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A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

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Final Report on

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

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ABSTRACT

The pharmacokinetics and urinary excretion of perfluorohexanesulfonate 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 perfluorohexanesulfonate, potassium salt (T-7504). At various times after dosing, serum and urine (24-hour collections) samples were obtained and analyzed by HPLC/MS/MS for levels of intact perfluorohexanesulfonate. The lower limit of detection of the analytical method was 5 ng/mL for serum samples and 1 ng/mL for urine samples. Peak serum concentrations of perfluorohexanesulfonate were similar in male and female monkeys and ranged from 104,600 to 140,900 ng/mL in male monkeys and from 116,400 to 174,000 ng/mL in female monkeys. Serum concentrations of perfluorohexanesulfonate in each monkey decreased relatively rapidly during the first 8-24 hours after dosing. At 24 hours, serum concentrations of perfluorohexanesulfonate ranged from 24,870 to 40,405 ng/mL in male monkeys and from 27,115 to 54,300 ng/mL in female monkeys. Subsequently, serum concentrations of perfluorohexanesulfonate decreased at a slower rate between 24 hours and the end of sample collection (171 days). On Day 171, serum concentrations of perfluorohexanesulfonate ranged from 13,415 to 21,725 ng/mL in male monkeys and from 3,249 to 20,220 ng/mL in female monkeys. The serum concentration versus time data were subjected to non-compartmental pharmacokinetic analysis. The serum elimination half-life of perfluorohexanesulfonate ranged from 100 to 200 days (mean: 141 days) in male monkeys and from 49 to 140 days (mean: 87 days) in female monkeys. The total body clearance of perfluorohexanesulfonate was 1.1 to 1.5 mL/day/kg in male monkeys and 1.2 to 2.6 mL/day/kg in female monkeys. The volume of distribution of perfluorohexanesulfonate ranged from 223 to 391 mL/kg and from 160 to 255 mL/kg in male and female monkeys, respectively. Apparent differences among individual monkeys in the estimated serum half-life and clearance of the compound were likely attributable to an overestimation of the extrapolated $AUC_{0\text{-infinity}}$ values for two of the female monkeys. Only very low levels (<0.01 to 0.11% of the administered dose) of perfluorohexanesulfonate were measured in urine during any given 24-hour period of sample collection between Day 1 and Day 70 after dosing. The results of this study suggested that the pharmacokinetics of perfluorohexanesulfonate were similar in male and female monkeys. Perfluorohexanesulfonate was eliminated in urine by both male and female monkeys at low levels for a prolonged period of time (≥70 days) after iv administration.

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Reviewed by:

Charles D. Hébert 4-22-03

Charles D. Hébert, Ph.D., D.A.B.T. — Date
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.

4/22/03

Gregory S. Gorman, Ph.D. — Date
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

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

4/22/03
Date

Study Schedule and Personnel

Study Dates:

Study Initiation:	February 7, 2001
Day of Dosing:	February 9, 2001
Last Day of Sample Collection:	July 29, 2001
Study Completion:	April 22, 2003

Study Personnel:

Patricia E. Noker, Ph.D., D.A.B.T.	Study Director
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LaJuana A. Durbin, B.S.	Supervisor, Large Animal Laboratory
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 perfluorohexanesulfonate 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 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 3-4 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 67.7-72.8 °F and a relative humidity of 29-74%. 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). Monkey 2053 was naïve.

2.2 Test Article and Vehicle

Test Article: One bottle containing 5 grams of potassium perfluorohexanesulfonate (T-7504; expiration date not supplied; SRI E06/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 perfluorohexanesulfonate was sterile saline, USP (Phoenix Pharmaceutical Company; St. Joseph, MO; Lot 0081050, expiration date August 2003). 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 perfluorohexanesulfonate 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 1 day 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 perfluorohexanesulfonate 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, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119.

Urine and Feces Collection: Urine and feces were collected for 24-hour intervals on the following days: prior to dose administration (Day -1; baseline), on Day 1 (0-24 hours postdose), on Day 2 (24-48 hours postdose), and on Days 7, 14, 21, 28, 42, 56, and 70. 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 Perfluorohexanesulfonate: 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, and 171 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 perfluorohexanesulfonate using a previously validated HPLC/MS/MS method (Appendix B).

Data Analyses: The serum concentration data for unchanged perfluorohexanesulfonate were subjected to non-compartmental 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 perfluorohexanesulfonate 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 –1 and Day 119 (last day during the study that body weights were obtained).

