WILRESEARCH

FINAL REPORT

Study Title:	A Single (Oral Gavage) Dose Toxicokinetic Study of NaPFHx in Rats and Mice
Laboratory Project ID:	WIL-534022
Author:	Jennifer A. Thomas, PhD
Test Guideline:	Not Applicable
Study Initiation Date:	25 October 2011
Study Completion Date:	28 November 2012
Performing Analytical Laboratory:	WIL Research 1407 George Road Ashland, OH 44805-8946
Sponsor:	AGC Chemicals Asahi Glass Company, Ltd. 10 Goikaigan, Ichihara-shi Chiba 290-8566, JAPAN
Total Number of Pages:	240

COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study, WIL-534022, and those required by the United States EPA GLP Standards 40 CFR Part 792 (18 September 1989); the OECD Principles of GLP [C(97) 186/Final] (26 November 1997); the WIL Research SOPs; and the protocol and amendments as approved by the Sponsor. A Certificate of Analysis was provided by the Sponsor (presented in Appendix B); the characterization analyses were conducted according to unknown standards.

- Analyst® version 1.4.2, a widely used commercial software package for AB Sciex mass spectrometers, was used on this study for data acquisition. Validation of Analyst® version 1.4.1 has been performed. Upon upgrading to version 1.4.2, a gap analysis and additional validation testing was not completed prior to use, however, adequate functioning of the software was demonstrated with each analysis via acceptable assay performance as per WIL Research SOP AC-167 and/or AC-168. Therefore, use of Analyst® version 1.4.2 would not be expected to affect the quality or integrity of the data or the outcome of the study.

Study Director: Jennifer Thomas — Signature — 28 NOV 2012 — Date

Typed Name of Signer: Jennifer A. Thomas, PhD

Typed Name of Laboratory: WIL Research

Sponsor: _____ Signature _____ Date

Typed Name of Signer: Motoki Shinohara

Typed Name of Company: AGC Chemicals

Submitter: _____ Signature _____ Date

Typed Name of Signer: _____

Typed Name of Company: _____

534022

FLAGGING STATEMENT

I have applied the criteria of 40 CFR Part 792 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Submitter: _____ _____
Signature Date

Typed Name of Signer: _____

Typed Name of Company: _____

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1. SUMMARY

1.1. OBJECTIVES

The objective of this study was to measure the serum levels of Perfluorohexanoic Acid (PFHxA) following oral dosing of Perfluorohexanoic Acid Sodium Salt (NaPFHx) and to compare and contrast the toxicokinetic behavior in males versus females and in rats versus mice.

1.2. STUDY DESIGN

The test substance, NaPFHx, was supplied as an aqueous 20% (w/w) solution. The solution was diluted to a target dosage concentration of 30 mg/mL and administered as a single oral gavage dose to male and female Sprague Dawley rats and to male and female CD-1 mice. The target dosage level was 300 mg/kg, administered at a dose volume of 10 mL/kg. Blood samples were collected from 4 animals/sex/species prior to dosing and 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours after dose administration. Blood samples were collected into tubes containing no anticoagulant, allowed to clot at room temperature, and processed to serum. Following the final blood collection, animals were euthanized by CO_2 inhalation and discarded. Serum samples were analyzed for PFHxA concentration, by the Analytical Chemistry Department at WIL Research using a validated method.

1.3. RESULTS

The mean dose of NaPFHx for male and female rats and mice was 99% to 100% of the target 300 mg/kg. Oral administration of NaPFHx to mice and rats resulted in systemic exposure to PFHxA. At 300 mg/kg, mean plasma PFHxA concentrations increased through 0.5 hour post-dosing and then generally decreased through 48 hours post-dosing in both species and sexes. Toxicokinetic results are summarized in the following table:

Toxicokinetic Results for PFHxA in Mice and Rats

	AUC_{last} (ng•h/mL)	AUC_{inf} (ng•h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
			Mice		
Males	3629157	3633476	576750	0.5	5.3
Females	2089691	2091339†	684250	0.5	4.0†
			Rats		
Males	1317688	1318240†	328750	0.5	3.7†
Females	745868	746589	376750	0.5	11

† = Approximate value.

Exposure to PFHxA, in terms of AUC_{last} and C_{max}, was approximately 2- to 3-fold higher for mice than for rats in both sexes. Exposure to PFHxA, in terms of AUC_{last}, was approximately 2-fold higher for males than for females in rats and mice. In terms of C_{max}, exposure was slightly higher for females than for males in both species, but the difference was less than 2-fold.

After oral dosing of NaPFHx, T_{max} was 0.5 hour post-dosing for both sexes in mice and rats. A secondary peak in PFHxA concentration was observed for male mice at 2 hours post-dosing, but this may have been due to variability in the concentration results among the animals. Reportable half-life values for PFHxA were 5.3 hours for male mice and 11 hours for female rats.

