spearman Correlation Coefficient (between blood and urine normalized by urine volume);

55 R3-Spearman Correlation Coefficient (between blood and urine normalized by urinary creatinine).

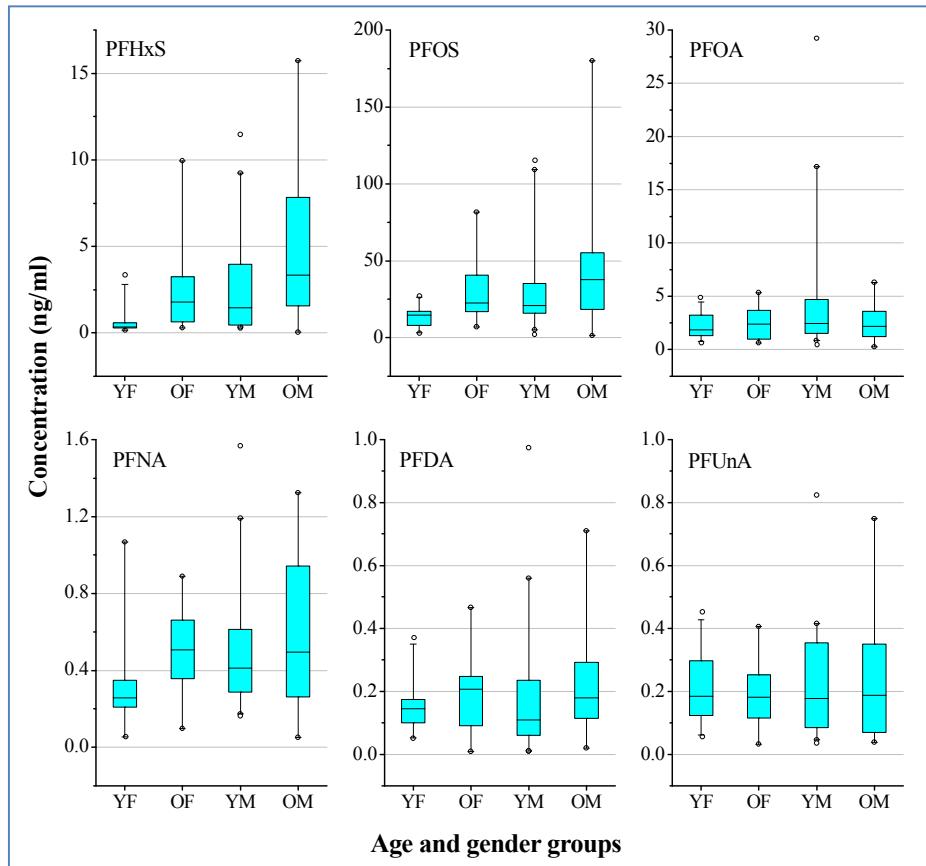


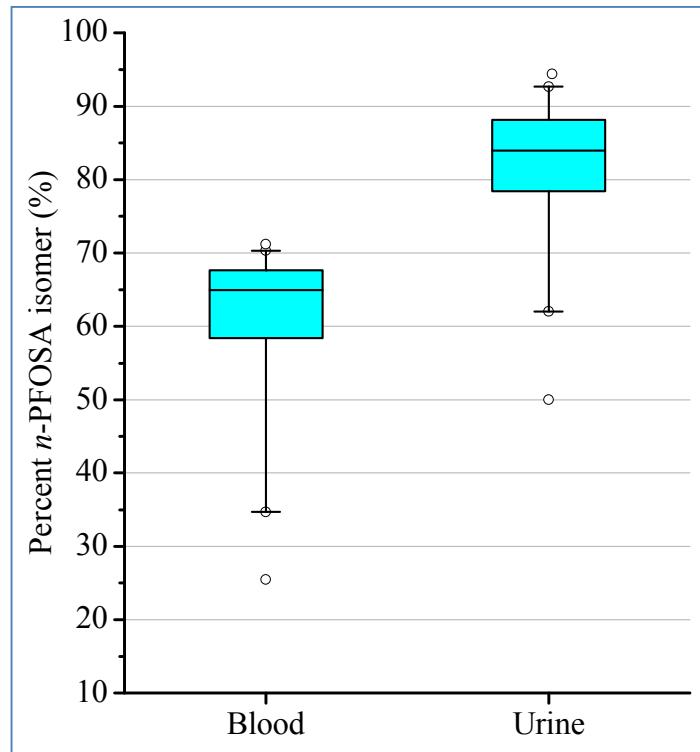
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58 **Figure S1.** Chromatogram of brPFOSK and TPFOA isomers and chromatograms of PFOSA  
59 in the No.1 paired blood and urine samples.

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61 **Figure S2.** PFAA concentrations in the blood of the four categories; young females (YF),  
 62 older females (OF), young males (YM) and older males (OM). The lower and upper bounds  
 63 of the boxes indicate the 25th and 75th percentiles, respectively. The horizontal lines within  
 64 the boxes indicate median values. The lower and upper limits of the whiskers indicate 5 %  
 65 and 95 % values, respectively, and circles below or above the whiskers indicate outlier values.





68 **Figure S3.** Percent of *n*-PFOSA peak area in total PFOSA area in the 30 paired whole  
69 blood-urine samples. The horizontal lines within the boxes indicate median values. The lower  
70 and upper limits of the circles indicate 5 % and 95 % values, respectively, and the circles  
71 below or above the whiskers indicate outlier values. Significantly higher *n*-PFOSA ratio in  
72 urine than in blood was observed.  
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74      **References**

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