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Final Report on

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey T-7507.1

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ABSTRACT

The pharmacokinetics and urinary excretion of perfluorooctanoate were investigated in male and female cynomolgus monkeys. Three male and three female monkeys were administered a single iv bolus dose of 10 mg/kg of perfluorooctanoate, potassium salt. At various times after dosing, serum and urine (24-hour collections) samples were obtained and analyzed by HPLC/MS/MS for levels of intact perfluorooctanoate. The lower limit of quantitation of the analytical method was 20 ng/mL for serum samples and 10 ng/mL for urine samples. At 0.5 hours after dosing, serum concentrations of perfluorocctanoate were similar in male and female monkeys and ranged from 91,130 to 96,660 ng/mL in male monkeys and from 88,940 to 96,400 ng/mL in female monkeys. Serum concentrations of perfluorooctanoate subsequently declined but, beyond the first few days after dosing, the rate of decrease appeared to be faster in male monkeys than in female monkeys. On Day 28, the concentrations of perfluorooctanoate in serum ranged from 1863 to 27,140 ng/mL in the male monkeys and from 7,145 to 33,680 ng/mL in the female monkeys. Due to the relatively high serum concentrations of the compound on this day, the length of serum collection was extended through Day 123. On Day 123, the serum concentration of perfluorooctanoate was at or only slightly above the limit of quantitation (20 ng/mL) in male monkeys and between 885 and 4701 ng/mL in female monkeys. The serum concentration versus time data were subjected to non-compartmental pharmacokinetic analysis. The terminal half-life of perfluorooctanoate in serum was 13.6, 13.7, and 35.3 days in the three male monkeys and 26.8, 29.3, and 41.7 days in the three female monkeys. The total body clearance was 4.0, 15.8, and 17.5 mL/day/kg for male monkeys and 3.1, 3.9, and 9.1 mL/day/kg for female monkeys. These estimated values for half-life and clearance indicated that two of the three male monkeys eliminated perfluorooctanoate at a faster rate than did the female

monkeys. The volume of distribution of the compound at steady state (Vd_{ss}) was similar for both sexes and ranged from 168 to 192 mL/kg for male monkeys and 133 to 270 mL/kg for female monkeys. Perfluorooctanoate was slowly excreted in urine by both male and female monkeys; levels of perfluorooctanoate, representing as much as 1% of the administered dose, were present in urine 28 days after dosing. The results of this study suggested that the pharmacokinetics of perfluorooctanoate may be different in male and female monkeys.

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A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Patricia E. Noker, Ph.D., D.A.B.T. — Date: 1/14/03
Study Director
Supervisor, ADME & Pharmacokinetics

Reviewed by:

Charles D. Hébert, Ph.D., D.A.B.T. — Date: 1-14-03
Director, Safety Assessment

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, Ph.D. — Date: 1-14-03
Manager, Bioanalytical Chemistry Group

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

1/14/03

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

Date

Study Schedule and Personnel

Study Dates:

Study Initiation:	October 4, 2000
Day of Dosing:	October 9, 2000
Last Day of Sample Collection:	February 9, 2001
Study Completion:	January 14, 2003

Study Personnel:

Patricia E. Noker, Ph.D., D.A.B.T.	Study Director
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D. Wayne May, LATG	Supervisor, Animal Care
Carolyn R. Oliver, B.S.	Supervisor, Study Coordination

1.0 Introduction

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

2.0 Materials and Methods

2.1 Test System

The 3 male and 3 female 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 3-4 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 68.0-70.3 °F and a relative humidity of 22.2-65.7%. 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. 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 (IACUC). Southern Research is fully accredited by the American Associate for Accreditation of Laboratory Animal Care International (AAALAC).

The monkeys used on this study were previously given a single IV bolus dose of perfluorobutanesulfonate (10 mg/kg) on April 10, 2000 (Southern Research Study No. 9921.1); a single IV bolus dose of potassium perfluorobutanoate (10 mg/kg) on June 13, 2000 (Southern Research Study No. 9921.2); and a single IV bolus dose of potassium perfluorohexanoate (10 mg/kg) on July 31, 2000 (Southern Research Study No. 9921.3).

2.2 Test Article and Vehicle

Test Article: One bottle containing 5.2 grams of potassium perfluorooctanoate (T-7507; expiration date not supplied; Southern Research Lot No. E09/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 perfluorooctanoate was sterile saline, USP (Phoenix Pharmaceutical Company; St. Joseph, MO; Lot 8070655, expiration date July 2001). 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 perfluorooctanoate prepared at 5 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 within 4 days after 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 perfluorooctanoate at 10 mg/kg by injection into a superficial arm or leg vein. Doses were based upon the Day -1 individual body weights. 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.

Body Weights: Each primate was weighed on Days -1, 4, 7, 14, 21, and 28.

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, 14, 21, and 28. 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 Perfluorooctanoate: Blood samples (approximately 3 mL) were collected from each primate at approximately 0 (predose) minutes; 0.5, 2, 4, 8, and 24 hours; and on Days 2, 4, 7, 11, 14, 21, 28, 57, 79, 87, and 123 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: A bioanalytical method was developed for the analysis of perfluorooctanoate in serum and urine matrices. The method was validated for accuracy. This method was used for the analysis of serum and urine samples collected during the study (see Appendices B and C).

Data Analyses: The serum concentration data for unchanged perfluorooctanoate were subjected to non-compartment 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 perfluorooctanoate 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

No test article-related deaths occurred during the study. One of the three male monkeys (2052) was euthanized on Day 79 because of repeated episodes of self-mutilation; there was no indication that the self-mutilation was related to administration of perfluorooctanoate.

3.2 Clinical Observations

No adverse drug-related clinical signs of toxicity were observed for any monkey. Male monkey 2052 was observed on Day 57 of the study to have several wounds on the right leg. The attending veterinarian diagnosed these wounds to be self-inflicted lacerations. The wounds were cleaned and subsequently flushed with a weak betadine solution every 3-4 days for 4 courses of treatment. The social enrichment of the monkey was also augmented in order to provide the monkey additional stimulation and to change his focus of attention. On Day 79, the monkey was examined and several severe lacerations were observed on the leg and foot of the animal. These lacerations were diagnosed as self-inflicted wounds. Due to the repeated instances of self-mutilation and the likelihood of continued episodes of self-mutilation, the monkey was euthanized on Day 79 for humane reasons.

3.3 Body Weights

Body weights for individual monkeys are presented in Table 1. The body weight of each monkey remained the same between Days -1 and 28.

