TRADE SECRET

Study Title

H-24648 and H-24020: Biopersistence Screening
20-Dose Oral Gavage Study in Rats with Recovery Period

Laboratory Project ID: DuPont-5207

AUTHOR: Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.

STUDY COMPLETED ON: August 9, 2001

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

[redacted]

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by: _____ 09-Aug-2001
Matthew S. Bogdanffy, Ph.D., D.A.B.T. Date
Director, Biochemical Toxicology

Reviewed by: _____ 09-Aug-2001
Paul M. Hinderliter, Ph.D. Date
Postdoctoral Fellow

Reviewed by: _____ 09-Aug-2001
Shawn A. Gannon, B.S. Date
Senior Staff Scientist

Reviewed by: _____ 09-AUG-2001
Judith C. Stadler, Ph.D., D.A.B.T. Date
Director, General Toxicology

Issued by Study Director: _____ 09-AUG-2001
Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T. Date
Senior Research Scientist

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STUDY INFORMATION

Test Substance No. 1

9th Collective Nomenclature: Octanoic acid, pentadecafluoro-, ammonium salt

Synonyms/Codes: [redacted]

- APFO
- H-24648

[redacted]

Haskell Number: 24648

CAS Registry Number: [redacted]

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Test Substance No. 2

9th Collective Nomenclature: Octanoic acid, pentadecafluoro-, ammonium salt

Synonyms/Codes: [redacted]

- APFO
- Perfluorooctanoate, ammonium salt
- C-8
- H-24020

[redacted]

Haskell Number: 24020

CAS Registry Number: [redacted]

STUDY INFORMATION (Continued)

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: White solid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: Daikin Industries, Ltd.
1-1, Nishi-Hitotsuya, Settsu-shi
Osaka 566-8585 Japan

Study Initiated/Completed: November 16, 2000 / (see report cover page)

In-Life Initiated/Completed: November 20, 2000 / March 5, 2001

STUDY PERSONNEL

Study Director: Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.
Management: Judith C. Stadler, Ph.D., D.A.B.T.
Primary Technician: Nita B. Baker

Biochemical Toxicologist: Shawn A. Gannon, B.S.
Management: Matthew S. Bogdanffy, Ph.D., D.A.B.T.

Blood Data Analysis: Paul M. Hinderliter, Ph.D.
Management: Matthew S. Bogdanffy, Ph.D., D.A.B.T.

Toxicology Report Preparation: Wanda F. Dinbokowitz

Laboratory Veterinarian: William Singleton, D.V.M., A.C.L.A.M.
Wanda L. West, D.V.M., A.C.L.A.M.

SUMMARY

The purpose of this study was to compare plasma concentrations of perfluorooctanoic acid (PFOA) in rats exposed to either H-24648 or H-24020. These test substances represent different isomeric mixtures of perfluorooctanoate ammonium salt (APFO). H-24648 contains [redacted] while H-24020 contains [redacted] Three groups of 5 male and 5 female Crl:CD®(SD)IGS BR rats each were exposed by oral gavage to 20 mg/kg/day of either H-24648 or H-24020 in deionized water, or to deionized water (control). Rats were weighed daily during the 20-dose exposure phase, then once a week during the recovery phase. Clinical signs of toxicity were evaluated weekly. Blood was collected from each rat on test days -3 (pretest), 1, 5, 10, 15, 18, 20, 24, 30, 37, 51, and 106, for evaluation of plasma concentration of PFOA. During the exposure phase, blood was collected approximately 2 hours after dosing. PFOA was extracted from the plasma by solid phase extraction, then the concentration of PFOA was determined by HPLC-MS.

Mild reductions in body weight and body weight gain (compared to control) were observed in male rats exposed to H-24648 or H-24020. The magnitude of effect was similar with both test substances, although more complete reversal (recovery) from effects occurred in the males exposed to H-24648. Body weights in female rats were not affected by exposure to either test substance. No compound-related deaths or clinical observations resulted from exposure to either test substance. Effects on body weight gain were not considered to have affected interpretation of pharmacokinetics.

No PFOA was detected in plasma collected prior to the start of test substance administration. Kinetic analysis of PFOA plasma levels demonstrated no significant differences between the two test substances in plasma biopersistence. Terminal half-life in male rats was 9.6 ± 1.5 days and 10.4 ± 1.6 days for H-24648 and H-24020, respectively. Terminal half-life in female rats could not be calculated (< 1 day) for either test substance.

There was a statistically significant difference in the plasma concentrations achieved in the male and female rats during dosing with the two test substances. In males the total internal exposure to H-24020 was higher compared to H-24648. In females the total internal exposure to H-24648 was higher compared to H-24020.

Under the conditions of this study, in rats of the same gender dosed with the two test substances, no difference in biopersistence (half-life) of PFOA was observed. Differences in internal exposure were observed between compounds for males and females, although these differences were not consistent. In males, dosing with H-24020 resulted in greater exposure to PFOA, compared to dosing with H-24648. In females, dosing with H-24648 resulted in greater exposure to PFOA compared to dosing with H-24020. Differences between genders were observed in the pharmacokinetics of PFOA in rats dosed with the two test substances. Female rats dosed with either test substance had lower peak plasma levels and total internal dose, and a shorter half-life of PFOA than males dosed with the same test substance.

INTRODUCTION

The objective of this study is to compare the internal exposure to and biopersistence of perfluorooctanoic acid (PFOA) in rats with test substances containing straight-chain ammonium perfluorooctanoate (APFO) or a mixture of APFO isomers. The rat was selected for these comparisons because of its use in related toxicity studies and the oral route of exposure was selected because it is relevant for human health risk extrapolation. Internal exposure was determined by measuring plasma area-under-the-curve (AUC) of total PFOA. AUC, which is simply the integral of plasma PFOA concentration over time, is the most common means for expressing internal dose. Biopersistence was assessed by quantifying terminal elimination plasma half-life ($T_{½}$).

A dosage of 20 mg/kg was selected for each test substance. This dosage was expected to produce less than a 10% difference in mean body weight over 20 days of exposure (when compared to vehicle control) for both test substances based on previously reported data from studies conducted with H-24020.[1]

MATERIALS AND METHODS

A. Test Substances

The test substance, H-24648, was supplied by Daikin Industries, Ltd. as a colorless liquid consisting of a 10% solution in water. The test substance, H-24020, was supplied by DuPont Chemical Solutions Enterprise as a white solid. The test substances appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

B. Test Species

Male and female Crl:CD®(SD)IGS BR rats, with an assigned birth date of October 2, 2000, were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The Crl:CD®(SD)IGS BR rat was selected on the basis of extensive experience with this strain and its suitability with respect to longevity, hardiness, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

1. Housing Environment

Rats were housed singly in stainless steel, wire-mesh cages suspended above cage boards. Animal rooms were maintained on an approximate 12-hour light/dark cycle (fluorescent light) and at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20%.

2. Feed and Water

Tap water was provided *ad libitum*. All rats were fed PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 chow *ad libitum*. The feed is guaranteed by the manufacturer to meet specified nutritional requirements and to be free of specified contaminants.

3. Identification

Prior to assignment to groups, each rat was temporarily identified by cage identification. After assignment to groups, an individual identification number was tattooed on the tail of each rat. The information on the cage labels included the unique 6-digit Haskell animal number assigned to each rat.

4. Animal Health 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 will not be included in the final report. Evaluation of these data did not indicate any conditions that affected the validity of the study.

D. Quarantine and Pretest

Upon arrival at Haskell Laboratory, the rats were removed from shipping cartons and quarantined for 7 days. The rats were weighed 3 times during the pretest period and examined daily for any clinically apparent signs of disease or injury. The rats were observed daily for mortality and signs of illness, injury, or abnormal behavior.

On the bases of acceptable body weight gains and freedom from clinically apparent signs of disease or injury, the rats were released from quarantine on test day –4 by the laboratory animal veterinarian designee.

E. Study Design

The study design is as follows:

Group		Animal Numbers[a]					
Male	Female	Male	Female	#/Group	Dose (mg/kg/day)	Dose Solution Concentration (mg/ml)	Test Substance
I	II	101-105	201-205	5	0 (control)	0	Deionized water
III	IV	301-305	401-405	5	20	2	H-24648
V	VI	501-505	601-605	5	20	2	H-24020

a Each rat was assigned its own Haskell animal number.

