DOCUMENT 30

Evaluation of the Half-life ($T_{1/2}$) of Elimination of Perfluorooctanesulfonate (PFOS), Perfluorohexanesulfonate (PFHS) and Perfluorooctanoate (PFOA) from Human Serum

G. Olsen,[1] D. Ehresman,[1] J. Froehlich,[2] J. Burris,[1] J. Butenhoff[1] [1]3M Company, St. Paul, MN; [2]Pace Analytical Services, Inc., St. Paul, MN

Abstract

PFOS is well-absorbed orally and very slowly eliminated from the body, and these combined properties can result in the accumulation of PFOS body burden from various sources and pathways of exposure. Elimination half-lives after i.v. injection in rats and monkeys are currently estimated to be in the range of 100 days to 150 days. Enterohepatic circulation likely plays a predominant role in the long elimination half-life of PFOS. Serum elimination half-lives for PFHS in cynomolgus monkeys have been estimated at approximately two-thirds less than PFOS, and limited data in rats also suggests a shorter elimination. Marked sex and species differences occur in the elimination of PFOA. Urine is the primary route of excretion for PFOA. The elimination half-life in male rats is 4-6 days and 2-4 hours in females, and is approximately 21 and 30 days in male and female monkeys, respectively. Sex hormones may modulate differential expression of organic anion transporters involved in the urine elimination of PFOA in rats.

To investigate the half-life of serum elimination in humans of PFOS, PFHS and PFOA, 27 retirees (25 males, 2 females) from two fluorochemical manufacturing plants were followed for up to 5.5 years with periodic blood collections. One retiree's samples were excluded due to the likelihood of occupational exposure during follow-up. The analyses used a primary extraction in combination with an alkaline back extraction technique. A 5 µL injection was introduced to the mass spectrometer through a high performance liquid chromatography system. All quantitative calculations for PFOS and PFHS were based on the ion ratios between PFOS or PFHS and the internal standard (dual substituted $^{18}O_2$-PFOS). All quantitative calculations for PFOA were based on the ion ratios between PFOA and the internal standard (dual substituted $^{13}C_2$-PFOA). Individual serum elimination rates were calculated with Pharsight WinNonlin® software.

Initial serum concentrations for the 26 subjects ranged between 150 – 3490 ng/mL for PFOS, 20 – 1300 ng/mL for PFHS and 70 – 5100 ng/mL for PFOA. The mean half-lives of serum elimination for PFOS, PFHS and PFOA were 5.4 years (95% CI 3.9 – 6.9), 8.8 years (95% CI 6.7 – 10.9) and 3.8 years (95% CI 3.1 – 4.4), respectively. The half-life of serum elimination for each fluorochemical was not associated with initial concentration, age or sex of retiree, years worked at the manufacturing facility or the time between retirement and first blood collection.

Introduction/Objective

In the general population, perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHS) and perfluorooctanoate (PFOA) have been measured in the serum of adults in the US with geometric means approximating 35 ng/mL, 2 ng/mL and 5 ng/mL, respectively (Olsen *et al.* 2003). The highest upper bound estimate of the geometric mean concentration of these three fluorochemicals at the 95th percentile were 100 ng/mL, 11 ng/mL and 14 ng/mL, respectively. The source(s) of exposure have not been definitively determined in the general population but would include both environmental as well as consumer-related exposures. For example, exposure possibilities for PFOA include it as a possible by-product in perfluorooctanesulfonyl fluoride (POSF) production and materials (Olsen *et al.* 2003), exposure to nonrelated fluorochemical consumer products (Moriwaki *et al.* 2003) and the degradation of fluorotelomer alcohols in sediment, water and atmospheric environments (Martin *et al.* 2004).

PFOS is well-absorbed orally and very slowly eliminated from the body, and these combined properties can result in the accumulation of PFOS body burden from various sources and pathways of exposure (3M Company 2003). The serum terminal half-life of PFOS ranged from 122 to 146 days (mean 132 days) in male cynomolgus monkeys given an intravenous administration (i.v.) bolus dose of 2 mg/kg of PFOS potassium salt and from 88 to 138 days (mean 110 days) in female monkeys. In both rats and monkeys, small and frequent external doses of PFOS or precursor chemicals above a threshold would be expected to result in an accumulation of PFOS body burden as reflected by serum PFOS concentration. PFOS is not metabolized in any of the species studied, although it can be formed metabolically from perfluorooctanesulfonyl-based precursors. PFOS does not preferentially distribute to fatty tissue, preferring instead to associate with proteins in blood and liver.

