13 pp

PROTOCOL

Perfluorooctanoic Acid:
Inhalation Repeated Dose Pharmacokinetic Study in the Rat

Project Number: DuPont-12944

Work Request Number: 14552

Service Code Number: 1017

Haskell Animal Welfare Committee No. BT152-P

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INTRODUCTION

Perfluorooctanoic acid (PFOA) is a surfactant in industrial processes. In rat metabolism studies with PFOA, whole body elimination of the compound was found to be much more rapid in females compared to males following oral dosing.[1-2] There is a large pharmacokinetic data set describing the behavior of PFOA following oral exposure. The purpose of this work is to generate plasma PFOA data to bridge the oral and inhalation exposure data sets.

OBJECTIVE

The objective of this study is to generate plasma PFOA concentrations following repeated inhalation exposure at three concentrations.

SPONSOR AND TEST FACILITY

This study is sponsored by the Association of Plastics Manufacturers in Europe (APME), Brussels, Belgium. The sponsor's approval was effective the date the sponsor authorized the work on the Agreement between Haskell and the APME.

The study will be conducted at Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Company, Newark, Delaware in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards.[3]

MATERIALS AND METHODS

A. Test Substance

PFOA will be obtained from Aldrich Chemicals (Milwaukee, Wisconsin) and assigned a unique Haskell Laboratory Number upon receipt. Available information on the purity, composition, contaminants, synonyms, hazards, and hazardous material classification(s) will be provided by the vendor and documented in the study records and/or report.

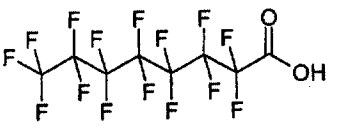
Since this study is utilizing the inhalation route of exposure, no attempt will be made to establish the actual dose each rat received. All effects will therefore be reported as a function of the exposure concentration(s) rather than a function of dose.

CAS Registry Number: 335-67-1

Molecular Weight: 414.1

Molecular Formula: $C_8F_{15}O_2H$

Structure:



B. Test System

Male and female Crl:CD®(SD)IGS BR rats will be obtained from Charles River Laboratories, Inc., Raleigh, North Carolina. The Sprague-Dawley rat was chosen for this study because of the extensive experience with this strain and its suitability with respect to longevity, sensitivity, and low incidence of spontaneous diseases. Furthermore, the Sprague-Dawley rat has been used previously for toxicokinetic testing of PFOA and other fluorinated test materials.[1,4,5]

Upon arrival at Haskell Laboratory, all rats will be housed singly, sexes separate, in quarantine. The rats will be:

- quarantined for at least 5 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine and once prior to initiation of exposures.
- observed with respect to weight gain and any gross signs of disease or injury.

The rats will be released from quarantine by the laboratory animal veterinarian or designee on the basis of body weights and clinical signs.

Rats that are accidentally killed or removed from study during the pretest period will be discarded without necropsy. Rats that are found dead or are sacrificed in extremis during the pretest period will be given a gross examination to check for the presence of disease. Dependant upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be included in the final report unless considered significant to the evaluation of the study.

At the time of dosing, rats should be approximately 6-8 weeks of age and the weight variation should not exceed ± 20% of the mean weight by exposure group. Each animal will be assigned a unique identification number to be used throughout the study. The last 3 digits of the animal identification number will be marked on the tail of each animal in indelible ink.

C. Animal Husbandry

1. Animal Housing

Except during exposure, rats will be housed singly in stainless steel, wire-mesh cages suspended above cage boards. Each cage rack will contain only rats of one sex.

2. Environmental Conditions

Rats will be housed in proximity to the inhalation chambers. Animal room(s) will be maintained at a temperature of 18-26°C (targeted to 22-24°C) and a relative humidity of 30-70% (targeted to 40-60%). Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle. Unless judged by the study director or the laboratory veterinarian to

have significantly affected the results of the study, the relative humidity and temperature ranges in the housing rooms will be recorded but will not be included in the final report.

3. Feed and Water

Except during exposure, PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and tap water will be available *ad libitum*.