3.4 Serum and Urine Concentrations of Perfluorohexanesulfonate

Serum concentrations of perfluorohexanesulfonate in three male and three female monkeys at various times through Day 171 after administration of a single iv dose of 10 mg/kg are presented in Table 2 and Figure 1. No sex-related differences were apparent in serum concentrations of perfluorohexanesulfonate at any time of sample collection. Peak serum concentrations of perfluorohexanesulfonate were observed in 1/3 male monkeys and 3/3 female monkeys at 0.5 hours (earliest time point) after dosing. Serum concentrations of perfluorohexanesulfonate at this time ranged from 116,400 to 140,900 ng/mL among these four monkeys. For the other 2 male monkeys (2053 and 2211), peak serum concentrations of perfluorohexanesulfonate (104,600 and 128,500 ng/mL) were not observed until 2 hours after dosing. The possibility was investigated that the 0.5- and 2-hour serum samples collected from these two monkeys were switched during collection and/or analysis; however, it could not be determined if the switching of the samples had occurred. Subsequent to the time of peak levels, serum concentrations of perfluorohexanesulfonate in each monkey decreased relatively rapidly during the first 8-24 hours after dosing. At 24 hours, serum concentrations of perfluorohexanesulfonate ranged from 24,870 to 40,405 ng/mL in the three male monkeys and from 27,115 to 54,300 ng/mL in the three female monkeys. Serum

concentrations of perfluorohexanesulfonate decreased at a slow rate between 24 hours and the end of sample collection (171 days). During this period, fluctuations were observed in the serum concentrations of perfluorohexanesulfonate among individual monkeys. In that the fluctuations appeared to be random among the monkeys, they may have been attributable to analytical error introduced as a consequence of the large dilution of each sample that was required prior to HPLC/MS/MS analysis. On Day 171, serum concentrations of perfluorohexanesulfonate ranged from 13,415 to 21,725 ng/mL in the male monkeys and from 3,249 to 20,220 ng/mL in the female monkeys.

Pharmacokinetic parameters calculated from serum concentrations of perfluorohexanesulfonate in individual monkeys are presented in Table 3. The values were derived from non-compartmental analysis of the data. No distinct differences were apparent between male and female monkeys in the estimated parameters; however, for two of the three female monkeys, the serum half-life and clearance of perfluorohexanesulfonate were shorter and faster, respectively, than observed for the three male monkeys and the other female monkey. $AUC_{0\text{-infinity}}$ values ranged from 6456 to 8745 μg•day/mL in male monkeys and from 3849 to 8501 μg•day/mL in female monkeys. The terminal half-life of perfluorohexanesulfonate in serum ranged from 100 to 200 days (mean: 141 days) in the three male monkeys and from 49 to 140 days (mean: 87 days) in the three female monkeys. The total body clearance of perfluorohexanesulfonate was 1.1 to 1.5 mL/day/kg in male monkeys and 1.2 to 2.6 mL/day/kg in female monkeys. The volume of distribution of perfluorohexanesulfonate ranged from 223 to 391 mL/kg in male monkeys and from 160 to 255 mL/kg in female monkeys.

The amount of perfluorohexanesulfonate eliminated in urine by individual monkeys at various times after dosing is presented in Table 4. Only very low levels (<0.01 to 0.11% of the administered dose) of perfluorohexanesulfonate were measured in urine during any given 24-hour period of sample collection between Day 1 and Day 70 after dosing. There was no clear indication of a sex-related difference in the urinary excretion of perfluorohexanesulfonate. The urinary excretion of perfluorohexanesulfonate was

prolonged; detectable levels of the compound were present in urine on Day 70 (last day of urine collection) after dosing.

4.0 Discussion

The results of this study indicated that perfluorohexanesulfonate was slowly eliminated by male and female monkeys given a single iv dose of 10 mg/kg. Throughout the period of sample collection, serum concentrations of perfluorohexanesulfonate were similar at each sample time in both male and female monkeys. In addition, the rate of urinary excretion of perfluorohexanesulfonate appeared to be similar for male and female monkeys. Thus, there was no clear indication from the data that there was a sex-related difference in the elimination of the compound.

