John B.

July 30, 1998

Fax to: Dr. Jack Moore — Fax # 202-289-8530
Dr. Joe Rodricks — Fax # 703.516.2393

From: John L. Butenhoff — Fax # 651.733-1773
Tel # 651.733-1962

I have attached our rationale for dose setting on the six month chronic oral study of PFOS in Cynomolgus Monkeys.

You will also find attached the report from the environmental laboratory of serum levels for the four week range finder animals.

Serum levels are reported for days -7, 2, 3, 14 and 29. We have done an analysis of the slopes of the cumulative dose vs. serum concentration for these range finder animals at the low dose of 0.02 mg per kg per day. This is also attached.

By the end of the day, we will have an analysis of the slopes for the two high dose animals. I will fax that to you when available.

The interesting thing to know from this analysis is the extreme linearity of the cumulative dose vs. serum concentration.

We will need to produce new charts because of a slight error in the data analysis. For example: If a serum value is reported for day two, there has only been one dose since collection of serum is always prior to dosing on the day of serum collection.

The value that I state in the rationale (5.8 ppm PFOS in serum per 1 mg per kg of PFOS dose), has taken that fact into consideration.

We look forward to discussing this dose-setting exercise with you on August 3, at 5:30 pm (Eeastern) during our conference call (Dial-In # (303)633-6052).

4:30 Central

Original to L. Z.

9 pgs.

Dose-Setting Rationale for Six-Month Chronic Oral Study in Cynomolgus Monkeys

Prepared by John Butenhoff
July 29, 1998

Recommended Dose Groups: 0.0, 0.02, 0.15 and 0.75 mg/kg/day

Rationale:

Results of analyses for serum concentrations of PFOS from the four-week rangefinder (attached) suggest a highly linear relationship between total accumulated dose and serum concentration. A significant male/female difference is not apparent. The consistency of the ratio of serum [PFOS] to total accumulated dose at either dose level and all time points gives confidence in using this preliminary data to set dose levels for the six-month definitive study based on estimated serum concentrations.

The data provided by the Environmental Lab suggest that serum PFOS concentration increases incrementally with each daily dose of PFOS in direct proportion to the magnitude of the dose. The data give us a good degree of confidence in deriving a factor relating accumulated dose in mg/kg to ppm PFOS serum. This relationship suggests that 1 mg/kg results in a serum PFOS concentration increase of approximately 5.8 ± 0.39 ppm. Assuming that this zero-order accumulation kinetic will hold at the higher dose over the course of a six-month study, this allows us to predict serum concentrations that would be attained with chronic dosing. The apparent precision of this is invaluable in setting dose levels that will result in serum PFOS concentrations which can be compared directly to known human serum PFOS concentrations for risk characterization.

In addition, we now have the data to estimate the serum concentrations that were associated with effects observed in the previous 90-day rhesus studies. Going back to the original 90-day rhesus studies, the LOEL, which we believe to be close to a NOEL, of 0.5 mg/kg/day would have resulted in an estimated serum PFOS concentration of 261 ppm. At the 1.5 mg/kg/day dose, 783 ppm would have been attained after 90 days. Death resulted within seven weeks of dosing at 4.5 mg/kg/day, which is now estimated to have produced serum PFOS concentrations of approximately 1305 ppm.

We have the following primary objectives for the six-month study: 1) to establish a NOAEL for chronic exposure; 2) to determine threshold effects for health surveillance; 3) to provide a framework for interspecies comparison; and, 4) to provide a reference dose for human risk characterization. It is not our objective to study higher levels of exposure which would result in serious illness in the test monkeys. Dose levels for the six-month study should be set to allow us to achieve the stated objectives:

Low Dose: The low dose needs to be a confirmed NOEL. If we use the low dose from the rangefinder study, we expect to achieve approximately 21 ppm PFOS in the serum of the low dose animals at the end of six months. After the first two weeks of dosing, the animals will be at and above the average serum concentration of our workforce. At study term, these animals will have serum concentrations that are approximately an order of magnitude above average worker serum concentrations. We are suggesting that we keep 0.02 mg/kg/day dose as our low dose.

