Perfluorooctanoic Acid: Relationship Between Repeated Inhalation Exposures and Plasma PFOA Concentration in the Rat

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Abstract

A large pharmacokinetic database exists describing the behavior of perfluorooctanoic acid (PFOA) following oral exposure. The objective of this study was to quantify plasma PFOA concentrations in rats, following single and repeated inhalation exposures at three airborne concentrations, for the purpose of bridging the oral and inhalation exposure data sets. The study was comprised of two separate experiments: Phase I consisted of a single 6-hour nose only exposure while Phase II consisted of repeated exposures (6 hours per day, 5 days per week for three weeks). In both experiments, male and female rats were exposed nose only to an aqueous aerosol of 0, 1, 10, or 25 mg/m³ PFOA. Levels were selected to produce plasma concentrations similar to those observed in previous oral gavage studies. In Phase I, blood was drawn via the tail vein pre-exposure, during exposure at 0.5, 1, 3 and 6 hours, and post-exposure at 1, 3, 6, 12, 18 and 24 hours. Rats were not removed from the exposure chamber during the exposure period and blood was taken from the tail vein while the animals remained in the nose-only restrainers attached to the exposure chamber. For Phase II, blood was collected immediately before and after the daily inhalation exposure period three days per week. Plasma derived from the whole blood samples was analyzed by liquid chromatography-mass spectrometry (LC-MS).

PFOA appears rapidly in the blood of both male and female rats exposed via the inhalation route. PFOA elimination is sex-dependent, with female rats eliminating PFOA from the plasma much more efficiently than male rats. PFOA plasma concentrations are proportional to the atmospheric exposure concentrations from 1 to 25 mg/m³. Repeated daily inhalation exposures produce little plasma carryover in female rats, but significant carryover in male rats. Male rats reach a steady state plasma concentration by three weeks with plasma concentrations of 8, 21, and 36 µg/mL respectively when exposed to 1, 10, and 25 mg/m³ PFOA. Female rats reached post exposure plasma concentrations of 1, 2, and 4 µg/mL respectively when exposed to 1, 10, and 25 mg/m3 PFOA but returned to baseline levels for the pre-exposure time points. This study provides the data necessary to relate external atmospheric concentrations of PFOA to PFOA levels in male and female rat plasma.

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Materials and Methods

•Linear perfluorooctanoic acid (PFOA) was obtained from Aldrich Chemicals. Since this study utilized the inhalation route of exposure, no attempt was made to establish the actual dose each rat received and all results were therefore reported as a function of the group exposure concentration(s) rather than a function of dose.

•Male and female Crl:CD®(SD)IGS BR rats were obtained from Charles River Laboratories, Inc., Raleigh, North Carolina. At the time of exposure, rats were approximately 6-8 weeks of age and the weight variation did not exceed ± 20% of the mean weight by exposure group.

•All exposure chambers were constructed of stainless steel and glass (NYU style) with a nominal internal volume of 150 L with a dispersion plate inside the chamber to promote uniform chamber distribution of the test atmosphere. Animals were individually restrained in polycarbonate (Phase I) or perforated stainless steel cylinders (Phase II) with conical nose pieces. During exposure, the animals were kept warm with a heat lamp (Figure 1).

•A 5% (weight/weight) solution was prepared and adjusted to a pH of 6.0 to 8.0. Chamber atmospheres were generated by aerosolization of the 5% test substance solution in air with a Spraying Systems nebulizer. The pump and flow controller were controlled and monitored by the Canule Inhalation Toxicology Automated Data System (CITADS). Chamber concentrations were controlled by varying the syringe infusion rate to the nebulizer or by using dilutional air. The atmospheric concentration of PFOA was determined by gravimetric analysis at approximately 60-minute intervals during each exposure.

•In order to determine if the gravimetric analytical method used to quantitate the airborne test substance concentration included the test substance plus water, air samples were taken from chamber atmospheres that ranged from 1 to 25 mg/m³ test substance. The filters were weighed immediately post-sampling, placed in a desiccation chamber for 24 hours and re-weighed. The filters lost <0.03% of their mass, indicating that the filter reflected the total mass of suspended aerosol in the exposure chambers.

