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Evaluation of PFHx- Pharmacokinetics in Mouse, Rat, Microminipig, Pig, Monkey and Human

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Summary

PFHx- elimination kinetics have been determined from published reports for multiple mammalian species: mouse, rat, microminipig, pig, monkey and human.

Based on this interspecies comparison, the following conclusions can be drawn:

(1) PFHx- elimination kinetics in mammals are consistent with a rapid initial (alpha) phase followed by a slower terminal (beta) phase.

(2) The PFHx-alpha phase elimination has a first-order half-life range of 1-2 hours in mouse, rat, microminipig, pig, monkey and human. The alpha phase elimination in mammals accounts for over 99.7% of the total PFHx-elimination in mammals.

(3) PFHx- elimination is extensive in the rapid alpha phase and there are no significant pharmacokinetic differences across mammals: mice, rats, monkeys, pigs and humans.

The overall conclusion of this kinetic assessment is that PFHx- does not appear to exhibit significantly different elimination kinetics across a wide range of mammalian species.

1. Introduction

Numerous research studies have been published to characterize the pharmacokinetic behavior of perfluorohexanoic acid (PFHxA) in mammalian species including mouse, rat, microminipig, domestic pig, monkey and human. The exposures in these studies vary in terms of dosage, route of administration (i.e., intravenous, oral, inhalation) as well as frequency and duration of exposure. Evaluation of the pharmacokinetics of PFHxA is performed by analysis of the concentrations of the anion PFHx- in serum, plasma or blood. The design of individual pharmacokinetic studies varied depending upon the type of test substance (C^{14}-labelled or non-labelled), the analytical method employed, the level of quantitation (LOQ) and the frequency of sampling.

This analysis of the elimination rate of PFHx- clearly shows the biphasic elimination profile that is typically observed for this substance from a wide range of mammals including humans. Specifically, the initial and terminal rates of elimination (i.e. the alpha- and beta phases, Figure 1) are calculated, as possible, for each species and compared. In some published studies, the alpha phase is incorrectly identified as the beta phase either due to the relatively short duration of the study or due to an elevated LOQ which masked the biphasic elimination behavior. In addition, in order to provide a meaningful comparison of the elimination kinetics across species, it is critically important to assess the proportion of PFHx- that is eliminated during each phase of elimination.

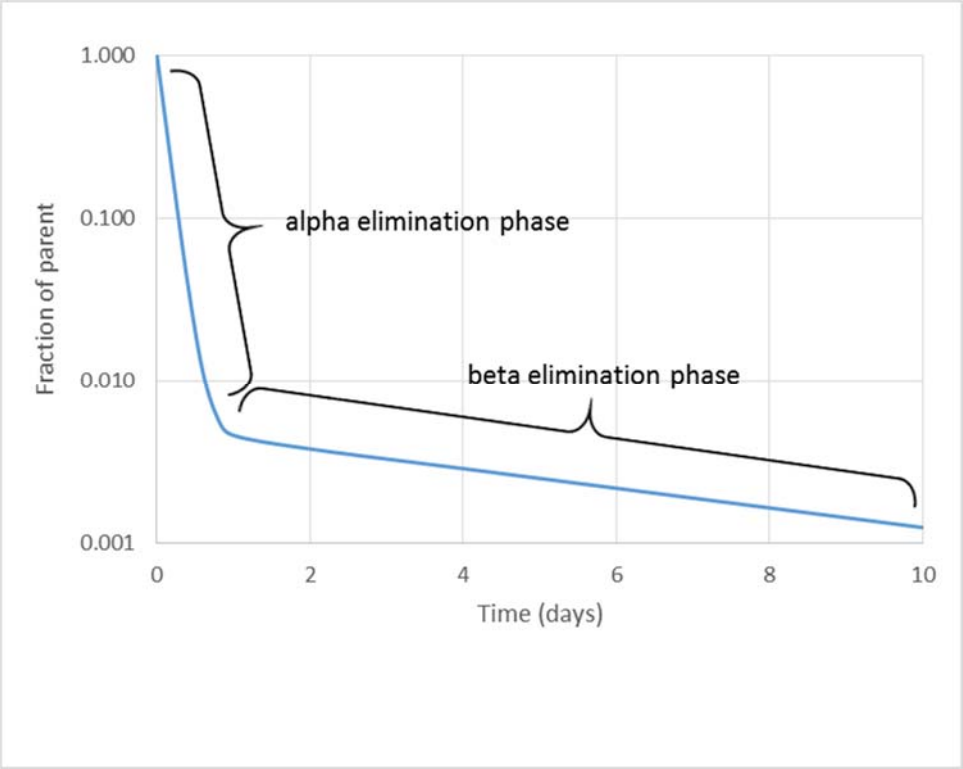

Figure 1. Illustration of the alpha and beta phases in a two-compartment model

This review provides a detailed compilation of the elimination pharmacokinetics of PFHx- in mouse, rat, microminipig, pig, monkey and human. From the available data, the alpha and beta phase elimination rates are summarized together with the proportion of PFHx- that is eliminated during each phase.

For each dataset, the following biphasic equation has been fitted to the experimental data:

$$C(t)/C_0 = A*\exp(-k_\alpha*t) + B*\exp(-k_\beta*t) \qquad \text{Equation 1}$$

where $C(t)$ = concentration of PFHx- in blood, serum or plasma with time (experimental data)
C_0 = initial concentration of PFHx- in blood, serum or plasma (experimental data)
A = proportion of PFHx- that is eliminated in alpha phase (i.e., faster initial rate)
B = proportion of PFHx- that is eliminated in beta phase (i.e., slower terminal rate)
k_α = elimination rate constant in alpha phase (hr^{-1})
k_β = elimination rate constant in beta phase (hr^{-1})
t = time after exposure (hr) (experimental data)

This biphasic equation is a "double first-order parallel" or DFOP equation, indicating that the elimination of PFHx- follows two simultaneous first-order equations.

All pharmacokinetic parameters have been calculated using PKPlus™, a computational module of GastroPlus™. Similar calculations can readily be performed with other software including Excel™, CAKE (Computer Assisted Kinetic Evalution) or KINGUI2 (Kinetic Graphic User Interface) with similar results.

2. PFHx- elimination results for each mammalian species

2.1 PFHx- elimination from mouse

An ADME (adsorption, distribution, metabolism and elimination) study of ^{14}C-labelled PFHx- was performed in the mouse dosed once orally at 2 or 100 mg/kg (Gannon et al., 2011). Blood was collected prior to dosing and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours and then every 24 hours until Day 7. Samples were processed to plasma and then analyzed for PFHx-.