3.4 Serum and Urine Concentrations of Perfluorooctanoate

During the study, an HPLC/MS/MS method was developed and validated for the analysis of perfluorooctanoate in monkey serum and urine. Details of the method validation procedure are provided in Appendix B. Details of the analytical method are provided in Appendix C. The lower limit of quantitation of the method was 20 ng/mL for serum samples and 10 ng/mL for urine samples.

Serum concentrations of perfluorooctanoate in three male and three female monkeys at various times through Day 123 after administration of a single iv dose of 10 mg/kg are presented in Table 2 and plotted in Figure 1. At 0.5 hours after dosing, serum concentrations of perfluorooctanoate were similar in male and female monkeys and ranged from 91,130 to 96,660 ng/mL in the male monkeys and from 88,940 to 96,400 ng/mL in the female monkeys. Serum concentrations of perfluorooctanoate subsequently declined at a slow rate; the rate of decline appeared to be similar in male and female monkeys through at least the

first two days after dosing. At 48 hours, serum concentrations of perfluorooctanoate were between 48,530 and 65,810 ng/mL in the male monkeys and 39,820 and 61,600 ng/mL in the female monkeys. Beyond this time, serum concentrations of perfluorooctanoate in two of the three male monkeys decreased at a faster rate than that observed for the third male monkey and for the three female monkeys. On Day 28, the concentrations of perfluorooctanoate in serum ranged from 1863 to 27,140 ng/mL in the male monkeys and from 7,145 to 33,680 ng/mL in the female monkeys; due to these relatively high serum concentrations of the compound on this day, the length of serum collection was extended through Day 123. On Day 123, the serum concentration of perfluorooctanoate was at or only slightly above the limit of quantitation (20 ng/mL) in the two surviving male monkeys and between 885 and 4701 ng/mL in the three female monkeys.

Pharmacokinetic parameters calculated from serum concentrations of perfluorooctanoate in individual monkeys are presented in Table 3. The values were derived from non-compartmental pharmacokinetic analysis of the data. $AUC_{0\text{-infinity}}$ values ranged from 571 to 2501 μg•day/mL (mean: 1235 μg•day/mL) for male monkeys and from 1094 to 3224 μg•day/mL (mean: 2293 μg•day/mL) for female monkeys. The terminal half-life of perfluorooctanoate in serum was 13.6, 13.7, and 35.3 days in the three male monkeys and 26.8, 29.3, and 41.7 days in the three female monkeys. The total body clearance was 4.0, 15.8, and 17.5 mL/day/kg for male monkeys and 3.1, 3.9, and 9.1 mL/day/kg for female monkeys. These estimated values for half-life and clearance indicated that two of the three male monkeys eliminated perfluorooctanoate at a faster rate than did the female monkeys. The volume of distribution of the compound at steady state (Vd_{ss}) was similar for both sexes and ranged from 168 to 192 mL/kg for the male monkeys and 133 to 270 mL/kg for the female monkeys.

The amount of perfluorooctanoate eliminated in urine by individual monkeys at various times after dosing is presented in Table 4. Sex differences in the urinary excretion of perfluorooctanoate were not readily apparent prior to Day 21; on Day 21 and Day 28, the female monkeys appeared to eliminate a higher percentage of the dose in urine than did the male monkeys. The urinary elimination of perfluorooctanoate was prolonged for both the male and female monkeys; on Day 28 from 0.1 to1% of the administered dose was recovered in urine collected from the individual monkeys.

4.0 Discussion

Perfluorooctanoate was slowly eliminated by male and female monkeys given a single iv dose of 10 mg/kg. The results of this study indicated that beginning on around Day 7 and continuing throughout the remaining period of sample collection, serum concentrations of perfluorooctanoate were lower in two of the three male monkeys than in the three female monkeys. On the final day of serum collection (Day 123), serum concentrations of perfluorooctanoate were below or only slightly above the quantitation limit (20 ng/mL) in the two surviving male monkeys, whereas, on the same day, serum concentrations of perfluorooctanoate ranged from 885 to 4701 ng/mL in the three female monkeys. These differences in the serum concentrations of perfluorooctanoate were reflected in a shorter serum half-life of perfluorooctanoate in these two male monkeys (13.6 or 13.7 days) than observed for the three female monkeys (range: 26.8 to 41.7 days). The clearance of perfluorooctanoate also appeared to be higher in two of the male monkeys than in the three female monkeys. Although the number of monkeys was limited, these results suggested that the kinetics of perfluorooctanoate may have been different in male and female monkeys.

It was observed that the serum concentration data for one of the male monkeys (2052) more closely paralleled the serum concentration profile observed for the female monkeys than that observed for the other two male monkeys. It is possible that the study procedures (e.g., handling, short-term restraint in a chair) or other unknown factors may have produced stress in this monkey resulting in the release of high levels of cortisol. Cortisol is known to affect various physiological changes including changes in carbohydrate, protein, and/or lipid metabolism, shifts in electrolyte and water balance, and increases in plasma proteins. Such changes could have possibly led to changes in the

disposition of perfluorooctanoate in this male monkey. It is also possible that the self-mutilation activities of this monkey, which lead to the eventual euthanasia of the animal, were a response to stress.

Although the serum levels of perfluorooctanoate indicated that at least two of the three male monkeys may have eliminated perfluorooctanoate at a faster rate than did female monkeys, it was difficult to discern a difference in the urinary excretion of the compound by male and female monkeys. This may have been related to the fact that the rate of urinary excretion of the compound by all the monkeys on study was slow. Less than 20% of the administered dose was excreted in urine by either male or female monkeys within the first 48 hours after dosing. Subsequently, on Day 28, perfluorooctanoate was present in urine collected from male or female monkeys at levels that represented as much as 1.0% of the dose.

5.0 Conclusions

The mean terminal serum half-life of perfluorooctanoate was 22.0 days in male monkeys and 32.6 days in female monkeys. Unchanged perfluorooctanoate was slowly excreted in urine at a slow rate by male and female monkeys.

