A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

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Final Report on

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

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ABSTRACT

The pharmacokinetics and urinary excretion of perfluorooctanesulfonate were investigated in male and female cynomolgus monkeys. Three male and three female monkeys were administered a single iv bolus dose of 2 mg/kg of perfluorooctanesulfonate, potassium salt (T-6295). At various times after dosing, serum and urine (24-hour collections) samples were obtained and analyzed by HPLC/MS/MS for levels of intact perfluorooctanesulfonate. The lower limit of quantitation of the analytical method was 10 ng/mL for serum samples and 5 ng/mL for urine samples. At 0.5 hours after dosing (earliest time point), serum concentrations of perfluorooctanesulfonate appeared to be slightly higher in male monkeys than in female monkeys and ranged from 13,790 to 16,250 ng/mL in male monkeys and from 7,871 to 11,790 ng/mL in female monkeys. Serum concentrations of perfluorooctanesulfonate then decreased relatively rapidly in each monkey through the first 24 hours after dosing and, at 24 hours, ranged from 7,039 to 9,538 ng/mL in male monkeys and 5,042 to 7,294 ng/mL in female monkeys. With the exception of one male and one female monkey, serum concentrations of perfluorooctanesulfonate remained essentially constant between Days 7 and 70. Thereafter, serum concentrations of perfluorooctanesulfonate decreased in all animals and ranged from 3,803 to 3,840 ng/mL in the male monkeys and from 2,019 to 2,781 ng/mL in the female monkeys on Day 161. The serum concentration versus time data were subjected to non-compartmental pharmacokinetic analysis. The serum terminal half-life of perfluorooctanesulfonate ranged from 122 to 146 days (mean: 132 days) in male monkeys and from 88 to 138 days (mean: 110 days) in female monkeys. The total body clearance of perfluorooctanesulfonate was 1.0 to 1.2 mL/day/kg in male monkeys and 1.6 to 2.1 mL/day/kg in female monkeys. The volume of distribution of perfluorooctanesulfonate ranged from 178 to 220 mL/kg and from 231 to 327 mL/kg in male and female monkeys, respectively. Only very low levels (≤0.1% of the administered dose) of perfluorooctanesulfonate were measured in urine during any given 24-hour period of sample collection between Day 1 and Day 91 after dosing. The results of this study provided no clear indication that the pharmacokinetics of perfluorooctanesulfonate were different in male and female monkeys. Perfluorooctanesulfonate was eliminated in urine by both male and female monkeys at low levels for a prolonged period of time (≥91 days) after iv administration.

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Good Laboratory Practices Disclaimer

This study described in this final report was not conducted in strict compliance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations (21 CFR Part 58), and neither this report nor the raw data were reviewed by the Southern Research Quality Assurance Unit. However, the study was conducted according to the protocol and amendments and the applicable standard operating procedures, and all study procedures, data recording, and reporting were performed in a manner consistent with the standard of GLPs. The final report accurately reflects the raw data obtained during the performance of the study. There were no adverse circumstances that affected the quality or integrity of the study.

Patricia E. Noker 4/22/03

Patricia E. Noker, Ph.D., D.A.B.T. Date
Study Director

Signature Page

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Patricia E. Noker, Ph.D., D.A.B.T. Date
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We, the undersigned, were responsible for the conduct of the work and reporting of the results in the listed sections. We concur with the views relative to our body of work as expressed in the discussion and conclusions.

Gregory S. Gorman 4/22/03

Gregory S. Gorman, Ph.D. Date
Manager, Bioanalytical Chemistry Group

Study Schedule and Personnel

Study Dates:

Study Initiation:	July 26, 2001
Day of Dosing:	July 30, 2001
Last Day of Sample Collection:	January 7, 2002
Study Completion:	April 22, 2003

Study Personnel:

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Carolyn R. Oliver, B.S.	Supervisor, Study Coordination

1.0 Introduction

The objectives of this study were to determine the concentration of potassium perfluorooctanesulfonate in serum and to estimate urinary clearance at various times following administration of a single intravenous dose to monkeys. A copy of the protocol can be found in Appendix A.

2.0 Materials and Methods

2.1 Test System

The three male and three female cynomolgus monkeys designated for use in this study were selected from an in-house colony of monkeys that were housed at Southern Research Institute (Southern Research) prior to use on this study. These monkeys were purchased from Charles River BRF, Inc. (Houston, TX) and were an estimated 3.5-4.5 years of age when placed on study. Individual animal identification was by chest tattoo. The cynomolgus monkey is an accepted species to support clinical studies of drugs used or intended for use in humans.

During the quarantine period, a complete physical examination including a fecal examination for internal parasites, complete blood count (CBC), body weight, and rectal temperature was performed on each of the monkeys. The following procedures were performed on the monkeys during quarantine: (1) Three tuberculin tests were administered to each animal at 2-week intervals. All tuberculin tests were administered intrapalpebrally. The three tuberculin tests were negative for all monkeys. (2) Blood was drawn for CBC and B virus titer. (3) Fecal cultures (screening for *Salmonella* and *Shigella*) were obtained, and fecal flotation tests were performed. (4) In general, primates were examined at least once weekly by an approved veterinarian and were observed (cage-side observations recorded by exception only) twice daily for abnormal clinical signs and mortality/moribundity. Housing, feed, water, and socialization procedures remained the same during the quarantine, holding, and study periods.

Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St. Louis, MO) was fed to the monkeys 2-3 times each day. The quantity of the daily ration was sufficient to meet nutritional requirements. In addition, the diet was supplemented with fresh fruit/treats several times each week. Tap water (Birmingham public water supply) was available to the monkeys *ad libitum* during the quarantine and study periods. The monkeys were housed individually in stainless steel cages during the quarantine and the study periods. From Day 0 to the end of the study, the monkeys were housed in a room that was maintained at a temperature of 66.4-71.5 °F and a relative humidity of 25-74%. The humidity was within the required range (30-70%) over 90% of the time during the study; excursions below the recommended humidity range were of short duration and had no impact on animal health or the outcome of the study. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600 to 1800 hours, CST) and 12 hours of dark per day. Cage size and animal care conformed to the guidelines of the *Guide for the Care and Use of Laboratory Animals*, 7th edition[1] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198) and to the applicable Standard Operating Procedures (SOPs) of Southern Research. The study design was approved by Southern Research's Institute Animal Care and Use Committee. Southern Research is fully accredited by the American Association for Accreditation of Laboratory Animal Care International.

With the exception of animal 2053, all of the monkeys used on this study were previously given a single iv bolus dose of perfluorobutanesulfonate (10 mg/kg) on 4/10/00 (Southern Research Study No. 9921.1); a single iv bolus dose of potassium perfluorobutanoate (10 mg/kg) on 6/13/00 (Southern Research Study No. 9921.2); a single iv bolus dose of potassium perfluorohexanoate (10 mg/kg) on 7/31/00 (Southern Research Study No. 9921.3); and a single iv bolus dose of potassium perfluorooctanoate (10 mg/kg) on 10/9/00 (Southern Research Study No. 9921.4). In addition, all monkeys used on this study were given a single iv bolus dose of potassium perfluorohexanesulfonate (10 mg/kg) on 2/9/01 (Southern Research Study No. 9921.5).

2.2 Test Article and Vehicle

Test Article: One bottle containing 6.65 grams of potassium perfluorooctanesulfonate (T-6295; expiration date not supplied; SRI E05/L-1) was supplied by 3M (St. Paul, MN) and received on May 15, 2000. The test article was stored at room temperature until used. Stability of the test article was the responsibility of the Sponsor.

Vehicle: The vehicle used for the preparation of the dose formulation of potassium perfluorooctanesulfonate was sterile saline, USP (Phoenix Pharmaceutical Company; St. Joseph, MO; Lot 102049F, expiration date February 2004). The vehicle was stored at room temperature and was considered to be stable when stored according to these conditions.

Dose Formulation Preparation: For the single dose formulation of potassium perfluorooctanesulfonate prepared at 1 mg/mL, the required amount of test article was weighed out in a volumetric flask. Sterile saline was added and the formulation was stirred until in solution. The formulation was stored refrigerated and used for dosing on the same day of preparation; it was considered stable during this period.

Dose Formulation Analyses: Dose concentration and homogeneity analyses were not required to be performed.

2.3 Experimental Design

Group Assignment and Dose Procedure: As only one treatment group was used in this study, no formal randomization was required. On Day 0, each of the three male and three female monkeys received a single intravenous (iv) dose of perfluorooctanesulfonate at 2 mg/kg by injection into a superficial arm or leg vein. Doses were based upon the individual body weights obtained on the day of dosing. Doses were administered at a volume of 2 mL/kg.

Clinical Observations: All animals were observed twice daily for signs of mortality/moribundity. Each primate was examined shortly after dose administration for

clinical signs of toxicity. Additional clinical observations were performed on days of blood collection, with the exception of Day 21 when clinical observations were not performed due to technician error.

Body Weights: Each primate was weighed on Days 0, 4, 7, 14, 21, 28, 42, 56, 70, 85, 105, 126, and 151.

Urine and Feces Collection: Urine and feces were collected for 24-hour intervals on the following days: prior to dose administration (Day -3; baseline), on Day 1 (0-24 hours postdose), on Day 2 (24-48 hours postdose), and on Days 7, 15, 21, 28, 42, 56, 70, and 91. The volume of each urine sample was measured upon collection. Urine and feces samples were stored frozen (approximately -20 °C or below). Fecal samples will not be analyzed unless specifically requested by the Sponsor.

