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PROTOCOL

Ammonium Perfluorooctanoate: Age Effect on the Plasma Concentration in Post-Weaning Rats Following Oral Gavage

Project Number: DuPont-13267

Work Request Number: 14762*

Service Code Number: 1389

Haskell Animal Welfare Committee No. BTT155-P

*Note: Work request number 14654 and service code 1368 were used for the scoping purpose of this study.

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INTRODUCTION

Ammonium perfluorooctanoate (PFOA) is a synthetic perfluorinated octanoic acid used as an industrial surfactant. Elimination of PFOA in rats is sex-dependent and hormone regulated. PFOA elimination is much faster in female rats than in male rats,[1] down-regulated by testosterone in both female and castrated male rats,[2-3] and up-regulated by estradiol in the male rats.[4] However, these studies were done on sexually mature rats. The objective of the current study is to determine if immature male and female rats eliminate PFOA from blood at a rate different than that observed in mature rats. Male and female post-weaning rats at different ages will be dosed with PFOA and the plasma concentrations of PFOA at 24 hours post-dosing will be compared. This time point was chosen because 24 hours after dosing, the sex difference in PFOA elimination in mature rats can be easily distinguished via the plasma concentration of PFOA.[5] This study is not designed to fully characterize the pharmacokinetics of PFOA in mature rats, and is not designed for use in determining definitive elimination rate constants.

SPONSOR AND TESTING FACILITY

This study is co-sponsored by 3M, St. Paul, MN, and E.I. du Pont de Nemours and Company, Wilmington, Delaware. The sponsor's approval was effective the date the sponsor authorized the work on the contract.

The study will be conducted at Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Company, Newark, Delaware, USA.

REGULATORY COMPLIANCE

This study will be conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards.[6]

MATERIALS AND METHODS

A. Test Substance

PFOA will be obtained from 3M (St. Paul, MN) and assigned Haskell Laboratory Number H-24921 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.

CAS Registry Number: 335-67-1

Molecular Weight: 414.1

Molecular Formula: $C_8F_{15}O_2H$

B. Test System

Male and female Crl:CD®(SD)IGSBR 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.[2, 5, 7-8]

At the time of dosing, rats should be approximately 4, 5, 6, 7, and 8 weeks of age and the weight variation should not exceed ±20% of the mean weight by dose group and sex. Information on the cage labels will include the Haskell animal number and an individual identification number assigned to each rat. The individual identification number will be placed on the tail of each rat.

C. Animal Husbandry

1. Animal Housing

Upon arrival at Haskell Laboratory, rats will be removed from shipping cartons and housed in appropriate cages according to Standard Operating Procedures unless specified in the study records. Animals will be maintained under quarantine for at least three days unless approved otherwise by the site veterinarian, have at least one recorded weight gain, and no abnormalities detected. After the quarantine period, rats will be selected for study.

Throughout the dosing period with test substance, the rats will be housed individually in appropriate cages according to the SOP.

2. Environmental Conditions

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

All animals will be provided tap water *ad libitum* and fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*. Animals will be fasted overnight for approximately 12-15 hours before dosing with test substance. Food will be returned approximately two hours post-dose.

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. Main Study

1. Animals

The experiments will be composed of five animal groups:

Group	Age*		Number of Rats	
	Weeks	Days	Male	Female
I	4	28	10	10
II	5	35	10	10
III	6	42	10	10
IV	7	49	10	10
V	8	56	10	10

* Animals will be ±1 day from the target age at the time of dosing. The actual age at dosing will be documented in the study record and/or final report.

2. Dose Preparation, Analysis, and Rates

The test substance will be administered by single oral gavage. This route was chosen because it is the route most commonly used for toxicity studies of PFOA. PFOA will be mixed with water to achieve the target dose concentrations. For all experiments, a single dose level will be used equivalent to 10 mg PFOA/kg body weight and the dose volume will be approximately 4 mL/kg body weight.

Actual dose level may be modified at the Study Director's discretion, will be documented in the study records, and presented in the final report. Dose may be prepared on the day of use or prior to the day of use and stored under appropriate conditions.

HPLC-MS methods will be used to confirm PFOA concentration in the dose solutions.

3. Sacrifice and Blood Sampling

Rats will be sacrificed by CO_2 asphyxiation and exsanguinated by cardiac puncture.

4. Experimental Procedure

Rats will be obtained from the vendor and administered PFOA at a dose level described above. Twenty-four hours after PFOA administration, animals will be sacrificed and whole blood samples will be collected by cardiac puncture. Whole blood samples will be placed into EDTA tubes and maintained on wet ice. Plasma will be separated by centrifugation and frozen at <-10°C until analysis.

Kidneys and livers will be collected at sacrifice and will be flash frozen in liquid nitrogen and stored at <-60°C for possible future biochemical analysis.

Rats that are found dead or are sacrificed *in extremis* will be given a gross examination to check for the presence of disease. Dependent upon the 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.

5. PFOA Analysis

HPLC-MS method will be used to determine plasma concentration of PFOA. A description of the method selected will be included in the final report.

E. Statistical Analysis

Group data will be represented at Mean ± SD. Where appropriate, statistical significance will be assessed by the Student's t-test. Other statistical evaluations may be performed, if necessary.

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:	June 30, 2003
Proposed Experimental Termination:	August 29, 2003
Proposed Study Completion:	December 18, 2003

REFERENCES

1. Ohmori K., Kudo, H., Katayama, K., and Kawashima, Y. (2003). Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. *Toxicology* **184**, 135-140.

2. 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. Toxicol.* 7, 31-36.

3. Kudo, N., Suzuki, E., Katakura, M., Ohmori, K., Noshiro, R., and Kawashima, Y. (2001) Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. *Chem. Biol. Interact.* **134,** 203-216.

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

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SIGNATURES

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27-Jun-2003
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