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 Perfluorooctanoate in the Cynomolgus Monkey

Individual Body Weights

Animal ID	Dose Level	Body Weight (kg) on Study Day						
		0	4	7	11	14	21	28
Males								
2052	10 mg/kg	6.6	6.5	6.4	6.4	6.4	6.7	6.6
2054	10 mg/kg	6.4	6.3	6.3	6.4	6.3	6.6	6.2
2211	10 mg/kg	5.1	5.1	5.1	5.1	5.1	5.4	5.4
Females								
2058	10 mg/kg	3.4	3.4	3.5	3.5	3.5	3.4	3.5
2059	10 mg/kg	3.5	3.5	3.5	3.5	3.4	3.6	3.6
2061	10 mg/kg	4.1	4.0	4.0	4.1	4.1	4.1	4.1

Table 2

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Serum Concentrations of Perfluorooctanoate

	Serum Concentration (ng/mL)					
	Males			Females		
Timepoint	2052	2054	2211	2058	2059	2061
0	115	BQL	20	57	123	33
0.5 hrs	91,130	96,660	91,370	96,400	88,940	94,080
2 hrs	67,900	91,630	90,680	74,280	77,870	71,600
4 hrs	74,000	89,160	71,960	70,120	81,620	69,380
8 hrs	71,200	60,860	73,650	79,570	61,520	64,170
24 hrs	50,210	50,930	64,160	62,890	60,760	41,940
48 hrs	65,810	48,530	48,930	61,600	49,670	39,820
Day 4	43,370	42,940	30,490	70,520	41,640	44,370
Day 7	45,840	27,910	29,100	59,820	44,600	36,760
Day 11	39,450	19,320	23,640	38,980	53,980	37,500
Day 14	37,360	11,220	13,430	40,450	45,790	21,910
Day 21	33,600	3,985	5,939	38,750	37,850	10,460
Day 28	27,140	1,863	3,241	33,680	24,300	7,145
Day 57	15,010	467	780	31,690	16,870	4,992
Day 79	9915[a]	--	--	--	--	--
Day 87	--	95	111	8,585	5,453	2,123
Day 123	--	BQL	35	3,651	4,701	885

[a] Sample taken prior to humane sacrifice of animal.

BQL = Below the quantitation limit (<20 ng/mL)

Table 3

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Pharmacokinetic Parameters Calculated from Serum Concentrations of Perfluorooctanoate

Parameter	Male					Female				
	2052	2054	2211	Average	SD	2058	2059	2061	Average	SD
Cmax (μg/mL)[a]	101	98.4	91.6	97.0	4.9	105	93.0	103	100	6.0
$t_{1/2}$ (day)[b]	35.3	13.7	13.6	20.9	12.5	26.8	41.7	29.3	32.6	8.0
$AUC_{0\text{-last}}$ (μg•day/mL)[c]	1999	569	633	1067	808	3083	2314	1056	2151	1023
$AUC_{0\text{-infinity}}$ (μg•day/mL)[d]	2501	571	634	1235	1097	3224	2560	1094	2293	1090
Cl (mL/day/kg)[e]	4.0	17.5	15.8	12.4	7.4	3.1	3.9	9.1	5.4	3.3
Vd_{ss} (mL/kg)[f]	192	168	184	181	12	133	190	270	198	69

[a]Maximum concentration in serum
[b]Half-life of the terminal elimination phase
[c]Area under the serum concentration versus time curve calculated from 0 to the last time point
[d]Area under the serum concentration versus time curve calculated from 0 to infinity
[e]Total body clearance
[f]Volume of distribution at steady state

Table 4

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Urinary Excretion of Perfluorooctanoate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Baseline						
2052	M	BQL	960	--	66,000	--
2054	M	BQL	840	--	64,000	--
2211	M	BQL	490	--	51,000	--
2058	F	BQL	205	--	34,000	--
2059	F	BQL	700	--	35,000	--
2061	F	BQL	170	--	41,000	--
Day 1						
2052	M	6,150	200	1,230	66,000	1.9
2054	M	9,283	420	3,899	64,000	6.1
2211[a]	M	27,150	350	9,503	51,000	18.6
2058	F	5,527	190	1,050	34,000	3.1
2059	F	1,924	860	1,655	35,000	4.7
2061	F	b	80	--	41,000	--
Day 2						
2052	M	3047	250	762	66,000	1.2
2054	M	1174	170	200	64,000	0.3
2211	M	2766	120	332	51,000	0.7
2058	F	3783	150	567	34,000	1.7
2059	F	2085	660	1,376	35,000	3.9
2061	F	3486	60	209	41,000	0.5
Day 7						
2052	M	3906	350	1,367	66,000	2.1
2054[c]	M	10350	870	9,005	64,000	14.1
2211[c]	M	10770	300	3,231	51,000	6.3
2058	F	4347	200	869	34,000	2.6
2059	F	4253	110	468	35,000	1.3
2061[a]	F	18190	80	1,455	41,000	3.5

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

[a] Concentration of perfluorooctanoate in the sample greatly exceeded the highest concentration in the standard curve; therefore, urine concentration value may contain significant error

[b] Due to technician error, urine was discarded after the volume was obtained.

[c] Concentration of perfluorooctanoate in the sample was slightly above the highest concentration in the standard curve; urine concentration value should have no significant error.

Table 4 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Urinary Excretion of Perfluorooctanoate

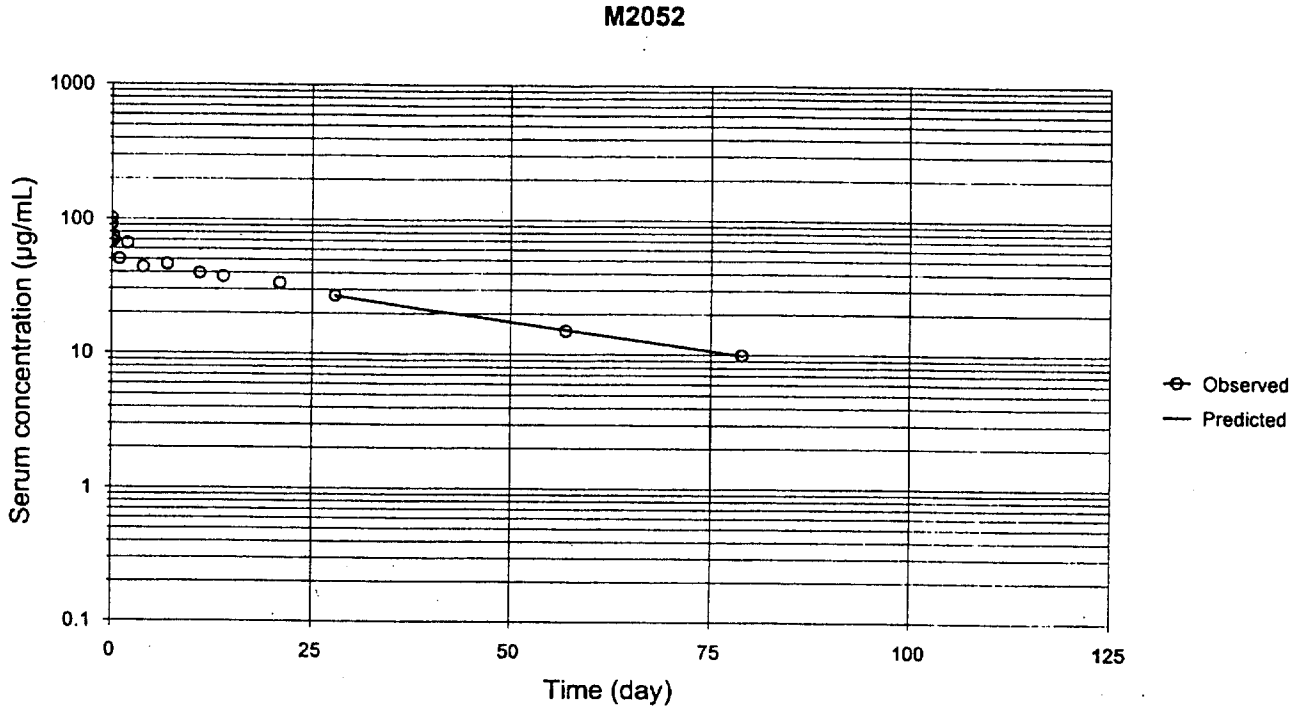
Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 14						
2052	M	1066	1010	1,077	66,000	1.6
2054	M	776	1060	823	64,000	1.3
2211	M	819	390	319	51,000	0.6
2058	F	1673	170	284	34,000	0.8
2059	F	5550	130	722	35,000	2.1
2061	F	6151	160	984	41,000	2.4
Day 21						
2052	M	389	1000	389	66,000	0.6
2054	M	575	710	408	64,000	0.6
2211	M	298	880	262	51,000	0.5
2058	F	2,278	140	319	34,000	0.9
2059	F	2,347	150	352	35,000	1.0
2061	F	6,052	100	605	41,000	1.5
Day 28						
2052	M	417	680	284	66,000	0.4
2054	M	79	660	52	64,000	0.1
2211	M	185	520	96	51,000	0.2
2058	F	665	410	273	34,000	0.8
2059	F	2286	150	343	35,000	1.0
2061	F	3126	105	328	41,000	0.8

