COMPARATIVE PHARMACOKINETICS OF PERFLUOROHEXANESULFONATE (PFHxS) IN RATS AND MONKEYS

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Abstract
Perfluorohexanesulfonate (PFHxS) has been found in biological samples from wildlife and humans. The geometric mean half-life of serum elimination of PFHxS in humans has been estimated to be 7.3 years (95% CI 5.8-9.2 years). We undertook a series of studies to establish pharmacokinetic parameters in non-human species. Male (M) and female (F) monkeys were given a single intravenous (IV) dose of K^+PFHxS and serum and urine PFHxS concentrations were followed for 171 d. M and F rats were given single oral doses of 1, 10, or 100 mg/kg K^+PFHxS and urine and feces were collected over 96 h and serum and liver collected at 96 h. Jugular-cannulated M and F rats were given either IV or oral single 10 mg/kg doses of K^+PFHxS and serum concentrations of PFHxS were followed for 24 h. M and F rats were given a single IV dose of 10 mg/kg, and serum, feces, and urine were collected weekly for 10 weeks. All PFHxS analyses utilized LC-MS\MS methods. Pharmacokinetic parameters were determined by WinNonlin® software. Volumes of distribution indicated predominant extracellular distribution. Mean serum elimination half-lives were 141 and 87 d for M and F monkeys and ca. 30 and 1.5 d for M and F rats.

Introduction

Perfluorohexanesulfonate (PFHxS) is a perfluorinated surfactant in which all hydrogens have been replaced with fluorine. As such, PFHxS is one of a number of functionalized, polyfluorinated compounds that have been produced for over half a century for use in specialized applications[1] as well as becoming the subject of increasing investigation with regard to environmental and health-related properties[2]. The unique properties of this and other perfluorinated surfactants, such as high surface activity, exceptional stability to degradation, density, solubility characteristics, and low intermolecular interactions, have been exploited in numerous industrial and consumer applications[3]. However, these same properties also create challenges for managing these materials in the environment. Exceptional stability to environmental and metabolic degradation together with poor elimination from the body in the case of several perfluorinated surfactants[4], including PFHxS, create a potential for accumulation and biomagnification. The geometric mean serum PFHxS elimination half-life in 26 retired production workers was estimated to be approximately 7.3 years (95% CI = 5.8 - 9.2)[4]. Spliethoff et al. estimated the serum elimination half-life for PFHxS to be 8.2 years (95% CI = 5.4 - 16.2) using PFHxS concentration data from neonatal screening blood spots[5]. Although the reported half-life values from the latter two articles are imprecise, they are consistent in suggesting poor elimination of PFHxS in humans. Due to these properties, it may not be surprising that PFHxS originally was found in pooled serum from the United States general population[6], and numerous later biomonitoring studies have found PFHxS widely distributed at low ng/mL concentrations in individual samples from the general population[7-9]. As limited pharmacokinetic and toxicological data were available for PFHxS[10], the purpose of this study was to establish pharmacokinetic parameters for PFHxS in a non-human-primate (cynomolgus monkey) and a rodent (Sprague Dawley rat) species.

Materials and Methods

The potassium salt of PFHxS (K^+PFHxS, linear, >99% pure) was supplied by 3M Company (St. Paul, MN). All studies were performed in laboratories accredited by International Association for the Accreditation of Laboratory Animal Care. All procedures involving animals were reviewed and approved by Institutional Animal Care and Use Committees associated with the facilities. Animal care and procedures followed the US Department of Health and Human Services Institute of Laboratory Animal Resources (ILAR) guidance[11].