1.4. CONCLUSIONS

At 300 mg/kg, mean plasma PFHxA concentrations increased through 0.5 hour post-dosing and then generally decreased through 48 hours post-dosing in both species and sexes. Mice and rats have comparable sex differences in exposure to PFHxA after a single oral administration of NaPFHx as measured by AUC_{last}. For the purpose of determining safety, mouse exposure (AUC_{last}) is generally higher than the rat of the same sex by approximately 2- to 3-fold and may therefore present a more conservative evaluation of the safety and toxicology of NaPFHx in subsequent studies.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A Single (Oral Gavage) dose Toxicokinetic Study of NaPFHx in Rats and Mice." The study was performed at WIL Research, Ashland, OH as Study Number WIL-534022. The Study Director was Jennifer A. Thomas, PhD, Senior Research Scientist and Head, *In Vitro* Metabolism.

The objective of this study was to measure the serum levels of Perfluorohexanoic Acid (PFHxA) following oral dosing of Perfluorohexanoic Acid Sodium Salt (NaPFHx) and to compare and contrast the toxicokinetic behavior in males versus females and in rats versus mice.

For the data collection process, each phase of the study was separated into independent WIL Research computer protocols. The computer protocol reference numbers and types of data collected were identified as follows:

Computer Protocol	Type of Data Collected
WIL-534022M	Main Study Data - Mice
WIL-534022P	Pretest Data - Rat
WIL-534022Q	Pretest Data - Mice
WIL-534022R	Main Study Data - Rat

The study protocol and deviations from the protocol are presented in Appendix A.

A list of abbreviations potentially used in this report is presented in Section 10. (Abbreviations).

2.2. KEY STUDY DATES

Date(s)	Event(s)
9 February 2009	Receipt of Test Substance
25 October 2011	Experimental Starting Date (Receipt of Animals)
1 November 2011	Experimental Start Date (First Day Test Substance was Applied)
5 November 2011	Last Day of Sample Collection
12 October 2012	Experimental Termination/Completion Date (Last Day of Data Collection)

2.3. WIL RESEARCH KEY STUDY PERSONNEL

Eric S. Bodle, PhD	Assistant Director, Analytical Chemistry
Scott D. Freer, BA	Manager/Research Chemist, Analytical Chemistry
Justin Godsey, BS, LATG	Associate Research Scientist, Metabolism
Janet M. House, BS, RQAP-GLP	Data Coordinator, Metabolism
Stephanie L. Ledyard, AS	Quality Control Coordinator, Metabolism
William (Clue) Nethero, BA	Research Chemist, Bioanalytical Chemistry
Timothy B. Riehl Jr., BS, LATG	Group Supervisor, Metabolism
Katie A. Alonso, BS	Senior Publishing Specialist, Reporting & Technical Support Services
Gregory A. Hawks, AS	Group Supervisor, Reporting & Technical Support Services

3. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

3.1. TEST SUBSTANCE: DESCRIPTION AND SOURCE

The test substance, NaPFHx (Perfluorohexanoic Acid Sodium Salt), was supplied as a 20% NaPFHx solution by the Sponsor. The Certificate of Analysis is presented in Appendix B. The translucent plastic bottles containing NaPFHx were received at ambient temperature at WIL Research by the Formulations Department from AGC Chemical on 9 February 2009. The material appeared as a clear, colorless liquid. One bottle containing NaPFHx (reference no. 090018-002, lot no. 81102, expiration date 30 November 2013) was transferred to the Metabolism department on 31 October 2011. The test material was stored at room temperature. According to the Certificate of Analysis, the purity in solids as measured was 98.2% by gas chromatography after

methyl-esterification. A retention sample of NaPFHx (reference no. 090018-001) was collected on 30 March 2009 and stored according to WIL Research SOPs.

3.2. PREPARATION OF THE DOSING FORMULATION

3.2.1. FORMULATION ADMINISTERED TO MICE

On 31 October 2011, 20% NaPFHx solution (reference 090018-002) was placed on a stir plate and stirred with a magnetic stir bar for 4 hours and 4 minutes. The solution was assigned reference no. 534022MA1-1-1. Using a transfer pipette, 6.42 g of 20% NaPFHx solution (reference no. 534022MA1-1-1) was placed into a tared glass jar, calibrated to contain 40 mL with a magnetic stir bar. Deionized (DI) water was added to bring the total volume to 40 mL. The solution was assigned reference no. 534022MA1-1-2. The solution was stirred overnight at room temperature. The final concentration was 32.1 mg/mL NaPFHx, or 30 mg/mL free acid (PFHxA). Following dosing, the solution was stored at room temperature.

3.2.2. FORMULATION ADMINISTERED TO RATS

On 2 November 2011, 20% NaPFHx solution (reference no. 534022MA1-1-1) was placed on a stir plate and stirred with a magnetic stir bar for 4 hours and 57 minutes. The solution was assigned reference no. 534022MA1-2-1. The 20% NaPFHx solution (reference no. 534022MA1-2-1), 24.08 g, was placed into a tared glass jar calibrated to contain 150 mL with a magnetic stir bar. DI water was added to bring the total volume to 150 mL. The solution was assigned reference no. 534022MA1-2-2. The solution was stirred overnight at room temperature. The final concentration was 32.1 mg/mL NaPFHx, or 30 mg/mL PFHxA. Following dosing, the solution was stored at room temperature.