For two of the three female monkeys on the current study, the estimated serum half-life and total body clearance of perfluorohexanesulfonate appeared to be shorter and faster, respectively, than observed for the third female money and for the three male monkeys. These apparent differences may have been artifactual in that both parameters (half-life and clearance) were calculated from $AUC_{0\text{-infinitity}}$ values; these latter values were obtained from extrapolation of the serum concentration time curve and may have contained considerable error. That the $AUC_{0\text{-infinity}}$ values for two of the three female monkeys may have been overestimated is supported by the observation that estimated $AUC_{0\text{-last}}$ values [0 to the last time point (Day 171)] for all three male and all three female monkeys were similar.

5.0 Conclusions

No sex differences were apparent in serum concentrations and the urinary excretion of perfluorohexanesulfonate among male and female monkeys given an iv dose. The mean terminal serum half-life of perfluorohexanesulfonate was 141 days in male monkeys and 87 days in female monkeys. Perfluorohexanesulfonate was eliminated in urine by both male and female monkeys at low levels for a prolonged period of time (≥70 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 Perfluorohexanesulfonate in the Cynomolgus Monkey

Body Weights: Males

		Day numbers relative to Start Date									
Group Sex	Animal Number	-1	4	7	14	21	28	35	42	49	56
1M	2053	6.0	6.1	6.0	6.4	6.1	6.1	6.2	6.3	6.0	6.1
	2054	6.2	6.4	6.4	6.6	6.5	6.6	6.6	6.6	6.7	6.7
	2211	5.4	5.5	5.6	5.8	5.6	5.7	5.7	5.9	5.9	6.0
	Mean	5.87	6.00	6.00	6.27	6.07	6.13	6.17	6.27	6.20	6.27
	S.D.	0.42	0.46	0.40	0.42	0.45	0.45	0.45	0.35	0.44	0.38
	N	3	3	3	3	3	3	3	3	3	3

Table 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Body Weights: Males

		Day numbers relative to Start Date								
Group Sex	Animal Number	63	70	77	84	91	98	105	112	119
1M	2053	6.3	6.4	6.5	6.5	6.7	6.4	6.5	6.6	6.6
	2054	6.8	7.0	6.9	6.9	6.9	7.0	6.7	6.5	6.5
	2211	6.2	6.3	6.2	6.7	6.5	6.5	6.4	6.5	6.7
	Mean	6.43	6.57	6.53	6.70	6.70	6.63	6.53	6.53	6.60
	S.D.	0.32	0.38	0.35	0.20	0.20	0.32	0.15	0.06	0.10
	N	3	3	3	3	3	3	3	3	3

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Table 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Body Weights: Females

Group Sex	Animal Number	Day numbers relative to Start Date: -1	4	7	14	21	28	35	42	49	56
1F	2058	3.6	3.7	3.7	3.8	3.5	3.6	3.6	3.7	3.7	3.7
	2059	3.7	3.6	3.6	3.8	3.6	3.5	3.5	3.7	3.6	3.6
	2061	4.1	4.1	4.0	4.3	4.1	4.1	4.1	4.2	4.3	4.2
	Mean	3.80	3.80	3.77	3.97	3.73	3.73	3.73	3.87	3.87	3.83
	S.D.	0.26	0.26	0.21	0.29	0.32	0.32	0.32	0.29	0.38	0.32
	N	3	3	3	3	3	3	3	3	3	3

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Table 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Body Weights: Females

Group Sex	Animal Number	Day numbers relative to Start Date: 63	70	77	84	91	98	105	112	119
1F	2058	3.8	3.9	3.7	3.7	3.8	3.9	3.7	3.6	3.6
	2059	3.6	3.7	3.6	3.7	3.6	3.6	3.6	3.6	3.5
	2061	4.2	4.3	4.4	4.4	4.3	4.3	4.4	4.4	4.3
	Mean	3.87	3.97	3.90	3.93	3.90	3.93	3.90	3.87	3.80
	S.D.	0.31	0.31	0.44	0.40	0.36	0.35	0.44	0.46	0.44
	N	3	3	3	3	3	3	3	3	3

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Table 2

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Serum Concentrations of Perfluorohexanesulfonate