Intravenous Study in Monkeys
Animals and Husbandry: This portion of the study was conducted at Southern Research Institute (Birmingham, AL). Three male and 3 female monkeys (all purchased from Charles River BRF, Inc. Houston, TX) designated for use in this study were selected from an in-house colony of monkeys that were housed at Southern Research Institute. These monkeys were at an estimated 3-4 years of age when placed on study. Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St. Louis, MO) was fed to the monkeys 2-3 times each day and the

diet was supplemented with fresh fruit/treats several times each week. Tap water (Birmingham, Alabama public water supply) was available to the monkeys *ad libitum*. The monkeys were housed in a room that was maintained at a temperature of 20.0-21.3ºC and a relative humidity of 22.2-65.7%. An automatic timer was set to control room lights with 12 hours of light and 12 hours of dark. With one exception, the same monkeys were used in four prior IV elimination studies with perfluorinated alkyls conducted in the following order: 1) perfluorobutanesulfonate; 2) perfluorobutyrate; 3) perfluorohexanoate; and 4) perfluorooctanoate. Prior to study, each monkey was tested for prior compounds and the new compound would only be administered if the previous compounds were below the method quantitation limit. One male monkey was replaced for this study with PFHxS.

Materials and Dose Preparation: Potassium salt of PFHxS dosing solutions were prepared in USP sterile saline at 5 mg/mL. Dose preparations were stored refrigerated and used within 4 days after preparation and were considered stable during this period. The vehicle was stored at room temperature.

Experimental Design: On Day 0, each of the 3 male and 3 female monkeys received a single IV dose of the potassium salt of the PFHxS at 10 mg/kg into a superficial arm or leg vein at a dosing volume of 2 mL/kg. All monkeys were observed twice daily for clinical signs. Body weights were taken the day before dosing and at least weekly after the dose was administered. Urine was collected in standard metabolism cages for 24-hour intervals prior to dose administration, on Day 1 (0-24 hours post-dose) and, thereafter, on selected days. The volume of each urine sample was measured. Urine samples were stored frozen (approximately –20ºC or below). Approximately 3 mL of blood samples (processed to serum via clotting and centrifugation at room temperature) were collected from a superficial arm or leg vein of each monkey in a restraint chair at selected times. Serum and urine samples were stored frozen (approximately –20ºC or below) until analyzed.

LC/MS-MS Analysis of PFHxS: The analytical method used for extraction and quantitative determination of the PFHxS in serum was based on a previously published LC-MS/MS method using ion-pairing extraction [6]. For urine samples, the same ion-pairing LC-MS/MS methodology was followed for the extraction and analysis and is described briefly here. A small aliquot of the urine sample was mixed with a buffered solution containing tetrabutylammonium hydrogen sulfate. After mixing, ethyl acetate was added to the extract and shaken thoroughly. The sample was centrifuged prior to transferring the organic layer to a clean polypropylene tube and evaporated to dryness under a stream of nitrogen. The extract was reconstituted in methanol or in a methanol/water mixture and transferred to an autovial through a syringe filter. Appropriate internal standards were added to each sample prior to the first extraction step. Method blanks, matrix blanks, and matrix spike samples were prepared along with each set of samples for quality control purposes. Standard curves used for quantitative determination of perfluorinated acid levels were spiked in and extracted from samples of blank primate matrix along with the samples. The lower limit of quantitation for perfluorinated acids determined in each matrix serum and urine is approximately 0.010-0.020 μg/mL.

Studies in Rats

Animals and husbandry: This portion of the study was conducted in the Medical Department laboratory facilities of 3M Company. Environmental controls for the laboratory animal rooms were set to maintain a temperature of 72 ± 3°F, humidity of 22-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose effect on elimination: To determine the effect of increasing dose on the clearance profile of PFHxS in the serum, liver, urine, and feces of male and female Sprague Dawley rats within 96 hours following a single oral dose, rats (N = 4/sex/group) were given single oral doses of 1, 10, or 100 mg K^+PFHxS/kg in a dose volume of 5 mL/kg. The rats were placed in metabolism caging and urine and feces were collected every 24 h after dosing for 96 h. Clinical observations were made after dosing and periodically until euthanasia by CO_2 asphyxiation at 96 h, at which time gross necropsies were performed and blood (for serum) and livers were collected. All samples were stored at -70ºC pending LC/MS-MS analysis.