3.3. CONCENTRATION ANALYSES OF THE NAPFHX DOSE FORMULATIONS

Quadruplicate samples were collected from each formulation using a syringe and dosing cannula and placed in individual polypropylene tubes. Single 1.0 mL aliquots from 2 of the dosing formulation samples from each quadruplicate set were analyzed for

concentration of the test substance. The concentration of the NaPFHx dosing formulations (534022MA1-1-2 [Mice] and 534022MA1-2-2 [Rat]) was assessed by LC/MS/MS. The methods are presented in Appendix C, Analyses of Dosing Formulations. Analyzed formulations used for dose administration were measured at a mean concentration of 99.0% and 99.8% of target for Group 1 and Group 2 (Table 3, Appendix C), respectively. These results met the applicable WIL Research SOP acceptance criteria for test substance concentration acceptability, thus indicating stability.

3.4. TEST SYSTEM, ANIMAL RECEIPT, AND ANIMAL HOUSING

Twenty-three male and 23 female Sprague Dawley, Crl:CD(SD), rats were received from Charles River Laboratories (Portage, MI) on 25 October 2011. According to the supplier, the male rats were approximately 7 weeks old and female rats were approximately 6 weeks old at the time of receipt. Upon arrival at the laboratory, each animal was inspected by a qualified technician. The following day, animals were uniquely identified by a metal ear tag displaying a permanent animal number and weighed. The male rats ranged in weight from 182 g to 200 g and the female rats ranged in weight from 134 g to 167 g, (Table D1 and Table D3, Appendix D). The animals were judged to be in good health and were immediately placed in quarantine/acclimation. Acclimation lasted for approximately 9 days.

During the quarantine/acclimation period, all animals were housed individually in suspended wire-mesh cages in an environmentally controlled room with at least 10 fresh air changes per hour. The cages were elevated above cage-board or other suitable material, which was changed at least 3 times per week. Individual cage cards were affixed to each cage displaying the permanent animal number, study number, and group number. All animals were observed for mortality and morbidity twice daily.

Temperature and humidity in the animal room used for the study were monitored continuously and scheduled for automatic data capture on an hourly basis. The animals were housed in room B118. The mean daily temperature ranged from 70.7°F to 72.8°F

(21.5°C to 22.7°C), and the mean daily relative humidity ranged from 34.2% to 43.9%. Light timers were used to provide a daily photoperiod of 12 hours of light followed by 12 hours of darkness. The light status (on or off) was recorded once every 15 minutes. The light/dark cycle was interrupted for room activities.

Twenty-three male and 23 female CD-1, Crl:CD-1(ICR), mice were received from Charles River Laboratories (Raleigh, NC) on 25 October 2011. According to the supplier, the male mice were approximately 10 weeks old and the female mice were approximately 17 weeks old at the time of receipt. Upon arrival at the laboratory, each animal was inspected by a qualified technician. The following day, animals were uniquely identified by a metal ear tag displaying a permanent animal number, and weighed. The male mice ranged in weight from 23.2 g to 32.2 g and the female mice range in weight from 23.3 g to 27.1 g (Table D2 and Table D4, Appendix D). The animals were judged to be in good health and were immediately placed in quarantine/acclimation. Acclimation lasted for approximately 7 days.

Upon arrival, all mice were triple-housed or double-housed per cage for approximately 1 day. Thereafter, during the quarantine/acclimation period, all animals were housed individually in suspended wire-mesh cages in an environmentally controlled room with at least 10 fresh air changes per hour. The cages were elevated above cage-board or other suitable material, which was changed at least 3 times per week. Individual cage cards were affixed to each cage displaying the permanent animal number, study number, and group number. All animals were observed for mortality and morbidity twice daily.

Temperature and humidity in the animal room used for the study were monitored continuously and scheduled for automatic data capture on an hourly basis. The animals were housed in room B119. The mean daily temperature ranged from 70.1°F to 70.6°F (21.2°C to 21.4°C), and the mean daily relative humidity ranged from 46.4% to 52.6%. Light timers were used to provide a daily photoperiod of 12 hours of light followed by

12 hours of darkness. The light status (on or off) was recorded once every 15 minutes. The light/dark cycle was interrupted for room activities.

Animals were maintained in accordance with Guide for the Care and Use of Laboratory Animals (National Research council, 1996). The animal facilities at WIL Research are accredited by AALAC International. Enrichment devices were provided to all animals as appropriate throughout the study for environmental enrichment and to aid in maintaining the animals' oral health.