	Serum Concentration (ng/mL)					
	Males			Females		
Timepoint	2053	2054	2211	2058	2059	2061
0	5.8	BQL	BQL	BQL	BQL	BQL
0.5 hrs	113,000	140,900	70,820	153,600	174,000	116,400
2 hrs	128,500	99,070	104,600	98,100	92,300	58,660
4 hrs	76,530	46,960	64,480	65,570	66,940	48,510
8 hrs	50,770	53,090	18,660	55,910	52,600	54,470
24 hrs	40,405	40,340	24,870	54,300	37,100	27,115
48 hrs	46,740	43,885	23,930	50,050	41,045	35,555
Day 4	37,930	54,850	16,640	44,850	37,250	48,200
Day 7	35,880	48,840	28,160	41,880	41,500	41,250
Day 14	45,585	48,475	29,400	41,045	33,300	37,405
Day 21	42,295	37,210	22,285	39,015	40,930	42,200
Day 28	42,845	43,820	23,745	42,460	38,105	34,800
Day 42	31,110	37,675	28,285	32,890	31,300	34,270
Day 56	16,960	24,990	19,960	16,010	27,220	22,050
Day 70	18,020	22,710	18,680	17,850	22,580	21,610
Day 171[a]	16,680	21,725	13,415	11,805	3,249	20,220

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

[a] Data represent an average of two samples.

Table 3

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Pharmacokinetic Parameters Calculated from Serum Concentrations of Potassium Perfluorohexanesulfonate

Parameter	Male					Female				
	2053	2054	2211[a]	Average	SD	2058	2059	2061[a]	Average	SD
C_0 (μg/mL)[b]	214	159	169	181	29	178	215	146	180	35
$AUC_{0\text{-last}}$ (μg•day/mL)[c]	4044	4864	3309	4072	778	3802	3619	4417	3946	418
$AUC_{0\text{-infinity}}$ (μg•day/mL)[d]	6456	8745	7186	7462	1169	5031	3849	8501	5794	2418
$t_{1/2}$ (day)[e]	100	124	200	141	52	72	49	140	87	47
Cl (mL/day/kg)[f]	1.5	1.1	1.4	1.3	0.2	2.0	2.6	1.2	1.9	0.7
Vd_{ss} (mL/kg)[g]	248	223	391	287	91	225	160	255	213	49

[a]Lambda_z ranges were specified for the estimation of the terminal half-life
[b]Serum concentration at time 0
[c]Area under the serum concentration time curve from 0 to the last time point
[d]Area under the serum concentration time curve from 0 to infinity
[e]Half-life of the terminal elimination phase
[f]Total body clearance
[g]Volume of distribution at steady state

Table 4

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorohexanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Baseline						
2053	M	BQL	740	--	60,000	--
2054	M	BQL	1000	--	62,000	--
2211	M	BQL	630	--	54,000	--
2058	F	BQL	290	--	36,000	--
2059	F	BQL	150	--	37,000	--
2061	F	BQL	250	--	41,000	--
Day 1						
2053	M	48	1000	48	60,000	0.08
2054	M	193	360	69	62,000	0.11
2211	M	79	640	51	54,000	0.09
2058	F	22	320	7	36,000	0.02
2059	F	60	150	9	37,000	0.02
2061	F	484	90	44	41,000	0.11
Day 2						
2053	M	65	610	40	60,000	0.07
2054	M	17	330	6	62,000	0.01
2211	M	10	340	3	54,000	0.01
2058	F	60	170	10	36,000	0.03
2059	F	193	60	12	37,000	0.03
2061	F	709	50	35	41,000	0.09
Day 7						
2053	M	28	490	14	66,000	0.02
2054	M	16	210	3	64,000	0.01
2211	M	182	210	38	51,000	0.07
2058	F	64	290	19	34,000	0.05
2059	F	15	180	3	35,000	0.01
2061	F	121	70	8	41,000	0.02

BQL = Below the quantitation limit (<1 ng/mL)

Table 4 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorohexanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 14						
2053*	M	5	280	1	60,000	<0.01
2054	M	8	740	6	62,000	0.01
2211	M	7	660	5	54,000	0.01
2058	F	167	170	28	36,000	0.08
2059	F	72	210	15	37,000	0.04
2061	F	129	110	14	41,000	0.03
Day 21						
2053	M	10	440	4	60,000	0.01
2054	M	27	320	9	62,000	0.01
2211	M	4	530	2	54,000	<0.01
2058	F	49	100	5	36,000	0.01
2059	F	237	110	26	37,000	0.07
2061	F	40	90	4	41,000	0.01
Day 28						
2053	M	5	660	3	60,000	0.01
2054	M	8	830	7	62,000	0.01
2211	M	10	520	5	54,000	0.01
2058	F	16	270	4	36,000	0.01
2059	F	25	200	5	37,000	0.01
2061	F	35	150	5	41,000	0.01
Day 42						
2053	M	34	450	15	60,000	0.03
2054	M	55	440	24	62,000	0.04
2211	M	24	820	20	54,000	0.04
2058	F	74	220	16	36,000	0.05
2059	F	28	160	4	37,000	0.01
2061	F	14	180	3	41,000	0.01