Study Parameters	Frequency
Body Weight and Clinical Observations	Daily during dosing. Weekly during recovery
Blood Collection for PFOA analysis	Test days –3, 1, 5, 10, 15, 18, 20, 24, 30, 37, 51, and 106
Dosing	Daily for 20 days
Recovery	86 days after last dose

F. Assignment to Groups and Study Start

After the quarantine period, the rats were selected on the bases of adequate body weight gain, freedom from any clinical signs of disease or injury, and a body weight within ± 20% of the mean. The selected rats were distributed by computerized, stratified randomization into 3 groups

of 5 male rats and 3 groups of 5 female rats, so that there were no statistically significant differences among group body weight means for each sex.

After assignment to groups, each rat was housed individually. The last 3 digits of the animal number were tattooed on the tail of each rat. The rats were approximately 7 weeks of age at the start of dosing. Dosing began on test day 1.

Rats that were not assigned to the study were released for other laboratory purposes or were sacrificed by carbon dioxide asphyxiation and discarded without pathology evaluation.

G. Dosing Material Preparation and Administration

1. Test Substance

H-24648, a 10% solution of active ingredient in water, as received, was diluted with deionized water to a concentration of 2 mg active ingredient/mL in the dosing solution. H-24020, a solid test substance, as received, was dissolved in deionized water to a concentration of 2 mg active ingredient/mL in dosing solution. Both test substances were dosed at a volume of 10 mL/kg body weight. The amount of test substance each rat received was based on the body weight collected on each day of dosing and the dosing solution concentration of 2 mg/mL. The dosing solution was stirred on a magnetic stir plate throughout the dosing procedure to maintain homogeneity.

2. Control

Deionized water was administered to the control groups because it was the vehicle for the test substance. Each control rat received 10 mL/kg dose volume.

H. Body Weights

All rats were weighed on each day of dosing during the dosing phase of the study and weekly during the recovery phase.

I. Mortality and Clinical Observations

Cage-site examinations to detect moribund or dead rats and abnormal behavior and appearance among rats were conducted at least once daily throughout the study. At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. After the final blood sample was drawn on test day 106, all rats were sacrificed by carbon dioxide overdose and discarded without necropsy.

J. Collection and Analysis of Blood

Blood samples (approximately 0.25 ml) were collected via orbital sinus bleeding while the rats were under carbon dioxide anesthesia. Blood was collected on the following test days: -3 (pretest), 1, 5, 10, 15, 18, 20, 24, 30, 37, 51, and 106. Test day 1 was defined as the first day of dosing. Blood was collected approximately 2 hours after dose administration during the dosing phase and at approximately the same time of day during the remainder of the in-life phase of the study. Blood was collected into EDTA tubes and stored on ice, then separated by centrifugation into plasma and packed red blood cells. Plasma was removed and refrigerated until analysis. Red blood cells were stored until the end of the study, but were not analyzed.

To prepare the plasma samples for solid phase extraction (SPE) a 100 µl aliquot of each plasma sample was transferred to a single well in a deep-well format 96-well microtiter plate. A 4 µl aliquot of internal standard (tridecafluoroheptanoic acid, initial concentration: 200 mg/ml) was added to each well followed by 96 µl 5M sulfuric acid. The plate was shaken to precipitate the plasma proteins.

A 96-well format SPE plate (Waters Oasis HLB 5 mg) was prepared by conditioning with 500 µl 0.5% sulfuric acid in acetonitrile followed by 500 µl of 0.5% sulfuric acid in water. Water (800 µl) was added to each well followed by an aliquot (50 – 100 µl) of the plasma sample described above. The samples were loaded onto the SPE bed and washed with 500 µl of water. The samples were then eluted from the SPE bed with two aliquots of acetonitrile (250-500 µl).

Following elution, the samples were analyzed for PFOA by LC-MS using a Waters 2690 HPLC Alliance HT connected to a Micromass Quattro Ultima with an electrospray source set in negative mode. A standardization function was calculated from the internal standard and a known linear PFOA standard (Aldrich Chemical). Since branched standards were not available, the MS detector response was assumed to be equal across PFOA isomers. The standardization function converted MS detector area counts into PFOA concentrations. Samples that contained a concentration of PFOA greater than the highest standard were diluted further and then reanalyzed. Details of analytical method are reported in Appendix C.

K. PFOA Data Analysis

Plasma concentration data for branched and straight-chain PFOA were combined to yield total PFOA. Noncompartmental analysis was conducted on total plasma PFOA data using WinNonlin Version 3.1 software (Pharsight Corporation, Mountain View, CA). WinNonlin software provided a means of computing derived pharmacokinetic parameters including terminal elimination half-life ($T_{½}$), maximal concentration (Cmax), time of maximal concentration (Tmax), and area under the curve (AUC). Two means of expressing AUC were calculated: area under the curve for all points (AUCall); and AUC measured from time 0 and extrapolated to infinity (AUCINF).

L. Statistics

The following methods were used to conduct statistical analysis of the data. Significance was judged at $p < 0.05$.

Body weight and body weight gain data from each test group was compared to the control group of the same gender using the following statistical methods. Levene's test[2] and Shapiro-Wilk test[3] were run to evaluate homogeneity and normality, respectively. If the preliminary tests were not significant, data were analyzed using a one-way analysis of variance,[4] followed with Dunnett's test.[5] If the preliminary tests were significant, data were analyzed using a Kruskal-Wallis test[6] followed with Dunn's test.[7] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used.

Comparisons of derived plasma kinetic parameters (Cmax, AUCall, and $T_{½}$) between the two substances, within the same gender, were analyzed using Student *t*-test.[4]

RESULTS AND DISCUSSION

A. Body Weight and Body Weight Gain
(Tables 1-4, Appendix A)

During the dosing phase of the study, exposure to both test substances produced statistically significant reductions (compared to control) in body weight and body weight gain in male rats. Recovery from these effects was demonstrated for both test substances. However, more complete recovery from the body weight effects was seen in male rats exposed to H-24648, compared to male rats exposed to H-24020. During recovery, mean body weight and body weight gain in the male H-24648 group exceeded that of controls, while mean body weight and body weight gain in the male H-24020 group remained below that of control. None of the differences during recovery was statistically significant.

During the dosing phase of the study, exposure to both test substances produced similar effects on body weight and body weight gain in female rats. In both groups, mean body weight and body weight gain were slightly higher than in control but none of the differences was statistically significant compared to control, and no notable differences between the two test groups were observed. Females exposed to both test substances demonstrated increased body weight gain (relative to control) during recovery, but the differences were not statistically significant.

Exposure to both test substances had similar effects on body weight and body weight gain. None of the body weight effects were considered to have affected the pharmacokinetic results.

B. Clinical Observations and Mortality
(Appendix B)

No compound-related deaths occurred and no compound-related clinical signs were observed in any test group. Most observed clinical signs were attributed to the orbital sinus bleeding process. None of the clinical observations were considered to have affected the pharmacokinetic results.

C. PFOA Data
(Tables 5-6, Figures 1-3, Appendix D)

1. Factors Influencing Interpretation of Analysis

The data used in the kinetic analysis were derived from a limited screen, and therefore several caveats and considerations are important. These considerations include (1) a single dose level was used and kinetics may or may not be linear with multiple dose levels, (2) the kinetics apply only to plasma, (3) steady-state may not have been achieved, (4) the small sample size may impact calculation of the terminal half-life, and (5) no analytical standard was available for the

branched chain molecules. For this analysis, it is assumed that the detector sensitivity is the same for all PFOA molecules.

2. Test Substances

Concentrations of PFOA were measured in rat plasma by LC/MS. Rat plasma concentration data are shown in Figures 1 and 2. Individual animal data are in Appendix D. Plasma concentration vs. time course data presented in Figures 1 and 2 are also summarized in Table 5.

No PFOA was detected in plasma collected during the pretest period (test day –3). PFOA was also not detected in the control group samples (Groups I and II).

Rats were dosed for 20 days with the intent of achieving steady state plasma PFOA levels. Figures 1 and 2 show an apparent plateau in plasma PFOA levels between days 10 and 20. However, a larger than expected degree of variability was observed among the analyses of samples collected on days 1, 5, and 10. This variability is likely attributable to an additional dilution step that was required to bring the concentration within the limits of the standard curve and analytical accuracy. For this reason it is difficult to determine if steady-state condition was, in fact, achieved. Sample availability precluded any re-analysis.

Difficulties extracting some samples collected on day 106 rendered them unanalyzable. These samples are indicated in the individual animal plasma data (Appendix D). Loss of these samples did not affect the interpretability of the data set or the conclusions drawn.