3.5. DIET AND DRINKING WATER

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 (meal) was made available *ad libitum* throughout acclimation and the biological phase of the study. Each lot used was documented. Analyses of the certified feed for the presence of heavy metals and pesticides were provided by the manufacturer. Feeders were changed and sanitized at least weekly.

Reverse osmosis-treated water was made available *ad libitum*. Water was supplied via an automatic watering system. The municipal water supplying the laboratory is sampled and analyzed for contaminants according to WIL Research SOPs.

3.6. SELECTION PROCEDURES AND GROUP ASSIGNMENT

One day prior to dosing, the animals were weighed and examined in detail for physical abnormalities. The male rats ranged in weight from 246 g to 276 g and the female rats ranged in weight from 170 g to 205 g, (Table D1 and Table D3, Appendix D). The male mice ranged in weight from 28.0 g to 34.8 g and the female mice ranged in weight from 24.3 g to 30.6 g, (Table D2 and Table D4, Appendix D). Animals that were deemed suitable for testing were randomly assigned to groups based on body weight stratification into a block design using a computer program according to the following table. Body weights at randomization were within 20% of the mean for each sex.

Group	Species	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dose Conc. (mg/mL)	Number of Animals	Sample Collection
1	Rat	300	10	30	20 M/20 F	Blood
2	Mouse	300	10	30	20 M/20 F	Blood

M = Male
F = Female

The group assignments and scheduled blood collection intervals for individual animals are indicated in Table D1, Table D2, Table D3, and Table D4 (Appendix D).

3.7. DOSE ADMINISTRATION

On the day of dose administration, each animal was weighed. The male rats ranged in weight from 254 g to 283 g and the female rats ranged in weight from 181 g to 215 g (Table D1 and Table D3, Appendix D). The male mice ranged in weight from 28.7 g to 33.7 g and the female mice ranged in weight from 23.7 g to 30.1 g (Table D2 and Table D4, Appendix D). The male rats were approximately 8 weeks old and the female rats were approximately 7 weeks old at initiation of dosing. The male mice were approximately 11 weeks old and the female mice were approximately 18 weeks old at initiation of dosing. Doses were administered once by oral gavage at a dose volume of 10 mL/kg. Calculations of individual doses are presented in Table E1, Table E2, Table E3, and Table E4 (Appendix E). The dosing formulation was maintained at room temperature throughout the dose administration period. The rats were dosed on 3 November 2011 and the mice were dosed on 1 November 2011.

3.8. BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples were collected for the determination of PFHxA concentration in serum at approximately 1 hour prior to dosing and approximately 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dosing. Blood collections were rotated among 4 animals/sex/species, such that each animal (rat or mouse) was sampled no more than 2 times throughout the entire collection period. Blood samples (target volume of 0.5 mL for rats and 0.25 mL for

mice) were collected from the jugular vein into non-chilled tubes containing no anticoagulant. Blood samples were allowed to clot at room temperature and centrifuged to isolate serum within 30 minutes of collection for approximately 10 minutes at 2500 rpm and 4°C. The isolated serum was collected and transferred to a Nunc© plastic vial and stored at approximately -20°C.

3.9. ANIMAL DISPOSITION

Following the final sample collection, all animals placed on study were euthanized by CO_2 inhalation and the carcasses discarded. Mice not placed on study were transferred to the WIL Research stock colony. Rats not placed on study were euthanized by CO_2 inhalation and the carcasses discarded.

3.10. SAMPLE ANALYSIS

Sample analysis was conducted by LC/MS/MS. A summary of the methods and results are presented in Appendix F, Determination of Perfluorohexanoic Acid Concentrations in Serum Samples.

3.11. CALCULATIONS

Summary calculations for this report were performed with Microsoft Excel® spreadsheets using full floating decimal point calculations. Calculations were performed on the data as collected or displayed or output by instruments, with no censoring for quantitation limits or significant figures. With the exception of descriptive statistics such as mean and standard deviation (SD), equations for calculations are presented with the tables. Slightly different results can be expected if calculations are based on the values as presented in the tables because some numbers have been rounded for display.

3.12. TOXICOKINETIC CALCULATIONS

Toxicokinetic parameters were calculated using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA). Graphical presentations were created using Prism 5 for Windows, Version 5.02, (GraphPad Inc., La Jolla, CA).

All toxicokinetic parameters were calculated from the individual plasma concentration data as indicated in the following table; all values were used with sparse sampling (no potential outliers were excluded). Descriptive statistics (mean, standard deviation [SD], and relative standard deviation [RSD]) were calculated for the serum concentration data. For all calculations, samples were assigned a value of zero if the concentration was below the LLOQ.