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

[a] Concentration of perfluorooctanoate in the sample greatly exceeded the highest concentration in the standard curve; therefore, urine concentration value may contain significant error

[b] Due to technician error, urine was discarded after the volume was obtained.

[c] Concentration of perfluorooctanoate in the sample was slightly above the highest concentration in the standard curve; urine concentration value should have no significant error.



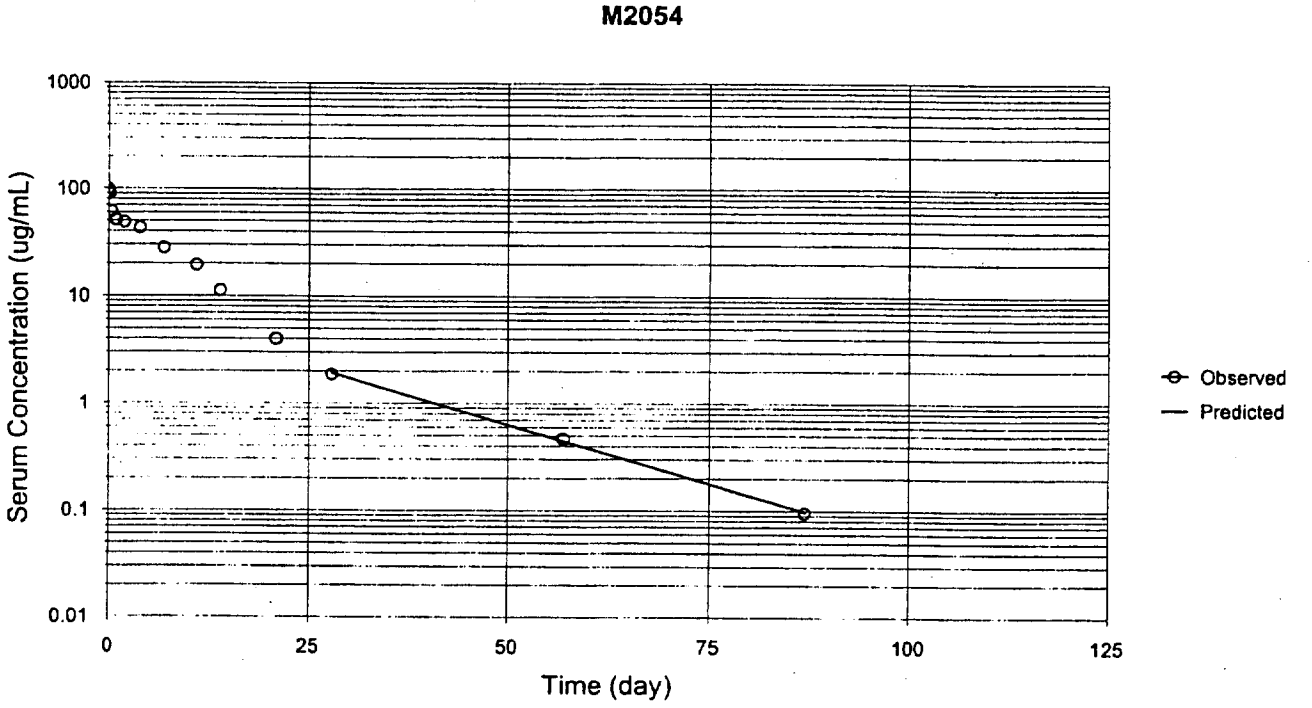
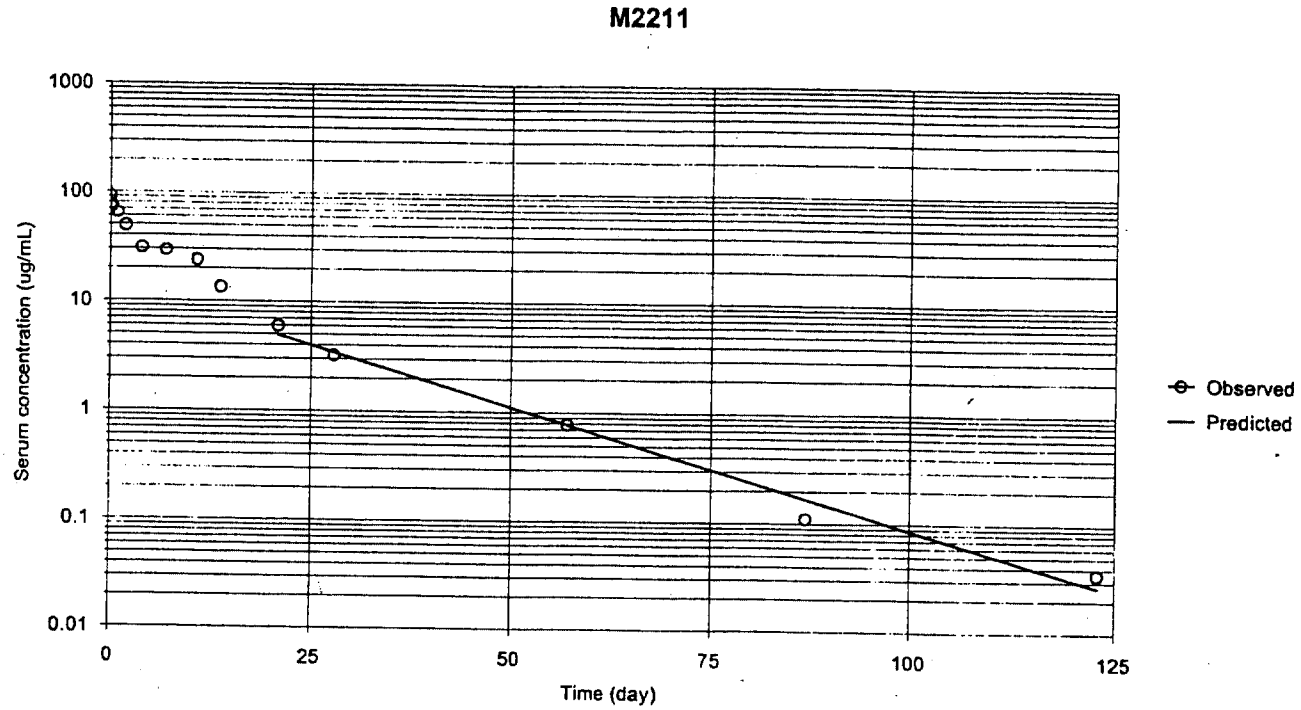