BQL = Below the quantitation limit (<1 ng/mL)

Table 4 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Urinary Excretion of Perfluorohexanesulfonate

Animal ID	Sex	Urine Concentration (ng/mL)	Urine Volume (mL)	Total (μg)	Dose (μg)	Percent of Dose in Urine (%)
Day 56						
2053[a]	M	15	510	8	60,000	0.01
2054	M	48	450	22	62,000	0.03
2211	M	7	510	4	54,000	0.01
2058	F	8	330	3	36,000	0.01
2059	F	11	160	2	37,000	<0.01
2061	F	70	200	14	41,000	0.03
Day 70						
2053	M	6	510	3	60,000	0.01
2054	M	38	430	16	62,000	0.03
2211	M	3	640	2	54,000	<0.01
2058	F	20	220	4	36,000	0.01
2059	F	28	150	4	37,000	0.01
2061	F	177	170	30	41,000	0.07

BQL = Below the quantitation limit (<1 ng/mL)

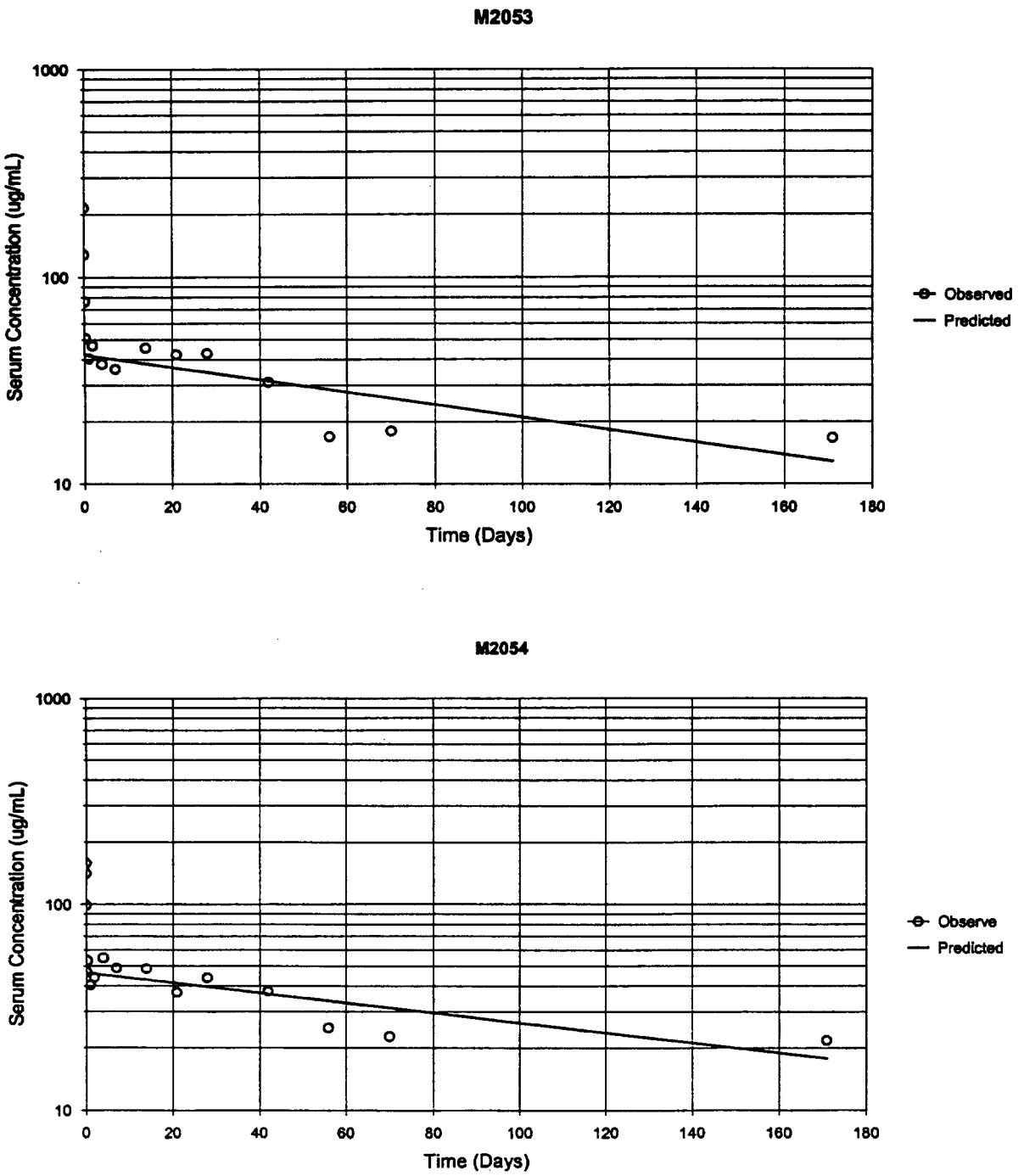


Figure 1

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorohexanesulfonate

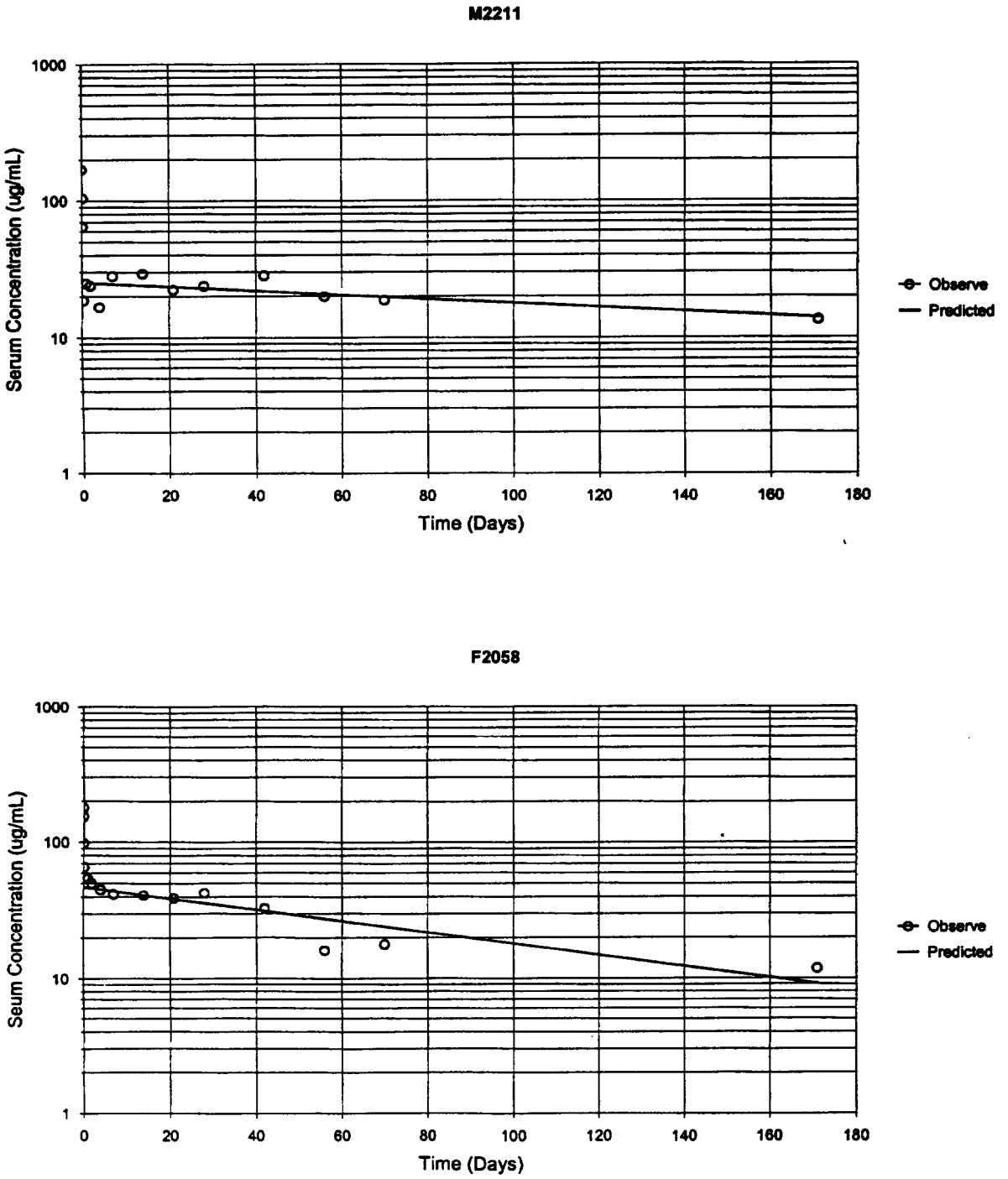


Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorohexanesulfonate

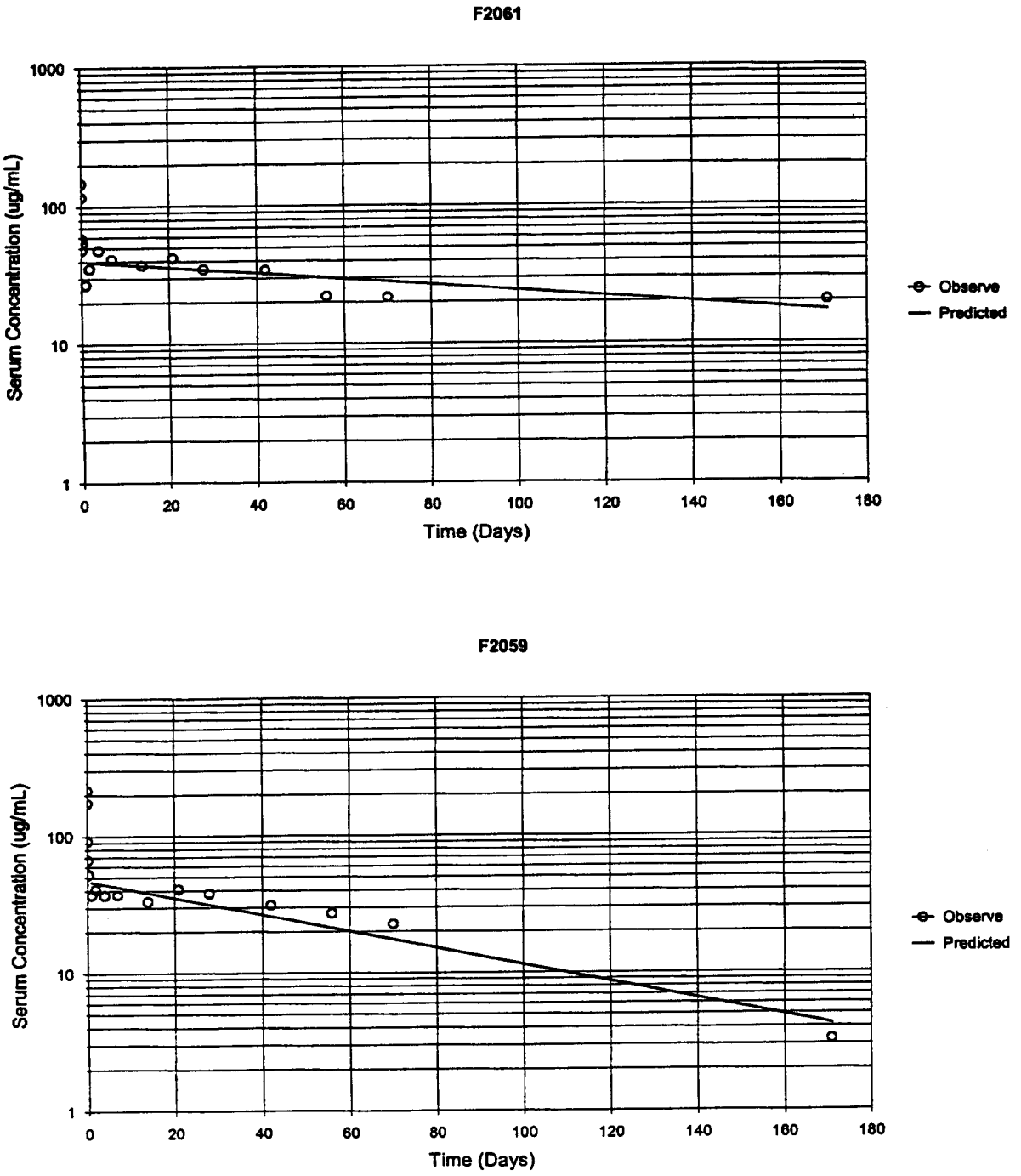


Figure 1 (Continued)

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Serum Concentration Profile of Perfluorohexanesulfonate

Appendix A

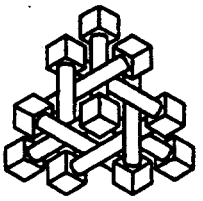
Study Protocol

Study Protocol:

A Pharmacokinetic Study of Potassium Perfluorohexanesulfonate in the Cynomolgus Monkey