Pharmacokinetic analysis of the plasma PFOA data is presented in Table 6. Biopersistence was evaluated by quantifying the half-life of total PFOA during the terminal elimination phase. The half-life for total PFOA in plasma of males was 9.6 ± 1.5 days for group III (H-24648 males) and 10.4 ± 1.6 days for group V (H-24020 males). These values were not statistically different from each other. The half-life for the females could not be estimated because the PFOA levels at the first post-dosing collection time (day 24) were below analytical detection.

Maximal plasma concentration (Cmax) would be expected on the day (Tmax) steady state is achieved which was expected to be at the end of dosing. For all groups, except Group V (male, H-24020), Tmax values were similar (approximately 13-15 days). For group V, the Tmax estimate of 8 days is likely an artifact of the variability observed in the day 5 sample analysis. Cmax for females dosed with either test substance was lower than the corresponding males. Same sex Cmax differences were not statistically significant.

Internal exposure was assessed by calculating AUC. Two means of expressing AUC were calculated: area under the curve for all points (AUCall); and AUC measured from time 0 and extrapolated to infinity (AUCINF). For these analyses, AUCall is the most appropriate measure of internal exposure because AUCINF could not be calculated for Groups IV and VI. A comparison of AUCall for the four test groups is presented in Figure 3.

Differences in internal exposure were observed between compounds for males and females although these differences were not consistent. For males, dosing with H-24020 resulted in greater exposure to PFOA compared to dosing with H-24648. For females dosing with H-24648

resulted in greater exposure to PFOA compared to dosing with H-24020. For both test substances, exposure was approximately 2.5-4 fold higher for males compared to females.

CONCLUSIONS

Under the conditions of this study, dosing with either test substance caused mild reductions in body weight and body weight gain in male rats. There were no effects on body weight or body weight gain in females. The differences noted for males were not considered to have affected interpretation of the pharmacokinetic analyses. In rats of the same gender, no difference was observed in biopersistence of total PFOA from either test substance. For both test substances total internal exposure was higher in males compared to females. Males also had higher plasma Cmax values, compared to females when dosed with either test substance. For males, dosing with H-24020 resulted in greater exposure to PFOA compared to dosing with H-24648. For females dosing with H-24648 resulted in greater exposure to PFOA compared to dosing with H-24020.

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.

REFERENCES

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TABLES

TABLE 1

MEAN BODY WEIGHTS (g) FOR MALES

		Test Substance	
Test Days	Control	H-24648	H-24020
1	214.6	219.1	211.4
2	216.5	218.8	210.4
3	226.5	227.2	217.9
4	236.2	224.9	218.5
5	242.1	223.8	220.3
6	246.1	218.1	219.0
7	253.9	221.8*	229.3
8	263.1	234.3	240.2
9	268.3	240.4	246.8
10	277.5	249.3	251.0
11	281.6	249.3*	253.1
12	288.1	258.3	260.0
13	295.3	265.6	266.7
14	298.3	269.1	269.4
15	305.4	275.3	272.3*
16	306.7	276.0	273.3*
17	313.7	279.2	276.7*
18	319.3	283.4*	281.9*
19	321.2	291.1	284.4*
20	323.5	290.9	286.4*
21	328.2	288.3*	286.2*
22	333.4	293.9	287.1*
32	384.6	362.0	342.9
39	409.8	399.7	369.2
46	443.0	435.8	397.4
53	451.5	445.5	400.6
60	470.8	466.1	418.6
67	494.9	494.1	440.4
74	502.8	506.4	453.7
81	521.0	521.7	468.6
88	534.1	533.5	481.6
94	554.1	545.4	494.8
102	562.2	556.6	501.4
106	561.5	560.1	502.1

* Statistically significant difference from control by parametric comparison (Dunnett/Tamhane-Dunnett) ($p < 0.05$).

TABLE 2

MEAN BODY WEIGHTS (g) FOR FEMALES

		Test Substance	
Test Days	Control	H-24648	H-24020
1	173.3	174.1	178.5
2	173.4	172.0	177.3
3	177.8	178.8	182.1
4	183.3	183.5	187.9
5	185.6	185.3	192.7
6	185.9	188.1	194.3
7	188.6	192.7	196.5
8	195.6	197.6	203.0
9	197.4	197.5	204.7
10	199.2	203.0	209.3
11	199.8	203.6	209.6
12	204.2	209.3	215.9
13	208.2	209.6	220.3
14	208.1	212.1	220.3
15	210.4	217.6	222.7
16	213.1	217.4	225.0
17	215.8	218.4	227.8
18	216.2	219.1	231.3
19	216.6	224.3	233.1
20	219.7	224.9	234.0
21	221.6	226.8	235.9
22	223.5	227.5	237.4
32	243.6	255.0	263.5
39	252.0	265.0	269.7
46	261.1	275.1	286.7
53	262.5	280.5	290.8
60	270.9	283.7	300.2
67	278.1	294.5	312.7
74	277.3	297.1	314.9
81	285.5	310.3	328.4
88	292.0	320.7	333.3
95	297.5	329.5	339.8
102	297.8	332.5	347.4
106	298.7	333.7	347.5

There were no statistically significant differences from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett.

TABLE 3

MEAN BODY WEIGHT GAINS (g) FOR MALES

Test		Test Substance	
Days	Control	H-24648	H-24020
1-21	113.6	69.2*	74.8*
21-106	233.3	271.8	215.9

* Statistically significant difference from control by parametric comparison (Dunnett/Tamhane-Dunnett) ($p < 0.05$).

TABLE 4

MEAN BODY WEIGHT GAINS (g) FOR FEMALES

Test		Test Substance	
Days	Control	H-24648	H-24020
1-21	48.3	52.7	57.4
21-106	77.0	107.0	111.6

There were no statistically significant differences from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett.

TABLE 5

PFOA CONCENTRATION IN RAT PLASMA (μmol/L)

Mean ± Standard Deviation

days	Group III male, H-24648	Group V male, H-24020	Group IV female, H-24648	Group VI female, H-24020
1	61 ± 59[a]	129 ± 31	82 ± 35	92 ± 10
5	284 ± 129	513 ± 151	115 ± 28	108 ± 11
10	193 ± 125	295 ± 142	144 ± 35	88 ± 14
15	255 ± 60	332 ± 49	174 ± 91	123 ± 22
18	376 ± 45	279 ± 55	147 ± 19	128 ± 25
20	258 ± 25	348 ± 49	141 ± 19	117 ± 23
24	127 ± 30	161 ± 24	0.4 ± 0.9[b]	0.3 ± 0.7[b]
30	52 ± 33	74 ± 15	0.0 ± 0.0	0.0 ± 0.0
37	33 ± 5[a]	49 ± 22	0.0 ± 0.0	0.0 ± 0.0
51	15 ± 8	20 ± 8	0.0 ± 0.0	0.1 ± 0.1
106	0.5 ± 0.4[b,c]	0.2 [b,d]	0.0 [d]	0.0 ± 0.0[c]

a N = 4
b Only one sample above limit of detection.
c N = 3
d N = 1

Note: N = 5 unless noted otherwise

TABLE 6

RESULTS OF KINETIC ANALYSIS OF PFOA DATA

	Group III male, H-24648	Group V male, H-24020	Group IV female, H-24648	Group VI female, H-24020
$T_{1/2}$ (days)	9.6 ± 1.5	10.4 ± 1.6	< 1	< 1
C_{max} (μM)	379 ± 44	519 ± 139	200 ± 78	134 ± 21
T_{max} (days)	15.4 ± 5.8	8.0 ± 6.7	14.0 ± 4.2	13.4 ± 7.1
AUCall (μM x days)	6913 ± 1329	9181 ± 1839[a]	2932 ± 306	2326 ± 176[b]
AUCINF (μM x days)	6968 ± 1337	9457 ± 1988[a]	(1)	(1)

Mean ± Standard Deviation
(1) Values not calculated
a Statistically significant difference from group III by Student t-test ($p < 0.05$).
b Statistically significant difference from group IV by Student t-test ($p < 0.05$).