Figure 1

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Serum Concentration Profiles of Perfluorooctanoate in Monkeys



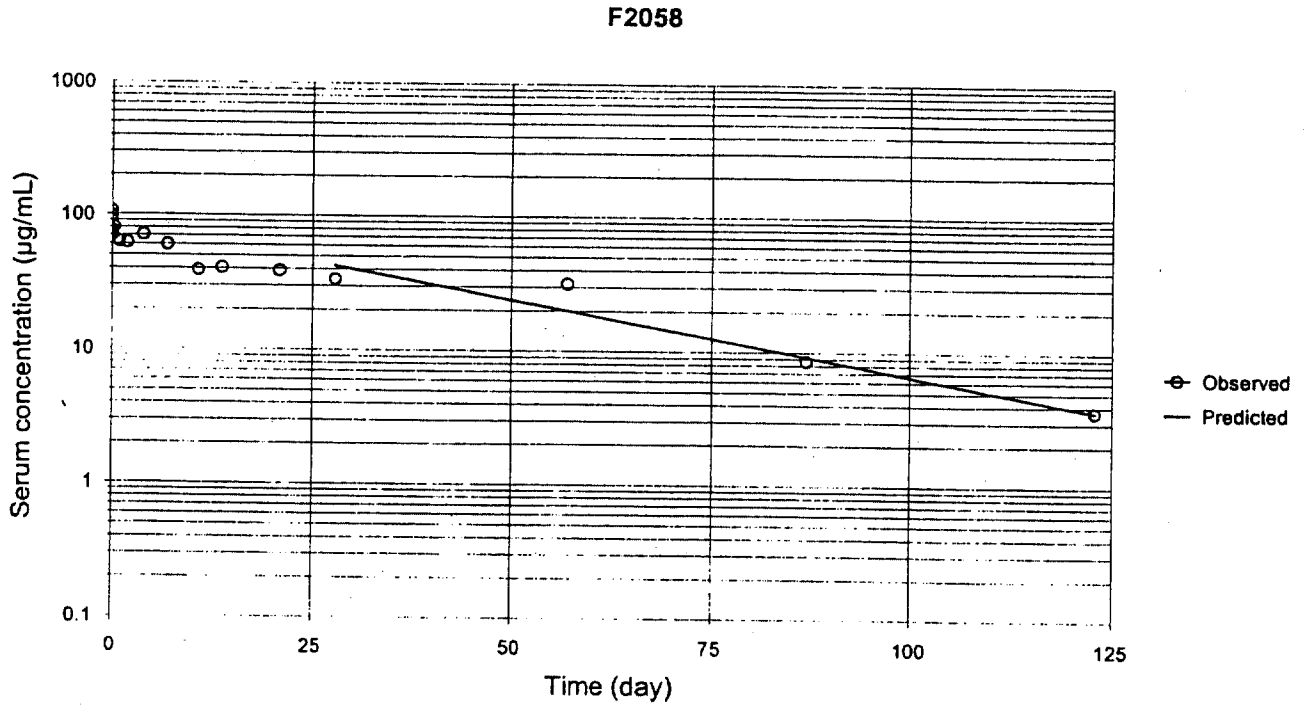


Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Serum Concentration Profiles of Perfluorooctanoate in Monkeys

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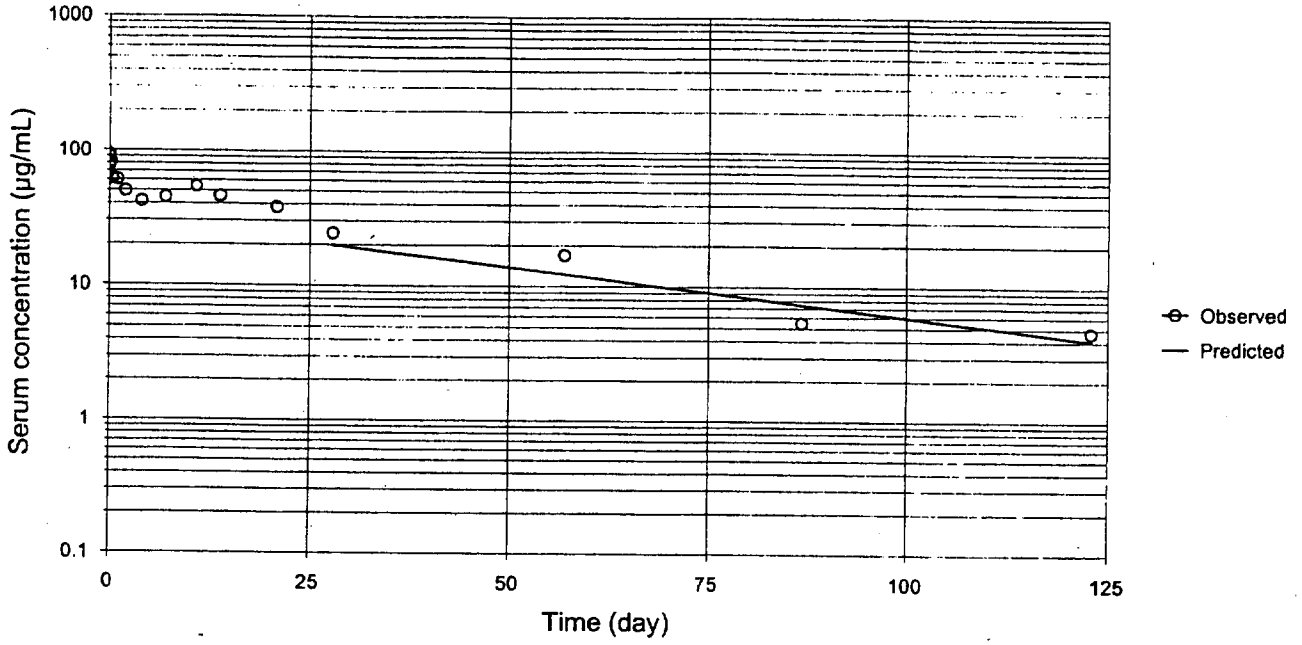