C_{max}	The maximum measured concentration of the analyte in serum.
T_{max}	The sampling time at which C_{max} was reached.
T_{last}	The sampling time of the last serum analyte concentration >LLOQ.
C_{last}	The concentration of the last serum analyte concentration >LLOQ.
AUC_{last}	The area under the serum analyte concentration vs. time curve from the time of dosing to the time of the last concentration >LLOQ. The values were calculated by linear trapezoidal summation using the equation: $$AUC_{last} = \sum_{0}^{T_{last}} \frac{C_1 + C_2}{2} \times \Delta t$$ where C_1 and C_2 are successive serum compound concentrations and Δt is the sampling interval, in hours, between C_1 and C_2.
AUC_{inf}	The estimate of the area under the serum concentration vs. time curve from the time of dosing to infinity. The values were calculated using the formula: $$AUC_{inf} = AUC_{last} + C_{last} / \lambda_z$$ where AUC_{last} was defined previously, C_{last} is the serum concentration at T_{last} and λ_z is defined subsequently. Extrapolations of more than 15% of the total AUC are reported as approximations.

AUC % Extrapolated	Percentage of AUC_{inf} due to extrapolation from T_{last} to infinity. The values were calculated using the formula: $$AUC\%\,Extr. = \frac{AUC - AUC_{last}}{AUC} \times 100$$ where $AUC = AUC_{inf}$.
Lambda Z (λ_z)	The terminal elimination rate constant for the analyte in serum. The values were calculated using the equation: $$\lambda_z = -\ln[10] \times b$$ where b is the slope of the least-squares linear regression line of the log serum concentrations through at least three time points after T_{max}.
$t_{1/2}$	The half-life for the analyte in serum. The values were calculated using the formula: $$t_{1/2} = -\ln[0.5]/\lambda_z$$ where λ_z is defined previously. The following requirements had to be met for an acceptable calculation of $t_{1/2}$: 1) the coefficient of determination for λ_z (r^2) was at least 0.90; 2) the span of time points used in $t_{1/2}$ determination was at least twice the calculated value of $t_{1/2}$. Values that did not meet these criteria are reported as approximations.

3.13. DATA ACQUISITION AND ANALYSIS

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval of information for archived materials (*e.g.*, lab books, study data, wet tissues, slides, *etc.*)
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF)
Master Schedule	Maintains the master schedule for the company.
Metasys DDC Electronic Environmental Control System	Controls and monitors animal room environmental conditions.
Microsoft® Office 2002 and 2007; GraphPad Prism® 2008	Used in conjunction with the publishing software to generate study reports.
WIL Formulations Dispense System (WFDS)	In-house developed system for use in conjunction with Provantis Dispense™ to ensure proper storage and use of formulations.
WIL Toxicology Data Management System™ (WTDMS™)	In-house developed system used for collection and reporting of in-life and postmortem data.

4. RESULTS

4.1. DOSING FORMULATION ANALYSIS AND ADMINISTERED DOSAGE

Summary Data: Table 1, Table 2

The concentration of NaPFHx in the dosing formulations was determined by the WIL Research Analytical Chemistry Department using a GLP validated LC/MS/MS method. A contributing report containing a detailed description of the validation and analytical results is provided in Appendix C. The NaPFHx formulation was prepared to provide a target dose of 300 mg/kg PFHxA delivered at a rate of 10 mL/kg or 30 mg/mL PFHxA. The mean analyzed concentration for rats was 29.7 mg/mL PFHxA, or 99.0% of the target concentration (Table 3, Appendix C). The mean analyzed concentration for mice was 29.9 mg/mL PFHxA, or 99.8% of the target concentration (Table 3, Appendix C). The mean dose for male rats was 297 mg/kg and for female rats was 299 mg/kg, or 99% and 100% of target, respectively (Table 1). The mean dose for male and female mice was

299 mg/kg, or 100% of target (Table 2). These analytical results met the WIL Research SOP acceptance criteria for concentration acceptability for solution formulations (90% to 110%).

4.2. SERUM CONCENTRATION OF PFHXA

Summary Data: Table 3, Table 4, Figure 1, Figure 2, Figure 3, Figure 4

Mean (±SD, %CV) serum concentrations of PFHxA following oral (gavage) administration of 300 mg/kg of NaPFHx to male and female mice and rats are presented in Table 3 and Table 4, respectively. Individual and Mean (±SD) serum concentrations of PFHxA versus time are illustrated in Figure 1 (mice), Figure 2 (mice), Figure 3 (rats), and Figure 4 (rats).

At 300 mg/kg, mean plasma PFHxA concentrations increased through 0.5 hour post-dosing and then generally decreased through 48 hours post-dosing in both species and sexes. At 0.5 hours post-dosing, the concentration of PFHxA was approximately 577,000 ng/mL in male mice, 684,000 ng/mL in female mice, 329,000 ng/mL in male rats, and 377,000 ng/mL in female rats. A secondary peak in PFHxA concentration was observed for male mice at 2 hours post-dosing, but this may have been due to variability in the concentration results among the animals. Overall, the variability was comparable between rats and mice of both sexes based on the overall range and distribution of the %CV values among each group.

4.3. TOXICOKINETICS OF PFHXA

Summary Data: Table 5

The toxicokinetic results calculated from the mean serum PFHxA concentration data in male and female mice and rats are presented in Table 5.