The elimination of PFHx- following a single oral dose of 2 or 100 mg/kg in the mouse is shown in Figure 2 and the experimental data are provided in Tables 1 and 2. Regression of Equation 1 to the experimental data using both the high and low dose data for the male mice resulted in the following equation and individual kinetic parameters:

$$C(t)/C_0 = 0.9972 * \exp(-0.451*t) + 0.0028 * \exp(-0.011*t) \qquad \text{Equation 2}$$

$A = 0.9972$ $k_\alpha = 0.451\ hr^{-1}$ $B = 0.0028$ $k_\beta = 0.011\ hr^{-1}$

The elimination data was determined in male mice only because the clearance rate is slightly slower in males than females. From the regressed biphasic equation, the percentage of PFHx- eliminated from the male mouse in the alpha phase was 99.7% (i.e. A * 100).

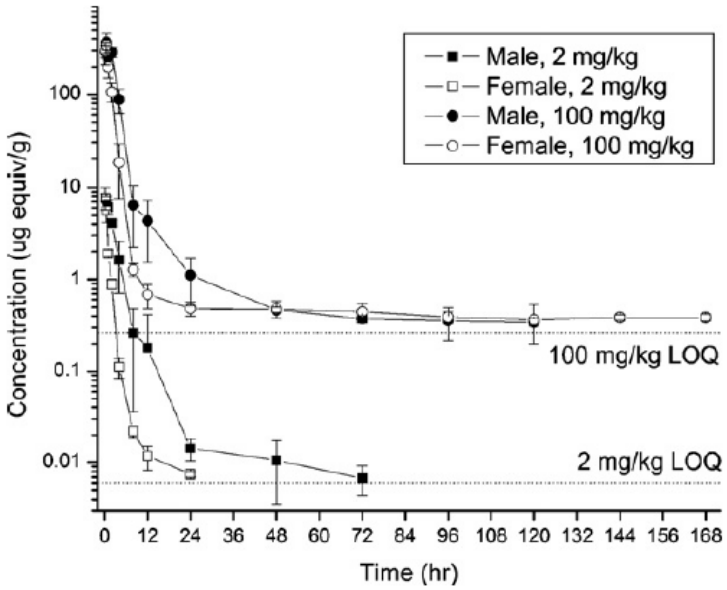

Figure 2. Concentration of PFHx- in mouse plasma following a single oral dose at either 2 or 100 mg/kg.

Table 1. Concentration of PFHx- in mouse plasma following a single oral dose of 2 mg/kg

Dose (mg/kg)	Time (hr)	Male		Female	
		Concentration (µg/g)	Std Deviation (µg/g)	Concentration (µg/g)	Std Deviation (µg/g)
2	0.25	2.23	0.31	1.78	0.63
	0.5	1.89	0.34	1.24	0.37
	1	1.43	0.35	0.451	0.025
	2	0.916	0.133	0.232	0.046
	4	0.368	0.211	0.0335	0.0070
	8	0.0811	0.0693	***0.0034***	NA
	12	0.0522	0.0719	< LOQ	NA
	24	***0.0034***	NA	< LOQ	NA
	48	< LOQ	NA	< LOQ	NA
	72	< LOQ	NA	< LOQ	NA
	96	< LOQ	NA	< LOQ	NA
	120	< LOQ	NA	< LOQ	NA
	144	< LOQ	NA	< LOQ	NA
	168	< LOQ	NA	< LOQ	NA

LOQ = 0.00686 µg/g (three times scintillation average background)
NA = not applicable
First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

Table 2. Concentration of PFHx- in mouse plasma following a single oral dose of 100 mg/kg

Dose (mg/kg)	Time (hr)	Male		Female	
		Concentration (µg/g)	Std Deviation (µg/g)	Concentration (µg/g)	Std Deviation (µg/g)
100	0.25	288	77	296	46
	0.5	368	97	342	38
	1	257	29	198	49
	2	288	35	106	25
	4	87.8	25.5	18.1	10.5
	8	6.41	4.14	1.28	0.21
	12	4.35	2.82	0.683	0.200
	24	1.11	0.59	0.483	0.085
	48	0.467	0.034	0.478	0.104
	72	0.374	0.031	0.444	0.105
	96	0.356	0.141	0.390	0.055
	120	0.344	0.032	0.365	0.166
	144	***0.169***	NA	0.386	0.028
	168	< LOQ	NA	0.387	0.030

LOQ = 0.338 µg/g (three times scintillation average background)
NA = not applicable
First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

2.2 PFHx- elimination from rat

In parallel with the mouse study, ADME results were also reported for ^{14}C-labelled PFHx- in the rat following a single oral dose of 2 or 100 mg/kg (Gannon et al., 2011). Blood was collected prior to dosing and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours and then every 24 hours until Day 7. Samples were processed to plasma and then analyzed for PFHx-.

The elimination of PFHx- following a single 2 or 100 mg/kg single dose in the rat is shown in Figure 3 and the experimental data are provided in Tables 3 and 4. Because rats eliminate PFHx- so quickly, only the alpha phase was observed before the concentration declined below the limit of quantitation. As a result, only the alpha phase of elimination was calculated from the reported data.

Regression of Equation 1 to the experimental data determined using both the high and low dose data for the male rats results in the following equation and individual kinetic parameters:

$$C(t)/C_0 = 1.000 * \exp(-0.38*t) \qquad \text{Equation 3}$$

$A = 1.000$ $k_\alpha = 0.38\ hr^{-1}$ $B = ND$ $k_\beta = ND\ hr^{-1}$

The elimination data was determined in male rats only because the clearance rate is slightly slower in males than females. From the monophasic equation (i.e. alpha phase only), 100% of the PFHx- eliminated from the mouse in the alpha phase (i.e. A * 100).

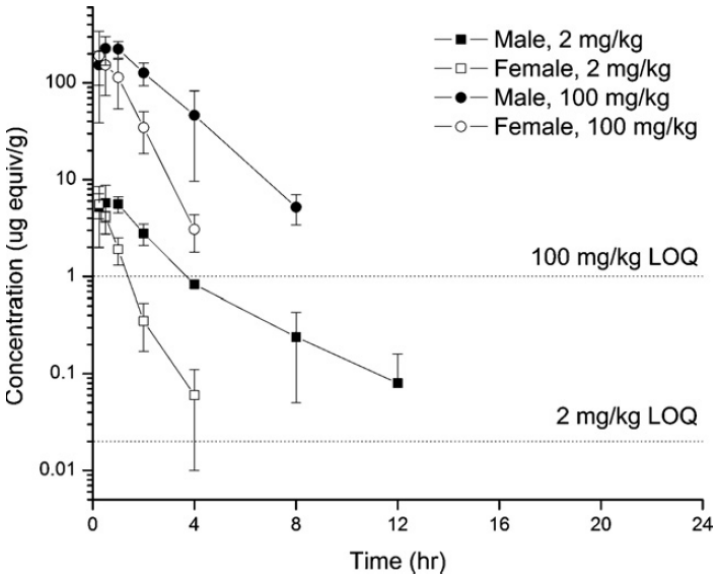

Figure 3. Concentration of PFHx- in rat plasma following a single oral dose of 2 or 100 mg/kg.