F2061

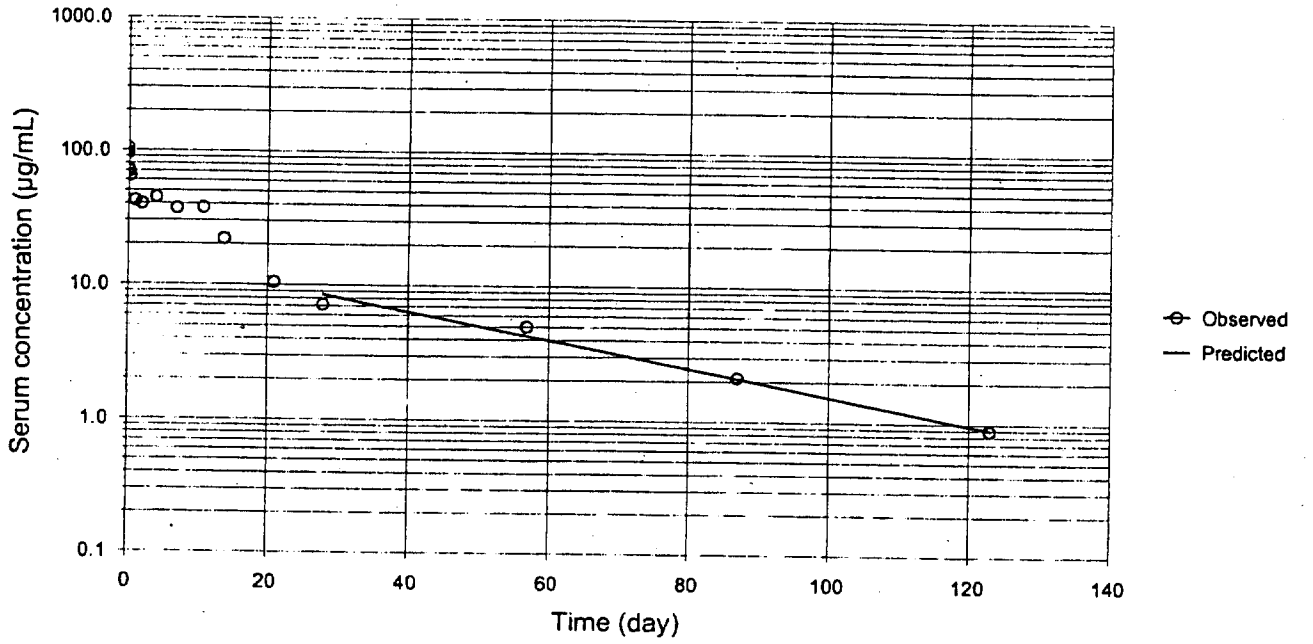


Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Serum Concentration Profiles of Perfluorooctanoate in Monkeys

Appendix A

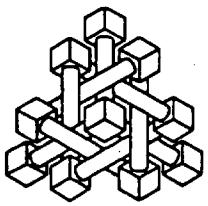
Study Protocol and Amendments

Study Protocol:

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

Southern Research Study ID: 9921.4

October 4, 2000



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~~ 1000
(651) 733-1962; FAX: (651) 733-1773

Protocol Approval:
(Initial last page also)

John L. Butenhoff 10/5/2000
John L. Butenhoff Date

Test Article:

Perfluorooctanoate, potassium salt

Ship Unused Test Article to:

D. Hakes
Building B236
3M
P.O. Box 33327 • 55133-3327
3M Center, 224-IN-04
St. Paul, Minnesota 55144-1000

2.0 TITLE:

A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomolgus Monkey

3.0 OBJECTIVE:

The objectives of this study are to determine the concentration of perfluorooctanoate in serum and urine at various times following administration of a single intravenous dose of potassium perfluorooctanoate 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 2000
Day of Treatment	0	**10/9/00**
Urine and Feces Collections	-3	10/6/00
	1	10/10/00
	2	10/11/00
	7	10/16/00
	14	10/23/00
	21	10/30/00
	28	11/6/00
Serum Drug Levels	0: 0 (predose), 0.5, 2, 4, 8 and 24 hours postdose	10/9 – 10/00
	2	10/11/00
	4	10/13/00
	7	10/16/00
	11	10/20/00
	14	10/23/00
	21	10/30/00
	28	11/6/00
Draft Report Due	60 Calendar days after completion of the in-life phase	**1/5/01**
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:	**Perfluorooctanoate, potassium salt**
Identification:	T-7507
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 5 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, and potassium perfluorohexanoate in study 9921.3.

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 interfere with or affect the outcome of the study.

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 will have been quarantined for a minimum of 35 days prior to study start. No prophylactic or therapeutic treatments will be administered during the quarantine period. Standard procedures to be conducted during the quarantine period are as follows:

a) A complete physical examination including a fecal examination for internal parasites, complete blood count (CBC), body weight, and body (rectal) temperature will be performed; b) three tuberculin tests at 2-week intervals (administered intrapalpebrally, using alternate eyelids for each test) will be performed on each primate (primates must test negative to all three tests prior to release from quarantine; primates that respond positively to a tuberculin test will be euthanized immediately, while non-responding primates housed in the same room will be held in quarantine for an additional 90 days); c) the blood sample drawn for CBC will also be used for measuring of B virus titer (primates found to have a positive B virus titer will be euthanized immediately); d) a fecal sample for culture (screening for *Salmonella* and *Shigella*) will be obtained and submitted to an independent laboratory for analysis (if any primates are found to be positive for *Salmonella, Shigella*, or parasites, the Study Director, in conjunction with the staff veterinarian, will determine the course of action); and e) all primates will be examined at least once weekly by a veterinarian and observed (cage-side observations) twice daily for abnormal clinical observations and mortality/moribundity.