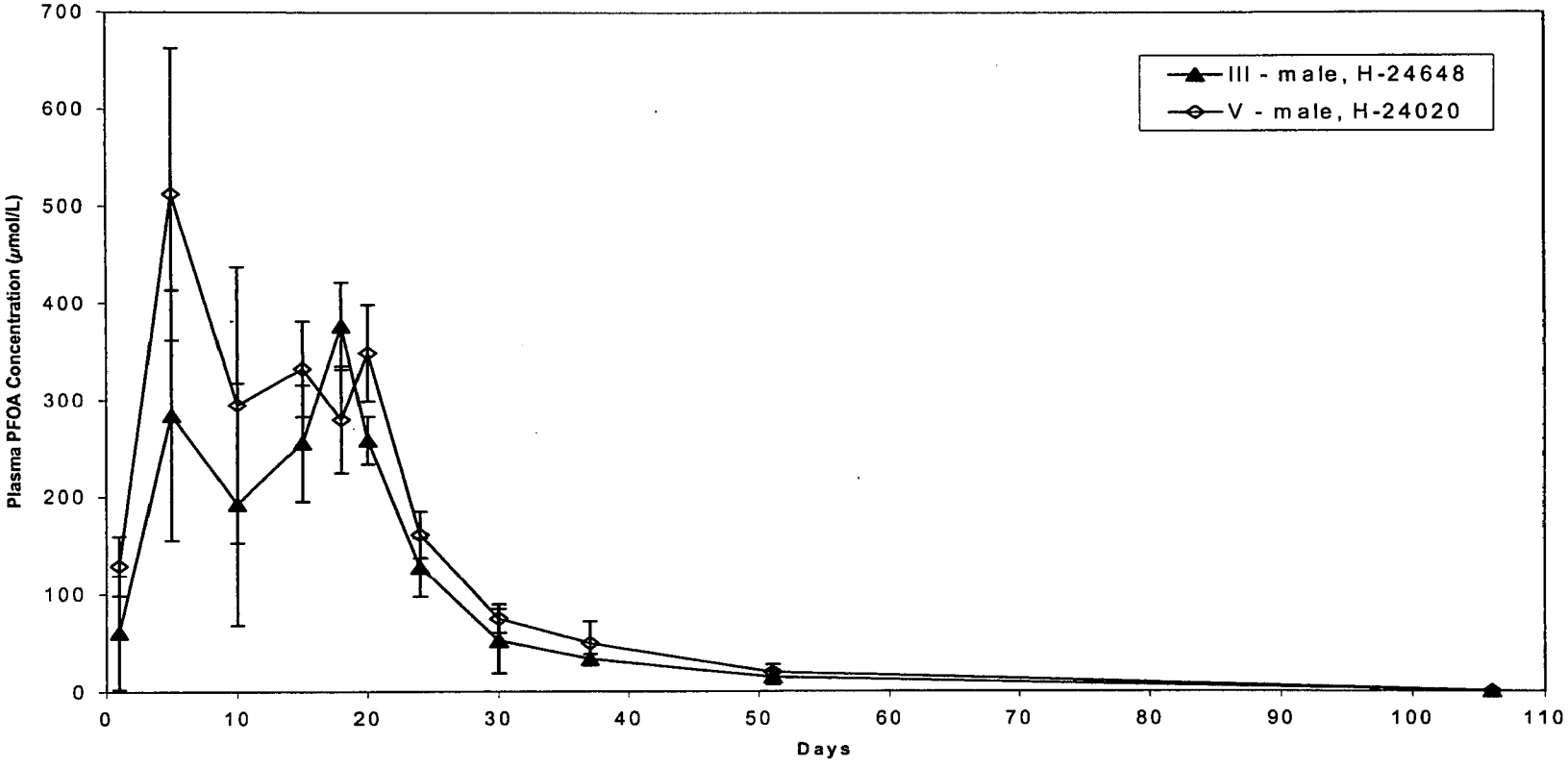
Abbreviations:

$T_{1/2}$ Terminal elimination half-life
C_{max} Maximum plasma concentration
T_{max} Time of maximum concentration
AUCall Area under the curve of all data points
AUCINF Area under the curve from 0 to infinity

FIGURES

FIGURE 1

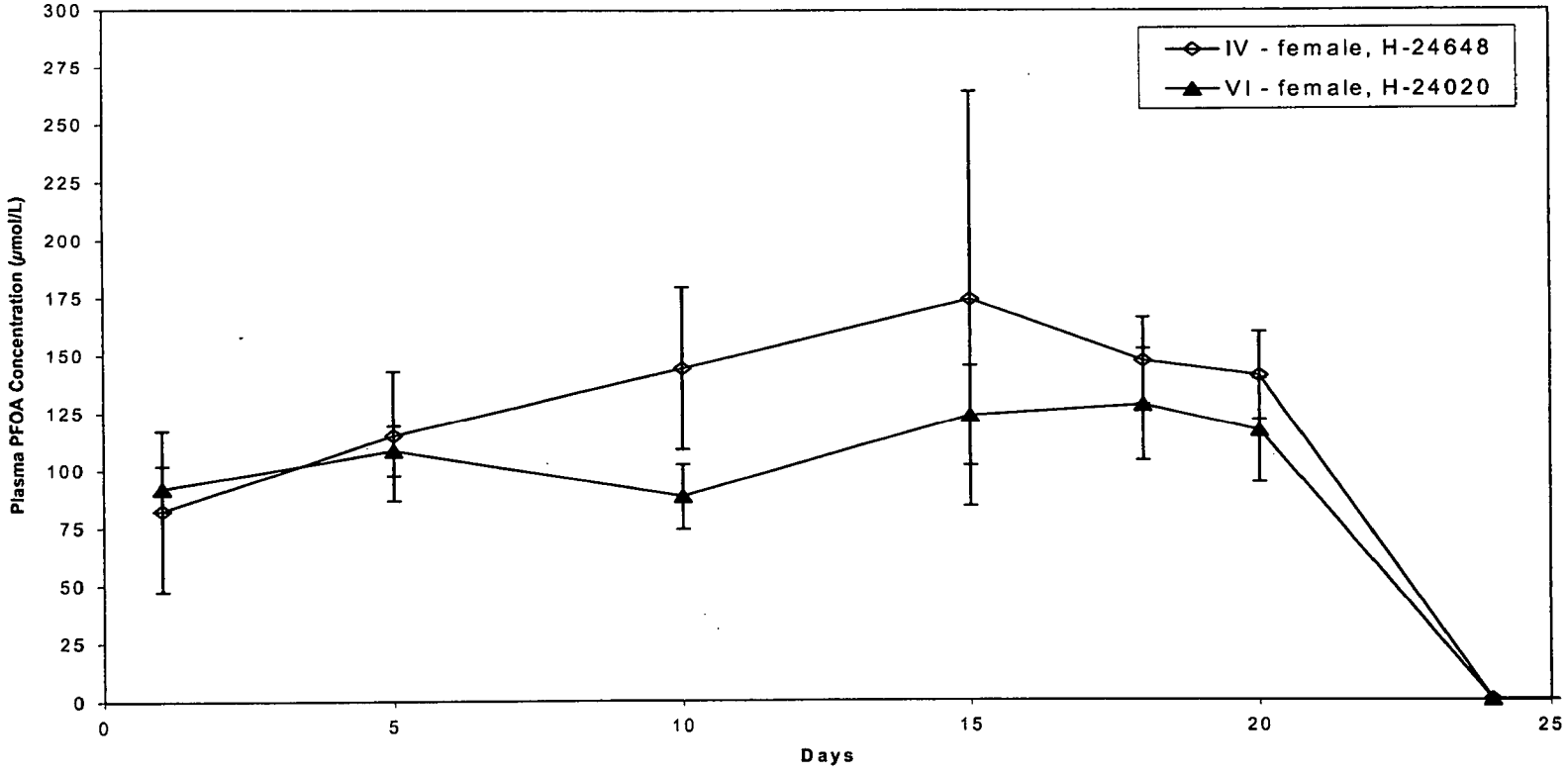
PFOA CONCENTRATION IN MALE RAT PLASMA



Plasma PFOA concentration in male rats dosed with H-24648 or H-24020. Rats received single oral doses daily up to day 20. Error bars represent standard deviations of the mean. On test day 106, several plasma samples were unanalyzable. Plasma data shown for test day 106 are the mean of 3 or 1 sample for groups III and V, respectively. Plasma data for test days 1 and 37 are the means of 4 samples for group III. All other data points are the means of 5 samples.