Oral administration of NaPFHx to mice and rats resulted in systemic exposure to PFHxA. Exposure to PFHxA, in terms of AUC_{last} and C_{max}, was approximately 2- to 3-fold higher for mice than for rats in both genders.

Exposure to PFHxA, in terms of AUC_{last}, was approximately 2-fold higher for males than for females in rats and mice. In terms of C_{max}, exposure was slightly higher for females than for males in both species, but the difference was less than 2-fold.

After oral dosing of NaPFHx, T_{max} was 0.5 hour post-dosing for both genders in mice and rats. A secondary peak in PFHxA concentration was observed for male mice at 2 hours post-dosing, but this may have been due to variability in the concentration results among the animals. Half-life values for PFHxA were 4.0 hours for female mice, 5.3 hours for male mice, 11 hours for female rats, and 3.7 hours for male rats, although values for the female mice and male rats were considered estimates because the coefficient of determination for λ_z (r^2) was <0.90.

5. CONCLUSIONS

At 300 mg/kg, mean plasma PFHxA concentrations increased through 0.5 hour post-dosing and then generally decreased through 48 hours post-dosing in both species and sexes. At 0.5 hours post-dosing, the concentration of PFHxA was approximately 630,500 ng/mL in mice and 353,000 ng/mL in rats, with less than a 2-fold difference in C_{max} between genders within a species. Mice and rats have comparable sex differences in exposure to PFHxA after a single oral administration of NaPFHx as measured by AUC_{last} approximately 2-fold higher for males than for females. For the purpose of determining safety, mouse exposure (C_{max} and AUC_{last}) is generally higher than the rat of the same sex by approximately 2- to 3-fold and may therefore present a more conservative evaluation of the safety and toxicology of NaPFHx in subsequent studies.

6. REPORT REVIEW AND APPROVAL

Report Authored and Approved by:

Jennifer A. Thomas, PhD
Senior Research Scientist and
Head, *In Vitro* Metabolism
Study Director

28 Nov 2012
Date

Report Prepared by:

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Project Specialist, Metabolism
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28 Nov 2012
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28 Nov 2012
Date

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Stephanie L. Ledyard, AS
Quality Control Coordinator, Metabolism

7. QUALITY ASSURANCE STATEMENT

7.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management
25-Oct-2011	Protocol	25-Oct-2011	28-Nov-2011
01-Nov-2011	Blood Collection and Processing	01-Nov-2011	20-Dec-2011
16-Mar-2012, 19-Mar-2012, 22-Mar-2012, 23-Mar-2012	Study Records (A-1)	23-Mar-2012	23-Mar-2012
23-Mar-2012, 26-Mar-2012, 10-Apr-2012	Analytical Chemistry Report	10-Apr-2012	10-Apr-2012
15-May-2012	Test Article Analysis	15-May-2012	15-May-2012
26-Apr-2012, 27-Apr-2012, 30-Apr-2012, 24-May-2012, 25-May-2012	Study Records (MI-1)	25-May-2012	25-May-2012
26-Apr-2012, 25-May-2012	Study Records (MA-1)	25-May-2012	25-May-2012
26-Apr-2012, 27-Apr-2012, 30-Apr-2012, 24-May-2012, 25-May-2012	Draft Report (excluding Analyses of Dosing Formulations)	25-May-2012	25-May-2012
22-Jun-2012	Audited Analytical Chemistry Report	22-Jun-2012	22-Jun-2012
27-Jun-2012	Audited Report (excluding Analyses of Dosing Formulations)	27-Jun-2012	27-Jun-2012
10-Aug-2012, 13-Aug-2012	Study Records (B-1, B-2)	13-Aug-2012	13-Aug-2012
15-Aug-2012, 16-Aug-2012	Bioanalytical Chemistry Report - Mouse Serum	16-Aug-2012	16-Aug-2012

16-Aug-2012, 20-Aug-2012	Bioanalytical Chemistry Report - Rat Serum	20-Aug-2012	20-Aug-2012
06-Sep-2012	Audited Bioanalytical Chemistry Report - Rat Serum	06-Sep-2012	06-Sep-2012
06-Sep-2012	Audited Bioanalytical Chemistry Report - Mouse Serum	06-Sep-2012	06-Sep-2012
30-Oct-2012	Study Records (B-2: Long-term stability)	30-Oct-2012	30-Oct-2012
13-Nov-2012	Revised Audited Bionanalytical Chemistry Report - Mouse Serum	13-Nov-2012	13-Nov-2012
26-Nov-2012	Final Report	26-Nov-2012	26-Nov-2012

This study was inspected in accordance with the applicable GLP Regulations, the WIL Research SOPs, and the protocol and protocol amendments, with the following exceptions. The data located in Appendix B (Certificate of Analysis) was the responsibility of the Sponsor. A yearly internal facility inspection is conducted by the WIL Research Quality Assurance Department.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and the WIL Research SOPs.

