13 pp

Perfluorooctanoic Acid: Lactational and Placental Transport Pharmacokinetic Study in Rats

Work Request Number 14787

Service Code 1569

Protocol

Haskell Animal Welfare Committee Number: <>

TABLE OF CONTENTS

INTRODUCTION

The test substance, Perfluorooctanoic acid, 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, however, available data on the transport concentrations of the test material when administered to pregnant females is limited in scope and there is no existing data on the concentration of the test material transported via the milk from lactating females.[1,2]

OBJECTIVE

The objective of this study is to evaluate the concentrations of PFOA in maternal milk and blood plasma, placental, embryonic, fetal, and neonatal tissue(s) following repeated oral dosing of the dam at three concentrations.

SPONSOR AND TEST FACILITY

This study is co-sponsored by 3M, St. Paul, Minnesota, 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.

REGULATORY COMPLIANCE

This study will be conducted in compliance with all applicable Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan Number 3850.[3,4,5]

STUDY DESIGN

The study design is as follows:

Group	Dose (mg/kg/day)[a]	Suspension Concentration (mg/mL)[b]	Time-Mated Females
I	0[c]	0	20
II	3	0.6	20
III	10	2.0	20
IV	30	6.0	20

a Formulations of test substance in deionized water will be administered once daily by oral gavage at a dosing volume of 5 mL/kg.
b To achieve these concentrations of active ingredient, the formulations will be adjusted for sample purity (%).
c The control group animals will receive vehicle deionized water only at 5 mL/kg.

A. Dose Group Subsets and Dosing Schedules

Group	Female Subset A Dose Days 4 – 10G[a]	Female Subset B Dose Days 4 – 15G	Female Subset C Dose Days 4 – 21G	Female Subset D Dose Days 4G – 21PP[b]
I	5	5	5	5
II	5	5	5	5
III	5	5	5	5
IV	5	5	5	5

a Gestation
b Postpartum

The first 5 animals in each group will be designated as Subset A, the second 5 as Subset B, the third 5 as subset C, and the remaining 5 as Subset D.

B. Selection of Dose Levels

Dose levels selected for this study were based upon the results of a two-generation reproduction study in rats.[6] Groups of 30 male and 30 female rats were dosed by oral gavage at 0, 1, 3, 10, or 30 mg/kg/day for approximately 70 days before cohabitation. Dosing of the P0 females continued throughout mating, gestation, and lactation or approximately 112 days total. With the exception of significantly reduced kidney weight and kidney weight to terminal body weight and to brain weight ratios, no test substance related maternal toxicity was observed at doses up to 30 mg/kg/day. Offspring mortality, attributed to failure to thrive, was observed in F_1 generation weanlings at 30 mg/kg/day.

C. Gestation Subset Sacrifice Schedule And Sample Collection

Subset	Terminal Sacrifice	Samples Collected
A	10G after dosing	Maternal Blood, Amniotic Fluid, Placentas, Whole Embryos
B	15G after dosing	Maternal Blood, Amniotic Fluid, Placentas, Whole Embryos
C	21G after dosing	Maternal Blood, Amniotic Fluid, Placentas, Whole fetuses, Fetal Blood

- Maternal blood sample volume 0.5 mL per animal collected in tubes containing heparin
- Amniotic Fluid sample minimum volume approximately 100 µL collected in glass tubes
- Fetal blood sample minimum volume approximately 100 µL collected in tubes containing heparin
- All embryos and fetuses collected in cryotox tubes

D. Lactation Subset Sample Collection And Sacrifice Schedule

Subset D	Number of Females/Litters	Maternal Samples	Number of Pups for Terminal Sacrifice	Pup Samples
Day 3pp after dosing	5/group	Milk, Blood	Minimum of 1 per litter[a]	Blood
Day 7pp after dosing	5/group	Milk, Blood	Minimum of 1 per litter	Blood
Day 14pp after dosing	5/group	Milk, Blood	Minimum of 1 per litter	Blood
Day 21pp – after dosing[b]	5/group	Milk, Blood	Minimum of 1 per litter	Blood

a At the discretion of the study director, the number of pups sacrificed may be increased to ensure sufficient sample volumes for analysis.
b Maternal Terminal Sacrifice

- Maternal milk sample minimum volume approximately 100 µL collected in glass tubes
- Maternal blood sample volume 0.5 mL per animal collected in tubes containing heparin
- Fetal blood sample minimum volume approximately 100 µL collected in tubes containing heparin

MATERIALS AND METHODS

A. Test Substance

1. Identification

The test substance will be obtained from 3M, St. Paul, Minnesota, and was 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 report.

2. Purity

The vendor reported purity was 95.2 %.

3. Test Substance Stability

The stability of the test substance over the course of the study will be confirmed by purity analyses conducted near the beginning and end of the study.

B. Vehicle

The vehicle will be Nano-Pure® water.