FIGURE 2

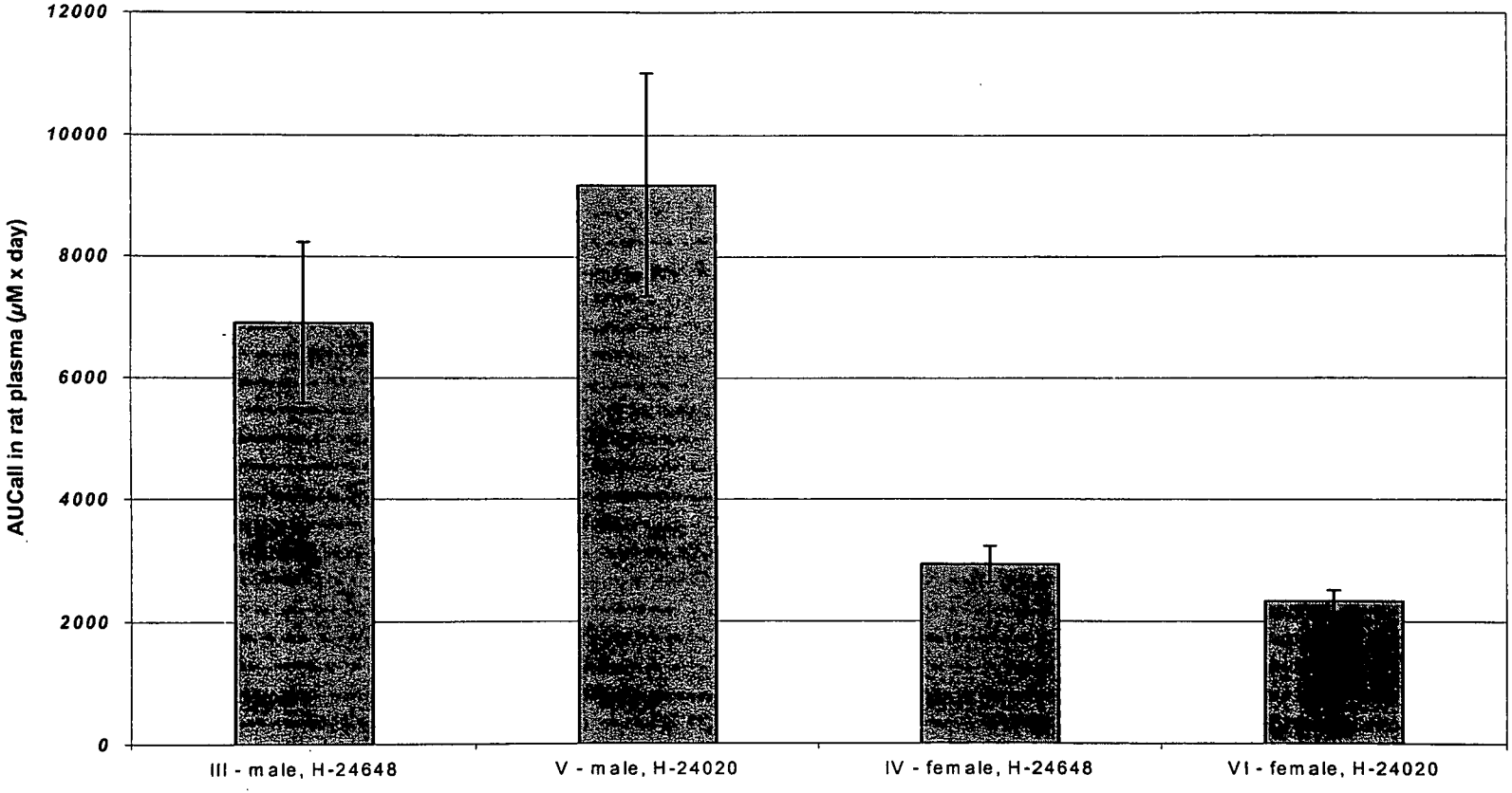
PFOA CONCENTRATION IN FEMALE RAT PLASMA



Plasma PFOA concentration in female rats dosed with H-24648 or H-24020. Rats received single oral doses daily up to day 20. Error bars represent standard deviations of the mean (N = 5).

FIGURE 3

AUCall IN RAT PLASMA RESULTING FROM A 20-DAY ORAL GAVAGE



Summary of area-under-the-curve (AUCall) for male and female rats dosed with H-24648 or H-24020. Error bars represent standard deviations (N = 5).

APPENDICES

APPENDIX A

Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

SD - sacrificed by design

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP I (Control)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643111	229.4	230.0	241.4	249.3	259.7	264.8	270.1	281.4	285.9
643112	210.6	213.0	221.4	234.0	238.2	242.1	252.0	259.5	267.3
643113	217.6	218.1	228.4	239.4	244.4	247.7	255.5	265.3	269.4
643114	218.5	222.3	230.3	240.1	247.6	253.7	259.3	267.8	273.1
643115	196.9	199.1	210.9	218.1	220.6	222.2	232.4	241.3	245.9

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643111	297.9	300.7	312.9	318.9	317.4	329.0	328.5	338.4	345.2
643112	274.6	280.0	284.0	292.6	299.8	307.0	308.5	317.0	323.5
643113	276.6	280.9	282.3	290.4	289.7	296.7	300.6	303.0	308.5
643114	284.3	288.8	297.7	303.8	309.5	314.2	315.3	322.0	327.2
643115	254.1	257.6	263.8	270.6	275.1	280.0	280.7	288.0	292.1

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643111	346.4	346.0	353.2	356.7	415.1	443.3	477.5	484.8	505.4
643112	324.3	328.5	331.7	341.4	392.8	420.2	454.3	453.2	466.3
643113	309.9	309.8	314.8	317.0	367.2	389.7	424.4	437.8	460.2
643114	334.6	338.9	342.7	348.8	403.1	430.8	463.7	471.0	493.6
643115	290.6	294.2	298.5	303.2	345.0	364.8	395.3	410.7	428.5

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106	
643111	526.0	536.5	547.4	561.0	573.0	572.3	572.6	SD Test day 106
643112	494.6	500.3	513.0	515.9	534.4	542.9	539.1	SD Test day 106
643113	492.5	482.5	512.3	536.5	565.2	589.2	589.5	SD Test day 106
643114	508.1	530.1	548.3	555.5	580.4	573.2	568.0	SD Test day 106
643115	453.4	464.8	483.9	501.4	517.7	533.4	538.2	SD Test day 106

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP III (H-24648)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643116	233.0	231.1	235.3	219.0	210.0	191.8	187.9	196.2	203.0
643118	211.2	211.4	222.0	222.5	225.6	228.0	235.7	250.8	253.2
643119	218.4	217.8	225.7	223.7	214.8	199.2	202.6	216.7	231.0
643120	204.3	202.2	210.4	214.5	216.9	217.6	222.8	234.6	240.2
643126	228.6	231.3	242.5	244.7	251.6	254.0	260.0	273.0	274.7

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643116	217.9	224.3	235.7	251.0	262.9	274.8	277.1	283.1	293.5
643118	262.2	254.8	262.1	273.2	274.7	281.1	280.8	291.0	290.1
643119	241.9	239.4	250.8	258.6	257.5	261.6	253.8	248.0	255.6
643120	244.4	245.7	254.5	253.8	255.6	259.7	266.0	266.0	269.9
643126	279.9	282.2	288.6	291.2	294.9	299.5	302.2	308.1	307.9

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643116	305.3	300.7	290.3	303.8	384.7	435.1	483.0	488.8	519.4
643118	298.7	301.0	304.7	306.7	369.0	401.3	435.8	451.0	465.5
643119	266.7	271.1	261.1	259.7	329.7	373.4	408.9	416.2	440.0
643120	274.1	271.3	273.4	282.5	341.4	371.8	401.0	414.6	437.4
643126	310.5	310.6	312.0	316.9	385.0	417.0	450.4	456.7	468.2

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106	
643116	547.1	561.7	582.2	598.5	616.1	625.5	628.1	SD test day 106
643118	493.8	504.7	520.0	532.7	543.9	551.6	556.9	SD test day 106
643119	466.8	477.6	486.9	495.7	502.1	511.9	517.8	SD test day 106
643120	466.0	483.8	499.7	511.1	525.3	539.2	542.8	SD test day 106
643126	497.0	504.4	519.6	529.5	539.5	554.7	555.0	SD test day 106

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP V (H-24020)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643121	230.0	231.6	239.8	239.6	252.8	245.0	242.6	263.0	270.7
643122	201.9	204.4	208.7	206.0	202.6	199.7	213.7	221.7	230.1
643123	215.3	212.6	221.9	222.4	228.1	231.7	240.8	249.4	256.0
643124	215.0	210.7	218.0	222.0	221.4	222.0	236.3	244.6	252.6
643125	194.7	192.7	201.1	202.4	196.4	196.6	212.9	222.4	224.8