Table 3. Concentration of PFHx- in rat plasma following a single oral dose of 2 mg/kg.

Dose (mg/kg)	Time (hr)	Male		Female	
		Concentration (µg/g)	Std Deviation (µg/g)	Concentration (µg/g)	Std Deviation (µg/g)
2	0.25	5.18	3.21	5.52	1.61
	0.5	5.69	2.97	4.12	0.52
	1	5.56	1.03	1.90	0.59
	2	2.77	0.70	0.351	0.181
	4	0.826	0.071	0.059	0.049
	8	0.240	0.192	***0.0205***	NA
	12	0.0841	0.0762	< LOQ	NA
	24	***0.0205***	NA	< LOQ	NA
	48	< LOQ	NA	< LOQ	NA
	72	< LOQ	NA	< LOQ	NA
	96	< LOQ	NA	< LOQ	NA
	120	< LOQ	NA	< LOQ	NA
	144	< LOQ	NA	< LOQ	NA
	168	< LOQ	NA	< LOQ	NA

LOQ = 0.0410 µg/g (three times scintillation average background)
NA = not applicable
First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

Table 4. Concentration of PFHx- in rat plasma following a single oral dose of 100 mg/kg.

Dose (mg/kg)	Time (hr)	Male		Female	
		Concentration (µg/g)	Std Deviation (µg/g)	Concentration (µg/g)	Std Deviation (µg/g)
100	0.25	154	59	191	152
	0.5	227	74	153	79
	1	225	44	114	60
	2	127	34	34.6	15.9
	4	46.3	36.6	3.07	1.28
	8	5.21	1.81	***1.03***	NA
	12	***1.03***	NA	< LOQ	NA
	24	< LOQ	NA	< LOQ	NA
	48	< LOQ	NA	< LOQ	NA
	72	< LOQ	NA	< LOQ	NA
	96	< LOQ	NA	< LOQ	NA
	120	< LOQ	NA	< LOQ	NA
	144	< LOQ	NA	< LOQ	NA
	168	< LOQ	NA	< LOQ	NA

LOQ = 2.066 µg/g (three times scintillation average background)
NA = not applicable
First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

2.3 PFHx- elimination from pigs

2.3.1 Microminipigs (MMpigs)

Microminipigs (weight 9-14 kg) were given a single oral dose of a mixture of perfluoroalkyl acids (PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA), each at 3 mg/kg (Guruge et al., 2015). Blood samples were collected prior to exposure and at ten subsequent time points: 3, 6, 12 and 24h and 2, 4, 8, 11, 15 and 21d.

The PFHx- blood concentrations in this study were fitted to a one compartment first-order elimination model using data beginning with the onset of depuration. The resulting elimination half-life was reported to be 2.7d (equivalent to 64 hr) by the study authors (Figure 4). It should be noted that the first sampling point used for this calculation appears to be 24h after dosing. The rapid initial rate of elimination in rats and mice occurred within the first few hours after dosing, depending upon the route of administration. As a result, it is reasonable to conclude that the observed elimination half-life of 2.7d for MMpig corresponds to the beta phase or terminal rate of elimination for these animals. Therefore, from this study the beta phase half-life is 64 hr, resulting in a k_β value of 0.011 hr^{-1}.

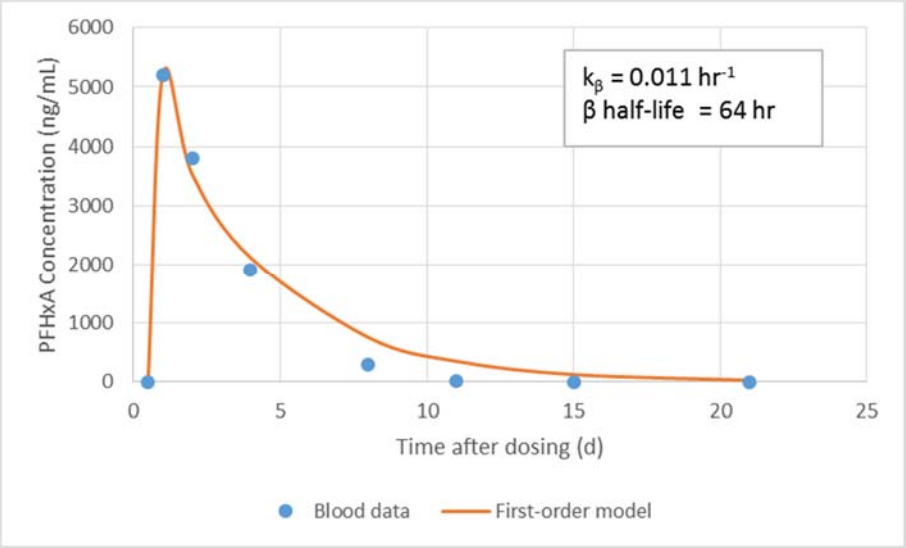

Figure 4. PFHx- elimination from MMpig (data recreated from Figure 1 of Guruge et al., 2015)

2.3.2 Domestic pigs

In a comparative assessment of the toxicokinetics (TK) of perfluoroalkyl acids (PFAAs) , three groups of fattening pigs (gilts (F), barrows (M) and young boars (M), nominally 65-120 kg) were continuously exposed to PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHxS, PFHpS, PFOS and PFDS in contaminated feed for a period of 21 days (Numata et al., 2014). The mean concentration of PFHxA in the feed was 47.8 ug/kg.

A two compartment TK model was developed, consisting of a central plasma compartment and an edible tissue compartment interconnected by first-order exchange rates with a single first-order elimination rate from the plasma compartment. Values for the kinetic constants were obtained by regressing the TK model to the experimental values in plasma and tissue over the 21d course of the study. Plasma samples were taken on days 4, 8, 11, 15, 18 and 22 and concentrations were determined on tissue samples taken on day 22 (Numata et al., 2014).

Using a two compartment model, the overall half-life of PFHx- was reported by the study authors to be 4.1d (equivalent to 98 hr or a k_β value of 0.007 hr^{-1}). This half-life was the most rapid elimination half-life of any of the PFAS evaluated in this this study. The plasma concentration of PFHx- approached a steady-state value by the end of the 21d exposure period (Figure 5). This kinetic behavior corresponds well with repeated dosing of a substance with a terminal (beta) elimination half-life of 4.1d (i.e., steady-state is typically approached within 5 half-lives).

