Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans

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Abstract: Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans: Yukiko Fuji, et al. Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine—Objectives: Perfluoroalkyl carboxylic acids (PFCAs) consist of analogs with various carbon chain lengths. Their toxicokinetics have remained unexplored except in the case of perfluorooctanoic acid (8 carbon chemicals). This study aimed to investigate the toxicokinetics of PFCAs with six to fourteen carbon atoms (C6 to C14) in mice and humans. Methods: We applied a two-compartment model to mice administered PFCAs intravenously or by gavage. The time courses of the serum concentration and tissue distribution and elimination were evaluated for 24 hours after treatment. For human samples, urine from healthy volunteers, bile from patients who underwent biliary drainage, and cerebral spinal fluid (CSF) from brain drainage were collected. Results: The mouse experiment showed that short-chained PFCAs (C6 and C7) were rapidly eliminated in the urine, whereas long-chain PFCAs (C8 to C14) accumulated in the liver and were excreted slowly in feces. Urinary clearance of PFCAs in humans also decreased with increasing alkyl chain lengths, while biliary clearances increased. C9 to C10 had the smallest total clearance for both mice and humans. However, disparities existed in the magnitude of the total clearance between mice and humans. A slightly higher partition ratio (brain/serum) was observed for long-chained PFCAs in mice, but this was not observed in the corresponding partition ratio in humans (CSF/serum). Conclusions: The large sequestration volumes of PFCAs in the liver seem to be attributable to the liver’s large binding capacity in both species. This will be useful in evaluating PFCA bioaccumulation in other species.

Key words: Human, Mice, Perfluoroalkyl carboxylates, Perfluorooctanoic acid, Toxicokinetics

Perfluorochemicals, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid [PFOA, chemicals with eight carbon atoms (C8)], have been detected in the environment, and their toxicokinetics have been examined extensively. Their biological half-lives are significantly longer in humans than in other laboratory animal models1, 2. The reason for the longer biological half-lives in humans remains unknown.

C8 PFOA has been found to cause hepatotoxicity, developmental toxicity, immunotoxicity and endocrine disruption3. Consequently, perfluoroalkyl carboxylic acids (PFCAs) other than C8 PFOA with shorter chain lengths, such as perfluorobutanoic acid and perfluorohexanoic acid (C4 to C6), have been used for commercial applications4. These short-chained PFCAs seemed to be less toxic than C8 PFOA5, 6, possibly stemming from their relatively short half-lives compared to the C8 PFOA7, 8. In contrast, long-chained PFCAs, such as perfluorononanoic acid (PFNA, C9) and perfluorodecanoic acid (PFDA, C10), showed relatively longer half-lives than PFOA in rodents9, 10. It is well known that straight-chain PFCAs are not metabolized biologically11. Furthermore, several in vitro studies have found that biological activities are dependent on the alkyl chain length of the parent...
compounds. Nevertheless, increasing levels of long-chained PFCAs have been found in the human serum and daily diet in recent decades.

The present study aimed to investigate the toxicokinetic differences of C6 to C14 PFCAs in mice and humans. Serum concentration and tissue distribution and elimination were evaluated for 24 hours after intravenous (IV) and gavage PFCA administration in mice. Urinary clearance, biliary clearance and cerebrospinal fluid (CSF) partitions of PFCAs in humans were examined for comparison. No such comparison has ever been reported, despite its toxicological importance.

Material and Methods

Animal experiments

1) Animals. All experiments were performed with mice aged 8–10 weeks (body weight 20–30 g). FVB/NJcl mice were purchased from CLEA Japan, Inc. (Tokyo, Japan), and housed in the Kyoto University Institute of Laboratory Animals. A standard commercial lab chow diet (F-2, 3.73 kcal/g, Funahashi Farm Corp., Chiba, Japan) was used. All animals were maintained at an ambient temperature of 24 ± 2°C and 50 ± 10% humidity with a 12-h light/dark cycle (lights on at 7:00 a.m.). Mice were individually placed in metabolic cages and were provided with free access tap water and food.