Unlike PFOS, there is substantial variation in the elimination rate of PFOA from the serum in different species as well as between sexes within some species (Butenhoff *et al.* (2004a, 2004b)). Upon oral or i.v. of experimental doses, female rats have serum elimination half-life of hours compared to several days in male rats (Hanhijarvi *et al.* 1982; Kemper 2003; Kojo *et al.* 1986; Kudo *et al.* 2001; Ophaug and Singer 1980; Vanden Heuvel *et al.* 1991; Ylinen *et al.* 1989). In dogs, the half-life of elimination in plasma following a single i.v. dose in two female dogs (202 hours and 305 hours) was cleared twice as quickly than in two male dogs (473 and 541 hours) (Hanhijarvi *et al.* 1988).

Based on repeat dose oral studies in male cynomolgus monkeys and i.v. studies with PFOA in male and female cynomolgus monkeys, the serum elimination half-life was approximately 14-42 days (Butenhoff *et al.* 2002, 2004b). Unlike rats, a distinct difference in clearance by sex was not observed in the monkeys. In the i.v. study, three monkeys per sex were given a single dose of 10 mg/kg PFOA (potassium salt). Terminal half-life of elimination in the serum was 13.6, 13.7 and 35.3 days in the three male monkeys and 26.8, 29.3 and 41.7 days in the three female monkeys. Volume of distribution at steady state was 181 ± 12 and 198 ± 69 mL/kg for males and females, respectively, which suggests distribution primarily in extra-cellular space. Urinary excretion of PFOA was slow in both sexes.

Introduction (Cont.)

Serum elimination half-life data from humans are sparse. The purpose of the present study was to more accurately determine the elimination half-life ($T_{1/2}$) of PFOS, PFOA and PFHS from human serum through the long-term evaluation of a group of retired fluorochemical production workers who were no longer occupationally exposed. This study was not designed to address pharmacokinetic parameters other than serum elimination half-life of these three fluorochemicals from serum.

Methods

Analytical Method Summary

Primary Extraction (Acid pH):
Add: Internal standard ($^{13}C_2$-PFOA and/or $^{18}O_2$-PFOS) to all tubes
250 uL serum
300 uL of 1N formic acid
300 uL of saturated ammonium sulfate, vortex
5.0 mL of acetonitrile: shake 30 minutes
Centrifuge at 2500 x *g* for 5 minutes
Decant acetonitrile, remove organic using LabConCo® vacuum evaporator

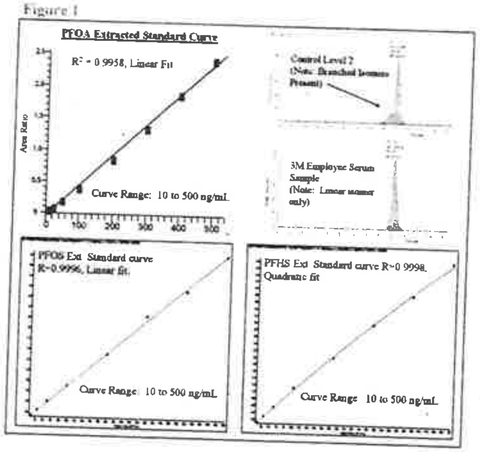
Back Extraction (Alkaline pH):
Add: 300 uL of Milli-Q purified H_2O
300 uL of 1.0 N KOH, vortex
7.0 mL of Methyl Tert-Butyl Ether (MTBE), shake 20 minutes
Centrifuge at 2500 x *g* for 5 minutes
Transfer MTBE. Dry to dryness with N_2 "N-Evap" drier
Add: 200 uL of 50 % acetonitrile / 50 % 10 mM ammonium acetate
Inject: LC-MS (PFOA) or LC-MS-MS (PFOS and PFHS) analysis

PFOA LC-MS Analysis (Finnigan TSQ-7000):
Column: MacMod ACE C-18 (100 x 2.1 mm id), 5 µm particle size; 3 µm guard column (10 x 2.1 mm id)
Flow Rate: Isocratic (0.25 mL/min); Optimized for separation of branched chain isomers; Acetonitrile / 10 mM ammonium acetate (~ 50 / 50 mixture)
Ions Monitored: PFOA - 413 amu; $^{13}C_2$-PFOA - 415 amu