Pharmacokinetics after single oral dose in jugular-cannulated rats: To determine the serum uptake and elimination of PFHxS within a 24-h period after a single oral dose, male and female, jugular-cannulated, rats were given a single oral dose of 10 mg K^+PFHxS/kg in a dose volume of 5 mL/kg. Each rat was observed immediately following dosing and periodically throughout the study for mortality and morbidity. Interim blood samples (approximately 200 uL) were collected from all rats at 0.25, 0.5, 1, 2, 4, 8, and 24 h post-dose via cannula and centrifuged for serum. At 24 h post-dose, all animals were euthanized via CO2 asphyxiation and liver samples were harvested. Both serum and liver samples were stored at -70ºC pending LC-MS/MS analysis.

Pharmacokinetics after single IV administration jugular-cannulated rats: To determine the serum elimination of PFHxS within a 24-hour period after a single IV dose, male and female jugular-cannulated rats were given a single IV dose of 10 mg K^+PFHxS/kg in dose volume of 1 mL/kg body weight was used for all animals. Each animal was observed immediately following dosing and periodically throughout the study for mortality and morbidity. Interim blood samples (approximately 200 uL) were collected from all animals at 0.25, 0.5, 1, 2, 4, 8, and 24 hours post-dose via cannula and centrifuged for serum. At 24 h post-dose, all animals were euthanized via CO2 asphyxiation and liver samples were harvested. Both serum and liver samples were stored at -70°C pending LC-MS/MS analysis

Ten-week elimination after single IV dose: To determine the clearance profile of PFHxS in the serum, urine, and feces of male and female Sprague Dawley rats ten weeks following a single IV dose, rats (N = 4/sex/group) were given a single IV dose of 10 mg K^+PFHxS/kg in a dose volume of 1 mL/kg. The rats were placed in metabolism caging and urine and feces were collected at selected 24-hour intervals throughout the study. Clinical observations were made after dosing and daily thereafter. Ten weeks post-dose, all rats were euthanized by CO_2 asphyxiation and gross necropsies were performed, and blood (for serum) and livers were collected. All serum, urine, feces, and liver samples were stored at -70°C pending LC-MS/MS analysis.

LC-MS/MS analysis of PFHxS: PFHxS concentrations were analyzed by LC-MS/MS in 3M Medical Department Bioanalytical Laboratory (St. Paul, MN). One hundred (100) µL of serum, urine or fecal extract samples were aliquoted into clean polypropylene tubes. The isotopically labeled ($^{18}O_3$) PFHxS was used as an internal standard by addition to each sample tube. One mL of 1.0 N formic acid was added to all tubes, followed by 100 µL saturated ammonium sulfate. Samples were vortexed between each addition. All samples were extracted using solid phase extraction (SPE cartridges, Waters Oasis® HLB). Final eluets were analyzed by LC-MS/MS as described in a prior publication[4].

Data Analyses

The serum concentration data for PFHxS were subjected to non-compartmental (monkeys) or two-compartmental (rats) pharmacokinetic analyses using WinNonlin (Standard Edition; Version 1.1; Scientific Consulting, Inc.; Cary, NC). Mean values and standard deviations for each parameter were calculated using Microsoft Excel® software (Microsoft Corporation; Irvine, CA).

Results and Discussion

Monkeys

Pharmacokinetic parameters determined for monkeys are presented in Table 1. A characteristic elimination curve is shown for a female monkey in Figure 1.

Table 1. Mean (± SD) pharmacokinetic parameters in male and female (3/sex) cynomolgus monkeys following an IV dose (10 mg/kg body weight) of potassium PFHxS.

Parameter	Males	Females
Serum [PFHS] @ 0.5 hrs	108±35	148±29
Serum [PFHS] @ 2 hrs	111±16	83±21
C_0 (µ/d•mL)[a]	181 ± 29	180 ± 35
$AUC_{0\text{-last}}$ (µg•d/mL)[b]	4072 ± 778	3946 ± 418
$AUC_{0\text{-infinity}}$(µg•d/mL)[c]	7462 ± 1169	5794 ± 2418
$T_{1/2}$ (d)[d]	141 ± 52	87 ± 47
CL(mL/d/kg)[e]	1.3 ± 0.2	1.9 ± 0.7
Vd_{ss} (mL/kg)[f]	287 ± 91	213 ± 49
24 h % of Dose in Urine	0.093±0015	0.017±.004

[a] Serum concentration at time 0; [b] Area under the serum concentration time curve from 0 to the last time point; [c]Area under the serum concentration time curve from 0 to infinity; [d] Half-life of the terminal elimination phase; [e] Clearance; [f] Volume of distribution at steady state.