Similar to the results observed with the microminipig, the observed elimination half-life of PFHx- in domestic pigs appears to be reflective of the beta phase or terminal elimination half-life. In the supplementary data for this study, the authors report that the first-order rate of elimination from the central compartment, ku, was determined to be 0.3522 day^{-1} (0.0147 hr^{-1}) which contributes to the initial rapid phase of elimination. Conversion of compartmental kinetics to a biphasic elimination expression results in the following equation (see Appendix 1):

$$C(t)/C_0 = 0.541 * \exp(-0.428*t) + 0.459 * \exp(-0.007*t) \qquad \text{Equation 4}$$

$A = 0.541 \quad k_\alpha = 0.428\ hr^{-1} \quad B = 0.459 \quad k_\beta = 0.007\ hr^{-1}$

The observed overall elimination half-life of 99 hours (k_β = 0.007 hr^{-1}) corresponds to the slower rate of elimination observed during the beta phase over the 21d course of this study.

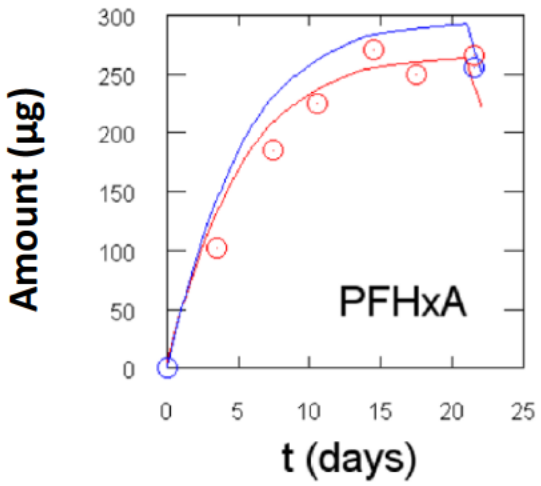

Figure 5. Pharmacokinetic behavior of PFHxA in plasma (plotted as µg versus time) in a representative pig during daily dosing via diet over a period of 21 days (Figure taken from Figure 3 in Numata et al., 2014).

2.4 PFHx- elimination from monkey (Noker, 2001)

Six cynomolgus monkeys (3F and 3M) were given a single IV dose of KPFHx at 10 mg/kg and serum samples were collected and analyzed at eleven time points after dosing: 2, 4, 8 and 24h and 2, 4, 7, 11, 14, 21 and 36 d (Noker, 2001). The experimental results for both monkey genders are summarized in Table 5.

Table 5. Concentration of PFHx- in monkey serum following a single IV dose of 10 mg/kg (Noker, 2001)

Time (hr)	Concentration (ng/g)									
	Male					Female				
	2052	2054	2211	Mean	Std Dev	2058	2059	2061	Mean	Std Dev
0	< LOQ	< LOQ	< LOQ	< LOQ	--	< LOQ	< LOQ	< LOQ	< LOQ	--
2	12,558	7,082	6,234	8,625	3433	14,240	29,780	16,388	20,136	8421
4	4,122	1,543	2,774	2,813	1290	5,640	4,958	5,062	5,220	367
8	1,171	314	a	743	606	114	1,085	671	623	487
24	28	13.5	27.8	23.1	8.3	49.3	50.8	51.7	50.6	1.2
48	4.6	2.2	5.5	4.1	1.7	6.5	11.4	13.2	10.4	3.5
96	1.5	1.4	2.7	1.9	0.7	2.1	1.4	3.9	2.5	1.3
168	1	b	1.5	1.3	0.4	2.4	1.3	< LOQ	1.9	--
264	1.7	1.9	1.7	1.8	0.1	1.8	1.5	1.8	1.7	0.2
336	***0.5***	***0.5***	***0.5***	***0.5***	--	***0.5***	***0.5***	***0.5***	***0.5***	--
504	< LOQ	< LOQ	< LOQ			< LOQ	< LOQ	< LOQ		
672	< LOQ	< LOQ	< LOQ			< LOQ	< LOQ	< LOQ		
864	< LOQ	< LOQ	< LOQ			< LOQ	< LOQ	< LOQ		

LOQ = 1 ng/mL

First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

In both the male and female monkey, the observed decline of PFHx- in serum was clearly biphasic with time. This kinetic behavior is shown in Figure 6.

In the study report, the author calculated the pharmacokinetic elimination parameters by fitting the observed blood data to a biphasic model using only data from the first four days after exposure (0 hr through 96 hr). However, this analysis excluded the additional data points after Day 4 (96 hr through 336 hr). As a result, the reported β-phase values are not reflective of the full range of data. The data have been reanalyzed using Equation 1 and the results of both the original and revised kinetic analyses are presented in Table 6. Due to generally similar elimination behavior for male and female monkeys (especially the alpha phase), a single regression was performed using the data from both genders.

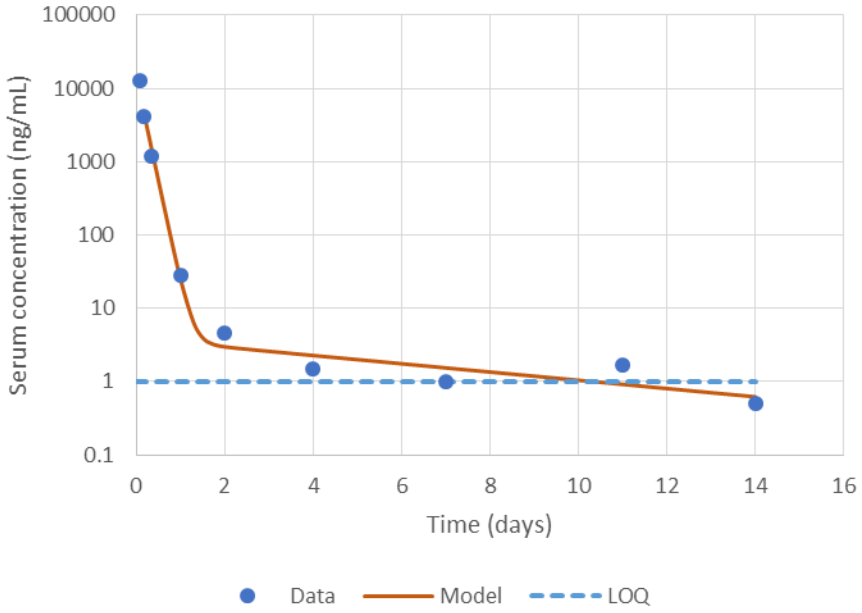

Figure 6. Example of biphasic elimination behavior of PFHx- in male monkey #2052

Table 6. Comparison of original and revised kinetic analyses of PFHx- elimination in monkeys