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:

During quarantine, 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 perfluorooctanoate 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 Procedures	-1[a]	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	21	28
Health Check	x																	
Dose Preparation	x																	
Dosing		x																
Clinical Observations		x	x	x		x			x				x			x	x	28
Body Weights	x					x			x							x	x	x
Urine & Feces	x		x	x					x							x	x	x
Serum Drug Levels		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 perfluorooctanoate (10 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 quarantine 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.

9.5 URINE AND FECES COLLECTIONS:

Urine and feces will be collected on Days –3, 1 (0-24 hours postdose), 2 (24-48 hours post dose), 7, 14, 21, and 28. 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, 11, 14, 21, and 28 days after dosing. 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) are to be developed for the determination of perfluorooctanoate in serum and urine matrices. The method will be validated for accuracy and should be sensitive to the 1 ppm or less level. If feces and other tissue methods need to be developed, such a decision would result in additional cost.

9.8 BIOANALYTICAL SAMPLE ANALYSIS:

The serum and urine samples from all monkeys will be analyzed for the concentrations of perfluorooctanoate using the previously validated method. The data will be expressed as equivalents of potassium perfluorooctanoate. The analytical data on samples collected through and including Day 14 should be available by Day 21 postdose. The Sponsor will use this information to make

decisions concerning possible blood sampling of animals beyond Day 28 postdose. 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.

10.0 DATA ANALYSIS:

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

The total amount of perfluorooctanoate 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 10/4/00

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

Sponsor's Monitor: PZB 10/5/2000

INITIALS ONLY (See page 2) — Date

Management Approval: Ward R. Richter 10/4/00

Ward R. Richter, M.S., D.V.M., D.A.C.V.P. — Date
Director, Safety Assessment Department, Southern Research Institute

Appendix B

Method Validation Report: Validation of Analytical Method for Determination of Perfluorooctanoate in Monkey Serum and Urine Using HPLC/MS/MS

METHOD VALIDATION REPORT

VALIDATION OF AN ANALYTICAL METHOD FOR DETERMINATION OF PERFLUOROOCTANECARBOXALATE IN MONKEY SERUM AND URINE USING HPLC/MS/MS

STUDY ID: 9921.4.2

Southern Research Institute
2000 Ninth Avenue South
P.O. Box 55537
Birmingham, AL 35255-5537

SUMMARY

Southern Research Institute has successfully validated for 3M an analytical method (BACG 3548) entitled "Determination of Perfluorooctanecarboxylate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)". Calibration standards were prepared by spiking samples of either blank monkey serum or human urine with known amounts of test article, perfluorooctanecarboxalate (PFOC) and internal standard, perfluorohexanesulfonate (PFHS). The concentration of test article in serum covered a combined range from 10 to 100,000 ng/mL while the urine extended from 5 to 500 ng/mL. Quantitation limits were set at 20 ng/mL for serum and 10 ng/mL for urine. Composite standard curves generated using internal standard quantitation were used to determine the correlation coefficients. For the serum, three composite curves composed of a total of 44 individual calibration standards extending over concentration ranges from 5000 – 100,000 ng/mL, 100 - 10,000 ng/mL and 10 – 500 ng/mL produced correlation coefficient of 0.9874, 0.9750 and 0.9977 respectively. A single composite curve prepared from urine over a concentration range of 5 – 500 ng/mL and containing 20 individual calibration standards produced a correlation coefficient of 0.9960.

KEY PERSONNEL

James D. Johnson, M.S., MBA
Manager
Bioanalytical Chemistry Group

Gregory S. Gorman, Ph.D.
Staff Chemist
Bioanalytical Chemistry Group

Lester Williams, B.S.
Associate Chemist II
Bioanalytical Chemistry Group

George Dollar
Associate Biologist I
Bioanalytical Chemistry Group

1. OBJECTIVE

The objective of this study was to provide a validated analytical method for the determination of perfluorooctanecarboxalate in monkey serum and urine.

2. SAFETY

All necessary procedures to ensure safety of the analysts were based on information contained in the Material Safety and Data Sheets (MSDS), provided by 3M for the test article used in this study.

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.

3. EXPERIMENTAL

3.1 Analytical Procedures

The sample preparation and analysis procedures as described in the analytical method entitled "Determination of Perfluorooctanecarboxalate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)" were employed for all analyses. For the preparation of the calibration standards, a known volume of blank serum or urine (e.g., 500 µL) was spiked with a known amount of test article (PFOC) and internal standard (PFHS). To each standard was added 500 µL of TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized water. This mixture was vortexed for approximately 5 seconds. Then 2.5 mL of ethyl acetate was added and the mixture was placed on a horizontal platform shaker at a low speed for 1 hour. The mixture was then centrifuged at approximately 2500 rpm for 5 minutes. The ethyl acetate layer was transferred to a second tube and evaporated to dryness at approximately 50°C under a stream of dry nitrogen. The residue was reconstituted in 5% 5mM ammonium acetate in deionized water : 95% methanol containing 1.5% formic acid and filtered through a 0.2 µm syringe filter.

3.2 Method Validation

Validation for BACG 3548 "Determination of Perfluorooctanecarboxalate in Monkey Serum and Urine: Sample Preparation and Analysis by HPLC Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS)" consisted of analyzing standard curves prepared in monkey serum or human

urine over various concentration ranges: 5,000 – 100,000 ng/mL, 100 – 10,000 ng/mL and 10 - 500 ng/mL for serum and 5 – 500 ng/mL for urine.

5,000 – 100,000 ng/mL (Serum)

One composite calibration curve comprising 3 sets of calibration standards in a single analytical run in the 5,000 – 100,000 ng/mL concentration range generated from a total of 22 calibration standards produced a correlation coefficient of 0.9874. A statistical summary for the composite curve is shown below:

STANDARD COMPOSITE CURVE 1 (ng/mL)

Conc. (ng/mL)	5000	10,000	20,000	25,000	50,000	75,000	100,000
# of Stds	3	2	2	3	5	4	3
Mean % Accur.	93.9	102	98.7	107	101	95.3	102
Std. Deviation	385	31.9	1193	3917	2016	3048	13580

100 – 10,000 ng/mL (Serum)

Two composite curves consisting of a total of 12 calibration standards encompassing a concentration range of 100 – 10,00 ng/mL produced correlation coefficient of 0.9750 The 5,000 ng/mL standards were dropped due to preparation error. A statistical summary for the composite curve is given below:

STANDARD COMPOSITE CURVE 2 (ng/mL)