Serum Levels of Perfluorooctanesulfonate: Blood samples (approximately 3 mL) were collected from each primate at approximately 0 (predose) minutes; 0.5, 2, 4, 8, 24 and 48 hours; and on Days 4, 7, 14, 21, 28, 42, 56, 70, 105, and 161 postdose. Samples were collected into tubes without anticoagulant and were allowed to clot at room temperature. The blood samples were then centrifuged, and the serum separated and stored frozen (approximately -20 °C or below) until analyzed.

Bioanalytical Method Development and Sample Analysis: Serum and urine samples were analyzed for perfluorooctanesulfonate using a previously validated HPLC/MS/MS method (Appendix B). The lower limit of quantitation of the method was 10 ng/mL for serum samples and 5 ng/mL for urine samples.

Data Analyses: The serum concentration data for unchanged perfluorooctanesulfonate were subjected to noncompartmental pharmacokinetic analysis using WinNonlin (Standard Edition; Version 1.1; Scientific Consulting Inc.; Cary, NC). Mean values and standard deviations for each parameter were calculated using Microsoft® Excel Software (Microsoft

Corporation; Irvine, CA). The urinary excretion of perfluorooctanesulfonate at each collection interval was calculated and expressed as a percent of the administered dose. No other statistical analyses of the data were performed.

3.0 Results

3.1 Mortality

All of the monkeys in this study survived to the end of the study.

3.2 Clinical Observations

No adverse drug-related clinical signs were noted for any monkey during the course of this study.

3.3 Body Weights

Body weights are presented in Table 1. Each monkey either gained weight or maintained essentially a constant weight between Day 0 and Day 151 (last day during the study that body weights were obtained).

3.4 Serum and Urine Concentrations of Perfluorooctanesulfonate

Serum concentrations of perfluorooctanesulfonate in three male and three female monkeys at various times through Day 161 after administration of a single iv dose of 2 mg/kg are presented in Table 2 and in Figure 1. At 0.5 hours after dosing (earliest time point), serum concentrations of perfluorooctanesulfonate appeared to be slightly higher in male monkeys than in female monkeys and ranged from 13,790 to 16,250 ng/mL in male monkeys and from 7,871 to 11,790 ng/mL in female monkeys. Serum concentrations of perfluorooctanesulfonate then decreased relatively rapidly in each monkey through the first 24 hours after dosing and ranged from 7,039 to 9,538 ng/mL in the three male monkeys and from 5,042 to 7,294 ng/mL in two of the female monkeys at 24 hours; data for the other female monkey was not obtained at this time point. A trough in serum concentrations of perfluorooctanesulfonate was observed in individual monkeys between Days 2 and 7. This trough was followed by an increase and subsequent plateau in serum concentrations of

perfluorooctanesulfonate; for all except one male and one female monkey (M2054 and F2058), serum concentrations of perfluorooctanesulfonate remained essentially constant between Days 7 and 70. Thereafter, serum concentrations of perfluorooctanesulfonate decreased in all monkeys and ranged from 3,803 to 3,840 ng/mL in the male monkeys and from 2,019 to 2,781 ng/mL in the female monkeys on Day 161.

To confirm the reproducibility of the analytical method, selected serum samples collected from individual monkeys at various times during the study were re-analyzed approximately 3 months after their initial analysis. The results of these determinations are presented in Table 3. The results of the first and second analyses of each serum sample were in good agreement (within 20% of the initially determined concentration) with each other.

Pharmacokinetic parameters calculated from serum concentrations of perfluorooctanesulfonate in individual monkeys are presented in Table 4. $AUC_{0\text{-infinity}}$ values ranged from 1666 to 1877 μg•day/mL in male monkeys and from 932 to 1259 μg•day/mL in female monkeys. The terminal half-life of perfluorooctanesulfonate in serum ranged from 122 to 146 days (mean: 132 days) in the three male monkeys and from 88 to 138 days (mean: 110 days) in the three female monkeys. The total body clearance of perfluorooctanesulfonate was 1.0 to 1.2 mL/day/kg in male monkeys and 1.6 to 2.1 mL/day/kg in female monkeys. The volume of distribution of perfluorooctanesulfonate ranged from 178 to 220 mL/kg in male monkeys and from 231 to 327 mL/kg in female monkeys.

The amount of perfluorooctanesulfonate eliminated in urine by individual monkeys at various times after dosing is presented in Table 5. Only very low levels (≤0.1% of the administered dose) of perfluorooctanesulfonate were measured in urine during any given 24-hour period of sample collection between Day 1 and Day 91 after dosing. There was no clear indication of a sex-related difference in the urinary excretion of perfluorooctanesulfonate. The urinary excretion of perfluorooctanesulfonate was prolonged; detectable levels of the compound were present in urine on Day 91 (last day of urine collection) after dosing.

4.0 Discussion

The results of this study indicated that perfluorooctanesulfonate was detectable in male and female monkeys for a prolonged period of time after administration of a single iv dose of 2 mg/kg. Among individual male and female monkeys, the apparent terminal elimination half-life of perfluorooctanesulfonate was estimated to be 88-146 days. In addition, it was found that only low levels of perfluorooctanesulfonate were excreted in urine by either male or female monkeys during any given 24-hour period of sample collection. For either sex, the urinary excretion of perfluorooctanesulfonate was prolonged; the compound was still present in urine at 91 days after dosing (longest time point that urine samples were collected). From the serum concentration versus time data and the urinary excretion data, there was no clear indication the pharmacokinetics of perfluorooctanesulfonate were different in male and female monkeys.

A summary of estimated pharmacokinetic parameters and urinary excretion data obtained during this and previous investigations with various perfluoro-compounds is presented in Appendix C. For the C6 and C8 carboxylated derivatives, and possibly the C4 carboxylated compound, the serum pharmacokinetics appeared to be different in male and female monkeys. Sex differences in pharmacokinetic parameters were less apparent for the C4, C6, and C8 sulfonated derivatives.

5.0 Conclusions

There was no clear indication that the serum kinetics of perfluorooctanesulfonate were different in male and female monkeys. Perfluorooctanesulfonate was eliminated in urine by both male and female monkeys at low levels for a prolonged period of time (≥91 days) after iv administration.

6.0 Record Archives

Data, specimens, and a copy of the final report from this study will be stored in the Archives at Southern Research for up to 1 year after acceptance of the final report by the Sponsor. After 1 year and with the permission of the Sponsor's Monitor, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. If materials are to be retained

in the archives beyond this date, such continued storage will be for a specific fee determined with the Sponsor. A copy of the final report will be retained in the central archives at Southern Research.

7.0 References

1. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington D.C.; 1996.

Table 1

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Individual Body Weights

Animal ID	Dose Level	Body Weight (kg) on Study Day						
		0	4	7	14	21	28	42
Males								
2053	2 mg/kg	6.5	6.8	6.6	6.7	6.7	6.7	6.6
2054	2 mg/kg	6.4	6.7	6.5	6.5	6.5	6.4	6.5
2211	2 mg/kg	6.6	6.6	6.6	6.6	6.6	6.5	6.6
Females								
2058	2 mg/kg	3.6	3.5	3.6	3.7	3.5	3.4	3.5
2059	2 mg/kg	3.5	3.5	3.4	3.5	3.4	3.5	3.5
2061	2 mg/kg	4.3	4.3	4.3	4.3	4.2	4.1	4.3

Animal ID	Dose Level	Body Weight (kg) on Study Day					
		56	70	85	105	126	151
Males							
2053	2 mg/kg	6.7	6.6	6.7	6.7	6.9	7.0
2054	2 mg/kg	6.4	6.4	6.4	6.5	6.6	6.7
2211	2 mg/kg	6.6	6.6	6.8	6.7	6.9	7.2
Females							
2058	2 mg/kg	3.5	3.5	3.7	3.6	3.7	3.8
2059	2 mg/kg	3.5	3.5	3.6	3.6	3.7	3.8
2061	2 mg/kg	4.3	4.3	4.5	4.6	4.6	4.8

Table 2

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Serum Concentrations of Perfluorooctanesulfonate

	Serum Concentration (ng/mL)					
	Males			Females		
Timepoint	2053	2054	2211	2058	2059	2061
0	BQL	BQL	BQL	5.8	BQL	BQL
0.5 hrs	16,250	15,120	13,790	7,871	10,755	11,790
2 hrs	11,390	12,480	11,280	7,991	9,500	9,207
4 hrs	10,273	11,308	9,585	6,080	9,490	9,200
8 hrs	9,578	11,473	10,015	7,358	10,525	8,717
24 hrs	8,345	9,538	7,039	5,042	a	7,294
48 hrs	7,998	7,636	7,501	5,165	7,860	7,436
Day 4	7,354	9,157	7,277	4,994	6,620	7,825
Day 7	7,589	10,090	7,592	5,163	5,485	6,873
Day 14	8,630	10,040	9,515	5,586	5,995	7,136
Day 21	8,547	7205[b]	8,380	6,972	5,675	8,023
Day 28	9186[b]	11,746	9089[b]	4,990	5,680	8,208
Day 42	9,485	10,580	8,358	6,403	5,730	6,134
Day 56	7,880	8,695	7,406	4,652	6,265	6,204
Day 70	9,038	9,885	6,624	4,457	5,735	6,925
Day 105	4,596	4,423	3,750	3,079	2,197	3,332
Day 161	3,803	3,805	3,840	2,584	2,019	2,781

BQL = Below the quantitation limit (<10 ng/mL)

[a] Initial analysis of the sample was problematic; there was an insufficient volume of sample for a reanalysis.