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643121	279.7	285.7	292.2	302.3	301.6	306.4	309.4	311.5	320.8
643122	232.8	238.3	245.8	253.2	254.9	260.7	263.0	264.8	265.9
643123	261.2	264.0	272.6	279.2	285.2	287.4	281.7	292.8	297.3
643124	249.0	243.8	248.3	251.9	254.6	252.9	257.1	253.7	258.8
643125	232.2	233.9	241.3	247.0	250.5	253.9	255.1	260.6	266.7

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643121	326.2	328.2	328.8	334.8	397.8	428.0	464.5	467.3	486.5
643122	269.4	272.1	274.0	275.2	327.3	347.7	378.9	377.0	390.0
643123	298.0	300.1	302.6	305.8	365.3	401.3	428.9	442.3	467.1
643124	258.5	259.4	254.8	246.3	316.2	338.3	363.1	365.0	386.5
643125	270.0	272.4	270.6	273.3	307.8	330.5	351.6	351.3	363.0

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106
643121	506.1	521.2	533.3	550.3	561.8	575.8	575.1 SD test day 106
643122	411.3	419.9	436.1	447.3	460.6	466.1	466.4 SD test day 106
643123	489.5	499.0	516.1	531.5	542.4	542.3	547.6 SD test day 106
643124	406.2	426.2	436.5	446.1	462.7	461.4	458.6 SD test day 106
643125	389.0	402.4	420.8	433.0	446.4	461.2	462.6 SD test day 106

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP II (Control)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643127	175.1	176.6	178.5	184.9	187.9	189.7	186.3	198.1	198.7
643128	179.0	176.2	181.4	188.7	190.7	189.1	194.2	201.7	202.5
643129	177.0	177.0	181.8	189.5	192.6	191.7	198.2	203.5	206.4
643130	167.9	170.7	176.5	180.5	182.0	180.9	182.4	188.8	193.7
643131	167.7	166.4	170.9	173.0	175.0	178.2	181.9	185.9	185.8

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643127	204.2	199.1	205.2	210.5	211.2	209.9	213.4	217.2	218.9
643128	201.1	208.8	213.0	214.2	211.9	219.3	219.2	222.0	218.9
643129	206.2	212.1	215.1	221.6	218.7	227.2	229.9	232.8	232.0
643130	195.1	189.0	196.2	201.9	204.0	198.5	204.6	206.0	211.1
643131	189.6	190.0	191.7	192.9	194.5	197.2	198.3	201.1	200.1

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643127	215.7	223.0	221.9	227.1	233.8	243.3	261.1	258.0	260.0
643128	219.4	224.6	229.3	225.8	257.4	264.4	265.1	274.9	280.6
643129	234.6	240.3	240.6	241.4	271.2	282.7	289.6	295.9	311.7
643130	211.6	206.3	210.3	216.0	230.2	236.1	245.1	245.5	254.6
643131	201.8	204.1	206.0	207.3	225.5	233.3	244.6	238.0	247.5

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106
643127	263.2	265.6	274.0	279.3	281.2	282.2	286.8 SD test day 106
643128	288.4	284.5	293.9	300.5	305.9	304.7	304.5 SD test day 106
643129	314.6	313.4	325.7	338.0	345.8	352.8	351.6 SD test day 106
643130	270.3	269.8	275.3	280.2	286.1	281.7	282.2 SD test day 106
643131	254.0	253.1	258.6	261.8	268.4	267.4	268.2 SD test day 106

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP IV (H-24648)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643132	178.7	177.6	184.9	190.6	195.2	197.5	200.0	208.8	210.4
643133	183.4	180.8	189.3	194.8	191.4	198.5	203.9	207.8	206.1
643134	162.8	164.5	168.5	170.8	172.3	177.4	181.5	182.2	183.4
643135	177.5	176.8	181.6	186.8	191.1	191.7	197.1	202.7	199.7
643136	168.0	160.4	169.5	174.5	176.6	175.5	180.8	186.3	187.8

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643132	213.6	216.1	222.4	225.4	224.1	232.3	232.0	236.0	234.7
643133	219.1	213.8	223.1	222.5	231.3	233.6	232.8	232.4	228.9
643134	188.9	188.5	192.1	192.8	194.0	197.7	195.5	196.2	201.2
643135	206.7	207.6	210.5	210.3	214.2	219.7	220.8	219.9	225.0
643136	186.5	192.1	198.6	197.2	197.1	204.6	205.7	207.6	205.8

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643132	239.8	243.5	247.2	250.0	277.1	284.2	304.3	307.1	319.6
643133	234.8	233.6	237.9	236.8	271.0	286.1	306.5	307.6	300.8
643134	206.6	203.2	203.7	208.0	221.3	228.5	227.8	245.9	243.0
643135	226.8	227.0	227.1	229.0	257.9	267.9	280.3	274.3	283.3
643136	213.3	217.0	218.0	213.8	247.8	258.3	256.8	267.7	271.8

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106
643132	330.2	334.3	349.5	358.1	369.8	372.2	376.9 SD test day 106
643133	316.4	328.1	350.8	370.2	383.3	391.2	390.8 SD test day 106
643134	252.2	256.9	259.5	264.8	277.7	276.7	275.4 SD test day 106
643135	297.8	293.2	302.5	315.1	316.8	322.4	321.2 SD test day 106
643136	275.9	272.8	289.0	295.3	300.0	299.9	304.4 SD test day 106

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP VI (H-24020)

ANIMAL NUMBER	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
643137	184.1	183.1	187.8	196.3	201.0	198.9	202.4	209.5	212.7
643138	195.2	195.2	198.8	207.1	212.7	216.0	216.4	222.6	225.4
643139	174.9	170.4	177.4	182.8	185.9	188.1	192.5	200.0	201.1
643140	165.6	164.4	166.4	171.0	178.1	181.2	179.9	188.8	190.4
643141	172.7	173.6	180.3	182.2	185.8	187.4	191.3	193.9	193.9

ANIMAL NUMBER	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
643137	220.6	220.4	225.9	232.1	230.7	236.2	236.5	238.8	245.4
643138	230.8	229.3	239.1	243.4	245.3	244.4	248.9	249.4	253.3
643139	198.2	202.5	210.4	209.4	208.0	214.5	215.4	219.4	218.4
643140	197.2	194.8	200.0	210.1	209.6	206.5	212.0	215.0	218.8
643141	199.8	201.0	204.3	206.6	208.0	211.7	212.1	216.5	220.6

ANIMAL NUMBER	Day 19	Day 20	Day 21	Day 22	Day 33	Day 39	Day 46	Day 53	Day 60
643137	245.2	247.5	249.1	248.7	280.5	284.7	293.2	303.6	312.8
643138	253.4	256.8	258.9	259.3	281.6	288.9	315.4	327.4	339.2
643139	224.6	225.1	223.5	221.3	248.5	256.0	261.8	268.5	280.4
643140	220.8	220.0	228.8	230.4	252.2	251.6	277.0	278.2	281.3
643141	221.3	220.7	219.4	227.3	254.8	267.5	286.2	276.2	287.1

ANIMAL NUMBER	Day 67	Day 74	Day 81	Day 88	Day 95	Day 102	Day 106
643137	326.4	336.6	351.2	356.7	358.6	368.8	369.8 SD test day 106
643138	359.5	356.9	378.2	379.0	394.7	401.0	404.1 SD test day 106
643139	291.0	291.2	297.8	304.7	311.3	314.6	316.8 SD test day 106
643140	285.4	288.1	305.4	307.8	308.3	321.6	315.2 SD test day 106
643141	301.2	301.9	309.3	318.5	326.1	331.1	331.6 SD test day 106

APPENDIX B

Individual Clinical Observations

INDIVIDUAL CLINICAL OBSERVATIONS

EXPLANATORY NOTES

Any clinical sign observed on consecutive data collection days is assumed to have been present during the interim between observation days, and will be reported as having been observed over the period from the first-last day observed.