Cynomolgus monkey (ID number)		Elimination half-life values (d)			
		Original (excluding data > 4d)		Revised (using all data)	
		α phase	β phase	α phase	β phase
M	2052	0.10	1.16		
	2054	0.06	1.73		
	2211	0.11	1.94		
	geomean:	0.09	1.57		
				0.07	2.1
F	2058	0.03	0.95		
	2059	0.06	0.59		
	2061	0.05	0.88		
	geomean:	0.04	0.79		

The revised kinetic analysis using the whole dataset for monkeys results in the following elimination equation:

$$C(t)/C_0 = 0.9975 * \exp(-0.415*t) + 0.0025 * \exp(-0.014*t) \qquad \text{Equation 5}$$

$A = 0.9975$ $k_\alpha = 0.415\ hr^{-1}$ $B = 0.0025$ $k_\beta = 0.014\ hr^{-1}$

Using the biphasic equation, the percentage of PFHx- eliminated from the monkey in the alpha phase is 99.8% (i.e., A * 100). This modeled % of dose eliminated agrees with the observed percent eliminated at 24 hr, $(C_0\text{-}C_{24})/C_0$, which is 99.7% for male and female monkeys (Table 10).

2.5 PFHx- elimination from monkey (Chengelis, 2009)

The elimination of PFHx- from monkeys was performed with monitoring of serum concentrations for times of up to 4 days after IV dosing (Chengelis et al., 2009). A summary of the measured serum concentrations is provided in Table 7. In the original report, a single first-order equation was fitted to the observed data through 8hr which resulted in calculated elimination half-lives of 1.0d in males and 0.42d in females.

Table 7. Concentration of PFHx- in monkey serum following a single IV dose of 10 mg/kg (Chengelis, 2009)

Time (hr)	Concentration (ng/g)									
	Male					Female				
	1	2	3	Mean	Std Dev	1	2	3	Mean	Std Dev
1	18,004	25,993	21,259	21,752	4017	25,140	17,469	20,004	20,871	3908
2	5,106	11,419	8,866	8,464	3176	7,447	5,086	7,898	6,810	1510
4	1,414	4,235	2,079	2,576	1475	2,332	2,006	3,078	2,472	550
8	972	1,902	567	1,147	684	341	272	438	350	83.4
24	108	32.8	30.3	57	44	***15***	***15***	84.3	38	40.0
48	***15***	***15***	35.5	22	--	0	0	***15***	5	--

LOQ = 30 ng/mL

First data point < LOQ was set equal to 0.5 LOQ, indicated by bold italics

Due to the short time-frame of this study (4d) and the assumption of a single first-order equation, the kinetic results from this study cannot be directly compared to the parameters obtained from fitting the data to a biphasic model. However, the mean serum concentrations reported for male monkeys for the entire 48 hours of the Chengelis study have been modeled using the biphasic parameters (A, B, α and β) determined from the more extensive Noker, 2001 data presented above. The only adjustable parameter in this regression was C_0, the initial concentration of PFHx- used to normalize the values of the subsequent serum concentrations.

A graph of the resulting fit of the biphasic model from Noker to the data for the male monkeys in the Chengelis study is provided in Figure 7. The excellent agreement between the Noker model and the Chengelis data indicates that both studies clearly support the biphasic elimination of PFHx- with the more extensive data of Noker providing a more sound basis for determining the kinetic rates in the alpha and beta phases of elimination.

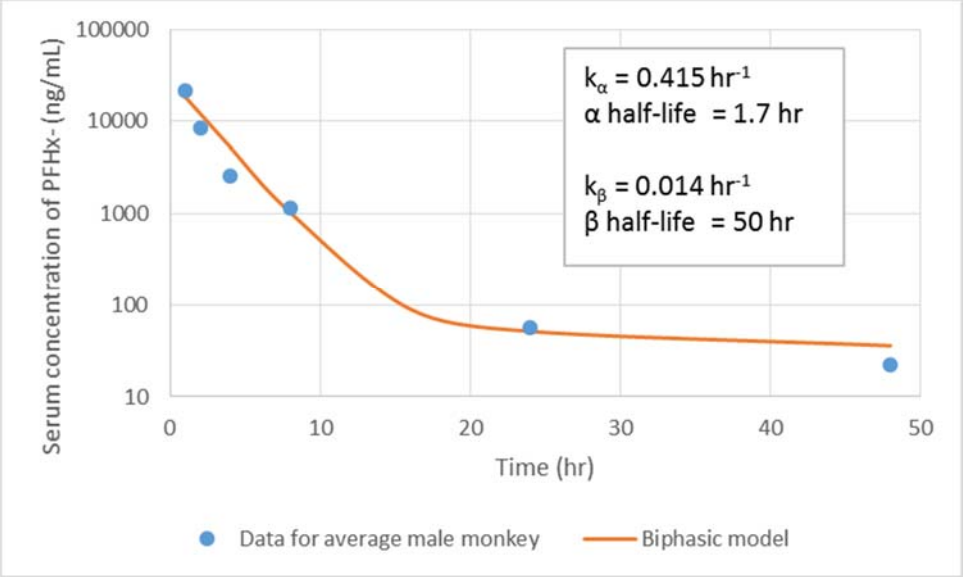

Figure 7. Regression of biphasic elimination model to average male monkey data of Chengelis et al., 2009

2.6 PFHx- Elimination from humans

A biomonitoring study of human exposure to a suite of perfluoroalkyl substances including PFHx- was conducted with a group of eleven professional ski wax technicans in Europe (Nilsson et al., 2013). The study consisted of periodic blood monitoring data extending from September 2007 to March 2011 with blood concentrations of PFHx- ranging between <0.05 (LOQ) and 12.2 ng/mL in individual sampling events. The exposure of the technicians resulted from inhalation of volatilized and particulate matter generated from the application of fluorinated ski waxes to racing skis using hot irons and scrapers.

In a subsequently published article, the temporal series of PFHx- concentrations in the blood of each technican was analyzed using only sets of data with 3 to 9 sequential samples that showed a consistent downward trend (Russell et al., 2013). The geomean elimination half-life of PFHx- determined from this analysis was 32d with a range of 14 to 49 days. The half-life in this analysis was reported to be an "apparent" elimination half-life since there may have been ongoing inhalation exposure of PFHx- and/or precursor compounds which metabolically form PFHx- during the selected period of elimination. As a result, the authors explicitly noted that the 32d value should be regarded as a highly conservative upper-bound estimate of the actual rate of PFHx- elimination from humans.