[b] Data represent an average of two analyses of the sample.

Table 3

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Replicate Analysis Comparisions for Potassium Perfluorooctanesulfonate in Monkey Serum

Sample[a]	Results from 10/15/01 (ng/mL)	Results from 1/9/02 (ng/mL)	Percent Difference
F2061 4 hr	9,200	7,512	-18.3
F2058 Day 14	5,586	5,674	1.6
F2061 24 hr	7,294	7,399	1.4
F2061 Day 7	6,873	6,737	-2.0
F2058 Day 42	6,403	5,216	-18.5

[a]These samples were randomly selected from a group of samples containing a sufficient volume of serum for analysis.

Table 4

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Pharmacokinetic Parameters Calculated from Serum Concentrations of Potassium Perfluorooctanesulfonate

Parameter	Male					Female				
	2053	2054	2211	Average	SD	2058[g]	2059	2061	Average	SD
Cmax (μg/mL)[a]	18.3	14.7	16.1	16.4	1.8	10.5	11.2	12.8	11.5	1.2
$AUC_{0\text{-last}}$ (μg*day/mL)[b]	1,078	962	1,165	1,068	102	672	677	841	730	96
$AUC_{0\text{-infinity}}$ (μg*day/mL)[c]	1,877	1,666	1,833	1,792	111	1,188	932	1,259	1,126	172
$t_{1/2e}$ (day)[d]	146	127	122	132	13	138	88	104	110	26
Clearance (mL/day/kg)[e]	1.0	1.2	1.1	1.1	0.1	1.7	2.1	1.6	1.8	0.3
Vd_{ss} (mL/kg)[f]	209	220	178	202	22	327	264	231	274	49

[a]Maximum concentration in serum

[b]Area under the serum concentration time curve from 0 to the last time point

[c]Area under the serum concentration time curve from 0 to infinity

[d]Half-life of the terminal elimination phase

[e]Total body clearance

[f]Volume of distribution at steady state

[g]Value for 0.5 hour time point was excluded from the pharmacokinetic analyses

Table 5

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorooctanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Baseline						
2053	M	BQL	740	--	26,000	--
2054	M	BQL	300	--	25,600	--
2211	M	BQL	350	--	26,400	--
2058	F	BQL	280	--	14,400	--
2059	F	BQL	150	--	14,000	--
2061	F	BQL	420	--	17,200	--
Day 1 (0-24 hours)						
2053	M	11	540	5.9	26,000	0.02
2054	M	33	210	6.9	25,600	0.03
2211	M	22	330	7.3	26,400	0.03
2058	F	29	170	4.9	14,400	0.03
2059	F	14	130	1.8	14,000	0.01
2061	F	10	350	3.5	17,200	0.02
Day 2 (24-48 hours)						
2053	M	15	450	6.8	26,000	0.03
2054	M	41	160	6.6	25,600	0.03
2211	M	24	180	4.3	26,400	0.02
2058	F	20	210	4.2	14,400	0.03
2059	F	22	160	3.5	14,000	0.03
2061	F	16	200	3.2	17,200	0.02
Day 7						
2053	M	31	330	10.2	26,000	0.04
2054	M	33	340	11.2	25,600	0.04
2211	M	27	220	5.9	26,400	0.02
2058	F	41	180	7.4	14,400	0.05
2059	F	46	140	6.4	14,000	0.05
2061	F	84	110	9.2	17,200	0.05

BQL = Below the quantitation limit (<5 ng/mL)

[a] One of these samples was mislabeled during collection; due to uncertainty regarding correct identification, the results are not reported.

Table 5 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorooctanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 15						
2053	M	32	400	12.8	26,000	0.05
2054	M	40	230	9.2	25,600	0.04
2211	M	31	320	9.9	26,400	0.04
2058	F	37	180	6.7	14,400	0.05
2059	F	40	90	3.6	14,000	0.03
2061	F	60	140	8.4	17,200	0.05
Day 21						
2053	M	14	450	6.3	26,000	0.02
2054	M	20	400	8.0	25,600	0.03
2211	M	21	560	11.8	26,400	0.04
2058	F	37	130	4.8	14,400	0.03
2059	F	75	180	13.5	14,000	0.10
2061	F	72	200	14.4	17,200	0.08
Day 28						
2053	M	31	410	12.7	26,000	0.05
2054	M	20	510	10.2	25,600	0.04
2211	M	28	300	8.4	26,400	0.03
2058	F	31	230	7.1	14,400	0.05
2059	F	38	180	6.8	14,000	0.05
2061	F	41	110	4.5	17,200	0.03
Day 42						
2053	M	6	810	4.9	26,000	0.02
2054	M	11	930	10.2	25,600	0.04
2211	M	11	730	8.0	26,400	0.03
2058	F	28	120	3.4	14,400	0.02
2059	F	34	130	4.4	14,000	0.03
2061	F	62	190	11.8	17,200	0.07

BQL = Below the quantitation limit (<5 ng/mL)

[a] One of these samples was mislabeled during collection; due to uncertainty regarding correct identification, the results are not reported.

Table 5 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorooctanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 56						
2053	M	a	620	--	26,000	--
2054	M	12	1,010	12.1	25,600	0.05
2211	M	12	560	6.7	26,400	0.03
2058	F	a	340	--	14,400	--
2059	F	20	90	1.8	14,000	0.01
2061	F	42	200	8.4	17,200	0.05
Day 70						
2053	M	13	600	7.8	26,000	0.03
2054	M	19	390	7.4	25,600	0.03
2211	M	11	510	5.6	26,400	0.02
2058	F	27	220	5.9	14,400	0.04
2059	F	23	190	4.4	14,000	0.03
2061	F	33	170	5.6	17,200	0.03
Day 91						
2053	M	8	660	5.3	26,000	0.02
2054	M	10	900	9.0	25,600	0.04
2211	M	9	400	3.6	26,400	0.01
2058	F	16	300	4.8	14,400	0.03
2059	F	20	90	1.8	14,000	0.01
2061	F	41	150	6.2	17,200	0.04

BQL = Below the quantitation limit (<5 ng/mL)

[a] One of these samples was mislabeled during collection; due to uncertainty regarding correct identification, the results are not reported.