2) Sample collection. Each PFCA was administered by IV or gavage. PFCAs were dissolved in ethanol/water/dimethyl sulfoxide (5:4:1) and prepared with Milli-Q water. In this study, both IV and gavage administration were applied to evaluate the absorbed ratios of PFCAs. Single doses of PFCAs were administered through the tail vein (IV dose 0.31 µmol/kg, injection volume 10 mL/kg) or orally (gavage dose 3.13 µmol/kg, injection volume 10 mL/kg). Each group contained 18 mice: 9 males and 9 females.

To observe the time course of the serum PFCAs concentrations, whole blood samples (10 µL) were collected from the tail veins at 0, 1, 3, 6, 12 and 24 hours after IV or gavage administration. An additional collection was made at 0.5 hours for IV administration. The study protocol is summarized in Table S1.

After 24 hours, urine and feces were collected in metabolic cages. Mice were then placed under sevoflurane anesthesia and euthanized by cervical dislocation. A portion of the whole blood was collected and centrifuged (370 g) to isolate the serum. Liver, kidney and brain tissues were collected and weighed. Adipose tissue was collected from the abdominal mesenteric fat. The total serum in the mice was estimated to be 56 mL/kg mouse body weight for male mice and 65 mL/kg mouse body weight for female mice

The total adipose tissue was assumed to be 2.3% of the total body weight of mice. All experimental procedures were approved by the Kyoto University Animal Research Committee (MedKyo11067).

Paired human samples: urine, bile and CSF serum pairs

All paired human samples (bile-serum, CSF-serum and urine-serum) were obtained from the archived samples in the Kyoto University Human Specimen Bank. The characteristics of the participants are summarized in Table S2. Bile samples were taken by nasobiliary drainage, percutaneous transhepatic biliary drainage or percutaneous transhepatic gallbladder drainage for 24 hours. Paired 5-ml blood samples were collected from the cubital vein into polypropylene tubes on the same day. CSF samples were taken by cerebral drainage, spinal drainage, ventriculoperitoneal shunt or duraplasty. Ten milliliters of blood was also donated from the donor on the same day. Healthy volunteers were requested to collect 24-h pooled urine samples and to donate 10 mL of blood at the end of urine collection. The research protocol was reviewed and approved by the ethics committee of Kyoto University (E25). Written informed consent was obtained from all participants before sample collection.

Determination of PFCA concentration in biological samples

1) Sample homogenization and preparation. Mouse tissue and feces were weighed and diluted with Milli-Q water/methanol (1:1) at a ratio of 15 ml water/methanol per gram of mouse tissue. The sample was homogenized using a homogenizer. Part of the homogenate (0.1–1 ml, depending on the concentration) was transferred into a 15-ml polypropylene tube. For whole blood, serum and urine samples, approximately 10–100 µL of each sample and 1 ml of methanol were placed in a 1.5 ml microcentrifuge tube and mixed for 3 hours. Part of the resulting solution (0.1–1 ml, depending on the concentration) was then transferred into a 15 ml polypropylene tube. For the human samples, approximately 0.5–30 ml of each sample was directly transferred into 15 or 50 ml polypropylene tubes.

2) Determination of PFCAs. Determination of PFCA concentrations in all samples was performed using a method previously reported. Target chemicals included perfluorohexanoic acid (PFHxa, C6), perfluoroheptanoic acid (PFHpA, C7), PFOA (C8), PFNA (C9), PFDA (C10), perfluoroundecanoic acid (PFUnDA, C11), perfluorododecanoic acid (PFDoDA, C12), perfluorotridecanoic acid (PFTrDA, C13) and perfluoro-
rotetradecanoic acid (PFTeDA, C14). Procedural blank controls were analyzed after every 10 samples. The method detection limit (MDL) was defined as the concentration that produced a signal three times that of the blank (Table S3). Total recoveries are shown in Table S4.

**Toxicokinetic analysis of PFCAs**

The ratio of PFCAs between whole blood and serum at 24 hours was used to convert PFCA concentrations in whole blood samples into serum PFCA concentrations. Serum concentration data were analyzed using a two-compartmental model described by the following equation:

\[ C(t) = C_0 \exp(-\lambda_1 \cdot t) + C_0 \exp(-\lambda_2 \cdot t). \]

To obtain \( C_1 \), \( C_2 \), \( \lambda_1 \) and \( \lambda_2 \), PFCA levels in the serum were fitted into a two-compartment toxicokinetic model by nonlinear optimization with a least-square approach\(^{21}\). The volume distribution in the IV injection study was defined as follows:

\[ \text{Volume distribution} = \frac{\text{Dose}}{C(0)}. \]

**PFCA clearance in mouse and human samples**

Mouse urinary clearance (CL\(_{u\text{-mice}}\)) was determined by dividing the total amount excreted in the urine during a 24-h period with the area under the curve (AUC) of the serum concentration of each PFCA between 0 to 24 hours. Mouse fecal clearance (CL\(_{f\text{-mice}}\)) was determined by dividing the total amount excreted in the feces during a 24-h period with the AUC of the serum concentration of each PFCA between 0 to 24 hours.