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	I	643111	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,4,10,20,24,30,37,106
			Eye Observations, Bled via Orbital for Clin Path, Right	15,18,51
			Sacrificed by design	106
M	I	643112	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,20,24,30,37,106
			Eye Observations, Bled via Orbital for Clin Path, Right	15,18,51
			Sacrificed by design	106
M	I	643113	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	51,106
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,20,24,30,37
			Eye Observations, Bled via Orbital for Clin Path, Right	15,18
			Sacrificed by design	106
M	I	643114	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	51
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,37,106
			Eye Observations, Bled via Orbital for Clin Path, Right	20,24,30
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	I	643115	General observation, No Abnormality Detected	1-11
			Eye Observations, Bled via Orbital for Clin Path, Left	51
			Eye Observations, Bled via Orbital for Clin Path, Right	1,5,10,15,18,20,24, 30,37
			Eye Observations, Bled via Orbital for Clin Path	106
			Discharge, Eye bilateral	21
			Discharge, Nose, Black	21
			Discharge, Eye right, Black	19-20
			Hair Loss, Forelimb, Bilateral	74-106
			Hair Loss, Forepaw, Bilateral	18-22,32,39-46,53-67
			Hair Loss, Forepaw, Left	12-17
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	III	643116	General observation, No Abnormality Detected	1-7,46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51,106
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Hair Loss, Abdomen, Medial	8-22,32,39
			Sacrificed by design	106
M	III	643118	General observation, No Abnormality Detected	1-10,21-22,32,39-46, 53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51,106
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Eye Observations, Dark, Left	11-20
			Sacrificed by design	106
M	III	643119	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	10
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,15,18,24,30, 37,51,106
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	III	643120	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	106
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Sacrificed by design	106
M	III	643126	General observation, No Abnormality Detected	1-18,20,22,32,39-46, 53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	24,37
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,30,106
			Discharge, Eye bilateral, Black	21
			Discharge, Eye left, Black	19
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	V	643121	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	37
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
M	V	643122	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
M	V	643123	General observation, No Abnormality Detected	1-22,32,34-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	106
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
M	V	643124	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
M	V	643125	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN FEMALE RATS

Sex	Group	Animal	Observation	Days
F	II	643127	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
F	II	643128	General observation, No Abnormality Detected	1-18,21-22,32,39-46, 53-81,95-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Discharge, Eye left, Black	19-20,88
			Sacrificed by design	106
F	II	643129	General observation, No Abnormality Detected	1-16
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Eye Observations, Corneal Opacity, Right	17-22,32,39-46,53-106
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN FEMALE RATS

Sex	Group	Animal	Observation	Days
F	II	643130	General observation, No Abnormality Detected	1-22,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Discharge, Nose, Black	32
			Discharge, Eye left, Black	32
			Sacrificed by design	106
F	II	643131	General observation, No Abnormality Detected	1-22,32,39
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Eye Observations, Corneal Opacity, Left	46,53-106
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN FEMALE RATS

Sex	Group	Animal	Observation	Days
F	IV	643132	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
F	IV	643133	General observation, No Abnormality Detected	1-6,9-10,16-22,32,60-74
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Eye Observations, Dark, Left	11-15,39-46,53
			Hair Loss, Forelimb, Bilateral	81-106
			Stain Fur/Skin, Perinasal, Brown	7-8
			Sacrificed by design	106
F	IV	643134	General observation, No Abnormality Detected	1-22,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Discharge, Eye bilateral, Black	32
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN FEMALE RATS

Sex	Group	Animal	Observation	Days
F	IV	643135	General observation, No Abnormality Detected	1-22,32,39-46,53-60
			Eye Observations, Exophthalmus, Left	72-106
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Eye Observations, Corneal Opacity, Left	67-106
			Hair Loss, Forepaw, Bilateral	81-106
			Sacrificed by design	106
F	IV	643136	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
F	VI	643137	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	24
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,30,37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
F	VI	643138	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	24
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,30,37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106
F	VI	643139	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

Sex	Group	Animal	Observation	Days
F	VI	643140	General observation, No Abnormality Detected	1-22,32
			Eye Observations, Partially Closed, Right	74-106
			Eye Observations, Enophthalmus, Right	46,53-106
			Eye Observations, Exophthalmus, Right	39
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	37
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 51,106
			Eye Observations, Bled via Orbital for Clin Path, Right	20
			Eye Observations, Corneal Opacity, Right	39-46,53-106
			Sacrificed by design	106
F	VI	643141	General observation, No Abnormality Detected	1-22,32,39-46,53-102
			Eye Observations, Bled via Orbital for Clin Path, Left	1,5,10,15,18,24,30, 37,51
			Eye Observations, Bled via Orbital for Clin Path, Right	20,106
			Sacrificed by design	106

APPENDIX C

ANALYTICAL METHODS

HPLC Method

Column: Waters Xterra MS C18 2.1X30 mm, 2.5 µm
Solvent A: 50mM ammonium acetate
Solvent B: acetonitrile
Gradient:

Time (min)	%A	%B
0	80	20
12	61	39
12.1	80	20
14	80	20

Flow rate: 200 µl/min
Column Temperature: 40° C
Sample Temperature: 8° C
Injection Volume: 5 µl

Mass spectrometer method

Temperatures:

Source: 125° C
Desolvation: 350° C

Gas Flows:

Desolvation: 558 l/hr
Cone: 144 l/hr

Settings:

Polarity: ES-
Cone voltage: 20 V
Aperture: 0 V
LM 1 Resolution: 15.0
Ion energy1: 1.0
Exit: 50
HM 2 Resolution: 12.0
Capillary voltage: 2.5 kV
Hexapole 1: 0 V
Hexapole 2: 0 V
HM 1 Resolution: 15.0
Entrance: 50
LM 2 Resolution: 12.0
Ion energy1: 1.0

Method:

SIR of 2 channels

Channel Mass	Dwell (sec)	Cone Voltage (V)
412.9	0.25	20.0
363.0	0.25	20.0

APPENDIX D

INDIVIDUAL PFOA PLASMA CONCENTRATION DATA

INDIVIDUAL PFOA PLASMA CONCENTRATION DATA

EXPLANATORY NOTES

All control rats had PFOA concentrations of 0 at all time points.

Footnote "a" on each table represents data points where concentration could not be calculated due to sample processing error.

Individual PFOA Plasma Concentration Data for Male Rats

Group III (H-24648)

Animal Number	Test Day	Total µg/mL	Total µM
643116	1	16.25	37.70
643116	5	82.38	191.13
643116	10	55.13	127.90
643116	15	115.14	267.15
643116	18	137.66	319.40
643116	20	102.92	238.79
643116	24	49.75	115.43
643116	30	28.01	64.99
643116	37	12.79	29.68
643116	51	4.45	10.31
643116	106	[a]	[a]
643118	1	63.84	148.12
643118	5	158.75	368.33
643118	10	158.50	367.75
643118	15	124.92	289.84
643118	18	153.50	356.15
643118	20	124.10	287.94
643118	24	50.10	116.24
643118	30	23.49	54.50
643118	37	13.44	31.18
643118	51	4.57	10.59
643118	106	[a]	[a]
643119	1	9.63	22.33
643119	5	166.38	386.02
643119	10	55.75	129.35
643119	15	122.76	284.83
643119	18	168.32	390.53
643119	20	113.58	263.53
643119	24	75.14	174.34
643119	30	38.75	89.91
643119	37	[a]	[a]
643119	51	12.12	28.12
643119	106	0.44	1.01

Individual PFOA Plasma Concentration Data for Male Rats

Group III (H-24648)

Animal Number	Test Day	Total µg/mL	Total µM
643120	1	15.25	35.38
643120	5	160.75	372.97
643120	10	27.50	63.81
643120	15	123.38	286.26
643120	18	161.14	373.87
643120	20	97.64	226.54
643120	24	40.83	94.73
643120	30	21.30	49.42
643120	37	13.91	32.27
643120	51	5.27	12.23
643120	106	0.13	0.29
643126	1	[a]	[a]
643126	5	44.75	103.83
643126	10	118.50	274.94
643126	15	64.28	149.14
643126	18	189.80	440.37
643126	20	117.28	272.11
643126	24	58.39	135.48
643126	30	0.00	0.00
643126	37	17.27	40.07
643126	51	5.47	12.69
643126	106	0.10	0.23

All rats had pretest (test day –3) PFOA concentration of 0.

Note – Only [redacted] present in H-24648.