Mid Dose: The mid-dose is the most problematic from a dose-setting perspective. From the rhesus study, we know that minor effects (questionable significance) occurred at 0.5 mg/kg/day over 90 days. We estimate that serum concentrations at term were 261 ppm. If we use this serum concentration as a reference for setting dose on a six-month study, we would assume that similar serum concentrations would be reached with 0.25 mg/kg/day. From a risk characterization perspective, 261 ppm serum PFOS is two orders of magnitude above average worker serum [PFOS] of 2-3 ppm. Achieving a NOAEL at this level of exposure would be desirable; however, confidence that 0.25 mg/kg/day would be a NOAEL is not as high as confidence that 0.15 mg/kg/day would be a NOAEL. The S. C. Johnson Wax 90-day drinking water study in rats yielded reduced serum triglycerides in males at 0.3 mg/kg/day. Females had increased liver weights down to 0.06 mg/kg/day; however, we know that the rat liver responds more than the monkey to PFOS. Setting the mid-dose at 0.15 mg/kg/day would achieve an estimated serum PFOS concentration of 156 ppm at the end of six months. This serum level is still reasonably close to two orders of magnitude above average employee serum levels and close to four orders of magnitude above currently understood general population values. Therefore, the 0.15 mg/kg/day dose seems to be the best fit for the mid-dose.

High Dose: The high dose should be designed to produce threshold effects. After one month of dosing at 2.0 mg/kg/day (resulting in an average of 306 ppm PFOS in serum), the male and female rangefinder monkeys (one per sex) demonstrated the expected reduction in serum cholesterol and triglycerides, which appears from rat and monkey studies to be an early marker of biologic effect. There were no remarkable histopathologic effects. Even though there were no major disturbances after one month at 2.0 mg/kg/day, six-months of exposure at this dose is now predicted to result in lethal body burdens. The rangefinder data tell us that threshold effects begin to occur at serum levels approximating 300 ppm. Using the serum concentrations estimated to have been produced in the 90-day rhesus study by 1.5 mg/kg/day (783 ppm) as a reference, a 0.75 mg/kg/day high dose would seem appropriate to achieve marginal effects on body weight and general energy level of the animals. Raising the dose to 1.0 mg/kg/day would result in serum levels of approximately 1044 ppm by term, which would approach fatal serum concentrations. Therefore, 0.75 mg/kg/day is the suggested high dose that will clearly take us beyond threshold effects of serum cholesterol and triglygeride reduction.

Comment: It is interesting to note that the consistent linearity of increasing serum [PFOS] and apparent lack of significant elimination may make it possible to give much

less frequent doses or even to give a single dose at the beginning of the study and adjust that dose periodically to maintain a relatively constant serum level. We will not reach a steady state in the study as it is currently designed and will be studying the effect of increasing body burden over time. Is there also value in attempting to hold animals at a relatively constant serum level over time? Also, it would be interesting to look at serum concentration at extremely low level exposure over time; e.g., 0.1 to 1.0 ug/kg/day.

AMDT# 041598.1
Covance# 6329-222

Study:	4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295 (PFOS)		
Matrix:	Monkey Serum		
Method/Revision:	FACT-M-3.0 & FACT-M-4.0		
Analytical Equipment System Number:	Amelia 062498		
Instrument Software/Version:	MassLynx 3.1		
Filename:	See Attachments		
R-Squared Value:	See Attachments		
Slope:	See Attachments		
Y-Intercept:	See Attachments		
Dates of Extraction/Analyst:	7/16/98 RWW	7/22/98 RWW	
Dates of Analysis/Analyst:	7/17/98 HOJ	7/24/98 HOJ	

	Sample #	Timepoint	PFOS Conc. ug/mL
Group 1 Control 0.0 mg/kg/day	105349M	Day -7	0.0173
		Day 2	0.0223
		Day 3	0.172
		Day 7	0.181
		Day 14	0.184
		Day 29	0.160
	105350M	Day -7	0.0121
		Day 2	0.0207
		Day 3	0.0202
		Day 7	0.0259
		Day 14	0.0178
		Day 29	0.0220
	105366F	Day -7	0.0118
		Day 2	0.0214
		Day 3	0.0232
		Day 7	0.0194
		Day 14	0.0183
		Day 29	0.0269
	105367F	Day -7	0.0135
		Day 2	0.0215
		Day 3	0.0175
		Day 7	0.0155
		Day 14	0.0333
		Day 29	0.118