Human urinary (CL\(_{u\text{-human}}\)) and biliary clearance (CL\(_{b\text{-human}}\)) of each PFCA was determined by dividing the cumulative urine or bile excretion in a 24-h period with the serum concentration of each PFCA.

**Statistical analysis**

Concentrations lower than the detection limits were given a value half that of the detection limit for statistical analyses. Differences between mean values of each PFCA in human CSF were tested using the Student’s \( t \)-test. Values of \( p < 0.05 \) were considered statistically significant.

**Results and Discussion**

**Toxicokinetic analyses in mice after IV administration**

The ratios of whole blood to the serum concentrations of each PFCA (mean ± SD) were 0.60 ± 0.1 for C8, 0.43 ± 0.1 for C9, 0.50 ± 0.1 for C10, 0.53 ± 0.1 for C11, 0.70 ± 0.2 for C12, 0.88 ± 0.2 for C13, and 1.05 ± 0.2 for C14. The mean ratio of each chemical was multiplied by the whole blood concentrations to calculate the corresponding serum concentration.

The time course and fitted curves for PFCAs in logarithmic scale are shown in Fig. 1. As C6 was not detected in the serum at even 1 hour after administration, its serum kinetics was not analyzed. For the other PFCAs (C7 to C14), the serum levels were above the MDLs. As shown in Fig. 1, C7 disappeared from the serum in a time-dependent manner. The other compounds (C8 to C14) demonstrated very unique kinetic profiles characterized by slow elimination from the serum (Table 1). The two-compartment model successfully described the kinetics of PFCAs in mice. The parameters obtained from the serum PFCA concentrations are depicted in Table 1.

The volume distributions of the PFCAs (C7 to C14) exhibited no differences between sexes, with the volume increasing as a function of length in both males and females (Fig. S1). The distributions corresponded roughly to the total volume of blood with C7, extracellular water with C8 and C9 and body water with C11 and C12. Tissue binding was suggested for C13 and C14. These results indicated that chain length was a determining factor for volume distribution (Table 1). The AUCs reached their maximums at C8 and decreased with increasing chain length (Table 1).

Table S5 shows the tissue distribution of PFCAs 24 hours after administration. Total recoveries for all C6 to C14 were greater than 76% in males and somewhat lower in females (greater than 58%). For C6 and C7 PFCAs, almost all of the administered doses were recovered in the urine after 24 hours (101 and 99% for males, 66 and 79% for females), with only a small portion excreted in the feces (5 and 3% for males, 16 and 13% for females). In contrast, only a small portion of C8 was excreted in the urine (6% for males, 7% for females), and even less was excreted in the feces (<1% for both sexes); the majority was retained in the serum and liver (80% for males, 62% for females), with a discernible amount retained in the kidney (1% for both sexes). For C9 to C14, the distribution pattern was similar to that of C8. However, C9 to C14 excretion in the urine and feces for both males and females was much lower than that of C8; most were retained in liver (64–80% for males, 46–55% for females).

**Toxicokinetics of PFCAs in mice after gavage administration**

After gavage administration, C6 was not detected in the serum at any sampling points. Thus, a two compartment analysis was not conducted for C6. As shown in Fig. 2, the time courses for C7 to C14 were well simulated by the two-compartment toxicokinetic models with no differences in sex (Table 1). The
AUCs increased with decreasing numbers of carbon, with C8 having the largest AUC. The adjusted AUC ratios of gavage to IV administration were close to 1 for C7 to C13 and less than 1 for C14 (Table 1).