Further analysis of the ski wax technican data provides additional insights into the "intrinsic" or actual rate of PFHx- elimination (i.e., elimination in the absence of ongoing exposure). The biomonitoring data from the ski wax technicians contain numerous instances of dramatic decreases in PFHx- blood concentrations (i.e., from 3-fold to 100-fold) between sequential

monthly blood sampling events. These extreme reductions provide an improved estimate of the actual rate of PFHx- elimination than the apparent rate which was calculated over an extended period of time that may have been biased by ongoing exposure.

A summary of these monthly or bimonthly elimination half-lives for PFHx- from the ski wax technicians is provided in Table 8. The shortest elimination half-life for each individual ranged between 3.9 days and 19.1 days, indicating rapid elimination over periods of one to two months. The five fastest bimonthly half-life values (for Technicians 1, 2 and 3) were for instances where the final blood concentration rapidly declined to a value less than the LOQ of 0.05 ng/mL. For these cases, a value corresponding to ½ LOQ was assumed for the final concentration.

An improved estimate of the intrinsic half-life of PFHx- from humans can be obtained from the sequential blood samples where the second value declined to a value less than the LOQ of 0.05 ng/mL. For these samples (shown highlighted in gray), the half-life ranged between 3.9 and 7.5 days with a geomean value of 5.1 days.

Table 8. Estimates of intrinsic elimination half-life of PFHx- from blood of ski wax technicians

Sampling Interval (days)	Sampling Interval (days)	Elimination Half-life of PFHxA from Human Blood (days) Technician Number						
		1	2	3	4	5	6	8
Dec-07 to Jan-08	30	13.2	3.9	7.4	10.9	19.2		
Jan-08 to Feb-08	30	392.6						25.6
Feb-08 to Mar-08	30		30.8	13.9	13.6	19.1	90.2	
Mar-08 to Apr-08	30		7.5	5.4	9.6		17.1	
Mar-08 to May-08	60	7.5						15.7
May-08 to Jun-08	30		16.2					17.9
Jan-09 to Mar-09	60	54.6						
Feb-09 to Mar-09	30		4.8	4.7				
Shortest HL for each technician:		7.5	3.9	4.7	9.6	19.1	17.1	15.7

For the five half-life estimates shown in gray, the lack of detection in the terminal concentration indicates that there is little or no ongoing exposure to PFHx- or its precursors during the sampling interval. Based on this premise, the geomean half-life of 5.1 days should be viewed as the current best estimate of the intrinsic elimination half-life of PFHx- from humans.

The 5.1d elimination half-life of PFHx- from the ski wax technicians can be put into context by comparing the daily dose of PFHx- calculated from the inhaled concentration in workplace air with the daily dose of PFHx- back calculated from the monitored blood concentration. Details of this calculation are provided in Appendix 2 and the results of this comparison for technicians 1 to 5 during Dec-07 to Jan-08 are as follows:

1. Average daily inhalation dose of PFHx- based on inhaled air: 95 ug/d

   (based on reported air monitoring data for PFHx- (Nilsson , 2010) together with typical inhalation rates - See Appendix Section A2.1 for details)

2. Average daily inhalation dose of PFHx- based on inverse modeling from blood: 90 ug/d

   (based on 99% of PFHx- rapidly eliminated, 1% of PFHx- eliminated with 5d beta elimination HL, 7.6-fold blood concentration increase from repeated exposure and use of a one compartment PK model for the terminal elimination phase – See Appendix Section A2.2 for details)

This comparison indicates that ~1% of the inhaled dose results in the observed concentrations of PFHx- in human blood. Based on this analysis, 1% of PFHx- is eliminated from humans with a 5.1 day half-life during the slower (beta) elimination phase while ~99% of PFHx- is eliminated in the rapid alpha phase. Therefore, the elimination of PFHx- from humans is clearly biphasic, with kinetic results very similar to the elimination behavior observed in other mammals.

3. Comparison of PFHx- Elimination Kinetics (PK) data across mammalian species

The temporal series of pharmacokinetic data for mouse, rat and monkey are sufficiently detailed to determine of both the initial (alpha) and terminal (beta) phases of elimination. These data an then be compared with the less detailed data available for MMpig, pig, and human. A comparison of the pharmacokinetic results across species is provided in Table 9.

3.1 Pharmacokinetic Parameters

The alpha elimination half-life is relatively invariant across mammalian species with a geomean value for mammals of 1.7 hr (range = 1.5-1.8 hr). The beta elimination half-life is also relatively invariant with a geomean for mammals of 75 days (range = 50-122 d).

Furthermore, the alpha elimination rate k_α is relatively invariant across mammalian species with a value of 0.4 hr^{-1}. The beta elimination rate k_β beta is relatively invariant across mammalian species with a value of 0.01 hr^{-1}.

Table 9. Comparison of pharmacokinetic elimination parameters across species

Pharmacokinetic Parameter	Mammalian Species					
	Mouse 0.03 kg	Rat 0.25 kg	Monkey ~5 kg	MMpig ~12 kg	Pig ~100 kg	Human ~80 kg
alpha elimination rate (hr^{-1})	0.451	0.38	0.415	ND	0.428	ND
beta elimination rate (hr^{-1})	0.011	ND	0.014	0.011	0.007	0.006
alpha elimination half-life (hr)	1.5	1.8	1.7	ND	1.6	ND
beta elimination half-life (hr)	63	ND	50	64	99	122

3.2 Proportion, % of Dose, of PFHx- Elimination

In addition to evaluating the alpha and beta half-lives of PFHx- from mammals it is important to consider the proportion of elimination that occurs during the alpha phase. There are two ways to evaluate the percentage of alpha phase elimination:

Method 1: Estimation from the biphasic model

Compile the A parameters from the biphasic equations derived for each species. The A parameter represents the fraction of the dose that is eliminated via the alpha phase.

Method 2: Estimation from experimental data

Compare the blood, plasma or serum concentration 24h after dosing with the initial concentration (or the first measured time point). This ratio indicates the proportion of the dose that is excreted during the rapid initial alpha phase of elimination.

A summary of both methods of evaluating the elimination of PFHx- from mammals is provided in Table 10. From this assessment, it is clear that well over 99% of the applied dose of PFHx- is rapidly eliminated by mouse, rat and monkey with only a fraction of 1% remaining in blood 24 hours after dosing.