Tracy S. DeVan Booth

Tracy S. DeVan Booth, AA, RQAP-GLP
Quality Assurance Representative

28 Nov 2012
Date

8. REFERENCES

National Research Council. Guide for the Care and use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences; National Academy Press: Washington, DC, 1996.

9. DATA RETENTION

The raw data, the retention sample(s) if applicable, pertinent electronic storage media, and the original final report are retained in the WIL Research Archives in compliance with regulatory requirements.

10. ABBREVIATIONS

The following abbreviations may apply to this report:

µ	-	micro
µL	-	microliter
AAALAC	-	Association for Assessment and Accreditation of Laboratory Animal Care
amu	-	atomic mass unit
AUC	-	area under the curve
Ci	-	curie
cm	-	centimeter
C_{max}	-	maximum measured concentration of the analyte in plasma
conc.	-	concentration
CO_2	-	carbon dioxide
cpm	-	counts per minute
DI	-	deionized
DMSO	-	dimethylsulfoxide
dpm	-	disintegrations per minute
EPA	-	Environmental Protection Agency
FA	-	formic acid
g	-	gram
GLP	-	Good Laboratory Practices
GMP	-	Good Manufacturing Practices
HPLC	-	high performance liquid chromatography
hr	-	hour(s)
IS	-	internal standard
kg	-	kilogram
L	-	liter
LLOQ	-	lower limit of quantitation
LSC	-	liquid scintillation counting
M	-	molar
MC	-	methylcellulose
MeOH	-	methanol
mg	-	milligram
mL	-	milliliter
mm	-	millimeter
ms	-	milliseconds
MS	-	mass spectrometry
mM	-	millimolar
NA	-	not applicable
ND	-	not detected

ng	-	nanogram
nm	-	nanometer
OECD	-	Organisation for Economic Cooperation and Development
ppm	-	parts per million
QC	-	quality control
%RE	-	percent relative error
RSD	-	relative standard deviation
SD	-	standard deviation
SOP	-	standard operating procedure
SPE	-	solid phase extraction
T_{max}	-	Sampling time at which C_{max} was achieved
UV		ultraviolet
v	-	volume
w	-	weight
WTDMS™	-	WIL Toxicology Data Management System

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TABLES 1 - 5

TABLE 1

Mean Doses* of NaPFHx Administered to Rats

Target Dose	Dose Route	Sex	mg/kg		% of Target†
			Mean	(±SD)	
300 mg/kg	Oral Gavage	Males	297.30	3.42	99
300 mg/kg	Oral Gavage	Females	298.91	3.42	100

* Derived from Table E1 and E3.

† $\%\ \text{of Target} = 100 \times \frac{\text{Actual Dose}}{\text{Target Dose}}$

TABLE 2

Mean Doses* of NaPFHx Administered to Mice

Target Dose	Dose Route	Sex	mg/kg		% of Target†
			Mean	(±SD)	
300 mg/kg	Oral Gavage	Males	299.33	2.88	100
300 mg/kg	Oral Gavage	Females	299.47	3.42	100

* Derived from Table E2 and E4.

† $\%\ \text{of Target} = 100 \times \frac{\text{Actual Dose}}{\text{Target Dose}}$

Table 3. Mean Concentrations of PFHxA in Serum of Male and Female Mice following Oral Administration of NaPFHx at 300 mg/kg

Hours Post-Dosing	Males			Females		
	Mean (ng/mL)	± SD	%CV	Mean (ng/mL)	± SD	%CV
0	0.00	0.00	NC	0.00	0.00	NC
0.5	576750	109192	19	684250	188970	28
1	464250	159508	34	617000	137957	22
2	561750	168804	30	401000	80283	20
4	521250	316251	61	205600	108325	53
6	112025	48822	44	41808	48624	116
8	129550	83213	64	29725	20645	69
12	46500	27114	58	11061	18311	166
24	6443	11773	183	412	115	28
48	566	118	21	286	310	108

N = 4.
NC=Not Calculable.

Table 4. Mean Concentrations of PFHxA in Serum of Male and Female Rats following Oral Administration of NaPFHx at 300 mg/kg

	Males			Females		
Hours Post-Dosing	Mean (ng/mL)	± SD	%CV	Mean (ng/mL)	± SD	%CV
0	0.00	0.00	NC	0.00	0.00	NC
0.5	328750	58397	18	376750	101346	27
1	328500	14617	4	295000	58992	20
2	246500	38232	16	139750	24254	17
4	144400	56693	39	40375	19159	47
6	90875	24245	27	12410	13202	106
8	13638	11016	81	4615	7727	167
12	2463	1527	62	538	180	33
24	267	239	90	130	163	126
48	102	50.4	49	46.5	47.5	102

N = 4.
NC=Not Calculable.