4. Animal Health and Environmental Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore, and fungal counts.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

D. Inhalation Exposure System

1. Atmosphere Generation

For this study, a 20% (weight/weight) solution will be prepared by diluting the powdered test substance in deionized water. Chamber atmospheres will be generated by aerosolization of the 20% test substance solution in air with a nebulizer. The test substance will be metered into the nebulizer with a syringe infusion pump. Filtered, high-pressure air, metered into the nebulizer by a mass flow controller, will carry the resulting atmosphere into the exposure chamber. The pump and flow controller will be controlled and monitored by the Camile Inhalation Toxicology Automated Data System (CITADS). Chamber concentrations of test substance will be controlled by varying the syringe infusion rate to the nebulizer and/or by introducing know volumes of dilutional air into the exposure chambers.

Test atmospheres will be exhausted through an MSA charcoal/HEPA filter cartridge prior to discharge into the fume hood. Air from the control chamber will be exhausted directly into the fume hood.

2. Chamber Construction and Design

All exposure chambers will be constructed of stainless steel and glass (NYU style) with a nominal internal volume of 150 L. A dispersion plate inside the chamber will promote uniform chamber distribution of the test atmosphere.

3. Exposure Mode

During exposure, animals will be individually restrained in perforated stainless steel cylinders with conical nose pieces. The restrainers will be inserted into a polymethylmethacrylate faceplate which will be attached to the exposure chamber so that the nose of each animal extends into the exposure chamber. Before the start of the exposure phase, animals will be placed in restrainers on 2 separate days for approximately 15 minutes each day to acclimate the animals to the restrainers.

E. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

The atmospheric concentration of test substance will be determined by gravimetric analysis at a minimum of approximately 60-minute intervals during each exposure. Known volumes of chamber atmosphere will be drawn from the sampling port through a filter cassette that contains a pre-weighed glass fiber filter. The filters will be weighed on an analytical balance. The filter weights will be automatically transferred to CITADS, which will calculate the chamber concentrations based on the difference in pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled. Gravimetric sample start- and stop-times for each sample will be controlled and recorded by CITADS.

2. Particle Size Determination

Samples to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 µm diameter) will be taken once per week from each test chamber with a Sierra® Series 210 cyclone preseparator/Cascade impactor and Sierra® series 110 constant flow air sampler.[6]

3. Environmental Monitoring

Chamber airflow will be set at the beginning of each exposure to achieve at least 10 air changes per hour and will be monitored continuously with a calibrated Brooks Mass Flow Controller. Chamber temperature will be targeted at 22 ± 3°C and measured with an Omega Thermocouple thermometer. Chamber relative humidity will be targeted at 50 ± 20% and measured with a Jumo wet-bulb/dry-bulb resistance temperature detector. Airflow, temperature and relative humidity will be monitored continuously with the Camile Inhalation Toxicology Automated Data System and recorded at 15-minute intervals during exposure. Chamber oxygen

concentration will be targeted to be at least 19%, measured with a Biosystems oxygen analyzer and recorded at least twice during each exposure. Ideally, the chamber conditions will be set so that temperature will be 20-24°C and humidity will be 30-70%. However, temperature and humidity variations outside of these ranges are commonly encountered due to the generation procedures used and the characteristics of the test substance itself. These variations are not expected to significantly affect the results of this type of study. Potentially adverse effects resulting from variations in temperature, relative humidity, or oxygen content will be addressed in the final report

F. Inhalation Exposure

To determine the proper exposure levels for the main study, four groups of 3 male and 3 female rats each will be exposed (nose only) for a single, 6-hour period to an aerosol of the test substance. One test group will be exposed to an atmospheric concentration of 1 mg/m^3, the second test group will be exposed to an atmospheric concentration of 10 mg/m^3, the third test group will be exposed to an atmospheric concentration of 25 mg/m^3. A control group of rats will be exposed to air only. Blood will be collected via the tail vein prior to exposure, at several time points during exposure, and at several time points after dosing, according to Haskell SOPs. To allow for timed blood collections, exposure start will be staggered less than 5 minutes for each animal. Approximately four samples will be drawn from each animal during the six hour inhalation exposure followed by approximately six additional blood draws during the 24-hour recovery period. The sample collection and exposure times will be documented in the study records. Rats will be sacrificed following the final blood collection.