Mass balance studies indicated lower total recoveries for C6, C7 and C13 to C14 with gavage administration than those with IV administration (Table S5 and Table S6). The exact reason for those lower recoveries is not well-known. The administrated PFCAs may be distributed in some part other than the collected samples (e.g., stomach and gut wall). The overall distribution profiles of gavage administration are close to those of IV administration: most of the C8 to C14 were recovered in the liver and serum, while C6 and C7 were recovered in the urine. Only small volumes of PFCAs were excreted in feces.

Fig. 1. Simulated serum concentrations in mice after IV administration (0 to 24 hours). Values derived from Table 1. Dots indicate the observed values with IV administration.
Table 1. Elimination of PFCAs determined by the two-compartment model in mice after IV or gavage administration

\[ C(t) = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t) \]

a. Intravenous injection with a target dose of 0.313 \( \mu \text{mol/kg} \)

<table>
<thead>
<tr>
<th></th>
<th>PFHpA (C7)</th>
<th>PFOA (C8)</th>
<th>PFNA (C9)</th>
<th>PFDA (C10)</th>
<th>PFUnDA (C11)</th>
<th>PFDoDA (C12)</th>
<th>PFTDA (C13)</th>
<th>PFTeDA (C14)</th>
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<tbody>
<tr>
<td>Male</td>
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</tr>
<tr>
<td>C1</td>
<td>( \mu \text{mol } h^{-1} )</td>
<td>0.8 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>( h^{-1} )</td>
<td>1.59 ± 0.50</td>
<td>0.03 ± 0.02</td>
<td>0.18 ± 0.11</td>
<td>1.20 ± 0.37</td>
<td>0.82 ± 0.25</td>
<td>0.75 ± 0.33</td>
<td>0.62 ± 0.32</td>
</tr>
<tr>
<td>C2</td>
<td>( \mu \text{mol } h^{-1} )</td>
<td>3.8 ± 0.9</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.4 ± 0.1</td>
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</tr>
<tr>
<td>( \lambda_2 )</td>
<td>( h^{-1} )</td>
<td>0.20 ± 0.1</td>
<td>0.0001 ± 0.0001</td>
<td>0.000046 ± 0.000046</td>
<td>0.00013 ± 0.000046</td>
<td>0.000052 ± 0.000004</td>
<td>0.00035 ± 0.000004</td>
<td>0.000058 ± 0.000003</td>
</tr>
<tr>
<td>AUC of 24 hours</td>
<td>( \mu \text{mol } \text{hour} )</td>
<td>22.2 ± 8.4</td>
<td>42.2 ± 9.9</td>
<td>33.2 ± 10.3</td>
<td>24.2 ± 6.0</td>
<td>17.6 ± 3.7</td>
<td>9.5 ± 3.1</td>
<td>9.2 ± 3.1</td>
</tr>
<tr>
<td>Volume distribution ( t )</td>
<td>( 1 ) ( \text{kg} )</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.04</td>
<td>0.22 ± 0.06</td>
<td>0.25 ± 0.06</td>
<td>0.33 ± 0.06</td>
<td>0.57 ± 0.21</td>
<td>0.58 ± 0.20</td>
</tr>
</tbody>
</table>

b. Gavage administration with a target dose of 3.13 \( \mu \text{mol/kg} \)

<table>
<thead>
<tr>
<th></th>
<th>PFHpA (C7)</th>
<th>PFOA (C8)</th>
<th>PFNA (C9)</th>
<th>PFDA (C10)</th>
<th>PFUnDA (C11)</th>
<th>PFDoDA (C12)</th>
<th>PFTDA (C13)</th>
<th>PFTeDA (C14)</th>
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<tr>
<td>Male</td>
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<td></td>
</tr>
<tr>
<td>C1</td>
<td>( \mu \text{mol } h^{-1} )</td>
<td>-19 ± 2</td>
<td>-20 ± 2</td>
<td>-19 ± 3</td>
<td>-18 ± 4</td>
<td>-15 ± 4</td>
<td>-11 ± 4</td>
<td>-9 ± 3</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>( h^{-1} )</td>
<td>0.8 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>C2</td>
<td>( \mu \text{mol } h^{-1} )</td>
<td>29 ± 6</td>
<td>23 ± 4</td>
<td>20 ± 4</td>
<td>19 ± 4</td>
<td>16 ± 4</td>
<td>11 ± 4</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>( h^{-1} )</td>
<td>0.18 ± 0.06</td>
<td>0.025 ± 0.004</td>
<td>0.014 ± 0.004</td>
<td>0.021 ± 0.001</td>
<td>0.033 ± 0.001</td>
<td>0.041 ± 0.001</td>
<td>0.042 ± 0.001</td>
</tr>
<tr>
<td>AUC of 24 hours</td>
<td>( \mu \text{mol } \text{hour} )</td>
<td>141 ± 51</td>
<td>348 ± 76</td>
<td>335 ± 63</td>
<td>277 ± 44</td>
<td>170 ± 30</td>
<td>90 ± 21</td>
<td>69 ± 21</td>
</tr>
<tr>
<td>Volume distribution ( t )</td>
<td>( 1 ) ( \text{kg} )</td>
<td>0.08 ± 0.02</td>
<td>0.15 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>0.20 ± 0.05</td>
<td>0.28 ± 0.08</td>
<td>0.35 ± 0.10</td>
<td>0.43 ± 0.14</td>
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</table>