Table 10. Percent PFHx- eliminated from mammals during alpha phase estimated from biphasic model and from experimental data

Species	Biphasic model		Experimental data (ug/mL)			
	Alpha phase (A*100)	Reference	Initial conc after IV dose	Conc 24h after dosing	% eliminated in 24 hrs	Reference
mouse	99.7%	Equation 2	288	1.11	99.6%	Table 2, male
			296	0.483	99.8%	Table 2, female
rat	100.0%	Equation 3	154	1.03*	> 99.3%	Table 4, male
			191	1.03*	> 99.5%	Table 4, female
monkey	99.8%	Equation 5	8.625	0.023	99.7%	Table 5, male
			20.136	0.056	99.7%	Table 5, female
				* < LOQ, 1/2 LOQ assumed		

A final useful comparison across mouse, rat and monkey is to run a model simulation of all three species for the same dose (10 mg/kg) using the biphasic equations derived for each species. A 10 mg/kg dose was used in the cynomolgus monkey study and is within the dose range tested in the rodents (2 – 100 mg/kg). The simulated dose route was intravenous to avoid complications caused by differences in absorption rate between the species. The results for this simulation are illustrated in Figure 8.

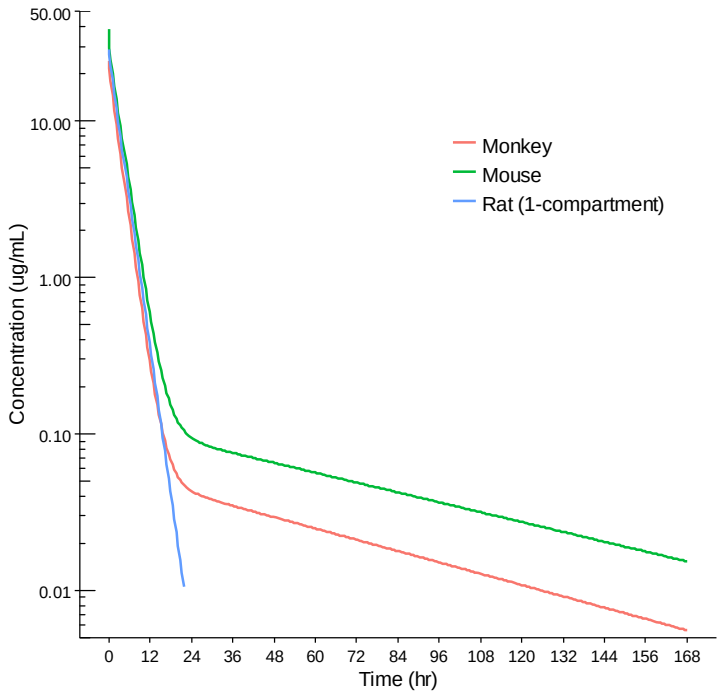

Figure 8. Simulated blood concentrations of PFHx- following a single 10 mg/kg intravenous dose in mouse, rat and cynomolgus monkey

As a result of the similar rapid alpha phase elimination, the initial pharmacokinetic rates of the mouse, rat and monkey are virtually identical, with concentrations declining almost three orders of magnitude before shifting to slower, beta phase elimination.

4. Discussion

The elimination kinetics of PFHx- are remarkably consistent across a wide range of mammalian species with only minor differences between typical laboratory species (mice, rats), larger mammals (microminipigs, pigs and monkeys) and humans.

The initial rate of elimination (i.e., alpha phase) of PFHx- from mammals is extremely rapid and accounts for elimination of more than 99.7% of this substance from blood in less than 24 hours in mice, rats and monkeys. Appropriate data to quantify the α-phase of elimination is not available for the microminipig, pig or human but, based on the consistent alpha and beta kinetic behavior shown across mammalian species, it is scientifically reasonable to expect similar elimination response for these species as well.

The terminal rate of elimination (i.e., beta phase) of PFHx- from mammals is slower, with half-lives of 50-122 hours (2-5 days) in mice, rats, microminipigs, pigs, monkeys and humans. However, almost all of the PFHx- has been eliminated in the alpha phase with this slower beta phase accounting for less than 0.3% of the PFHx- administered dose.

5. Conclusions

(1) PFHx- is consistently eliminated from a wide range of mammalian species with biphasic elimination kinetics.

(2) The initial elimination phase (alpha phase) of PFHx- in mammals is rapid with a half-life of less than 2 hours.

(3) Over 99.7% of PFHx- is rapidly eliminated from mammals during the alpha phase of elimination.

(4) Due to the extensive elimination of PFHx- in the rapid alpha phase, there are no significant pharmacokinetic differences across mice, rats, monkeys, pigs, and humans.

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Appendix 1

Derivation of a biphasic elimination equation from the results of a two-compartment model

A schematic diagram of a two compartment model is shown in Figure A1-1. The chemical is introduced into central compartment C1 at time zero and is eliminated only from compartment 1. In order to result in biphasic elimination behavior, k12 must be greater than or equal to ku. Otherwise, the rate of elimination from compartment 1 is so rapid relative to distribution that the effect of the peripheral compartment C2 is negligible and the elimination effectively occurs from a single compartment.

When distribution is significant, the concentration in compartment C1 initially decreases rapidly due to a combination of elimination plus distribution to the peripheral compartment C2 (i.e., the alpha phase). This process continues until a dynamic equilibrium is established between the central and peripheral compartments (i.e. k12*C1 = k21*C2). After this point, the net direction of transfer reverses with the rate of elimination now controlled by the concentration in C1 which is in pseudo-equilibrium with C2 in the beta phase of elimination.

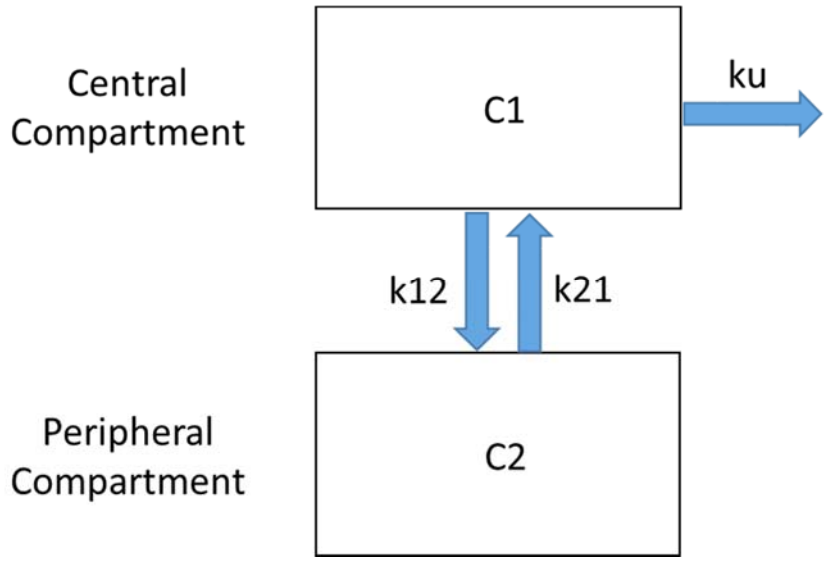

Figure A1-1. Schematic diagram of an open two compartment model

Mathematically, the following equations describe the concentration in the central compartment:

$$C(t)/C_0 = [(k21-\beta)*\exp(-\beta*t) - (k21-\alpha)*\exp(-\alpha*t)]/(\alpha-\beta) \quad \text{Equation A1-1}$$

where

$$\alpha = 0.5*[(k12+k21+ku) + sqrt((k12+k21+ku)^2 – 4*k21*ku)] \quad \text{Equation A1-2}$$

$$\beta = 0.5*[(k12+k21+ku) - sqrt((k12+k21+ku)^2 – 4*k21*ku)] \quad \text{Equation A1-3}$$