Individual PFOA Plasma Concentration Data for Male Rats

Group V (H-24020)

Animal Number	Test Day	Straight Chain µg/mL	Branched Chain µg/mL	Total µg/mL	Total µM
643121	1	35.79	11.08	46.87	108.74
643121	5	194.38	21.18	215.55	500.12
643121	10	156.13	12.62	168.74	391.52
643121	15	128.02	17.45	145.47	337.52
643121	18	104.50	11.38	115.88	268.87
643121	20	168.42	6.69	175.11	406.29
643121	24	67.89	0.00	67.89	157.52
643121	30	26.06	0.00	26.06	60.46
643121	37	36.93	0.00	36.93	85.68
643121	51	12.58	0.00	12.58	29.18
643121	106	[a]	[a]	[a]	[a]
643122	1	36.20	11.73	47.93	111.20
643122	5	185.25	23.71	208.96	484.82
643122	10	86.88	13.65	100.52	233.23
643122	15	104.86	13.96	118.82	275.69
643122	18	117.62	3.93	121.55	282.01
643122	20	115.98	4.55	120.53	279.65
643122	24	61.19	0.00	61.19	141.97
643122	30	35.39	0.00	35.39	82.11
643122	37	15.01	0.00	15.01	34.83
643122	51	4.99	0.00	4.99	11.58
643122	106	0.07	0.00	0.07	0.16
643123	1	35.95	10.95	46.90	108.82
643123	5	100.63	22.93	123.56	286.67
643123	10	21.38	16.21	37.58	87.20
643123	15	108.56	15.59	124.15	288.06
643123	18	123.88	5.14	129.02	299.34
643123	20	131.24	6.33	137.57	319.19
643123	24	58.32	0.00	58.32	135.31
643123	30	37.19	0.00	37.19	86.29
643123	37	17.19	0.00	17.19	39.88
643123	51	7.20	0.00	7.20	16.71
643123	106	[a]	[a]	[a]	[a]

Individual PFOA Plasma Concentration Data for Male Rats

Group V (H-24020)

Animal Number	Test Day	Straight Chain µg/mL	Branched Chain µg/mL	Total µg/mL	Total µM
643124	1	61.57	15.62	77.19	179.09
643124	5	271.00	25.15	296.15	687.13
643124	10	172.50	21.92	194.42	451.09
643124	15	144.80	18.54	163.34	378.97
643124	18	145.50	5.09	150.59	349.40
643124	20	154.94	7.08	162.02	375.92
643124	24	76.37	0.00	76.37	177.19
643124	30	37.14	0.00	37.14	86.17
643124	37	23.30	0.00	23.30	54.06
643124	51	11.72	0.00	11.72	27.19
643124	106	[a]	[a]	[a]	[a]
643125	1	46.88	13.00	59.88	138.93
643125	5	235.25	25.20	260.45	604.30
643125	10	118.50	15.88	134.38	311.78
643125	15	148.74	15.08	163.82	380.09
643125	18	82.46	2.57	85.03	197.29
643125	20	148.94	5.72	154.66	358.83
643125	24	82.77	0.00	82.77	192.04
643125	30	24.41	0.00	24.41	56.64
643125	37	13.29	0.00	13.29	30.84
643125	51	6.12	0.00	6.12	14.20
643125	106	[a]	[a]	[a]	[a]

All rats had pretest (test day –3) PFOA concentration of 0.

Individual PFOA Plasma Concentration Data for Female Rats

Group IV (H-24648)

Animal Number	Test Day	Total µg/mL	Total µM
643132	1	45.52	105.60
643132	5	35.88	83.24
643132	10	60.47	140.30
643132	15	144.70	335.73
643132	18	68.98	160.05
643132	20	52.94	122.83
643132	24	0.82	1.90
643132	30	0.00	0.00
643132	37	0.00	0.00
643132	51	0.04	0.08
643132	106	[a]	[a]
643133	1	37.92	87.98
643133	5	60.33	139.98
643133	10	73.63	170.82
643133	15	53.62	124.41
643133	18	53.10	123.20
643133	20	53.08	123.16
643133	24	0.00	0.00
643133	30	0.00	0.00
643133	37	0.00	0.00
643133	51	0.00	0.00
643133	106	0.00	0.00
643134	1	9.13	21.17
643134	5	37.75	87.59
643134	10	80.13	185.90
643134	15	56.10	130.16
643134	18	72.70	168.68
643134	20	69.04	160.19
643134	24	0.00	0.00
643134	30	0.00	0.00
643134	37	0.00	0.00
643134	51	0.00	0.00
643134	106	[a]	[a]

Individual PFOA Plasma Concentration Data for Female Rats

Group IV (H-24648)

Animal Number	Test Day	Total µg/mL	Total µM
643135	1	41.37	95.99
643135	5	51.50	119.49
643135	10	41.55	96.40
643135	15	59.22	137.40
643135	18	65.56	152.11
643135	20	69.68	161.67
643135	24	0.00	0.00
643135	30	0.00	0.00
643135	37	0.00	0.00
643135	51	0.00	0.00
643135	106	[a]	[a]
643136	1	43.35	100.58
643136	5	61.63	142.98
643136	10	54.78	127.09
643136	15	61.88	143.57
643136	18	57.28	132.90
643136	20	58.72	136.24
643136	24	0.00	0.00
643136	30	0.00	0.00
643136	37	0.00	0.00
643136	51	0.00	0.00
643136	106	[a]	[a]

All rats had pretest (test day –3) PFOA concentration of 0.

Note – Only [redacted] present in H-24648.

Individual PFOA Plasma Concentration Data for Female Rats

Group VI (H-24020)

Animal Number	Test Day	Straight Chain µg/mL	Branched Chain µg/mL	Total µg/mL	Total µM
643137	1	36.79	6.37	43.16	100.14
643137	5	34.49	6.13	40.61	94.23
643137	10	32.17	7.80	39.97	92.73
643137	15	35.70	3.52	39.22	90.99
643137	18	36.54	1.30	37.84	87.80
643137	20	40.30	1.57	41.87	97.15
643137	24	0.71	0.00	0.71	1.65
643137	30	0.00	0.00	0.00	0.00
643137	37	0.00	0.00	0.00	0.00
643137	51	0.09	0.00	0.09	0.20
643137	106	0.00	0.00	0.00	0.00
643138	1	36.38	6.73	43.11	100.02
643138	5	43.76	7.76	51.52	119.53
643138	10	27.99	6.21	34.20	79.34
643138	15	47.56	4.19	51.75	120.08
643138	18	60.12	3.96	64.08	148.68
643138	20	43.70	1.20	44.90	104.17
643138	24	0.00	0.00	0.00	0.00
643138	30	0.00	0.00	0.00	0.00
643138	37	0.00	0.00	0.00	0.00
643138	51	0.00	0.00	0.00	0.00
643138	106	[a]	[a]	[a]	[a]
643139	1	28.04	5.72	33.75	78.31
643139	5	42.17	9.06	51.23	118.85
643139	10	34.16	7.22	41.38	96.00
643139	15	49.00	7.06	56.06	130.07
643139	18	51.06	2.13	53.19	123.41
643139	20	42.50	0.94	43.44	100.79
643139	24	0.00	0.00	0.00	0.00
643139	30	0.00	0.00	0.00	0.00
643139	37	0.00	0.00	0.00	0.00
643139	51	0.02	0.00	0.02	0.05
643139	106	0.00	0.00	0.00	0.00

Individual PFOA Plasma Concentration Data for Female Rats

Group VI (H-24020)

Animal Number	Test Day	Straight Chain µg/mL	Branched Chain µg/mL	Total µg/mL	Total µM
643140	1	35.35	6.22	41.57	96.45
643140	5	37.86	7.38	45.24	104.97
643140	10	29.50		29.50	68.45
643140	15	58.04	7.27	65.31	151.54
643140	18	58.50	3.44	61.94	143.70
643140	20	61.56	2.06	63.62	147.61
643140	24	0.00	0.00	0.00	0.00
643140	30	0.00	0.00	0.00	0.00
643140	37	0.00	0.00	0.00	0.00
643140	51	0.08	0.00	0.08	0.17
643140	106	0.00	0.00	0.00	0.00
643141	1	30.30	6.21	36.51	84.71
643141	5	36.65	7.92	44.57	103.40
643141	10	33.71	11.08	44.79	103.91
643141	15	46.92	6.63	53.55	124.25
643141	18	55.42	3.95	59.37	137.74
643141	20	55.96	1.99	57.95	134.45
643141	24	0.00	0.00	0.00	0.00
643141	30	0.00	0.00	0.00	0.00
643141	37	0.00	0.00	0.00	0.00
643141	51	0.00	0.00	0.00	0.00
643141	106	0.00	0.00	0.00	0.00

All rats had pretest (test day –3) PFOA concentration of 0.