Plasma derived from the whole blood samples will be analyzed by LC-MS for PFOA concentration and the results compared to pharmacokinetic data from literature derived oral dosing studies. The exposure levels selected for the main study will be levels that produce plasma concentrations similar to plasma concentrations observed in a previous study.[2] The exposure levels will be documented in the study records.

After the exposure levels are selected, four groups of 5 male and 5 female rats each will be 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 each exposure will be defined as the time when the generation system is turned on. The ending time of each exposure will be defined as the time when the generation system is turned off. Rats will be exposed to the test substance during both the time it takes for the chamber to reach concentration, and the time it takes for the test substance to be purged from the chamber. After each exposure, rats will be returned to their cages. The exposure period will be defined as the period between initiation of the first exposure and completion of the last exposure. The control group of rats will be exposed to air only. Exposure start times will be staggered by 10-30 minutes by exposure concentration group to allow for blood collection time. The sample collection and exposure times will be documented in the study records. As in the pilot study, blood will be collected via the tail vein. Blood will be collected before and after the daily inhalation exposure period three days per week. Rats will be sacrificed following the final blood collection.

G. Statistical Analysis

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Exposure Concentration Data Environmental Data	None	Descriptive statistics (e.g., mean, standard deviation)	

Other methods will be used if appropriate, at the time of analysis. The statistical methods used will be described in the final report.

SAFETY AND HOUSEKEEPING

All chemicals used during this study will be handled according to the procedures specified in the MSDS and disposed of according to the Stine-Haskell Waste Disposal Guidelines and the area Safety, Health and Environmental (SHE) manual.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

PROPOSED STUDY DATES

Date of Approval:	Date Study Director signed Protocol
Proposed Experimental Start:	April 28, 2003
Proposed Experimental Termination:	July 15, 2003
Proposed Study Completion:	December 31, 2003

REFERENCES

1. Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J. and Peterson, R.E. (1991) Tissue Distribution, Metabolism, and Elimination of Perfluorooctanoic Acid in Male and Female Rats. J. Biochem. Toxicol. 6(2): 83-92.

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

3. U.S. EPA Good Laboratory Practice Standard, TSCA (40 CFR 792). (1989)

4. Biegel, L.B. (1997) Hazard Characterization for Human Health C8 Exposure. DuPont Haskell Laboratory.

5. Vanden Heuvel, J.P., Davis, J.W., Sommers, R., and Peterson, R.E. (1992). Renal Excretion of Perfluorooctanoic Acid in Male Rats: Inhibitory Effect of Testosterone. J Biochem Toxicology, 7(1): 31-36.

6. Calculation described in Sierra Instruments, Inc., Bulletin 7-79-219IM, Instruction Manual: Series 210 Ambient Cascade Impactors and Cyclone Preseparators.

SIGNATURES

Written and Approved for Issue by Study Director:

Paul M. Hinderliter, Ph.D.
Research Toxicologist

22-Apr-2003
Date

Approved by:

Gary W. Jepson, Ph.D.
Principal Research Toxicologist and Manager

22-APR-2003
Date

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PROTOCOL AMENDMENT

Amendment No.: 1

Work Request No.: 14552

Service Code: 1017

Project Number: DuPont-12944

Protocol Title: Perfluorooctanoic Acid: Inhalation Repeated Dose Pharmacokinetic Study in the Rat

DOCUMENTATION

Changes to be made to existing protocol:

1. Page 3, Test Substance, replace first sentence with:

   PFOA was obtained from Aldrich Chemicals (Milwaukee, Wisconsin) and assigned Haskell Laboratory Number 25811 upon receipt.

2. Page 5, D. Inhalation Exposure System, 1, Atmosphere Generation, replace first two sentences with:

   For this study, a 5% (weight/weight) solution will be prepared by diluting the powdered test substance in deionized water. The solution will be titrated with a concentrated ammonium hydroxide solution to achieve a test substance solution pH of approximately 6.0 to 8.0%. Chamber atmospheres will be generated by aerosolization of the 5% test substance solution in air with a nebulizer.