c. Ratio of dose-adjusted AUC (gavage average AUC / IV average AUC ratio, both adjusted with the administrated dose)

<table>
<thead>
<tr>
<th></th>
<th>PFHpA (C7)</th>
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<tr>
<td>PFHpA</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.7</td>
<td>0.5</td>
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<tr>
<td>Female</td>
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<td></td>
</tr>
<tr>
<td>PFHpA</td>
<td>0.9</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. a) See Eq (2).
PFOCA: perfluorooalkyl carboxylic acid.
confirming efficient absorption and enterohepatic circulation from the gut.

**Urinary and fecal clearance of PFCAs in mice**

Urinary and fecal clearance of PFCAs for both IV and gavage administration in mice are shown in Table S7. The urinary clearance of IV-administered C8 (males, 13.1 ml/day/kg; females, 9.8 ml/day/kg) was significantly smaller when compared with that of C7 (males, 336.7 ml/day/kg; females, 216.3 ml/day/kg) (Table S7). C7 had the highest fecal clearance, although the level was still smaller than the level of C7 urinary clearance, and C9 had the lowest level of fecal clearance. C7 had the greatest total clearance (males, 347.4 ml/day/kg; females, 265.7 ml/day/kg), and C10 had the lowest (males, 2.2 ml/day/
kg; females, 2.8 ml/day/kg). There were no marked differences between sexes.

Gavage-administered PFCAs had similar clearance patterns as IV-administered PFCAs. C8 had a significantly lower urinary clearance (males, 9.2 ml/day/kg; females, 6.6 ml/day/kg) than C7 (males, 248.8 ml/day/kg; females, 166.7 ml/day/kg) (Table S7). C7 had the highest fecal clearance, although it was still smaller than the urinary clearance, and C9 had the lowest fecal clearance. C7 had the highest total clearance (males, 292.5 ml/day/kg; females, 190.2 ml/day/kg), and C10 had the lowest (males, 3.9 ml/day/kg; females, 2.2 ml/day/kg).

When the fecal clearances of gavage- and IV-administered PFCAs were compared, disparities existed in the long-chain PFCAs (C13 and C14) (Table S7). The feces after 24 hours of gavage administration contained both PFCAs eliminated in the bile and unabsorbed PFCAs that passed through the gut. The actual fecal clearances of PFCAs were represented by the fecal clearances of IV-administered PFCAs. To evaluate the absorbed ratios of PFCAs, we calculated the theoretically absorbed portion using the following equation:

\[
\text{Theoretical absorbed portion (\%) = } 100 - \frac{\text{Fecal CL by gavage} - \text{Fecal CL by IV}}{\text{Fecal CL by gavage}} 
\]

Eq (3)

The results are recorded in Table S7. The theoretical absorbed portions for IV and gavage administration ranged from 94 to 104% for both males and females, suggesting that the PFCAs were efficiently absorbed in the gut.