PFOS and PFHS LC-MS-MS Analysis (Applied Biosystems® API4000):
Column: Same as above
Flow Rate: Isocratic (0.35 mL/min); Acetonitrile / 10 mM ammonium acetate (~ 50 / 50 mixture)
Transitions Monitored:
PFOS - 499 → 80 amu*; 499 → 99 amu
PFHS - 399 → 80 amu*; 399 → 99 amu
$^{18}O_2$-PFOS - 503 → 84 amu*; 503 → 103 amu

*Used for quantitation.

Figure 1



Methods (Cont.)

Extraction techniques employed were identical for both PFOA and the combined PFOS-PFHS extractions. Different internal standards were used for the two analyses ($^{13}C_2$-PFOA for PFOA extraction or $^{18}O_2$-PFOS for the combined PFOS and PFHS extraction). PFOA analysis was completed first using a Finnigan® TSQ-7000 mass spectrometer operating in Q1 (parent ion) mode. PFHS and PFOS analyses were completed simultaneously using an Applied Biosystems® API4000 mass spectrometer operating in MRM (product ion) mode. PFOS and PFHS extracts were re-evaluated using the Finnigan® TSQ-7000 (parent ion) for both validation of the new instrument and to justify choosing the product ion at 80 amu for quantitation.

PFOS and PFHS each form product ions at 80 and 99 amu, and both product ions were monitored during this study. Quantitation was based on the area ratio between the 80 amu product ion formed by the analytes and the internal standard product ion formed at 84 amu.

Twenty-four retirees from the 3M Decatur, Alabama facility and three retirees from the Cottage Grove, Minnesota facility participated in the study. Retirees were the population of choice because they did not have occupational exposure but had serum PFOA, PFHS and PFOS concentrations higher than the general population. This minimized any influence that nonoccupational sources of exposure might have had on the determination of the elimination rate.

Blood collection (approximately 10 mL/collection) occurred between November 1998 and March 2004 for the 24 Decatur retirees and June 1999 and March 2004 for the 3 Cottage Grove retirees. A brief questionnaire was administered to the study subjects at the time of each blood collection except for the initial November 1998 visit. Questions pertained to medication use and health conditions experienced at the time of the blood collection. Participants were also asked whether they had performed any contractual work for 3M in the fluorochemical production facilities since retirement. One retiree was excluded from the data analysis because of repeated contractual work.

At the time of the initial blood collection, the mean age of the 26 subjects was 61 years (range 55 – 75). Their mean years worked was 31 (range 20 – 36 years), and they had been retired, on average, 2.6 years (range 0.4 – 11.5 years). Two subjects died during the study, a fact that limited each of their length of follow-up to 4.2 years.

Version 4.1 WinNonlin® software (Pharsight Corporation, Mountain View, CA) was used to calculate the half-life elimination. The data yielded a straight line when plotted as the logarithm of serum concentration versus time (first order).

This study was reviewed and approved by the 3M Company IRB.

Results

Individual half-life of serum elimination results (years) are provided in Figure 2. Subject #1 did not have PFHS analyzed, due to low concentrations in multiple samples. Initial mean serum fluorochemical concentrations are shown in Table 1.

Mean half-life of elimination (years) were 5.4, 8.8 and 3.8, respectively (Table 2). Removal of two subjects' data as possible outliers (subject #25 for PFHS and subject #26 for PFOS) to see the effect of reducing the variance of this estimate, resulted in substantially narrower confidence intervals for PFOS (see lower half of Table 2).

The half-life of serum elimination was not associated with initial fluorochemical concentrations, age or sex of retiree, years worked at the manufacturing facility, or years since retirement.