The parameters in Equation A1-1 can then be reexpressed as the following biphasic equation which was used in the main text of this report:

$$C(t)/C_0 = A*exp(-\alpha*t) + B*exp(-\beta*t) \quad \text{Equation A1-4}$$

where

$A = (\alpha-k21)/(\alpha- \beta)$

$B = (k21-\beta)/(\alpha- \beta)$

In Equation A1-4, A represents the fraction of chemical eliminated in the faster or alpha phase of elimination and B represents the fraction of chemical eliminated in the slower or beta phase of elimination.

Calculation of biphasic equation parameters from two compartment parameters of Numata, 2014

From kinetic parameters reported for PFHxA in Table S8 of Numata et al, 2014:

$ku = 0.3522\ d^{-1} = 0.0147\ hr^{-1}$

$k21/k12 = 0.910$ (unitless)

In order to observe biphasic elimination behavior in a two compartment model, the rates of k21 and k12 must be much faster than ku.

The value of β calculated from Equation A1-3 is not sensitive to the individual values assumed for k12 and k21 as long as both values are significantly greater than ku and the ratio of 0.910 is maintained. In contrast, the value of α increases in proportion to the increases in the individual values of k12 and k21. This results from the fact that the initial elimination rate α reflects a combination of both the rate of elimination (ku) as well as the rate of distribution into the second compartment (k12) whereas β reflects the slower terminal rate of elimination after distribution equilibrium has been established between both compartments. As a result, α is much faster than ku and β is marginally slower than ku.

It is reasonable to assume values for k12 and k21 that are significantly faster than ku but not so fast as to create an unreasonably estimate of α.

If k12 is assumed to be 15-fold faster than ku, $k12 = 15*0.0147\ hr^{-1} = 0.220\ hr^{-1}$. k21 is then $0.91*0.220\ hr^{-1} = 0.200\ hr^{-1}$.

The resulting values of α and β are then:

$\alpha = 0.5*[(0.225+0.205+0.015) + sqrt((0.225+0.205+0.015)^2 – 4*0.205*0.015)] = 0.428\ hr^{-1}$

$\beta = 0.5*[(0.225+0.205+0.015) - sqrt((0.225+0.205+0.015)^2 – 4*0.205*0.015)] = 0.0069\ hr^{-1}$

$A = (\alpha-k21)/(\alpha- \beta) = (0.428-0.200)/(0.428-0.0069) = 0.541$

$B = (k21-\beta)/(\alpha- \beta) = (0.200-0.0069)/(0.428-0.0069) = 0.459$

The estimated overall biphasic equation for the pig is then:

$C(t)/C_0 = 0.541*\exp(-0.428*t) + 0.459*\exp(-0.007*t)$

The α elimination half-life corresponds to an estimated initial half-life of 1.6 hr while the beta elimination half-life corresponds to a terminal half-life of 4.1d which agrees with the value reported by Numata et al., 2014.

This summary of the mathematics associated with a two compartment model is based on the equations published in **Toxicants and Drugs: Kinetics and Dynamics**, by Ellen O'Flaherty, John Wiley & Sons (1981).

Appendix 2

PFHx- dose inhaled by ski wax technicans: Calculation from monitored air concentration compared with calculation from monitored blood concentration

The average concentration of PFHx- in the ambient air and in blood of ski wax technicians during the Dec-07 to Jan-08 work period is summarized in Table 1.

Table A2-1. Mean measured values of PFHx- in air and in technician blood

Location	Date	Technician	PFHx- in air (ng/m3)	Mean PFHx- in blood (ng/mL)	Body weight (kg)
Kuusamo, Finland	Dec-07 to Jan-08	1	7300	7.4	86
		2	12000	2.4	67
		3	6800	2.9	87
		4	5700	1.3	80
		5	7600	1.3	75
		Average:	7880	3.1	79

(data from Table 1 of Nilsson et al., 2010 and Supplementary Information of Nilsson et al., 2013)

A2.1 Average daily inhalation dose of PFHx- calculated from monitored air concentration

The mean rate of PFHx- inhalation during the Dec-Jan work period was:

$$\frac{7880\ ng}{m3} * \frac{1.5\ m3}{hr} * \frac{8\ hr}{day} * \frac{\mu g}{1000\ ng} = \textbf{95 µg PFHx- inhaled per day}$$

A2.2 Average daily inhalation dose of PFHx- calculated from monitored blood concentration

Assume ~99% of PFHx- is rapidly eliminated with a half-life of 1-2 hours, similar to all other mammalian species. Therefore ~1% of PFHx- is slowly eliminated with a half-life of ~5 days.

Repeated daily exposure to PFHx- results in higher concentrations in blood than a single exposure since the beta elimination half-life is ~5 days. The resulting concentration increase from repeated daily PFHx- exposure can be estimated with the following equation:

$$\text{Increase factor} = \frac{\text{Blood concentration after n days}}{\text{Blood concentration after 1 day}} = \frac{(1-\exp(-n*k))}{(1-\exp(-k))}$$

For repeated daily exposure over a period of 30 days, the resulting increase in the blood concentration of PFHx- is a factor of 7.6-fold higher than for a single exposure.

The observed mean blood concentration during the Dec-07 to Jan-08 sampling period was 3.1 ng/mL (see Table A2-1). The corresponding blood concentration for a single day of exposure is then 3.1/7.6 = 0.4 ng/mL for a single day of inhalation exposure.

For the beta period of elimination, a one-compartment PK model can be used to relate blood concentration to inhaled dose:

Dose = Cb * k * Vd * BW

$$= \frac{0.4\ \text{ng}}{\text{mL}} * \frac{\ln(2)}{5\text{d}} * \frac{0.2\ \text{L}}{\text{kg}} * 79\ \text{kg} * \frac{\text{ug}}{1000\ \text{ng}} * \frac{1000\ \text{mL}}{\text{L}}$$

= 0.9 ug PFHx- inhaled per day

Based on the assumptions stated above concerning the extent of beta phase elimination, this calculated dose corresponds to the 1% of the actual inhaled dose that is eliminated during the beta phase.

Therefore, the estimated total inhaled dose is 0.9/0.01 = **90 ug PFHx- inhaled per day**