**Toxicokinetic model evaluation**

The physiologically based pharmacokinetic model of PFOA in rats and monkeys was previously developed using chemical parameters obtained from several animal studies. For this study, we developed a simple two-compartment model based on the PFCA concentration in mouse serum. This model described the time courses of serum concentrations containing single 3.13 µmol (1.3 mg for PFOA)/kg doses via gavage administration. To evaluate this model, we applied it to the toxicokinetics of serum concentrations with repeated gavage doses of 20 mg/kg. The dose model, shown in Figure S2, using a gavage dose of 20 mg/kg was selected because single gavage doses of 40 mg/kg or more were necessary to observe any nonlinear pharmacokinetics for PFOA in mice. PFOA serum concentrations reached a steady state by about 8 days after the first dose, and the minimum and maximum serum levels were approximately 260 and 185 µg/ml, respectively, for males and 300 and 400 µg/ml, respectively, for females. A previous study showed that daily gavage doses of 20 mg/kg yielded serum PFOA concentrations of 181 µg/ml for males and 178 µg/ml for females after 7 days and 199 µg/ml for males and 171 µg/ml for females after 17 days. The simulated serum concentrations in this study revealed that males showed similar results, while females showed slightly higher results (Fig. S2). These results confirmed that repeated doses could be simulated using our simple two-compartment toxicokinetic model with only single doses of PFOA. Moreover, if the model could be applied to other PFCAs, it would be enable us to predict PFCA clearances in repeated gavage administration by using only single gavages. Second, the doses could also be scaled up from 1.3 to 20 mg/kg. Taken together, this model might be able to represent the toxicokinetics in both chronic and trace-level PFCA exposure. However, the experimental proof for this was not demonstrated in this study. Thus, our discussion at the current conditions awaits further confirmation.

Table 1 presents the numerical results of the model. The \( \lambda_2 \) values of the PFCA (≥C8) were much smaller than the \( \lambda_1 \) values for both IV and gavage administration, indicating that the PFCAs were distributed rapidly into the body tissue and might have equilibrated between the blood and tissues in their early phases. These results suggested that the first exponential equation would be negligible for long-term observations and that a one-compartment toxicokinetic model would be sufficient for predicting the toxicokinetics of PFCA (≥C8) in human serum.

**Urinary and biliary clearance in humans**

The human urinary and biliary clearances of PFCAs are shown in Table 2. The concentrations of PFCAs in human serum, bile and urine are shown in Table S8. C6 was not analyzed for clearances, as it was not detected in the human serum. Human urinary clearances for PFCA were more than 200 times smaller than those in mice and decreased with respect to chain length (Fig. 3). Biliary clearance was lowest for C9 and increased as a function of chain length for C9 to C14 PFCA (Table 2).

To calculate fecal excretion, we estimated the reabsorption rate of PFCA excreted in the bile, as PFCA is known to circulate enterohepatically and to be reabsorbed into the biliary. Assuming a volume distribution of 200 ml/kg (based on previous reported mouse experiments), a half-life of 3.8 years, and that C8 could only be excreted into the urine and feces via the bile, the reabsorption rate of bile excreting C8 was calculated as 0.98. We assumed that this reabsorption rate was applicable to the other PFCAs.
Table 2 shows the fecal clearances for PFCAs estimated from biliary clearances. If the mouse PFCA clearances by single gavage administration are comparable to those in chronic and trace level exposure (Section “Toxicokinetics model evaluation”), the estimated fecal clearances would be similarly two times smaller in humans than in mice. The total clearances (urinary plus fecal clearances) were similar between humans and mice: total clearance in humans decreased as a function of chain length for C7 to C14, and C9

Fig. 3. PFCA (perfluoroalkyl carboxylic acid) clearances in mice and humans (values are means ± SD).
Table 2. Urinary, biliary and fecal clearances of PFCAs in humans

