# THE EFFECT OF ONGOING BLOOD LOSS ON HUMAN SERUM CONCENTRATIONS OF PERFLUORINATED ACIDS

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## Introduction

Perfluorinated alkyl acids (PFAAs) have been detected at low concentrations in human serum from the general population of numerous countries. Often higher concentrations are observed in adult males relative to females, with a possible explanation that menstruation offers females an additional elimination route. In this study, we examined the significance of blood loss as an elimination route of PFAAs in humans, thus indirectly investigating the need for hypotheses other than menstruation to explain the gender differences observed in humans. We used both data and a simple pharmacokinetic (PK) model in this study. The data included blood measurements of PFAAs from males who had undergone regular blood removal as part of a medical condition, and also on male and female adults of the general population. The model was a simple, 1st order, 1-compartment PK model

## Methods

Pooled serum samples were collected from individuals undergoing regular blood withdrawals (termed venesections) for a medical condition and compared them to pooled samples from the general population. The pooling of 151 samples from venesection patients resulted in a total of 33 composite samples (23 male, 10 female), ages 33 to 87. The samples were taken once the full treatment of venesections had been completed. The grouping into composites considered sex, age (> or <= 60 years), number of venesections (< or > 10), and time since last venesection (> or <= 365 days). They were measured for PFOS, PFOA, PFNA, PFDA, and PFHxS. Each pool consisted of 6 individual samples, contributing 0.2 mL each. To provide a general population 'control' group for comparison, pooled serum samples collected from individuals living in South East Queensland[1] were used.

A simple pharmacokinetic model was applied to further study the effect of on-going blood loss on serum PFC concentrations. The model was applied to both PFOA and PFOS in males undergoing venesections, and to PFOA in the study of regular menstrual blood loss in women. These compounds were chosen because they were the most frequently detected compounds, and the necessary model parameters were available (i.e. intake estimate, elimination rate, volume of distribution). The pharmacokinetic model is a simple 1-compartment, first order model previously used to study accumulation of PFOA and PFOS in the general population in Australia[2]. At steady state, the simple 1-compartment model can be solved for concentration as, $C(ss) = [ D / (k * Vd) ]$, where C(ss) is the steady state concentration of a given PFC in the serum (ng/mL), D is the constant daily absorbed intake dose (ng/kg-day), k is if the first order elimination rate (day-1), and Vd is the volume of distribution (mL/kg). Values of 170 mL/kg and 230 mL/kg were assigned for the Vd's of PFOA and PFOS respectively, and elimination rate constants (k) of 0.0008 day-1 for PFOA and 0.0003 day-1 for PFOS were used[2]. The model was coded onto an Excel spreadsheet, and run on monthly time intervals. Mass of PFCs was maintained and concentrations, when needed, were calculated as the mass divided by Vd. Intakes included background exposure intakes and elimination was modeled as a first order process. The losses due to venesection and menstruation were modeled as discrete events. Given the general male population steady state blood concentrations of 5.9 ng/mL for PFOA and 16.6 ng/mL for PFOS, a steady state

intake, D, of 0.80 ng/kg-day for PFOA and 1.15 ng/kg-day for PFOS in males was derived. These steady state blood concentrations were also used to derive the initial mass reservoirs of PFOA and PFOS.

A single simulation was run for each of the 23 composite samples representing male venesections. For each composite the average number of venesections, interval times between each, and time from last venesection and sample were used in the simulation. Each venesection involved the withdrawal of 450 mL whole blood, or 225 mL of serum (assuming serum is one-half the volume of whole blood). Given that Vd is not equivalent to the volume of circulating blood, but rather a calibrated dilution reservoir that leads to an accurate prediction of PFOS or PFOA in blood[2] , venesection events were assumed to remove 9% (equal to the percentage ratio of 225 mL removed over a whole body reservoir of 2500 mL serum) of mass of PFOA or PFOS within the blood reservoir. The impact of menstruation was modeled with a similar strategy. The simulation began at a steady state background concentration derived for males at 5.9 ng/mL of PFOA. Then, regular monthly withdrawals by menstruation were modeled and after several years, the body burden equilibrated to a new steady state. Each month 0.7% (derived as half of an average monthly menstrual loss of 35 mL divided by whole body volume of 2500 mL serum) of the mass reservoir was removed. The question is how close was this new steady state to the background concentration of 4.5 ng/mL seen in the adult female population.