Table 5. PFHxA Toxicokinetic Parameters following Oral Administration of 300 mg/kg NaPFHx to Male and Female Mice and Rats

Gender	AUC_{last} (ng•h/mL)	AUC_{inf} (ng•h/mL)	AUC % Extrap.	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
			Mice			
Males	3629157	3633476	0.12	576750	0.5	5.3
Females	2089691	2091339†	0.08	684250	0.5	4.0†
			Rats			
Males	1317688	1318240†	0.04	328750	0.5	3.7†
Females	745868	746589	0.10	376750	0.5	11

Extrap. = Extrapolated;
† Approximate value.

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FIGURES 1 - 4

Figure 1. Mean Concentrations of PFHxA in Serum of Male and Female Mice following Oral Administration of NaPFHx at 300 mg/kg

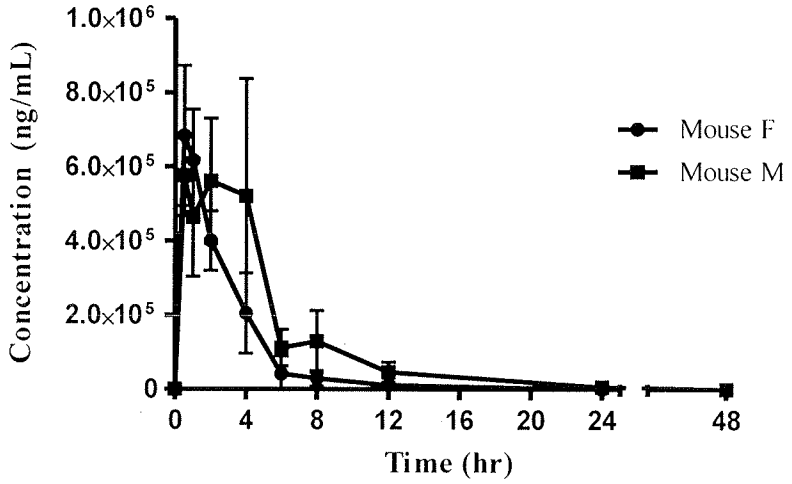

Figure 2. Individual Concentrations of PFHxA in Serum of Male and Female Mice following Oral Administration of NaPFHx at 300 mg/kg

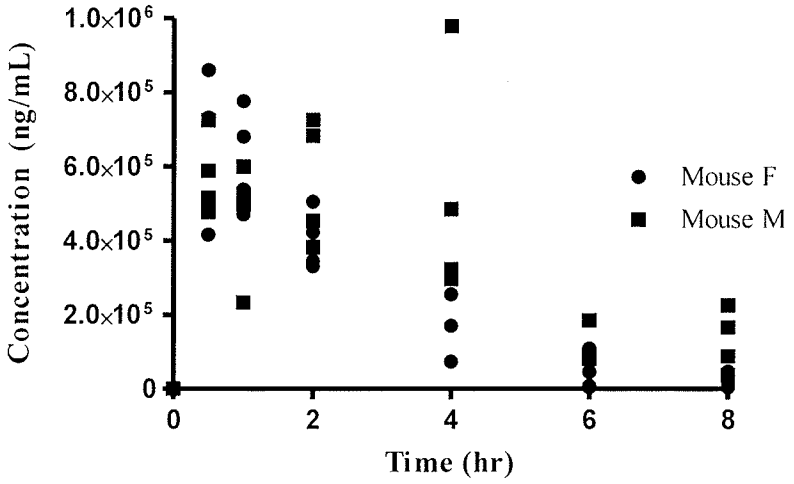

Figure 3. Mean Concentrations of PFHxA in Serum of Male and Female Rats following Oral Administration of NaPFHx at 300 mg/kg

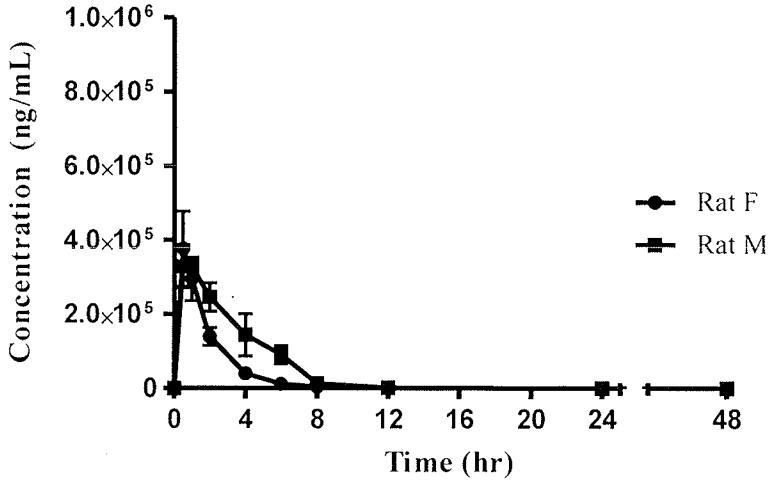

Figure 4. Individual Concentrations of PFHxA in Serum of Male and Female Rats following Oral Administration of NaPFHx at 300 mg/kg