Conc. (ng/mL)	100	200	500	1000	2000	10,000
# of Stds	2	2	2	2	2	2
Mean % Accur.	93.5	109	114	93.8	86.1	102
Std. Deviation	14.5	32.1	106	14.7	307	852

10 – 500 ng/mL (Serum)

A single composite curve consisting of 11 standards taken from an analytical run encompassing a concentration range of 10 – 500 ng/mL produced a correlation coefficient of 0.9977. No calibration standards were dropped from the run. A statistical summary for the composite curve is given below

STANDARD COMPOSITE CURVE 3 (ng/mL)

Conc. (ng/mL)	10	20	50	100	200	500
# of Stds	2	2	2	2	1	2
Mean % Accur.	96.6	100	101	106	90.6	101
Std. Deviation	0.51	0.97	1.04	2.08	----	38.1

5 – 500 ng/mL (Urine)

A composite curve prepared in urine consisting of 20 standards over a range of 5 – 500 ng/mL produced a correlation coefficient of 0.9960. Only one calibration standard, 5 ng/mL was dropped due to low internal standard response. A statistical summary for the composite curve is given below:

STANDARD COMPOSITE CURVE 4 (ng/mL)

Conc. (ng/mL)	5	10	20	50	100	200	500
# of Stds	2	3	3	3	3	3	3
Mean % Accur.	94.7	107	107	82.7	107	101	99.6
Std. Deviation	0.07	0.27	3.41	4.20	8.2	9.0	14.6

3.3 Calculations

Calculations were performed using TurboQuan (Version 1.0). The amount of analyte in the serum extracts (ng/mL) was back calculated using a calibration curve genèrated from a set of calibration standards. The calibration curve was generated by a regression analysis to determine the best fit curve (e.g., linear, quadratic etc.) and amount of weighting. A quadratic fit with 1/X weighting was determined to be the best fit :

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

where:

y = Peak height response of PFOC divided by peak height response of the IS (PFHS) in standards.

x = Concentration of the PFOC in standards.

a, b, c = Constants derived from the regression analysis.

4.0 Conclusion

A quantitative method (BAGC 3548) has been developed and validated for the determination of perfluorooctanecarboxalate in monkey serum utilizing LC/MS/MS. Quantitation for this method is based on an internal standard (PFHS) using a 1/x weighted quadratic equation. The total concentration range for a serum matrix is 20 – 100,000 ng/mL and 5 – 500 ng/mL for urine with limits of quantitation of 20 and 10 ng/mL respectively.

Appendix C

Analytical Method for Determination of Perfluorooctanoate in Monkey Serum and Urine

ANALYTICAL METHOD

Method No.: BACG-3548

Title: Determination of Perfluorooctanecarboxalate 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 Perfluorooctanecarboxalate (PFOC). The serum or urine (e.g., 0.5 mL) containing (PFOC) is fortified with an internal standard (IS), perfluorohexanesulfonate (PFHS). 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 100,000 ng/mL of PFOC in serum and from 10 to 500 ng/mL in urine. Samples containing PFOC at concentrations greater than 100,000 ng/mL may be diluted with control blank matrix so that the concentration of PFOC will be within the range of reliable results prior to analysis.

The mass spectrometry of PFOC and PFHS 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. In order to maximize sensitivity, this method is based on mixed reaction monitoring of the negative fragment ion $(M\text{-}COOH)^-$ for PFOC.

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

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

2.1.2 Methanol, HPLC grade

2.1.3 Perfluorooctanecarboxalate (analyte), as provided by the client

2.1.4 Perfluorohexanesulfonate (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

2.1.11 Sodium Hydroxide 50% solution, Certified grade or equivalent

2.1.12 Blank control monkey urine

2.1.13 Formic Acid

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

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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)

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

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.

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)

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3.7 Analytical balance

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

3.9 Disposable Pasteur pipets

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 PFOC ~1000 µg/mL

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

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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 (PFHS), ~200 μg/mL**

4.2.1 Prepare an ~200 μg/mL solution of PFHS 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 (PFHS), ~ 50μg/mL**

4.3.1 Prepare an ~ 50 μg/mL solution of PFHS 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 PFOC**

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 PFOC 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 PFOC in serum (ng/mL)
A	50 µL of 1000 µg/mL	100,000
B	37.5 µL of 1000 µg/mL	75,000
C	25 µL of 1000 µg/mL	50,000
D	12.5 µL of 1000 µg/mL	25,000
E	10 µL of 1000 µg/mL	20,000

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F	10 µL of 500,000 ng/mL	10,000
G	10 µL of 250,000 ng/mL	5,000
H	16 µL of 62,500 ng/mL	2,000
I	16 µL of 31,250 ng/mL	1,000
J	16 µL of 15,625 ng/mL	500
K	20 µL of 5,000 ng/mL	200
L	10 µL of 5,000 ng/mL	100
M	10 µL of 2,500 ng/mL	50
N	10 µL of 1,000 ng/mL	20
O	10 µL of 500 ng/mL	10
P	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

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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 To each tube add 500 µL of the TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds.

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 To each tube add 500 µL of the TBA ion-pairing solution, 1 mL of carbonate/bicarbonate buffer, and 1 mL of deionized organic free water. Vortex each tube for about 5 seconds.

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

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:	3 µL
Mobile phase:	A: 5mM ammonium acetate buffer B: 1.5% formic acid in methanol
Gradient Profile:	0 - 0.5 min. 50%A : 50%B 0.5 - 7.5 min. 10% A : 90% B linear gradient 7.5-8 min. 10%A : 90% B step gradient

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8-11 min. 50%A : 50%B

Temperature: Ambient

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

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	400
OR	-20
RNG	-120
Q0	5
IQ1	6
ST	10
RO1	6
IQ2	10
RO2	30
ST3	40
RO3	32
DF	250
CEM	1800

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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:

PFOC:

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

PFHS (IS)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell Time (ms)
398.9	398.9	200

Parameter	Start	Stop
RO2	50	50
ST3	60	60
RO3	52	52

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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
PFOC	412.9 to 368.9
PFHS	398.9 to 398.9

8.2 Plot the peak area response of PFOC divided by the peak area response of the IS (PFHS) 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 PFOC divided by peak height response of the IS (PFHS) in standards.
x = Concentration of the PFOC in standards.
a, b, c = Constants derived from the regression analysis.

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

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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.

Authors: Gregory S. Gorman 12-27-00

Gregory S. Gorman, Ph.D., Staff Chemist — Date
Bioanalytical Chemistry Group

Approved by: James D. Johnson 12-27-00

James D. Johnson MS, MBA, Manager — Date
Bioanalytical Chemistry Group

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