**Results**

Five of the investigated PFCs were consistently detected in the samples, PFOA, PFNA, PFDA, PFHxS and PFOS (Table 1). The results were consistent with the notion that blood loss will lower body burdens of PFCs. In general the male venesection patients had serum concentrations 30 – 50% less than males of the general public, both in terms of the data from pooled samples collected from the general public in the same year (Toms and Mueller 2010). For PFOS and PFOA, for example, the overall weighted average concentration in the pools of the general population males was 16.6 and 5.9 ng/mL respectively, while they were 11.2 and 3.4 ng/mL respectively, in the venesection subpopulation. This equates to a difference of 33% and 42% for PFOS and PFOA respectively. The difference between the female venesection patients and the corresponding general population was less pronounced. The overall weighted average of PFOS and PFOA for all females was 12.5 and 4.5 ng/mL, respectively, while it was 10.7 and 3.7 ng/mL, respectively, in the venesection patients. This equates to a difference of 14% and 18% for PFOS and PFOA respectively. Comparison of males and females in the general public population reinforces the typical gender differences described in the introduction. The female pools had lower concentrations than the males by 25% and 24% for PFOS and PFOA respectively. In contrast the gender difference was inconsistent when comparing male and female venesection patients. In the case of PFOS, the mean concentration in female patients was about 4% lower than in the males whereas for PFOA, the concentration in female patients was on average 9% higher than in their male counterparts. The only detected PFC which wasn't consistently lower in the venesection population was PFDA, which gave average results in two groups of male patients equivalent to the concentrations measured in the general public, 0.2 ng/mL each. This is potentially due to the concentrations of this compound being closer to the limits of quantification, and so further detail is obscured by an inability to measure with greater accuracy. Another explanation may be that the serum half-life for PFDA is shorter than that of other PFCs, making elimination via venesection less relevant.

The venesection modeling supports the hypothesis that blood loss is mostly (if not fully) responsible for the difference in PFOA and PFOS concentrations in the male venesection patients. The average PFOA concentration over the 23 simulations was 3.7 ng/mL, representing a decline from the initial assumed concentration of 5.9 ng/mL. The observed weighted average concentration in the venesection patients was 3.4 ng/mL. Hence for PFOA the model is in very good agreement with the mean of the measured data. For PFOS, the model predicts a concentration drop from 16.6 ng/mL serum to 7.9 ng/mL in the venesection group, where the mean measured concentration was substantially higher at 11.2 ng/mL. Graphs of measured and modeled data, displayed along with the corresponding averages are provided in Figures 1 and 2. As seen in those figures, there appeared to be a reasonable correlation

**Table 1 Mean concentrations of PFCs in venesection and general populations.**

| Analyte | Males, venesection | | Males, general population[2] | | Females, venesection | | Females, general population[2] | |
|---|---|---|---|---|---|---|---|---|
| | n[1] | ng/mL[3] | n | ng/mL | n[1] | ng/mL | n | ng/mL |
| PFOA | 23 | 3.4 | 12 | 5.9 | 10 | 3.6 | 12 | 4.5 |
| PFOS | 23 | 11.2 | 12 | 16.6 | 10 | 9.8 | 12 | 12.5 |
| PFDA | 23 | 0.3 | 12 | 0.3 | 10 | 0.3 | 12 | 0.3 |
| PFNA | 23 | 0.6 | 12 | 1.1 | 10 | 0.6 | 12 | 1.2 |
| PFHxS | 23 | 2.3 | 12 | 3.0 | 10 | 1.5 | 12 | 2.3 |

[1]n is the number of composite venesection samples; each composite is comprised of 6 individual samples; [2]from Toms and Mueller (2010), each sample is a pool of 100 donors. [3]detection limits: PFOA: 0.17 ng/mL; PFOS: 0.48 ng/mL; PFDA: 0.07 ng/mL; PFNA: 0.51 ng/mL; PFHxS: 0.48 ng/mL

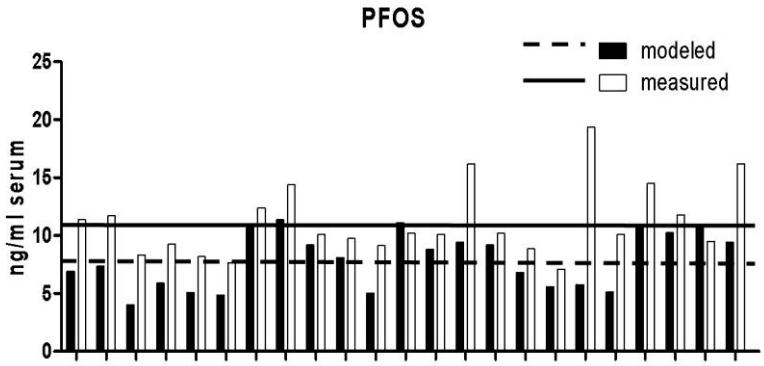



**Figure 1** Measured and modeled concentrations of PFOS (ng/mL in serum) across all 23 composite samples of male venesection patients, with horizontal lines representing average values for both