•A sample to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 µm diameter) was taken from each test exposure chamber at least once per week during the Phase II study with a Sierra® Series 210 cyclone preseparator/Cascade impactor and Sierra® series 110 constant flow air sampler. Chamber airflow was set to achieve at least 10 air changes per hour and was monitored continually. Chamber temperature, relative humidity and oxygen concentration were targeted at 22 ± 3°C, 40 to 60%, and 19%, respectively. Airflow, temperature, and relative humidity were monitored continuously and recorded at 15-minute intervals. Chamber oxygen concentration was recorded twice during each exposure

•Plasma samples were processed by protein precipitation (PPT) using Isolute Array protein precipitation columns (Argonaut Technologies, Foster City, CA). A 0.5 µg/ml solution of perfluorononanoic acid (Aldrich Chemicals) in ACN was used as an internal standard. Plasma samples were thawed, and a 20 µL aliquot of each sample was applied to the PPT array. The plasma samples were precipitated by adding appropriate dilution rate volumes of ACN/internal standard solution. Dilution rates, ranging from 1:4 (60 µL of internal standard solution) to 1:50 (980 µL of internal standard solution), were utilized in order to capture the sample concentrations within the range of the standard curve concentrations. The array was slowly eluted under vacuum into a 96-well receiver plate, centrifuged at ~3000 rpm for 5 minutes, and the extracts were analyzed by LC/MS.

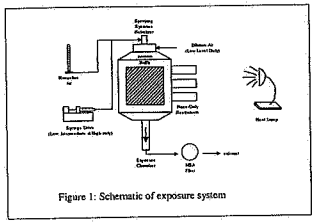

Figure 1: Schematic of exposure system

Experimental Design

•Phase I (Single Exposure)

To determine the proper exposure concentrations for Phase II, four groups of 3 male and 3 female rats each were exposed nose only for a single, 6-hour period to an aerosol of the test substance. The test groups were exposed to an atmospheric concentration of 1 mg/m³, 10 mg/m³, or 25 mg/m³. A control group of rats was exposed to air only. Blood was collected via the tail vein and was drawn pre-exposure, during exposure at 0.5, 1, 3 and 6 hours, and post-exposure at 1, 3, 6, 12, 18 and 24 hours. Individual animal exposure times were staggered by five minutes to allow time for blood collection. Rats were not removed from the exposure chamber during the exposure time. Blood was taken from the tail vein while the animals remained in the nose-only restrainers in the exposure chamber. Blood was collected in heparinized tubes and stored on wet ice. Plasma was separated by centrifugation. Rats were sacrificed following the final blood collection. Plasma derived from the whole blood samples was analyzed by LC-MS.

The exposure concentrations selected for Phase II were levels that produced plasma concentrations similar to plasma concentrations observed in a previous study of oral gavage dosing in rats.(1)

•Phase II (Repeated Exposure)

After the exposure levels were selected, four groups of 5 male and 5 female rats each were exposed (nose only) for a 6-hour period, 5 days per week (weekends excluded), for 3 weeks to an aerosol of the test substance. The starting time of the exposure is defined as the time when the nose only restrainers containing the animals are loaded into the exposure chamber. The exposure end time is defined as the time when the nose only restrainers are removed from the chamber. After each exposure, rats were returned to their cages. The control group of rats was exposed to air only. Exposure start times were staggered by 10-30 minutes by exposure concentration group to allow time for blood collection. As in Phase I, blood was collected via the tail vein. Blood was collected before and after the daily inhalation exposure period three days per week. Rats were sacrificed following the final blood collection.