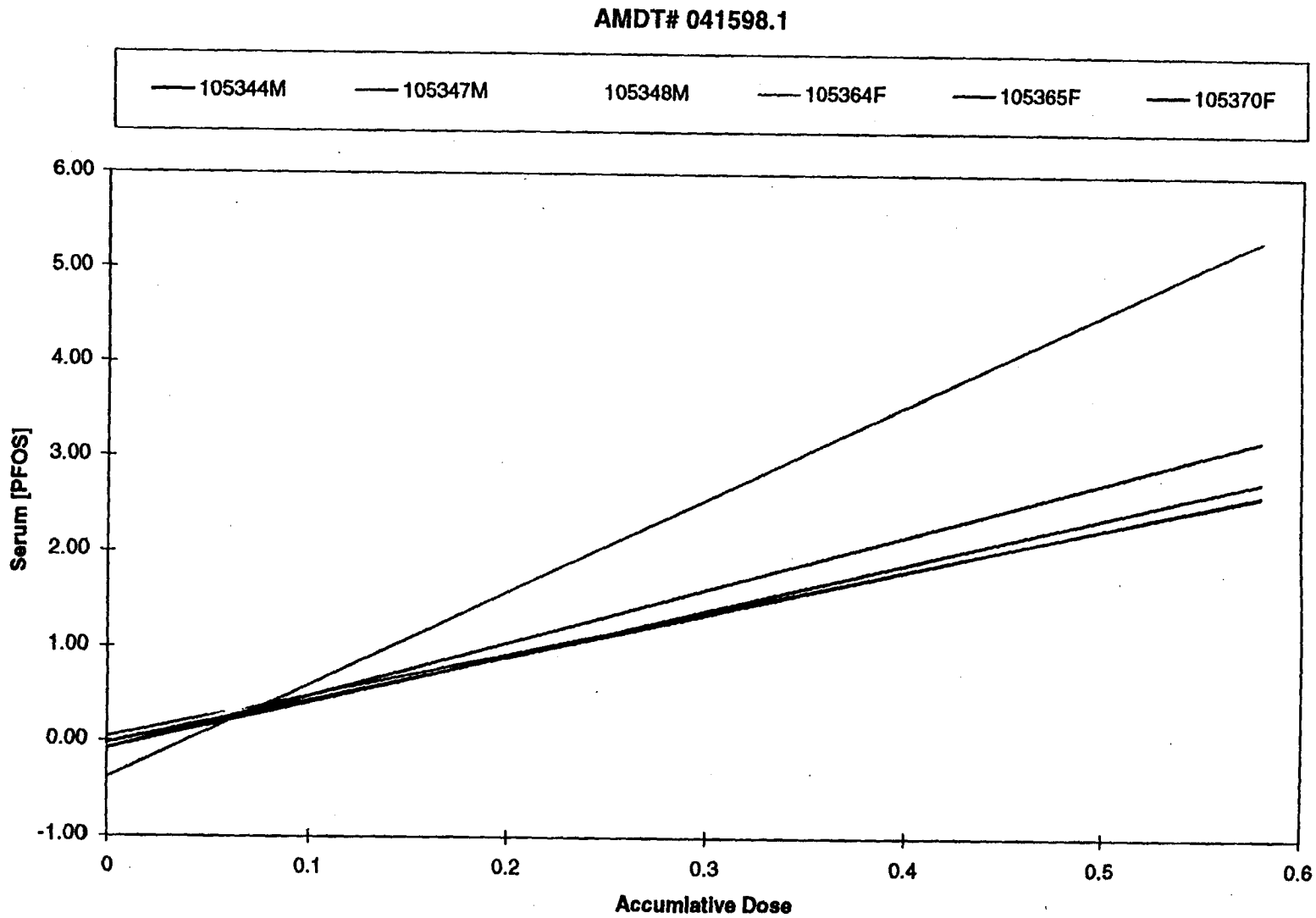
	Sample #	Timepoint	PFOS Conc. ug/mL
Group 2 Low Dose 0.02 mg/kg/day	105344M	Day -7	0.0120
		Day 2	0.157
		Day 3	0.174
		Day 7	0.449
		Day 14	1.37
		Day 29	2.78
	105347M	Day -7	0.0140
		Day 2	0.147
		Day 3	0.287
		Day 7	0.776
		Day 14	1.36
		Day 29	2.56
	105348M	Day -7	0.0100
		Day 2	0.161
		Day 3	0.289
		Day 7	0.874
		Day 14	1.46
		Day 29	3.57
	105364F	Day -7	0.0161
		Day 2	0.158
		Day 3	0.292
		Day 7	0.736
		Day 14	1.58
		Day 29	5.76
	105365F	Day -7	0.0144
		Day 2	0.117
		Day 3	0.267
		Day 7	0.628
		Day 14	1.22
		Day 29	2.65
	105370F	Day -7	0.0111
		Day 2	0.100
		Day 3	0.213
		Day 7	0.664
		Day 14	1.54
		Day 29	3.20