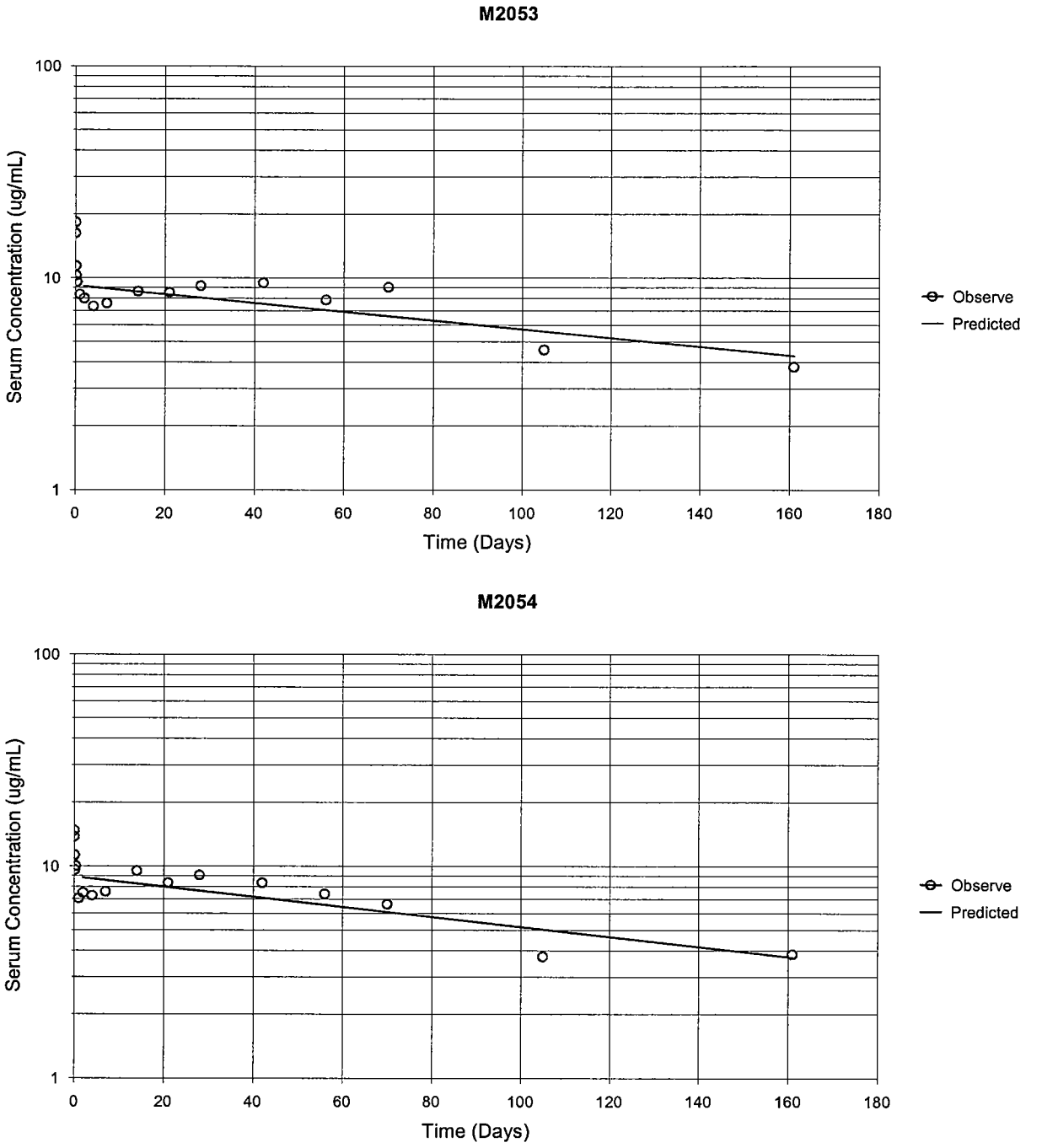

Figure 1

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorooctanesulfonate

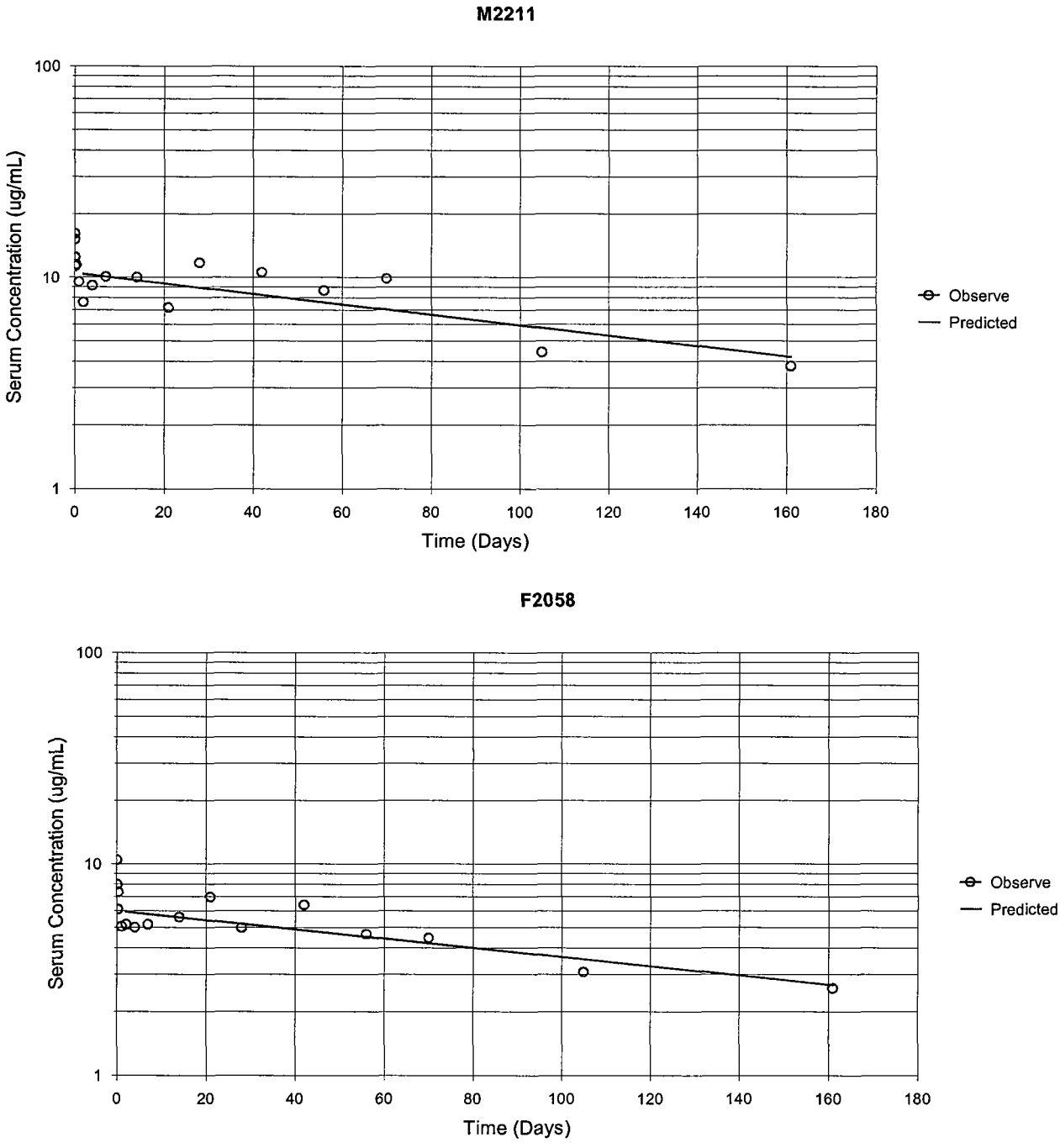

Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorooctanesulfonate

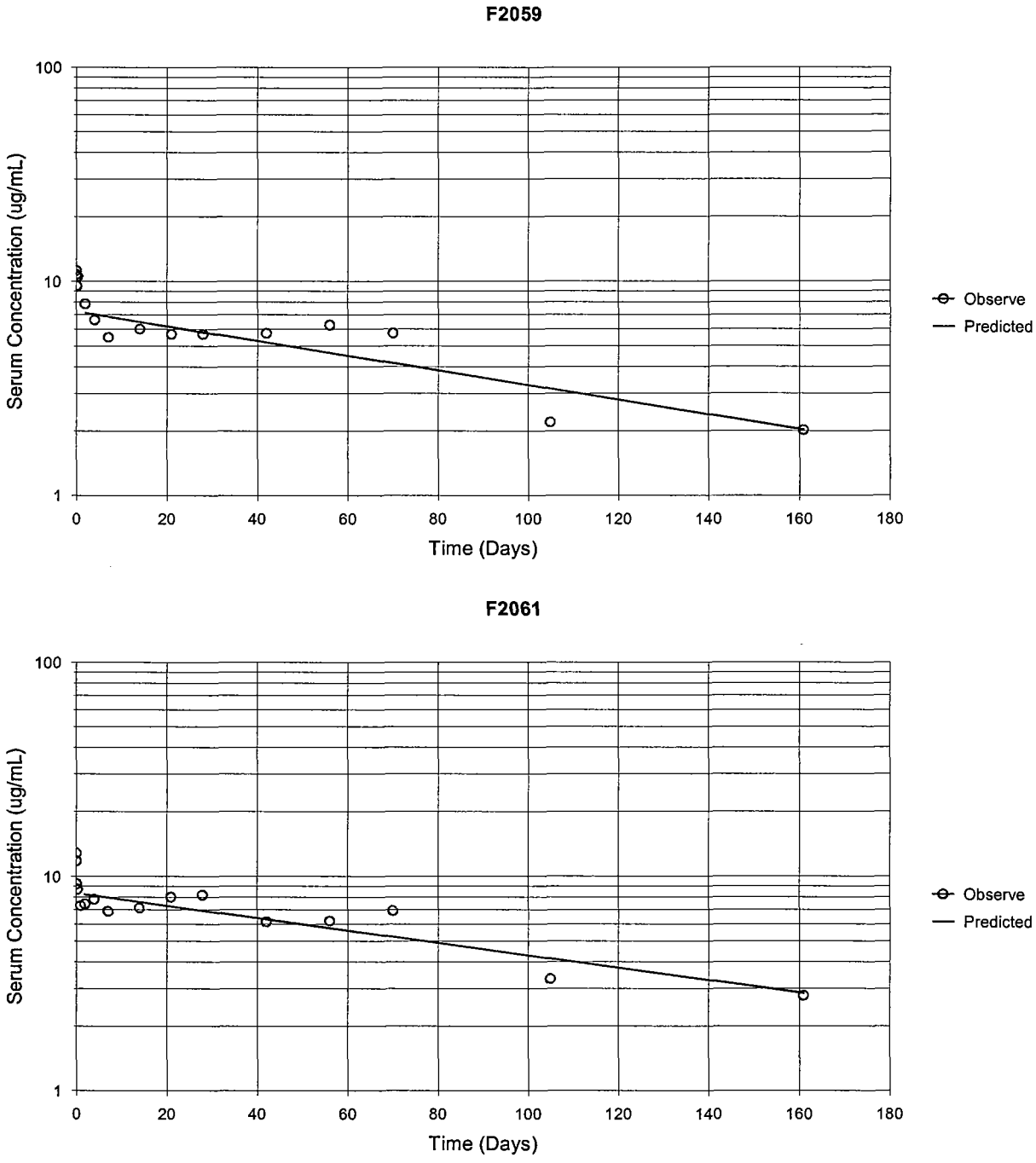

Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorooctanesulfonate

Appendix A

Study Protocol and Comments on Study Data

Study Protocol:

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Southern Research Study ID: 9921.6

July 26, 2001

SOUTHERN RESEARCH
INSTITUTE

1.0 SPONSOR REPRESENTATIVE AND CONTACTS:

Sponsor:

3M Center, 220-2E-02
P.O. Box 33220
St. Paul, Minnesota 55133-3220

Sponsor's Representative & Study Monitor:

John L. Butenhoff, Ph.D., D.A.B.T.
3M Center
Building 220-2E-02
St. Paul, Minnesota 55144-3220
(651) 733-1962; FAX: (651) 733-1773

Protocol Approval:
(Initial last page also)

John L. Butenhoff — April 7, 2003
John L. Butenhoff Date

Test Article:

Perfluorooctanesulfonate, potassium salt

Ship Unused Test Article to:

D. Hakes
Building B236
3M Center
P.O. Box 33327 • 55133-3327
St. Paul, Minnesota 55144-1000

2.0 TITLE:

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

3.0 OBJECTIVE:

The objectives of this study are to determine the concentration of perfluorooctanesulfonate in serum and urine at various times following administration of a single intravenous dose of potassium perfluorooctanesulfonate to monkeys.