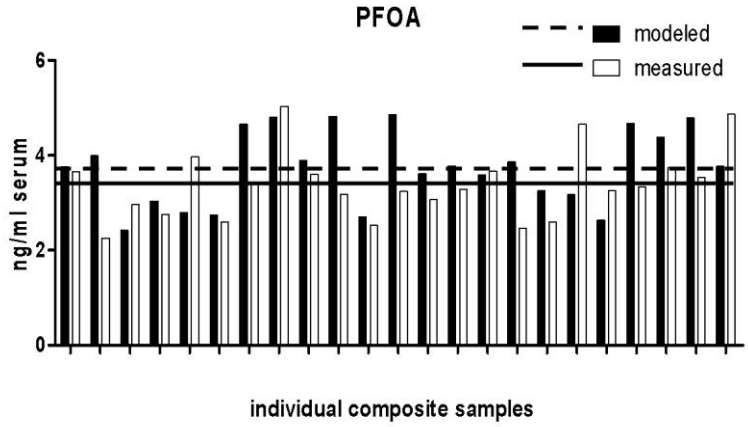


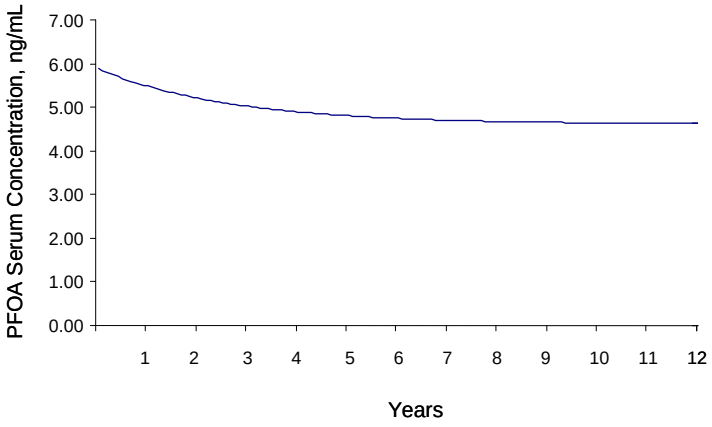

**Figure 2** Measured and modeled concentrations of PFOA (ng/mL in serum) across all 23 composite samples of male venesection patients

between measured and modeled serum concentrations, although there was up to a 2 ng/mL difference in several individuals for PFOA and up to a 15 ng/mL difference in one individual for PFOS. Overall, the correlation coefficient for PFOA, for observed versus predicted was 0.256 ($R^2$ = 0.065, Pearson correlation), and for PFOS, it was 0.403, ($R^2$ = 0.162) which improved to 0.643 ($R^2$ = 0.4133) upon removal of the one outlier with a measured

serum concentration >19 ng/ml. Although the model agreement was poorer for PFOS, overall, the modeling results suggest that the lower concentrations seen in venesection patients can be explained to a large part through the loss of PFCs via loss of blood during venesection.

The simulation of menstruation is shown in Figure 3. The body burden begins at 5.9 ng/mL, the presumption of what it might be without the menstrual loss. It is seen that steady state is regained after about 8 years at a new lower body burden of 4.6 ng/mL. This is in remarkably good agreement with the observed mean body burden in women of the general population of 4.5 ng/mL. It provides supportive evidence for the finding that menstrual losses alone could explain the difference in male and female body burdens. With this agreement, one can surmise that background intakes between men and women are the same, and that women's body burdens are lower because of menstruation. Then, one can return to the steady state equation and plug in values for C(ss) of 4.5 ng/mL, 170 mL/kg for Vd, and 0.8 ng/kg-day, in order to solve for a first-order elimination rate that would be applicable to women, in a manner of speaking. The k is solved as 0.001 $day^{-1}$, which is higher (meaning more rapid elimination) than the value of 0.0008 $day^{-1}$ used in the exercises above. Without this additional simulation of physical loss by menstruation, one might hypothesize that intake by women is less than men and instead use the steady state equation to derive a different "intake" for women versus men. In that case, the C(ss) is again 4.5 ng/mL, the Vd is 170 mL/kg, and the elimination rate is 0.0008 $day^{-1}$. The intake for women might mistakenly be calculated as 0.6 ng/kg-day, lower than the male intake modeled to be 0.8 ng/kg-day.

**Figure 3** Modeled reductions in PFOA serum concentration due to menstruation.

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**References**

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