Southern Research Study ID: 9921.5

February 7, 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 12 FEB 01

John L. Butenhoff Date

Test Article:

Perfluorohexanesulfonate, 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 Perfluorohexanesulfonate in the Cynomolgus Monkey

3.0 OBJECTIVE:

The objectives of this study are to determine the concentration of perfluorohexanesulfonate in serum and urine at various times following administration of a single intravenous dose of potassium perfluorohexanesulfonate 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**	**2/9/01**
Urine and Feces Collections	Baseline (predose)	2/9/01
	1	2/10/01
	2	2/11/01
	7	2/16/01
	14	2/23/01
	21	3/2/01
	28	3/9/01
	42	3/23/01
	56	4/6/01
	70	4/20/01
	91	5/11/01
Serum Drug Levels	0: 0 (predose), 0.5, 2, 4, 8 and 24 hours postdose	2/9-10/01
	2	2/11/01
	4	2/13/01
	7	2/16/01
	14	2/23/01
	21	3/2/01
	28	3/9/01
	42	3/23/01
	56	4/6/01
	70	4/20/01
	91	5/11/01
Draft Report Due	60 Calendar days after completion of the in-life phase	**7/10/01**
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:	**Perfluorohexanesulfonate, potassium salt**
Identification:	T-7504
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, potassium perfluorohexanoate in study 9921.3, and potassium perfluorooctanoate in study 9921.4.

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 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 perfluorohexanesulfonate 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.

Study Procecures	Day of Study																	
	-1[a]	0	1	2	4	7	14	21	28	35	42	49	56	63	70	77	84	91
Health Check	x																	
Dose Preparation	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	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

[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 perfluorohexanesulfonate (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 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, and weekly intervals thereafter through Day 91.

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, and 91 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) will be developed for the determination of perfluorohexanesulfonate 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 perfluorohexanesulfonate using the previously validated method. The data will be

expressed as equivalents of potassium perfluorohexanesulfonate. 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 perfluorohexanesulfonate, as appropriate and feasible, using a standard pharmacokinetic program.

The total amount of perfluorohexanesulfonate 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 2/7/01

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

Sponsor's Monitor: GZE

INITIALS ONLY (See page 2) — Date

Management Approval: for 02/07/01

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

Appendix B

Analytical Method for Determination of Perfluorohexanesulfonate in Monkey Serum and Urine

ANALYTICAL METHOD

Method No.: BACG-3606

Title: Determination of Perfluorohexanesulfonate 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 Perfluorohexanesulfonate (PFHS). The serum or urine (e.g., 0.5 mL) containing (PFHS) 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 5 to 20,000 ng/mL of PFHS in serum and from 10 to 500 ng/mL in urine. Samples containing PFHS at concentrations greater than 20,000 ng/mL may be diluted with control blank matrix so that the concentration of PFHS will be within the range of reliable results prior to analysis.

The mass spectrometry of PFHS 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-3606

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

2.1.3 Perfluorohexanesulfonate (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, Aldrich 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-3606

Title: Determination of Perfluorohexanesulfonate 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 approximately 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-3606

Title: Determination of Perfluorohexanesulfonate 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 PFHS ~1000 µg/mL

4.1.1 Prepare an ~1000 µg/mL solution of PFHS in deionized organic-free water (e.g., accurately weigh about 10 mg PFHS 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-3606

Title: Determination of Perfluorohexanesulfonate 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 PFHS**

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.

ANALYTICAL METHOD

Method No.: BACG-3606

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

Working Stock Level (WSL) Approximate Concentration (ng/mL)	Volume of PFHS 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
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

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4.4.2 Summary of concentrations of serum standards:

Standard Level	Volume and Spike Conc.	Approximate Concentration of PFHS in serum (ng/mL)
A	20 µL of 500,000 ng/mL	20,000
B	10 µL of 500,000 ng/mL	10,000
C	10 µL of 250,000 ng/mL	5,000
D	16 µL of 62,500 ng/mL	2,000
E	16 µL of 31,250 ng/mL	1,000
F	16 µL of 15,625 ng/mL	500
G	20 µL of 5,000 ng/mL	200
H	10 µL of 5,000 ng/mL	100
I	10 µL of 2,500 ng/mL	50
J	10 µL of 1,000 ng/mL	20
K	10 µL of 500 ng/mL	10
L	10 µL of 250 ng/mL	5

ANALYTICAL METHOD

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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.

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 Remove 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).