4.0 TESTING LABORATORY:

Southern Research Institute
2000 Ninth Avenue South • 35205
P.O. Box 55305
Birmingham, AL 35255-5305
(205) 581-2335; FAX: (205) 581-2044

5.0 KEY STUDY DATES:

Event	Sequence (Day)	Date(s) – Year 2001
Day of Treatment	**0**	7/30/01
Urine and Feces Collections	Baseline (predose)	7/27/01
	1 (0-24 hour)	7/30/01
	2 (24-48 hour)	7/31/01
	7	8/6/01
	14	8/13/01
	21	8/20/01
	28	8/27/01
	42	9/10/01
	56	9/24/01
	70	10/8/01
	91	10/29/01
Serum Drug Levels	0: 0 (predose), 0.5, 2, 4, and 8 hours postdose	7/30/01
	1: 24 hours postdose	7/31/01
	2	8/1/01
	4	8/3/01
	7	8/6/01
	14	8/13/01
	21	8/20/01
	28	8/27/01
	42	9/10/01
	56	9/24/01
	70	10/8/01
	105	11/12/01
	161	12/31/01
Draft Report Due	60 Calendar days after completion of the in-life phase	TBD
Final Report Due	15 days after final Sponsor comments received	---

6.0 STUDY PERSONNEL:

The following are the primary contributors and supervisory personnel participating in this study.

Study Director:	**Patricia E. Noker**	**Ph.D., D.A.B.T.**
Alternate Study Director:	James D. Johnson	M.S., M.B.A.
Director, Safety Assessment:	Ward R. Richter	D.V.M., M.S., D.A.C.V.P.
Associate Director:	Norman D. Jefferson	B.A.
Manager, Bioanalytical Chemistry:	James D. Johnson	M.S., M.B.A.
Supervisor, In-Life Laboratories:	LaJuana A. Durbin	A.A.S.
Veterinarian:	Darrell E. Hoskins	D.V.M., A.C.L.A.M. (Dipl.)

7.0 TEST & CONTROL ARTICLES:

The test article will be supplied by the Sponsor, who will be responsible for documentation of stability, as well as methods of synthesis, fabrication, or derivation. Upon completion of the study, residual bulk test article will be returned to the Sponsor.

7.1 IDENTITY OF THE TEST ARTICLE:

Name:	**Perfluorooctanesulfonate, potassium salt**
Identification:	T-6295
Supplier:	3M
Lot Number(s):	To be documented in the study data.
Special Handling:	None

Characterization: Documentation of the characterization of the test article, including identity, purity, strength, and composition, as well as methods of synthesis, fabrication, or derivation, is the responsibility of the Sponsor. Copies of characterization data have been provided to the testing laboratory.

Stability & Storage: The bulk test article will be stored at room temperature. Stability of the bulk test article is the responsibility of the Sponsor.

7.2 IDENTITY OF THE VEHICLE:

Name:	**Sterile Saline**
Supplier:	Commercial supplier
Lot Number(s):	To be documented in the study data.
Special Handling:	None

Characterization: Documentation of the characterization of the vehicle may be attained by recording all pertinent information from the container labels, or by retaining the container labels, or copies thereof, in the study data. The vehicle is a commercially available product.

Stability & Storage: Sterile saline is considered stable through the date(s) of expiration provided by the manufacturer when stored appropriately. The bulk vehicle will be stored in accordance with the manufacturer's instructions.

7.3 FORMULATION:

Preparation: The test article will be formulated in sterile saline at a concentration of 1 mg/mL for intravenous administration; briefly, the required amount of test article will be mixed with the required amount of sterile saline, and the mixture will be stirred until the test article is visibly in solution. Formulations will be stored refrigerated until used for dosing; formulations of the test article in sterile saline are expected to be stable for weeks when so stored.

Dose Formulation Concentration and Homogeneity Analyses: No analysis of dose formulation concentration and homogeneity will be conducted.

8.0 TEST SYSTEM:

Species & Strain:	**Cynomolgus monkeys** *(Macaca fascicularis)*
Supplier:	Charles River BRF, Inc. (Houston, TX)
Age on Day 1:	3-4 years of age (estimated)
Weight at randomization:	3-7 kg
Number on Study:	Males -3
	Females –3

Animals were previously dosed with potassium perfluorobutanesulfonate in study 9921.1, potassium perfluorobutanoate in study 9921.2, potassium perfluorohexanoate in study

9921.3, potassium perfluorooctanoate in study 9921.4, and potassium perflourohexanesulfonate in 9921.5.

8.1 JUSTIFICATION:

Primates are commonly used in preclinical pharmacological and toxicological evaluations of compounds used or intended for use in humans, or to which humans might be exposed.

8.2 HOUSING:

During quarantine/acclimation and study, animals will be individually housed in stainless steel, slat-bottom cages. All animals will be housed in a room that provides a minimum of 10 air exchanges per hour. Controls will be set to maintain the animal room at a temperature of 64-84 °F and a relative humidity of 30-70%. A 12-hour light/12-hour dark cycle will be routinely maintained. Animals will be acclimated in the same room used for study.

8.3 BEDDING:

None required for caging equipped with flushable pans. For cages equipped with excrement absorption pans, commercial heat-treated hardwood chip bedding will be used for excrement absorption. Analyses of the bedding, supplied by the vendor, will be reviewed by the Department of Veterinary Medicine and Bioresources (DVMB) of Southern Research to assure that no known contaminants are present that could affect the health of the animals.

8.4 DIET:

Diet will be commercial Certified Primate Chow #5048 (PMI Feeds, Inc.; St. Louis, MO). The primates will be offered feed twice daily, with approximately the recommended daily ration available at each feeding interval. In addition, the diet will be supplemented with fresh fruit offered daily and treats offered several times each week. The quantity of the daily ration will be sufficient to meet nutritional requirements. Analyses of the feed, supplied by the vendor, will be reviewed by the DVMB of Southern Research to assure that no known contaminants are present that could affect the health of the animals.

8.5 WATER:

Water (Birmingham public water supply) will be supplied *ad libitum* during the quarantine and study periods via an automatic watering system. Samples of water from the animal facility will be periodically analyzed, and the analyses will be reviewed by the DVMB of Southern Research to assure that no known contaminants are present that could affect the health of the animals.

8.6 QUARANTINE:

All primates were selected from stock animals that were quarantined for a minimum of 35 days upon receipt at Southern Research. No prophylactic or therapeutic treatments were administered during the quarantine period. Standard procedures conducted during the quarantine period were as follows:

a) A complete physical examination including a fecal examination for internal parasites, complete blood count (CBC), body weight, and body (rectal) temperature was performed; b) three tuberculin tests at 2-week intervals (administered intrapalpebrally, using alternate eyelids for each test) were performed on each primate. Primates tested negative to all three tests prior to release from quarantine. ; c) the blood sample drawn for CBC was also used for measurement of B virus titer; d) a fecal sample for culture (screening for *Salmonella* and *Shigella*) was obtained and submitted to an independent laboratory for analysis; and e) all primates were examined at least once weekly by a veterinarian and observed (cage-side observations) twice daily for abnormal clinical observations and mortality/moribundity.

Throughout the subsequent holding and study periods, monkeys will be maintained under conditions similar to those for quarantine. In addition, quarterly evaluations to be performed on each monkey will include tuberculin testing, body weight determination, and body temperature measurement. Primates that respond positively to a tuberculin test will be euthanized immediately.

8.7 PSYCHOLOGICAL WELL-BEING AND SOCIALIZATION:

Nonhuman primates will be provided a psychological well-being program for social enrichment as directed by a veterinarian and approved by the IACUC and in accordance with the appropriate SOP. Nonhuman primates will be provided cage and feeding regimen modifications daily for their psychological well-being. The modifications include, but are not limited to: swings, perches, Kong toys, clean 2-liter soft drink bottles, puzzle feeders, nutritionally sound primate treats, unshelled

peanuts, and raw fruit. Where possible, primates will be housed proximate to one another for visual and vocal contact.

8.8 ANIMAL IDENTIFICATION:

The primates will be individually identified by chest tattoo number or letter combination. Positive identification will be required after every cage change and prior to blood sampling, dose administration, and observation.

9.0 EXPERIMENTAL DESIGN:

As only one treatment group will be used in this study, no formal randomization will be required.

Doses will be administered by intravenous injection to determine the pharmacokinetics of the test article. Each primate (three males, three females) will receive a single dose of potassium perfluorooctanesulfonate by injection into a superficial arm or leg vein.

Blood samples for serum drug level determinations will be collected from each primate at selected time points during the study. Urine and feces will also be collected at pre-determined intervals.

A synopsis of the study design is presented in the following table.