<table>
<thead>
<tr>
<th></th>
<th>PFHpA (C7)</th>
<th>PFOA (C8)</th>
<th>PFNA (C9)</th>
<th>PFDA (C10)</th>
<th>PFUnDA (C11)</th>
<th>PFDoDA (C12)</th>
<th>PFTeDA (C13)</th>
<th>PFTdDA (C14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary clearance (Male, N=5; Female, N=5)</td>
<td>0.674 ± 0.374</td>
<td>0.044 ± 0.01</td>
<td>0.038 ± 0.01</td>
<td>0.015 ± 0.01</td>
<td>0.005 ± 0.00</td>
<td>0.005 ± 0.00</td>
<td>0.006 ± 0.01</td>
<td>&lt;MDL*</td>
</tr>
<tr>
<td>Biliary clearance (Male, N=3; Female, N=2)</td>
<td>2.62 ± 3.6</td>
<td>1.20 ± 1.2</td>
<td>2.51 ± 2.1</td>
<td>3.20 ± 3.0</td>
<td>3.72 ± 3.2</td>
<td>3.57 ± 3.3</td>
<td>11.22 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Estimated fecal clearance*</td>
<td>0.052 ± 0.05</td>
<td>0.024 ± 0.02</td>
<td>0.050 ± 0.04</td>
<td>0.060 ± 0.04</td>
<td>0.065 ± 0.04</td>
<td>0.071 ± 0.05</td>
<td>0.224 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Total clearance (Urinary clearance + Estimated fecal clearance)</td>
<td>0.674</td>
<td>0.096</td>
<td>0.062</td>
<td>0.066</td>
<td>0.065</td>
<td>0.070</td>
<td>0.077</td>
<td>0.224</td>
</tr>
</tbody>
</table>


showed the lowest total clearance (0.062 ml/day/kg) (Fig. 3). Nevertheless, the total clearances in humans were 50–100 times smaller than those in mice.

**PFCA in mouse and human central nervous systems**

PFOS and C8 PFOA are known to alter cell membrane potentials, which in turn affects channel gating properties. This suggests that PFCA may cause neurological toxicities. We have previously reported large concentration gradients of PFOS and C8 PFOA between the CSF and serum, suggesting that PFOS and C8 PFOA cannot enter the central nervous system through the blood-brain barrier. These reasons led us to evaluate the concentration gradients of PFCA between the brain and serum in mice (Table 3). The gradients generally increased with respect to chain length and were large for C8, C9 and C10 but small for C11 to C14. These results suggested that PFCA might not pass freely through the human blood-brain barrier.

In humans, the PFCA concentrations in CSF were smaller than those in the serum by two orders of magnitude (Table 3). The mean PFCA concentration in hydrocephalus patients ranged from 0.38 pg/ml to 37 pg/ml, whereas the mean concentrations in patients with cerebral hemorrhage and liquorhea ranged from 1.3 pg/ml to 70 pg/ml. The ratio of PFCA to serum was smaller for hydrocephalus patients than for patients with cerebral hemorrhage and liquorhea. It is of interest that significantly higher PFCA (C11 and C13) in the CSF were detected in patients with cerebral hemorrhage and liquorhea (p<0.05, Student’s t-test). This phenomenon may be associated with the direct infusion of serum into the central nervous system. However, generalization based on these patients only require further confirmations, as the number of patients in this study was relatively small.

**Implications of PFCA bioaccumulation**

This study clearly demonstrated that the toxicokinetics of PFCA was dichotomous: C6 and C7 PFCA were excreted rapidly from the body into the urine, while PFCA with alkyl chains longer than or equal to C8 were deposited mainly in the liver. Elimination by urine was more rapid than by the liver. Such toxicokinetic characteristics could predict whether PFCA accumulated in the body. The total clearances of C10 to C14 PFCA increased with respect to chain length, implying a link with the lipophilicity of PFCA. They were mainly cleared into the feces via the bile. Thus, C9 to C11 PFCA accumulated the most in mice. C6 and C7 PFCA, which were efficiently eliminated through the urine, had significantly shorter half-lives than the other longer-chain PFCA.

The biochemical mechanisms that cause bioaccumulation as a function of chain length are not well understood. Our study indicated that the volume distribution of PFCA increased with increasing chain length, which might be linked with the lipophilicity of PFCA. In addition to this, our study indicated that longer-chain PFCA had an increasing affinity for serum and liver fatty acid binding proteins. Our interpretation was supported by previous studies showing short-chain PFCA binding modestly to avian serum proteins and binding increasing with longer chains. These results suggest that unbound C6 and C7 PFCA were excreted by glomerular filtration in the kidney, while the ≥C8 PFCA were mainly eliminated by bile in the liver. At the same time, long alkyl-chain PFCA (≥C9) may accumulate preferentially in the liver because of their high affinity for liver fatty acid binding proteins. It is already a known fact that the binding affinity of PFCA increases with longer alkyl chains. Further studies are required to understand the large PFCA (≥C8) depositions in the liver.