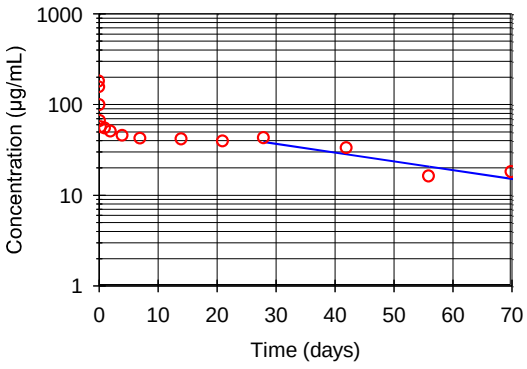

Figure 1. Characteristic serum PFHxS elimination curve from a female monkey given a single IV dose (10 mg/kg) of K^+PFHxS.

The apparent volume of distribution (Vdss) suggests predominant extracellular distribution. Although the mean serum PFHxS elimination half-life is less for females than males, this difference is not statistically significant (Student's t test, $p<0.05$) within the limits of study design. Less than 0.1 % of the given dose was recovered in urine within 24 h following the IV injection.

Rats

Dose effect on elimination: In male and female rats, mean serum PFHxS concentrations were non-linear in proportion to dose after 96 h (Table 2), and liver concentrations were significantly lower than corresponding serum concentrations. Female serum and liver concentrations were considerable lower than those of males.

Table 2. Mean (± SD) concentrations of PFHxS in serum and liver of male and female rats 96 h after a single oral dose of 1, 10, or 100 mg K^+PFHxS (*N* = 4/sex/dose; M = Male; F = Female).

	1 mg/kg		10 mg/kg		100 mg/kg	
	Serum	Liver	Serum	Liver	Serum	Liver
M	4.68 ± 0.35	5.58 ± 0.35	81.79 ± 5.56	25.71 ± 2.13	194.63 ± 15.63	118.13 ± 10.80
F	1.92 ± 0.21	0.45 ± 0.06	12.34 ± 2.19	2.18 ± 0.31	26.16 ± 2.53	5.70 ± 0.45

The percents of given dose recovered in serum, liver, urine, and feces, as well as the estimated total percents recovered in those four compartments by dose group and sex is presented in Figure 2.