	Day of Study																		
Study Procecures	-1[a]	0	1	2	4	7	14	21	28	35	42	56	70	84	91	105	126	147	161
Health Check	x																		
Dose Preparation	x																		
Dosing		x																	
Clinical Observations		x	x	x	x	x	x	x	x		x	x	x		x				
Body Weights	x				x	x	x	x	x	x	x	x	x	x		x	x	x	
Urine & Feces	x		x	x		x	x	x	x		x	x	x		x				
Serum Drug Levels		x	x	x	x	x	x	x	x		x	x	x		x	x			x

[a] Denotes week

9.1 RANDOMIZATION & GROUP ASSIGNMENT:

As only one treatment group will be used in this study, no formal randomization will be required.

9.2 DOSE PROCEDURE:

Each primate (three males, three females) will receive a single intravenous (IV) dose of potassium perfluorooctanesulfonate (2 mg/kg) by injection into a superficial arm or leg vein. Doses will be based upon the most recent individual body weights. Doses will be administered at a volume of 2 mL/kg. The day of dosing will be Day 0 of the study.

9.3 CLINICAL OBSERVATIONS:

Daily Observations: All monkeys will be observed once daily during the holding period and twice daily, morning and afternoon, at least 4 hours apart, during the study for signs of mortality/moribundity and overt toxicity. Animals found *in extremis* will be humanely sacrificed by an overdose of barbiturate followed by exsanguination with appropriate approval.

Detailed Observations: Each primate will be examined shortly after dose administration for detailed clinical signs of toxicity. All findings will be recorded. Additional clinical observations will be performed and recorded on days of blood collection.

9.4 BODY WEIGHTS:

Each primate will be weighed on Days –1, 4, 7, 14, 21, and 28 and at bi-weekly (Days 42, 56, 70, and 84) or tri-weekly (105, 126, 147) intervals thereafter through the end of the study.

9.5 URINE AND FECES COLLECTIONS:

Urine and feces will be collected for approximate 24 hour intervals prior to dosing and on Days 1 (0-24 hours postdose), 2 (24-48 hours post dose), 7, 14, 21, 28, 42, 56, 70 and 91. The volume of each urine sample will be measured upon collection. All samples collected will be stored frozen at –20 °C or below prior to analysis (urine) or until further notice by the Sponsor (feces).

9.6 SERUM DRUG LEVELS:

Blood samples (approximately 3 mL) will be collected from each primate at approximately 0 (predose) minutes; 0.5, 2, 4, 8, and 24 hours; and 2, 4, 7, 14, 21, 28, 42, 56, 70, 105, and 161 days after dosing. Additional blood samples may be taken at subsequent times if requested by the Sponsor. Samples will be collected into tubes

without anticoagulant and will be allowed to clot at room temperature. The blood samples will then be centrifuged, and the serum will be separated and stored frozen at –20 °C or below until analyzed.

9.7 BIOANALYTICAL METHOD DEVELOPMENT:

Bioanalytical method(s) will be developed for the determination of perfluorooctanesulfonate in serum and urine matrices. The method(s) will be validated for accuracy and ideally will be sensitive to the 1 ppm or less level. If feces and/or other tissues require analyses, these analyses will be negotiated with the Sponsor.

9.8 BIOANALYTICAL SAMPLE ANALYSIS:

The serum and urine samples from all monkeys will be analyzed for concentrations of perfluorooctanesulfonate using the previously validated method. The data will be expressed as equivalents of potassium perfluorooctanesulfonate. Feces will be analyzed only if requested by the Sponsor.

9.9 ANIMAL DISPOSITION:

At the end of the study, monkeys will be maintained in the stock colony. In the event untoward reactions or other conditions warrant the euthanasia of a monkey at any time during the sample collection period, a serum sample (5-10 mL) will be obtained from the animal prior to euthanasia. After euthanasia, the animal will be necropsied and the liver and bile (as much as possible) will be removed and stored at approximately -70 °C.

10.0 DATA ANALYSIS:

Pharmacokinetic parameters (e.g., AUC, half-life, clearance) will be estimated from serum concentrations of unchanged perfluorooctanesulfonate, as appropriate and feasible, using a standard pharmacokinetic program.

The total amount of perfluorooctanesulfonate in urine will be calculated and expressed in terms of percent of dose.

Mean values and standard deviations will be calculated for each time point and sample type, as appropriate. No other statistical analyses of the data will be performed.

11.0 RECORDS:

All raw data pertaining to the conduct of this study, and all samples/specimens collected in this study, will be stored in the Archives at Southern Research Institute for up to 1 year after acceptance of the final report by the Sponsor. After 1 year and with the permission of the Sponsor's Monitor, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. If materials are to be retained in the archives beyond this date, such continued storage will be for a specific fee determined with the Sponsor. A copy of the final report will be retained in the central archives at Southern Research.

12.0 FINAL REPORT:

A brief letter report summarizing the serum drug level results will be issued as soon as the information is available. A draft final report will be issued within 60 calendar days after completion of the in-life aspects of the study. The final report (electronic and hard copies) will be issued within 15 working days after receipt of the Sponsor's final review comments on the draft report. The final report for the present study will include, but not necessarily be limited to the following:

Dose formulation preparation
Clinical observations
Body weight data
Serum drug level data
Pharmacokinetic parameters
Urine excretion data

13.0 REGULATORY REFERENCES:

This study will be conducted in accordance with the protocol and the Standard Operating Procedures (SOPs) of Southern Research, and in accordance with the applicable regulatory requirements, as addressed below.

13.1 PROTOCOL AMENDMENTS AND DEVIATIONS:

Amendments: All changes in or revisions of the approved protocol and the reasons thereof will be documented, signed, and dated by the Study Director, and the Sponsor's Monitor. Amendments will be maintained with the protocol. Written approval (a fax signature or electronic communication, such as email) for changes in the protocol may be granted by the Sponsor's Monitor, but a written amendment will follow.

Deviations: All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures (SOPs) of Southern Research and/or the protocol, and any deviations from protocol or SOP will be documented.

13.2 REGULATORY COMPLIANCE:

Good Laboratory Practices: This nonclinical laboratory study will be conducted in the spirit of, but will not require strict compliance with, the U.S. Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) regulations (21 CFR Part 58). Data from this study may be submitted to the FDA in support of an IND/NDA application.

Quality Assurance Review: As this study will not be conducted in strict compliance with FDA's GLP regulations, neither the in-life activities nor the final report will be audited by the Quality Assurance Unit at Southern Research.

13.3 FACILITIES MANAGEMENT AND ANIMAL HUSBANDRY:

Animal care will be in compliance with the SOPs of Southern Research, the *Guidelines for the Care and Use of Laboratory Animals, 7th Edition* (Institute of Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington, DC; 1996), and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Southern Research Institute is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

13.4 ANIMAL WELFARE ACT COMPLIANCE:

By signing this protocol, the Sponsor signifies that there are no generally accepted alternatives to the use of animals, and that the study described by this protocol does not unnecessarily duplicate previously conducted or reported experiments.

Procedures used in this protocol are designed to conform to accepted practices and to minimize or avoid causing pain, distress, or discomfort in the animals. In those circumstances in which required study procedures are likely to cause more than momentary or slight pain or distress, the animals will receive appropriate analgesics or anesthetics unless the withholding of these agents has been justified in writing by the Study Director and/or Sponsor and approved by the IACUC.

The number of animals selected for use in this study is considered to be the minimum number necessary to meet scientific and regulatory guidelines for this type of study.

This study design was reviewed by the IACUC at Southern Research Institute and was approved on 07/26/2000; it was assigned IACUC tracking number **00-07-034**.

14.0 PROTOCOL APPROVALS:

This protocol has been reviewed and approved.

Study Director: Patricia E. Noker 4/4/03
① Patricia E. Noker 7/26/01
Patricia E. Noker, Ph.D., D.A.B.T. Date
Study Director

Sponsor's Monitor: John L. Butenhoff April 7, 2003
INITIALS ONLY (See page 2) Date

Management Approval: Ward R Richter 7/26/01
Ward R. Richter, M.S., D.V.M., D.A.C.V.P. Date
Director, Safety Assessment Department, Southern Research Institute

① The Study Monitor did not return original protocol. A copy of the original was signed again by the Study Director & forwarded to the monitor for signature. cw 4/4/03

Comments on Study Data

The following is a list of protocol and/or SOP deviations that occurred during this study. Unless otherwise noted, the Study Director determined these deviations to have no adverse impact on the outcome of the study.

The protocol required that urine collections be conducted on Day 14. In order to accommodate the laboratory schedule, the collections specified for Day 14 were made on Day 15.

Due to an oversight at protocol development, the protocol lists Day 161 as 12/31/01 in the "Serum Drug Levels" section of Section 5.0. Day 161 was actually on 1/7/02, and serum was collected on that day instead of 12/31/01.

Due to technician error, a clinical observation was not recorded on Day 21 (8/20/01).

In the data for this study, recording errors occurred but were corrected in accordance with the applicable SOPs.

Appendix B

Analytical Method for Determination of Perfluorooctanesulfonate in Monkey Serum and Urine

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

1.0 PRINCIPLE

Serum or urine samples are obtained from cynomolgus monkeys treated with Perfluorooctanesulfonate (PFOS). The serum or urine (e.g., 0.5 mL) containing (PFOS) is fortified with an internal standard (IS), Perfluorooctanecarboxalate (PFOC). The samples are then mixed with an ion-pairing reagent, buffer and water, followed by extraction with ethyl acetate. The ethyl acetate layer is removed, evaporated to dryness, reconstituted in 95% methanol containing 1.5 % formic acid, 5 % 5mM ammonium acetate, filtered, and transferred to autosampler vials, and analyzed by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS). The range of reliable results extends from about 20 to 10,000 ng/mL of PFOS in serum and from 10 to 500 ng/mL in urine. Samples containing PFOS at concentrations greater than 10,000 ng/mL may be diluted with control blank matrix so that the concentration of PFOS will be within the range of reliable results prior to analysis.