Table 1

Initial Mean Serum Fluorochemical Concentrations (ng/mL)

Fluorochemical	Mean	95% CI	Median	Range
PFOS	799	531 - 1067	626	145 - 3490
PFHS	290	174 - 407	193	16 - 1295
PFOA	691	284 - 1099	408	72 - 5100

Table 2

Mean, 95% CI, Median and Range of Half-life of Elimination (years) from Serum for PFOS, PFHS and PFOA

Fluorochemical	Mean	95% CI	Median	Range
PFOS	5.4	3.9-6.9	4.6	2.4-21.7
PFHS	8.8	6.7-10.9	7.7	2.8-27.0
PFOA	3.8	3.1-4.4	3.5	1.5-9.1

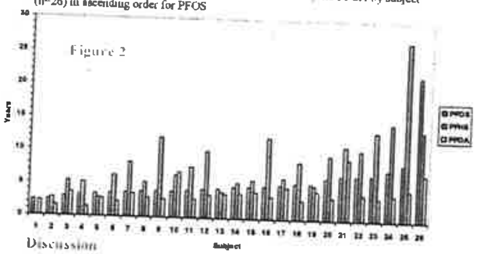
Without one possible 'high' outlier for PFHS and PFOS (Subject #25 for PFHS, Subject #26 for PFOS)

Fluorochemical	Mean	95% CI	Median	Range
PFOS	4.8	4.1-5.4	4.4	2.4-8.5
PFHS	8.0	6.5-9.5	7.1	2.8-14.6
PFOA	3.8	3.1-4.4	3.5	1.5-9.1

Results (Cont.)

Individual half-life of serum elimination for PFOS, PFHS, and PFOA by subject (n=26) in ascending order for PFOS

Figure 2



Discussion

Our findings confirm the long half-life of elimination of PFOS, PFHS and PFOA from human serum. Reasons for this long half-life are not understood but could involve multiple factors including but not limited to: limited excretion or increased re-absorption as mediated by organic anion transporters; differences in distribution of precursor molecules; or reconcile deep compartments.

As seen in Figure 1, the greater inter-individual variability in measurement occurred with PFHS. PFHS concentrations were lower than PFOS and PFOA, thus random error in a measurement would increase the bias. Analytically, PFHS has a higher response factor in the mass spectrometer and was calculated against the PFOS internal standard. Therefore, the curves were fit to a quadratic equation. As a result, we observed increased variability in the PFHS data.

Our results further demonstrate the substantial differences in pharmacokinetics for PFOA across species. The elimination half-life of PFOA from serum in the retired workers monitored in this study was quite long compared to the elimination half-life in other species. The marked difference in elimination of PFOA between sexes in rats has been proposed to be attributable to sex hormone regulation of the expression of certain organic anion transporters (OAT2, OAT3, and oatp1) in kidney (Kudo *et al.* 2002). They found OAT2 to be more highly expressed in female rat kidney and subject to up-regulation by estradiol. The diversity of proximal tubular organic anion transporters and potential for genetic variation (Eraly *et al.*, 2004; Lee and Kim, 2004; Ljubojevic *et al.*, 2004) suggest that it is possible that the long elimination half-life compared to other species studied may be due to differential expression of organic anion transporters and could be linked to either low-level transport into urine or increased tubular reabsorption. Harada *et al.* (2004) have recently published an analysis of the renal clearance of PFOA in humans based on their study subjects' serum and urine biomonitoring data. Regardless of sex, the renal clearance was estimated to be 0.001% that of the glomerular filtration rate in humans. This indicates the absence of active excretion of PFOA in human kidneys. Further work investigating the elimination of isomeric forms of PFOA in urine and the potential role of organic anion transporters in PFOA elimination would be of value in understanding species differences.

Conclusion

The purpose of this study was to determine the elimination half-life of PFOS, PFHS and PFOA from human serum. Twenty-six (24 male, 2 female) retired fluorochemical production workers had periodic blood samples collected over a five-year time period. Mass spectrometry quantitative calculations were based on the ion ratios between analyte and an internal standard ($^{13}C_2$-PFOA or $^{18}O_2$-PFOS). Initial serum concentrations for the 26 subjects ranged between 150 – 3490 ng/mL for PFOS, 20 – 1300 ng/mL for PFHS and 70 – 5100 ng/mL for PFOA. Assuming first-order elimination kinetics, the mean half-life of serum elimination for PFOS, PFHS and PFOA were 5.4 years (95% CI 3.9 – 6.9), 8.8 years (95% CI 6.7 – 10.9) and 3.8 years (95% CI 3.1 – 4.4), respectively. The half-life of serum elimination for each fluorochemical was not associated with initial concentration, age or sex of retiree, years worked at the manufacturing facility or the time between retirement and first blood collection.

References

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