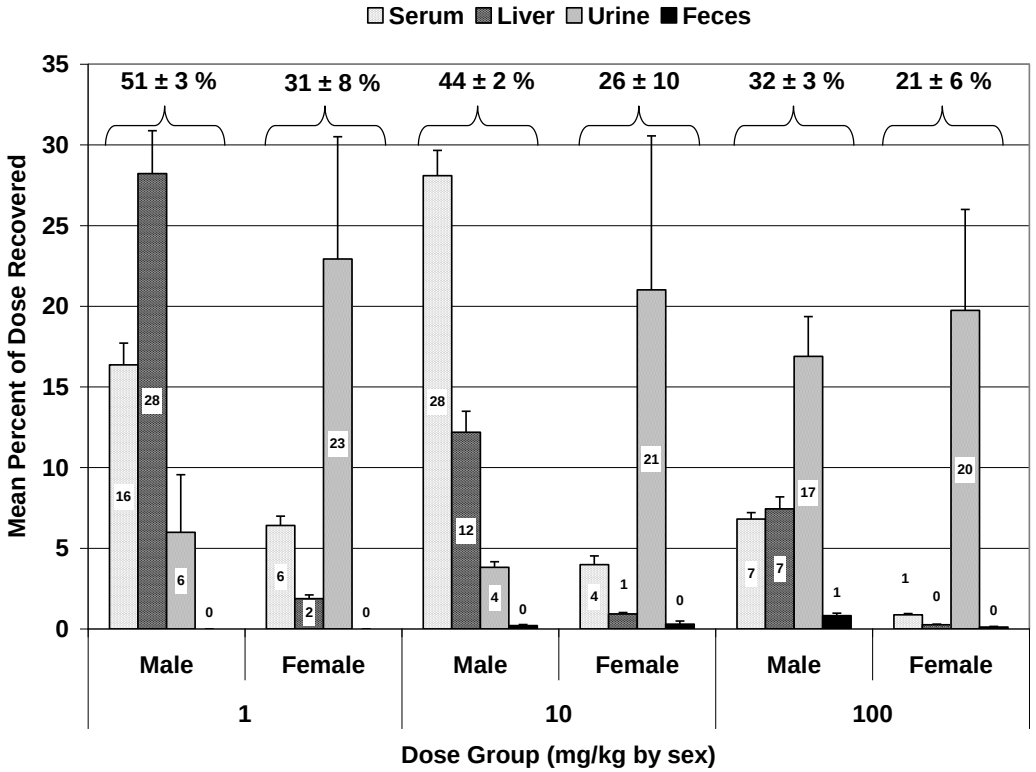

Figure 2. Mean percent of given dose recovered through 96 h following dosing in serum, liver, urine, and feces by K^+PFHxS dose group (mg/kg) and sex for male and female rats given 1, 10, or 100 mg K^+PFHxS/kg body weight. Estimated mean (± SD) total percent recovered in these four compartments is given above bracket linking bars by dose and sex. Error bars are SD. Numerical values are provided in center of each bar. Liver percent is based on weight of liver at 96 h sacrifice, and serum percent is based on estimate of 32 mL serum per kg body weight using terminal body weight at 96 h.

Urine was the major route of excretion in male and female rats. Females excreted approximately 20% of the dose in urine over 96 h regardless of dose; whereas, males excreted only about 5% of the dose in urine at the 1 and 10 mg/kg dose level, but excreted close to 20% at the 100 mg/kg dose level. The latter observation may suggest a saturable renal tubular reabsorption process associated with urinary elimination of PFHxS in the male, as has been identified for perfluorooctanoate[12] and hypothesized for perfluorooctanesulfonate[13]. For males, the

estimated mean total delivered dose recovered in the four compartments measured was almost twice that of females at the 1 and 100 mg/kg dose levels, even though female serum and liver concentrations were considerably lower. This may suggest a storage site in females not shared by males.

Pharmacokinetics after single oral dose in jugular-cannulated rats: Uptake and distribution to serum appeared to be more rapid in females than in males (Figure 3, left panel). In males, uptake appeared complete within several hours. Serum PFHxS elimination between 4 and 24 h appeared to be minimal for males (Figure 3, left panel), a fact precluding the reliable calculation of parameters. For females, mean values for parameters were: T_{max} (h) = 0.53 ± 0.42; C_{max} (µg/mL) = 63.6 ± 5.9; $T_{0.5}$ α (h) = 1.81 ± 1.68; $T_{0.5}$ β (h) = 32.9 ± 6.8; $AUC_{0\text{-}24\ h}$ (h·µg/mL) = 1022 ± 231.

Pharmacokinetics after single IV administration jugular-cannulated rats: After an initial approximately four-hour redistribution period following the IV dose, male rats exhibited a very low rate of serum PFHxS elimination (Figure 3, left panel). It was not possible to calculate parameters reliably for males. For females, mean values for parameters were as follows: C_{max} (µg/mL) = 58.7 ± 16.6; $T_{0.5}$ α (h) = 0.47 ± 0.24; $T_{0.5}$ β (h) = 44.0 ± 10.8; CL (mL/hr/kg) = 4.95 ± 3.43; Vd_{ss} (ml/kg) = 252 ± 114. CL and Vd_{ss} should be interpreted cautiously.