C. Test Substance Administration, Preparation, and Sampling

1. Administration

The test substance will be administered by oral gavage once daily at a dose volume of 5 mL/kg. Animals assigned to gestation subsets A, B, and C, will be dosed for 7, 12, and 18 days respectively. Animals assigned to the lactation subset (D) will be dosed for approximately 47 days. Females in the process of delivery or showing signs of delivery will not be dosed. Dose volumes will be adjusted daily based on body weights.

2. Preparation

Solutions of the test substance in the vehicle will be prepared daily and stored at room temperature until used. The method of mixing the test substance with the vehicle will be documented in the study records.

3. Sampling

Samples of each test solution (approximately 3 mL) will be taken 3 times during the study. If necessary, additional samples may be taken at the discretion of the study director or designee.

Analyses will address concentration, homogeneity, and stability. Samples will be submitted shortly after preparation to the Analytical Group at Haskell.

- Analysis of the first sampling will be taken from dose preparation near the beginning of study and will address uniformity of the mixture, concentration, and 5-hour room temperature stability.

  Three independent samples from each test solution will be collected. One sample of the vehicle and 2 samples from each test suspension will be analyzed immediately after submission. The third sample will be held for 5 hours at room temperature, then analyzed for stability.

- For the second and third samplings taken from dose preparation near the middle and end of the study, analysis will address concentration.

  Samples of the vehicle and 2 samples from each concentration of the solutions will be analyzed immediately following submission to verify concentration.

If samples cannot be analyzed at the specified times, they will be frozen until analyses can be conducted. On days samples are taken, the solutions remaining after dosing will be stored in the refrigerator for additional analysis. The analytical method used will be documented in the Analytical study records.

D. Animals

1. Species (Strain)

Crl:CD®(SD)IGS BR rats

2. Reason for Selection

The rat was selected for this study as it is a preferred species for reproductive toxicity testing as recommended by the guidelines. The Crl:CD®(SD)IGS BR strain was selected based on consistently acceptable health status and on extensive experience with this strain at Haskell Laboratory.

3. Description

Eighty nulliparous, time-mated females, will be received on July 8, 2003 (60 females) and July 11, 2003 (20 females) from Charles River Laboratories, Inc., Raleigh, North Carolina. The location of the supplier (city/state) will be documented in the study records and final report. The rats for this study were requested to be 63 days old and be at 1 day of gestation (day 0G) upon arrival. This age corresponds to an estimated weight range of 201-225 grams according to the vendor; however, the weight range is not a factor for the purposes of this study and, therefore, deviations outside of this range are expected not to have any impact on the study. Body weights

on the day the rats are mated, day 0G, will be supplied by the vendor. The day 0G body weights will be documented in the study records and provided in the final report.

Each rat is assigned a unique number and identified with an AVID Microchip implant by the supplier prior to shipping. Upon receipt, each animal will be assigned a Haskell animal number. Both the Haskell animal number and unique vendor animal number will be recorded on each cage card. A master list of unique vendor numbers, Haskell animal numbers, and corresponding unique AVID Microchip implant numbers will be maintained with the study records.

E. Animal Husbandry

1. Environmental Conditions

Animal rooms 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.

2. Housing

All rats will be housed individually in stainless steel, wire-mesh cages, suspended above cageboard until sacrifice or day 19G. Females selected for the lactation subset (D) will be housed in polycarbonate pans containing bedding material on day 20G through day 21pp.

3. Food

All rats will be provided tap water and pelleted PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*.

4. Water

Water from United Water Delaware will be available *ad libitum*.

5. Animal Health Monitoring

As specified in the DuPont 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.

6. Quarantine

Rats will be quarantined according to procedures outlined in Haskell Laboratory SOP LA003-P, and then released for the study upon approval of the Laboratory Animal Veterinarian or a designee.

Rats that die or are sacrificed *in extremis* during the quarantine period will be necropsied to check for the presence of disease. Dependent upon these findings, further diagnostic procedures may be employed at the discretion of the study director, the pathologist assigned to the study, or the laboratory veterinarian.

F. Randomization

Upon arrival, dams will be assigned to lots according to their gestation day. Then, they will be ranked within their respective lots on the basis of day 0G body weights and assigned to control and experimental groups by random sampling from the ranked list. The distribution should result in mean body weights for all groups that are not statistically different ($p > 0.05$). Dams that lose excessive weight or appear ill prior to the start of dosing will be removed from the study.

G. Adult Female Rats

1. In-life Observations of Females

a. Body Weight

Body weights will be recorded on the day after arrival and daily until the end of the study.

2. Clinical Observations

Clinical observations will be recorded on the day after arrival and daily until the end of the study.