   Rationale: The test substance (H-25811) is a free acid and is not as soluble in deionized water as initially anticipated. In order to get H-25811 to dissolve in water, the pH must be adjusted to approximately 6.0 to 8.0.

SIGNATURES

Written and Approved for Issue by Study Director:

Paul M. Hinderliter, Ph.D.
Research Toxicologist

28-Apr-2003
Date

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SH Swayze, HASK, H1
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PROTOCOL AMENDMENT

Amendment No.: 2

Work Request No.: 14552

Service Code: 1017

Project Number: DuPont-12944

Protocol Title: Perfluorooctanoic Acid: Inhalation Repeated Dose Pharmacokinetic Study in the Rat

DOCUMENTATION

Changes to be made to existing protocol:

1. Page 4, B. Test System, replace third bullet with:

   - weighed twice during the quarantine procedure,

   Rationale: Protocol 14552/1017 is a metabolic study, not a test of sub-acute toxicity of the test substance.

2. Page 4, B. Test System, last paragraph, first sentence, change "At the time of dosing" to "At the time of exposure".

   Rationale: To indicate proper terminology for an inhalation system.

3. Page 4, C. Animal Husbandry, 2. Environmental Conditions, change third sentence to:

   Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle, except on blood collection days where lights may be turned on as needed up to one hour extra per day.

   Rationale: Study personnel may need to enter the animal room before the end of the dark cycle to begin collecting the pre-exposure blood draw.

4. Page 6, D. Inhalation Exposure System, 3. Exposure Mode, replace entire paragraph with:

   During exposure, animals will be individually restrained in either perforated stainless steel or plastic cylinders with conical nose pieces. The restrainers will be inserted into a polymethylmethacrylate faceplate which will be attached to the exposure chamber so that the nose of each animal extends into the exposure chamber. Before the start of the exposure phase, animals will be placed in restrainers on 2 separate days for approximately 15 minutes each day to acclimate the animals to the restrainers. During exposure, the animals will be kept warm with a heat lamp placed behind the inhalation system.

   Rationale: The restrainer material will be documented in the study records. The animals also need to be kept warm during the exposure to minimize pharmacokinetic differences and to allow for efficient drawing of blood.

SIGNATURES

Written and Approved for Issue by Study Director:

Paul M. Hinderliter, Ph.D.
Research Toxicologist

28-May-2003
Date

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SH Swayze, HASK, H1
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JP Sloskey, HASK, H1
LS Ford, HASK, H1
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PROTOCOL AMENDMENT

Amendment No.:	3
Work Request No.:	14552
Service Code:	1017
Project Number:	DuPont-12944
Protocol Title:	Perfluorooctanoic Acid: Inhalation Repeated Dose Pharmacokinetic Study in the Rat

DOCUMENTATION

Changes to be made to existing protocol:

1. Amendment No. 1, item #2, remove "%" from the second sentence.

   Rationale: Typographical error.

2. Page 7, F. Inhalation Exposure, add the following to the end of the third paragraph:

   Rats that are found dead or are sacrificed *in extremis* will be given a gross examination to check for the presence of disease. Dependant upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be included in the final report unless considered significant to the evaluation of the study.

   Rationale: Clarification for procedures to handle rats that are found dead or sacrificed *in extremis*.

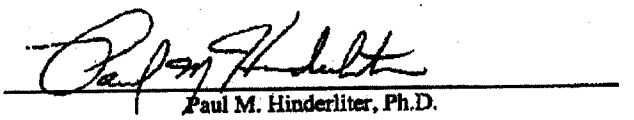
3. Page 7, F. Inhalation Exposure, add the following paragraph after the third paragraph:

   For the 3-week main study, all rats will be weighed at least once per week. These body weights are being measured for animal monitoring purposes only and will be recorded but not included in the final report.

   Rationale: Clarification on animal health monitoring.

SIGNATURES

Written and Approved for Issue by Study Director:



Paul M. Hinderliter, Ph.D.
Research Toxicologist

05 - Jun - 2003
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GW Jepson, HASK, H1	SA Gannon, HASK, H1	LG Burchfield, HASK, H1
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MP Delorme, HASK, H1	JP Sloskey, HASK, H1	