Results – Chamber Conditions

CHAMBER CONCENTRATIONS OF PFOA

PHASE I (SINGLE EXPOSURE)

Design Concentration (mg/m³)	Measured Concentration[a] (mg/m³) Mean	S.D.	Range	n
1	1.2	0.38	0.66 - 1.7	6
10	9.8	0.58	9.0 - 11	6
25	27	3.1	24 - 32	6

[a] Values represent the mean, standard deviation (S.D.), and range of the values obtained from 1 exposure.

PHASE II (REPEATED EXPOSURE)

Design Concentration (mg/m³)	Measured Concentration[b] (mg/m³) Mean	S.E.M.	Range	n
1	1.1	0.04	0.81 - 1.3	15
10	10	0.11	9.4 - 11	15
25	25	0.43	21 - 27	15

[b] Values represent the mean, standard error of the mean (S.E.M.), and range of the daily mean values obtained from n exposures.

PHASE II (REPEATED EXPOSURE)
PARTICLE SIZE DISTRIBUTION OF PFOA

Design Concentration (mg/m³)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation	% Particle by Mass <1 µm	<3 µm	<10 µm
1	1.6	1.7	18	88	100
10	1.5	1.8	23	87	100
25	1.8	1.8	15	83	100

CHAMBER ENVIRONMENTAL CONDITIONS

PHASE I (SINGLE EXPOSURE)

Design Concentrations (mg/m³)	Temperature (°C)[a] Mean	S.D.	Range	n	Relative Humidity (%)[a] Mean	S.D.	Range	n	Airflow (L/min)[a] Mean	S.D.	Range	n
0	26	0.62	24 - 27	27	37	1.1	36 - 40	27	28	0.025	28	27
1	25	1.2	22 - 27	28	34	4.5	31 - 48	16	70	0.05	70	28
10	25	1.5	22 - 26	30	40	1.7	38 - 45	30	28	0.04	28	30
25	24	0.93	21 - 25	33	34	2.5	30 - 38	32	27	0.6	26 - 28	32

[a] Values represent the mean, standard deviation (S.D.), and range of the values obtained

PHASE II (REPEATED EXPOSURE)

Design Concentrations (mg/m³)	Temperature (°C)[b] Mean	S.E.M.	Range	Relative Humidity (%)[b] Mean	S.E.M.	Range	Airflow (L/min)[b] Mean	S.E.M.	Range	n
0	25	0.07	25 - 26	39	0.39	36 - 42	28	0.066	28	15
1	25	0.16	24 - 26	35	1.6	27 - 47	70	0.014	70	15
10	25	0.09	24 - 26	38	0.57	33 - 43	28	0.031	28	15
25	24	0.08	23 - 24	33	0.58	29 - 37	28	0.09	27 - 29	15

[b] Values represent the mean, standard error of the mean (S.E.M.), and range of the daily mean values obtained from n exposures.

Results – Singe Exposure Pharmacokinetics

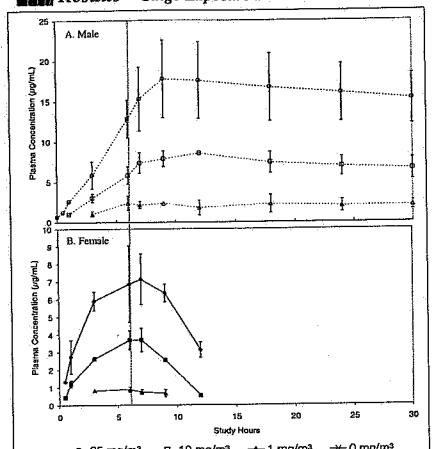

Figure 2: Plasma PFOA Concentration following a single 6 hour inhalation exposure

Phase I (Single Exposure)

For both sexes, PFOA plasma concentrations rose during the 6 hour inhalation exposure. A proportional relationship was produced in both the male and female rat maximum plasma concentrations (C_{max}) between 1 and 25 mg/m³ (Figure 2). The male C_{max} values were approximately 2-3 times higher than the female C_{max}. The female C_{max} occurred at the end of the exposure period or 1 hour post exposure while the male C_{max} occurred at the end of the exposure period up to 6 hours post exposure.