H. Gestation Procedures

On day 10, 15, and 21G, animals assigned to Subset A, B, and C, respectively, will be euthanized by carbon dioxide inhalation. For each female with visible implantation sites, the intrauterine location of each embryo (Day 10 and 15 G) or fetus, and implantation type (live or early resorption) will be recorded. A gross examination of maternal viscera will be done and lesions noted will be retained for further examination at the discretion of the study director. Blood samples from each dam will be collected from the vena cava approximately 2 hours (± 30 minutes) post-dose and processed to obtain plasma samples. Amniotic fluid from each implantation site, and all placentas and embryos/fetuses will be collected. On day 21G, fetuses will be euthanized with an overdose injection (ip) of sodium pentobarbital and fetal blood samples will be taken from a transverse cut made to the carotid artery.

Females that die or are sacrificed *in extremis* during the gestation or period will be necropsied and a gross postmortem examination performed. Lesions will be retained at the discretion of the study director, the pathologist assigned to the study, or the laboratory veterinarian. Dam carcasses will be discarded.

I. Lactation Procedures

The day when delivery is complete is designated day 0 postpartum. At each examination period (days 0, 3, 7, 14, and 21 postpartum), offspring will be individually handled and examined for abnormal behavior and appearance; any dead, missing, or abnormal pups will be recorded. Live and dead pups in each litter will be counted by sex as soon as possible after delivery is completed and litter weights will be recorded. Dead pups will be discarded.

On days 3, 7, and 14PP, animals assigned to Subset D, will be anesthetized by Isoflorane inhalation. During administration of anesthesia, milk and blood samples (orbital sinus) will be collected. Blood samples will be collected approximately 2 hours (± 30 minutes) post-dose. Dams will be removed from their litters approximately 1-2 hours before the milking procedure begins. Day 3 litters will be kept warm during the absence of the dam by the use of either a circulating hot water pad, heated gel packs, or heat lamp. Dams will be returned to their litters after recovery from anesthesia.

Randomly selected pups from these dams will be euthanized on the same postpartum days with an overdose injection (ip) of sodium pentobarbital. Pup blood samples will be taken from a transverse cut made to the carotid artery on days 3 and 7 or from the *vena cava* on day 14PP. Maternal and pup blood samples will be processed to obtain plasma samples and pups will be discarded.

On day 21PP, animals will be anesthetized by Isoflorane inhalation. During administration of anesthesia, milk samples will be collected. After milk collection but before recovery, dams will be euthanized by exsanguination. Blood samples will be taken from the vena cava approximately 2 hours (± 30 minutes) post-dose and the dam will be discarded. Randomly selected pups from these dams will be euthanized with an overdose injection (ip) of sodium pentobarbital. Pup

blood samples will be taken from the vena cava. Maternal and pup blood samples will be processed to obtain plasma samples and dams and pups will be discarded.

SAMPLE ANALYSES

Samples will be analyzed for test material concentration by the Haskell Toxicology group. Samples not requiring processing will be stored at approximately -15°C as soon as possible after collection and until analysis, otherwise, samples will be stored under the same conditions until processing and analysis. HPLC-MS or another suitable analytical method will be used to analyze samples. The method used will be documented in the study records.

STATISTICAL ANALYSES

Group means and concentration data will be represented as Mean ± SD. Other statistical evaluations may be performed if necessary.

SAFETY PRECAUTIONS AND DISPOSAL OF WASTE MATERIAL

Good housekeeping procedures will be practiced to avoid potential health hazards and contamination of dosing solution preparation facilities. To avoid skin contact, gloves will be worn when handling either the test substance or dosing solutions. In addition, the test substance will be handled in a chemical hood. Dosing solutions will be prepared in properly ventilated areas. Animal carcasses, feces, and unused dosing solutions and diet will be incinerated.

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.

STUDY PERSONNEL[MMW1]

Study Director: «SD»
«SDTitle»

Analytical Evaluation: <>
<>

«PP»

«PPTitle»

STUDY DATES

Initiation of Test Substance Administration July 11, 2003

In-Life Completion Date: August 22, 2003 (Approximate)

REFERENCES

1. Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J. and Peterson, Re. E. (1991). Tissue Distribution, Metabolism, and Elimination of Perfluorooctanoic Acid in Male and Female Rats. *J. Biochem. Toxicol.* **6920**, 83-92.

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

3. EPA/FIFRA Good Laboratory Practice Standards (40 CFR 160). (1989).

4. OECD Principles of Good Laboratory Practice (as revised in 1997, Published in ENV/MC/CHEM(98)17).

5. MAFF Japan Good Laboratory Practice Standards (59 NohSan Number 3850).

6. Argus Laboratories (2002). Oral (gavage) 2-generation (o new-litter per generation) reproduction study of ammonium perfluorooctanoic (APFO) in rats. Unpublished report.

SIGNATURES

Approved by: _____ _____

Eve Mylchreest
Management

Date

_____ _____

«sd»
Study Director

Date

cc: <Q.A. Contact> D.M. Hoban
<P.&S. Contacts> K. McIlhatton
<Primary Technician>
S.C. Craven
J.W. Green