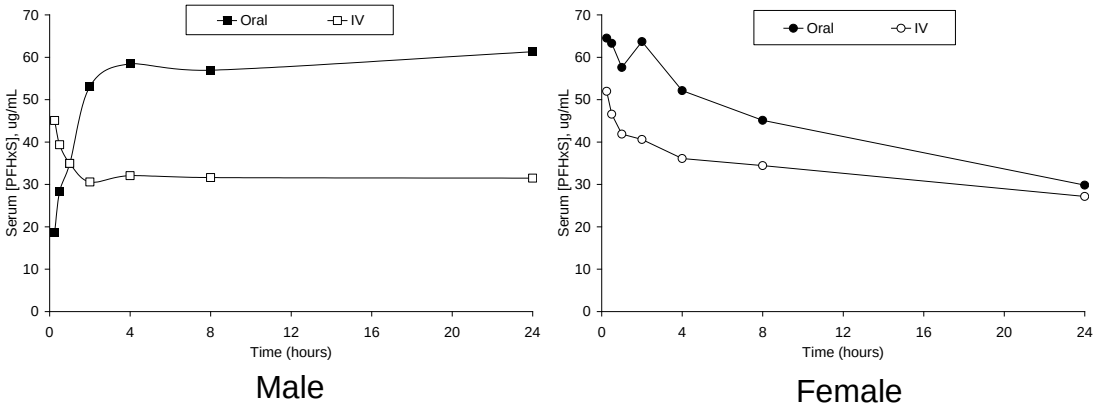

Figure 3. Serum PFHxS concentration in male (left panel) and female (right panel) rats given single oral (solid squares) or IV (open squares) doses (10 mg/kg) of K^+PFHxS over a 24 h period post-dose.

Ten-week elimination after single IV dose: Data are presented in Figure 4. After a single IV dose of K^+PFHxS, it appeared that PFHxS was being slowly eliminated from serum in male rats, especially compared to females. At the end of the 72 d follow-up period, mean serum PFHxS concentration in males were about 5 ug/mL, while the mean serum PFHxS concentration in females were below LOQ starting on study day 16 and beyond. Estimated male and female serum PFHxS elimination half-lives were approximately 30 and 1.5 d, respectively.

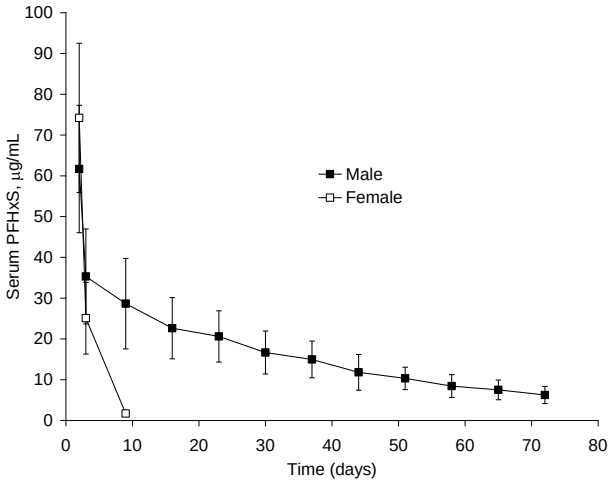

Figure 4. Serum PFHxS concentrations in male and females rats given a single IV dose of 10 mg K^+PFHxS/kg over a period of 72 days.

Conclusion: The difference in serum elimination half life between male and female monkeys was not statistically significant under study conditions. In contrast, large differences between male and female rats were evident. Female rats appeared to eliminate PFHxS more effectively in urine and had much lower serum and liver levels than male rats. However, when accounting for the percent of dose recovered in serum, liver, urine, and feces, less could be accounted for in females when totaling the percent of dose recovered in these four compartments. This may imply the existence of a compartment in female rats unique in comparison to male rats. In the rat, sex differences in renal organic anion transport have been noted for perfluorooctanoate[12], and this is an area we are exploring as a potential reason for the sex differences in pharmacokinetics observed with PFHxS in the rat. Also, female urinary elimination, as a percent of dose, did not appear to be dose-dependent or saturable, as it appeared to be in male rats. As exemplified by these data for PFHxS, differences in pharmacokinetic handling between species, and between sexes within species, underscore the importance of understanding pharmacokinetic handling when estimating exposure and risk.

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