The mass spectrometry of PFOS and PFOC is accomplished in the negative ion mode. The ion spray source voltage is set at - 2000 volts which is low enough to greatly reduce the formation of other potentially interfering ions extracted from the matrix.

CAUTION: Since primates may carry a number of zoonoses, all unpreserved tissues, including blood, plasma and serum, are to be considered as biohazards and handled with **universal precautions.** Refer to SOP number SRI 2-5-5 for a description of safety procedures to be used when handling unpreserved primate tissue.

2.0 REAGENTS AND SOLUTIONS

The listed reagents or their equivalents may be used.

2.1 Neat Reagents

2.1.1 Water, deionized and organic free (from in-house purification system; e.g., Ingalls 210N)

2.1.2 Methanol, HPLC grade

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

2.1.3 Perfluorooctanesulfonate (analyte), as provided by the client

2.1.4 Perfluorooctanecarboxalate (internal standard), 97%

2.1.5 Ammonium acetate, HPLC grade

2.1.6 Blank control monkey serum

2.1.7 Sodium Carbonate, Certified ACS Grade or equivalent

2.1.8 Sodium Bicarbonate, Certified ACS Grade or equivalent

2.1.9 Ethyl Acetate, HPLC grade

2.1.10 Tetrabutylammonium Hydrogen Sulfate, 97%

2.1.11 Sodium Hydroxide 50% solution, Certified grade or equivalent

2.1.12 Blank control monkey urine

2.1.13 Formic Acid, 88%

2.2 **Prepared Solutions**

Appropriate changes in the solutions may be made at the discretion of the analyst

2.2.1 5 mM Ammonium acetate in organic free water

2.2.1.1 For example, to prepare 4 liters, measure out ammonium acetate (e.g., 1.542 g) and add in organic-free water (e.g., 4 L). Mix well and filter through HPLC mobile phase filtration apparatus.

2.2.2 TBA Ion-Pairing Solution (0.5 M tetrabutylammonium hydroxide)

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

2.2.2.1 For example, to prepare 25 mL, dissolve 4.24 g of tetrabutylammonium hydrogen sulfate in deionized water and adjust the pH to 10 with 50% NaOH solution.
Note: a more dilute solution of NaOH in water may be used to effect smaller pH adjustments.

2.2.3 Carbonate/Bicarbonate Buffer Solution for Serum (0.25M/0.25M)

2.2.3.1 For example, to prepare 100 mL, dissolve approximately 2.65 g of sodium carbonate and approximately 2.10 g of sodium bicarbonate in 100 mL of deionized water. Mix well to ensure complete dissolution.

2.2.4 Carbonate/Bicarbonate Buffer Solution for Urine (1.0M/1.0M)

2.2.4.1 For example, to prepare 100 mL, dissolve approximately 10.6 g of sodium carbonate and approximately 8.4 g of sodium bicarbonate in 100 mL of deionized water. Mix well to ensure complete dissolution.

3.0 INSTRUMENTS, MATERIALS, AND APPARATUS

The following or their equivalents may be used.

3.1 HPLC pump(s), autosampler, and triple quadrupole mass spectrometer

3.2 Autosampler vials with inserts

3.3 Vortex mixers (e.g., touch mixer and IKA-Vibrax ® platform mixer)

3.4 Solvent-concentration apparatus (e.g., Zymark Turbo-Vap ® with source of nitrogen)

3.5 HPLC mobile phase filtration apparatus

3.6 Filters for HPLC mobile phase filtration apparatus (e.g., Nylon-66, 0.20 µm)

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

3.7 Analytical balance

3.8 Volumetric flasks (e.g., 10 and 25 mL)

3.9 Disposable Pasteur pipettes

3.10 Micropipettor(s) with tips

3.11 Culture tubes with teflon-lined caps

3.12 Centrifuge

3.13 Assorted glassware and syringes

3.14 Culture tubes (vials) for use with solvent-concentration apparatus

3.15 1 mL Plastic syringes with 0.2 µm PVDF syringe filters

3.16 Variable speed horizontal platform shaker

3.17 pH meter

4.0 PREPARATION OF STOCKS AND WORKING STOCKS

Appropriate changes in the concentrations of the solutions may be made at the discretion of the analyst. Actual dilutions will be documented on the preparation sheets.

4.1 Main Stock Solution of PFOS ~1000 µg/mL

4.1.1 Prepare an ~1000 µg/mL solution of PFOS in deionized organic-free water (e.g., accurately weigh about 10 mg PFOS into a 10-mL volumetric flask). Add deionized organic-free water to dissolve. Dilute to the mark. Alternatively, weigh the compound

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

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into an appropriate vessel (e.g., culture tube) and add 10 mL of deionized organic-free water. Mix well. Transfer the solution to a clean vessel if desired.

4.2 **Stock Solution of Internal Standard (PFOC), ~200 µg/mL**

4.2.1 Prepare an ~200µg/mL solution of PFOC in deionized organic-free water (e.g., accurately weigh about 10 mg into a 50-mL volumetric flask). Add deionized organic-free water to dissolve and dilute to the mark with deionized organic-free water. Alternatively, weigh the compound into a an appropriate vessel (e.g., culture tube) and add 50 mL of deionized organic-free water. Mix well. Transfer the solution to a clean vessel if desired.

4.3 **Spiking solution of Internal Standard (PFOC), ~ 50µg/mL**

4.3.1 Prepare an ~ 50 µg/mL solution of PFOC in deionized organic- free water by pipetting 2 mL of the 200 µg/mL solution into a culture tube and add 6 mL of deionized water. Mix well.

4.4 **Working Stock Solutions of PFOS**

4.4.1 To prepare working stock solutions, make the proper dilutions as shown in the following table. Prepare in 10-mL volumetric flasks or other appropriate glassware. If desired a modified dilution scheme can be used and documented in the study records.

Working Stock Level (WSL) Approximate Concentration (ng/mL)	Volume of PFOS solution	Final Volume in deionized organic free water (mL)
500,000	5 mL of 1000 µg/mL	10
250,000	5 mL of 500,000 ng/mL	10

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62,500	2.5 mL of 250,000 ng/mL	10
31,250	5 mL of 62,500 ng/mL	10
15,625	5 mL of 31,250 ng/ mL	10
5,000	50 µL of 1000 µg/mL stock	10
2,500	5 mL of 5000 ng/mL stock	10
1,000	4 mL of 2500 ng/mL stock	10
500	5 mL of 1000 ng/mL stock	10
250	5 mL of 500 ng/mL stock	10

4.4.2 Summary of concentrations of serum standards:

Standard Level	Volume and Spike Conc.	Approximate Concentration of PFOS in serum (ng/mL)
A	10 µL of 500,000 ng/mL	10,000
B	10 µL of 250,000 ng/mL	5,000
C	16 µL of 62,500 ng/mL	2,000
D	16 µL of 31,250 ng/mL	1,000

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E	16 µL of 15,625 ng/mL	500
F	20 µL of 5,000 ng/mL	200
G	10 µL of 5,000 ng/mL	100
H	10 µL of 2,500 ng/mL	50
I	10 µL of 1,000 ng/mL	20
J	10 µL of 500 ng/mL	10
K	10 µL of 250 ng/mL	5

5.0 PREPARATION OF SPIKED STANDARDS AND BLANKS

Appropriate changes in the concentrations of the solutions may be made at the discretion of the analyst.

5.1 Multiple (e.g., about three) sets of matrix standards and a matrix blank (blank + IS) are analyzed with each set of unknown samples. A matrix double blank (blank-IS) may also be analyzed if desired.

5.2 Into individual ~20-mL culture tubes, pipet blank matrix (e.g., 0.5 mL). Pipet in the appropriate volume as described in the table above for each standard. For the blanks, pipet 10 µL of organic-free water instead of the working stock solution. Add the 10 µL of internal standard stock (~50 µg/mL) to each tube except the blank-IS (pipet 10 µL of organic-free water instead) and vortex for ~5 seconds.

5.3 For serum samples add the following to each tube: 500 µL of the TBA ion-pairing solution, 1 mL of 0.25M/0.25M carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds. For urine samples add the following to each tube 1 mL of TBA ion-pairing solution, 1 mL of 1.0M/1.0M carbonate/bicarbonate buffer and 1 mL of deionized water. Vortex each tube for about 5 seconds.

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5.4 Add 2.5 mL of ethyl acetate and extract on horizontal mixer for 1 hour at a low speed setting.

5.5 Remove the tube from the shaker and place in a centrifuge (e.g., 2500 rpm) for about 5 minutes.

5.6 Take off the top ethyl acetate layer and put it into a clean tube and evaporate to dryness (e.g., ~ 50 minutes in the Turbo-Vap ®) with a gentle stream of nitrogen and moderate heat (e.g., 50 °C).

5.7 Reconstitute the residue in 500 µL of 5% 5 mM ammonium acetate: 95% methanol containing 1.5% formic acid and vortex briefly to mix. Filter the samples through 0.2 µm PVDF or Nylon syringe filters into autosampler vials.