Elimination of PFOA from female rat plasma was rapid and complete at all exposure levels in Phase I. Plasma concentrations dropped below the analytical limit of quantification (0.1 µg/mL) by 12 hours post exposure. Plasma elimination was much slower in male rats than female rats. After 24 hours of post exposure recovery, the plasma concentrations were approximately 90% of the peak concentrations at all tested exposure levels. The male and female elimination kinetics are consistent with the previously reported elimination kinetics of PFOA following oral dosing.(1) In addition, there did not appear to be a concentration dependence of the elimination rate in either male or female rats as the plasma curves are parallel on a log scale.

For the setting of exposure levels for Phase II of the study, male and female C_{max} values were compared to plasma C_{max} from an oral gavage study of PFOA.(1) This study examined single oral gavage doses from 0.1 to 25 mg PFOA/kg body weight. The C_{max} values for this study ranged from 0.598 to 160.0 µg/mL (male) and 0.67 to 132.7 (female). Because the C_{max} values from Phase I fell within the middle of the existing range and oral gavage data, the inhalation exposure concentrations were not changed for Phase II.

Results – Repeated Exposure Pharmacokinetics

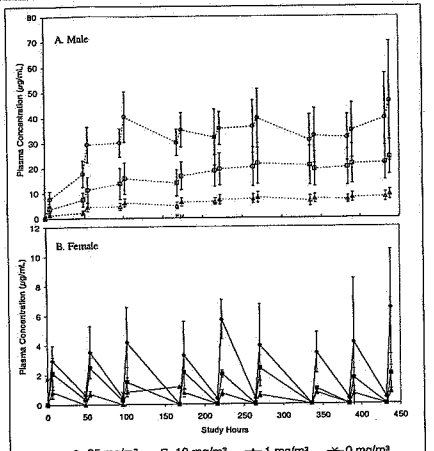

Figure 3: Plasma PFOA Concentration following a repeated 6 hour inhalation exposures

Phase II (Repeated Exposure)

Phase II plasma concentrations are shown in Figure 3. Exposures were conducted five days per week, with plasma samples taken before and after exposure on the first, third and fifth exposure day per week. The male and female concentration curves demonstrate the effects of the differing elimination kinetics on plasma levels following repeated exposure. In the female rats, there was little day-to-day carryover of PFOA in the plasma. There is no significant difference between the peak heights of the sampled exposure days. For the male rats, there is carryover between test days as expected from Phase I. Steady state was not achieved until week 3. This steady state value is approximately 2-3 times as high as the mean single dose C_{max}. As in Phase I, repeated exposures demonstrate a proportional relationship between exposure concentration and plasma concentrations in male and female rats. Male rats reach a steady state plasma concentration by three weeks with plasma concentrations of 8, 21, and 36 µg/mL respectively for the 1, 10, and 25 mg/m³ exposures. Female rats reached post-exposure plasma concentrations of 1, 2, and 4 µg/mL respectively for the 1, 10, and 25 mg/m³ exposures, but returned to baseline levels for the pre-exposure time points.

Conclusions

PFOA appears rapidly in the blood of both male and female rats exposed via the inhalation route. PFOA elimination is sex-dependent, with female rats eliminating PFOA from the plasma much more efficiently than male rats. At the tested concentrations from 1 to 25 mg/m³, PFOA plasma concentrations relate proportionally to the inhalation exposure concentration. Repeated daily inhalation exposures produce little plasma carryover in female rats, but significant carryover in male rats. Male rats reach a steady state plasma concentration by three weeks with plasma concentrations of 8, 21, and 36 µg/mL respectively for the 1, 10, and 25 mg/m³ exposures. Female rats reached post-exposure plasma concentrations of 1, 2, and 4 µg/mL respectively for the 1, 10, and 25 mg/m³ exposures, but returned to baseline levels for the pre-exposure time points.

Reference:

(1) DuPont Haskell Laboratory (2003). Perfluorooctanoic Acid: Toxicokinetics in the Rat. Unpublished report. DuPont-7473.