**Implications for species difference**

This study reports the toxicokinetic profile of PFCA with 9 different carbon chain lengths in humans and mice. We found that the total clearances (urinary plus fecal clearances) were similar between the two species despite large magnitude differences.
### Table 3. Concentration gradients of PFCAs between the serum and brain in mice and the serum and CSF in humans

<table>
<thead>
<tr>
<th>Compound (carbon atoms)</th>
<th>Brain tissue concentration (pmol/g)</th>
<th>Brain /serum ratio</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHpA (C7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFOA (C8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFNA (C9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFDA (C10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFUnDA (C11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFTrDA (C12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFTeDA (C13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFDoDA (C14)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Mouse experiment samples (male, N=9; female, N=9)

- **Disease:**
  - Total
  - Hydrocephalus (male, N=2; female, N=1)
  - Cerebral hemorrhage and liquorrhoea (male, N=2; female, N=1)

#### Human samples (male, N=6; female, N=1)

- **Disease:**
  - Hydrocephalus (male, N=4; female, N=1)

**Values are means ± SD. a) Brain tissues were collected 24 hours after IV injection (IV dose: 0.313 µmol/kg). b) Significant difference between the CSF/serum ratios (p<0.05).**

### Differences in the PFCA elimination rates between species have remained unclear. An epidemiological study of retired workers from a C8 PFOA production plant operated by 3M revealed that the serum elimination half-life was 3.8 years. Another study found that the serum elimination half-life of C8 PFOA was much shorter in mice (15–20 days), rats (<1–15 days) and Cynomolgus monkeys (20–35 days).

The reason for the extremely long half-life of PFCAs in humans is still not well understood. PFCAs are not metabolized biologically, thus these differences in half-lives are likely due to the difference in clearances. In the current study, the long half-lives of the PFCAs in humans were attributed to poor elimination from the kidney. Renal clearance of PFOA was reduced by probenecid, which decreases organic acid excretion in the urine, suggesting that organic anion transporters may have some important role for PFCA excretion. Other previous experiments studying transporters in rats and mice in vitro have also suggested that organic anion transporters in the proximal kidney tubules might be responsible. Indeed, the urinary clearances of C7 and C8 in mice in this study were 500 and 300 times larger than those in humans, respectively. Thus, the species differences are mainly due to the difference in urinary clearances. However, the fecal clearances also differed by one order of magnitude, indicating the other membrane transporters in the liver may also be involved. Furthermore, a slightly higher partition coefficient was observed for long-chain PFCAs in mice, but this was not observed in the corresponding partition ratio in humans (CSF/serum). PFCAs in the human CSF ranged from 1.0 to 2.5% of those in serum. This result indicated a species difference in the brain concentrations of PFCAs between human and mice. A previous study revealed that the blood-brain barrier in both humans and mice is maintained by several organic anion transporters, such as organic anion transporter 3, which may actively transport PFCAs from the CSF into the serum. However, there are some species differences in substance selectivity between mouse and human organic anion transporter 3. The species difference in this study may be explained by a difference in substrate selectivity of the transporters between humans and mice.

### Limitations of this study

This study had several limitations. First, the PFCA toxicokinetic model was for short-term observations. Nevertheless, our model could simulate both single and repeated doses and scale up C8 doses (Section “Toxicokinetic model evolution”). It is unknown whether the model is applicable to other PFCAs and in accordance with actual experimental data from
chronic and trace-level administration. This warrants further investigation. Second, several parameters, such as the rate of PFCA reabsorption into the human enterohepatic circulation and the serum to CSF ratio in mice, were estimated, and this therefore increases the uncertainty in the current study. Third, the number of human samples was relatively small, which may prevent generalization based on the current results.

Conclusion
The present study was a comprehensive toxicokinetics study of PFCAs in mice and humans. The highlight of this study was evaluation of various PFCAs of different alkyl chain lengths in mice and humans based on biological residual potency. The large deposition of PFCAs (≥C8) may suggest that fatty acid binding proteins in the liver have an important role for PFCA bioaccumulation. In addition, a simple two-compartment toxicokinetic model for PFOA was shown to simulate serum concentrations by both single or repeated dosing and small or large doses. This information will be useful for evaluating bioaccumulation of PFCAs in a variety of species.

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References
14) Upham BL, Deocampo ND, Wurl B, Trosko JE. Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int J Cancer 1998; 78: 491–5.