6.0 PREPARATION OF SAMPLES

6.1 Allow each serum sample to thaw to room temperature. Vortex each sample briefly, but vigorously. Pipet an aliquot of each sample (e.g., 0.5 mL) into individual ~20-mL culture tubes. If necessary, dilute an aliquot of any sample with blank matrix so that the expected concentration of the test article being analyzed will fall within the concentration range of the standard curve. Add 10 µL of internal standard stock (~ 50 µg/mL) to each tube and vortex for a couple of seconds.

6.2 For serum samples add the following to each tube: 500 µL of the TBA ion-pairing solution, 1 mL of 0.25M/0.25M carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds. For urine samples add the following to each tube 1 mL of TBA ion-pairing solution, 1 mL of 1.0M/1.0M carbonate/bicarbonate buffer and 1 mL of deionized water. Vortex each tube for about 5 seconds.

6.3 Add 2.5 mL of ethyl acetate and extract on horizontal mixer for 1 hour at a low speed setting.

ANALYTICAL METHOD

Method No.: BACG-3607

Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

6.4 Remove the tube from the shaker and place in a centrifuge (e.g., 2500 rpm) for about 5 minutes.

6.5 Take off the top ethyl acetate layer and put it into a clean tube and evaporate to dryness (e.g., ~ 50 minutes in the Turbo-Vap ®) with a gentle stream of nitrogen and moderate heat (e.g., 50 °C).

6.6 Reconstitute the residue in 500 µL of 5% 5 mM ammonium acetate: 95% methanol containing 1.5% formic acid and vortex briefly to mix. Filter the samples through 0.2 µm PVDF or Nylon syringe filters into autosampler vials. Cap vials for analysis.

7.0 ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY/MASS SPECTROMETRY (HPLC/MS/MS)

7.1 Conditions are to be optimized if necessary.

7.1.1 HPLC Conditions

Analytical Column: Keystone Scientific Aquasil C18, 150 mm x 2 mm ID, or equivalent

Guard Column:	Keystone Aquasil C18 10 mm x 2 mm	
Elution Flow rate:	400 µL/min.	
Injection volume:	5 µL	
Mobile phase:	A: 5mM ammonium acetate buffer	
	B: 1.5% formic acid in methanol	
Gradient Profile:	0 - 3 min.	50%A : 50%B
	3 - 5min.	20% A : 80% B linear gradient
	5 - 8 min.	10%A : 90% B step gradient
	8-10 min.	50%A : 50%B step gradient
Temperature:	Ambient	

7.1.2 PE Sciex API 3000 Triple Quadrupole Mass Spectrometer Conditions
Software: PE Sciex TurboQuan

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Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

Turboion Spray Source

Note: Values listed under "MS/MS Acquisition Conditions" override parameters in this table.

Auxiliary Gas: Air (e.g., Grade 0.1) at 85 pounds per square inch

Parameter	Value
IS	-2000
NC	0
TEM	450
OR	-20
RNG	-120
Q0	10
IQ1	11
ST	15
RO1	11
IQ2	20
RO2	50
ST3	60
RO3	52
DF	250
CEM	1800

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Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

Parameter	Value
NEB	15
CUR	6
CAD	5
QPE	0
POL	1
VCM	0
IPE	0

MS/MS Acquisition Conditions
Scan type: MRM
Polarity: Negative
Acquisition mode: Profile
Pause time: 5 milliseconds

Masses requested:

PFOS:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
498.9	498.9	200

PFOC (IS)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
412.9	368.9	200

Parameter	Start	Stop
RO2	35	35
ST3	45	45
RO3	37	37
QO	18	18
IQ1	19	19
ST	23	23

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Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

RO1	19	19
IQ2	20	20

8.0 CALCULATIONS

8.1 At the end of the analytical run, review each chromatogram to ensure the retention time, peak shape, and peak height and peak area determination of the test article and the IS are acceptable. The data may be smoothed as appropriate. For quantitation, use the ion profiles at the following mass-to-charge ratios:

Analyte	Ion Profile
PFOS	498.9 to 498.9
PFOC	412.9 to 368.9

8.2 Plot the peak area response of PFOS divided by the peak area response of the IS (PFOC) from all standards versus the concentration of the test article in the standards. Alternatively, the peak heights may be used instead of peak areas. Obtain the best curve fit of the data (e.g., quadratic fit weighted with 1/concentration of the test article or a quadratic fit). Note: The best curve fit may be dependent on the range of the standard curve and it may be necessary to have more than one standard curve for various concentration ranges using the following:

$$y = ax^2 + bx + c$$

where y = Peak height response of PFOS divided by peak height response of the IS (PFOC) in standards.
x = Concentration of the PFOS in standards.
a, b, c = Constants derived from the regression analysis.

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Title: Determination of Perfluorooctanesulfonate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)

8.3 Using the standard curve, calculate the level of PFHC in each unknown sample. Correct the results of samples for any dilutions.

NOTE: Due to unresolvable interferences with the test article and/or internal standard from the matrix, external standard quantitation may be used at the discretion of the supervising mass spectrometrist.

9.0 ACCEPTANCE AND REJECTION CRITERIA

9.1 Refer to SOP SRI 91-3 for acceptance/rejection criteria except acceptable accuracy for standards is 80-120% of theoretical.

10.0 REPORTING

10.1 Results of all analyses are tabulated, and the raw data, original chromatograms, and reports are to be filed in the appropriate study file.

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Appendix C

Summary of Pharmacokinetic Parameters Calculated from Serum Concentrations of PFOs and Urinary Excretion Data for PFOs

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Summary of Pharmacokinetic Parameters Calculated from Serum Concentrations of PFOs

PARAMETER	C4 CARBOXYLATE		C4 SULFONATE		C6 CARBOXYLATE	
	Male	Female	Male	Female	Male	Female
Study Number	9921.2	9921.2	9921.1	9921.1	9921.3	9921.3
PFO Identification	T-7505	T-7505	T-7485	T-7485	T-7506	T-7506
Dose (mg/kg)	10	10	10	10	10	10
AUC $_{0\text{-inf}}$ (µg•day/mL)	4.7	6.6	24.3	35.4	1.6	4.0
$t_{½e}$ (days)	1.7	1.7	4.0	3.5	1.5	0.8
Clearance (mL/day/kg)	2154	1515	511	368	6923	2494
Vdss (mL/kg)	526	443	254	255	953	235

PARAMETER	C6 SULFONATE		C8 CARBOXYLATE		C8 SULFONATE	
	Male	Female	Male	Female	Male	Female
Study Number	9921.5	9921.5	9921.4	9921.4	9921.6	9921.6
PFO Identification	T-7504	T-7504	T-7507	T-7507	T-6295	T-6295
Dose (mg/kg)	10	10	10	10	2	2
AUC $_{0\text{-inf}}$ (µg•day/mL)	7462	5794	1235	2293	1792	1126
$t_{½e}$ (days)	141	87	20.9	32.6	132	110
Clearance (mL/day/kg)	1.3	1.9	12.4	5.4	1.1	1.8
Vdss (mL/kg)	287	213	181	198	202	274

A Pharmacokinetic Study of Potassium Perfluorooctanesulfonate in the Cynomolgus Monkey

Summary of the Urinary Excretion of PFOs

	URINARY EXCRETION RATE (MEAN % OF DOSE/DAY)					
	C4 CARBOXYLATE		C4 SULFONATE		C6 CARBOXYLATE	
	Male	Female	Male	Female	Male	Female
Study Number	9921.2	9921.2	9921.1	9921.1	9921.3	9921.3
PFO Identification	T-7505	T-7505	T-7485	T-7485	T-7506	T-7506
Dose (mg/kg)	10	10	10	10	10	10
Day 1 (0-24 hours)	41.6	46.2	48.9	66.4	69.9	85.1
Day 2 (24-48 hours)	2.3	3.1	--[a]	--	--	--
Day 7	--	--	0.2	0.2	0.03	0.03
Day 14	0.02	0.02	0.01	0.02	--	--
Day 21	--	--	--	--	<0.01	<0.01
Day 28	--	--	--	--	<0.01	<0.01

	URINARY EXCRETION RATE (MEAN % OF DOSE/DAY)					
	C6 SULFONATE		C8 CARBOXYLATE		C8 SULFONATE	
	Male	Female	Male	Female	Male	Female
Study Number	9921.5	9921.5	9921.4	9921.4	9921.6	9921.6
PFO Identification	T-7504	T-7504	T-7507	T-7507	T-6295	T-6295
Dose (mg/kg)	10	10	10	10	2	2
Day 1 (0-24 hours)	0.09	0.05	8.9	3.9	0.03	0.02
Day 2 (24-48 hours)	0.03	0.05	0.7	2.0	0.03	0.03
Day 7	0.03	0.03	7.5	2.5	0.03	0.05
Day 14	<0.01	0.05	1.2	1.8	0.04[b]	0.04[b]
Day 21	<0.01	0.03	0.6	1.1	0.03	0.07
Day 28	0.01	0.01	0.2	0.9	0.04	0.04
Day 42	0.04	0.02	--	--	0.03	0.04
Day 56	0.02	<0.02	--	--	0.04	0.03
Day 70	<0.02	0.03	--	--	0.03	0.04
Day 91	--	--	--	--	0.02	0.03

[a]Urine was not collected at the indicated time

[b]Sample was collected on Day 15