	Sample #	Timepoint	PFOS Conc. ug/mL
Group 3 High Dose 2.0 mg/kg/day	105345M	Day -7	0.0128
		Day 2	12.6
		Day 3	29.7
		Day 7	71.8
		Day 14	129
		Day 29	313
	105369F	Day -7	0.0139
		Day 2	14.4
		Day 3	18.3
		Day 7	72.7
		Day 14	143
		Day 29	299

Practical Quantitation Limit (PQL): = PFOS = 25 ng/mL, PFOSA = 0.5 ng/mL, PFOSAA = 10 ng/mL

PFOS = Perfluorooctanesulfonate

Method Detection Limit (MDL): PFOS = 12.5 ng/mL, PFOSA = 0.25 ng/mL, PFOSAA = 5 ng/mL


AMDT# 041598.1
105344M
105347M
105348M
105364F
105365F
105370F
6.00
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
Serum [PFOS]
0
0.1
0.2
0.3
0.4
0.5
0.6
Accumlative Dose

105344M

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0120
0.04	0.1570
0.06	0.1740
0.14	0.4490
0.28	1.3700
0.58	2.7800

slope	4.92	-0.08	y-int
se (slope)	0.21	0.06	se (y-int)
r-square	0.99	0.10	se (y-est)
F-value	540.118	4	df
ss (reg)	5.749	0.043	ss (resid)

105347M

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0140
0.04	0.1470
0.06	0.2870
0.14	0.7760
0.28	1.3600
0.58	2.5600

slope	4.43	0.04	y-int
se (slope)	0.17	0.05	se (y-int)
r-square	0.99	0.08	se (y-est)
F-value	667.749	4	df
ss (reg)	4.671	0.028	ss (resid)

105348M

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0100
0.04	0.1610
0.06	0.2890
0.14	0.8740
0.28	1.4600
0.58	3.5700

slope	6.13	-0.06	y-int
se (slope)	0.24	0.07	se (y-int)
r-square	0.99	0.12	se (y-est)
F-value	642.745	4	df
ss (reg)	8.944	0.056	ss (resid)

105364F

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0161
0.04	0.1580
0.06	0.2920
0.14	0.7360
0.28	1.5800
0.58	5.7600

slope	9.85	-0.38	y-int
se (slope)	1.06	0.29	se (y-int)
r-square	0.96	0.52	se (y-est)
F-value	86.129	4	df
ss (reg)	23.092	1.072	ss (resid)

105365F

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0144
0.04	0.1170
0.06	0.2670
0.14	0.6280
0.28	1.2200
0.58	2.6500

slope	4.58	-0.02	y-int
se (slope)	0.08	0.02	se (y-int)
r-square	1.00	0.04	se (y-est)
F-value	3683.682	4	df
ss (reg)	4.989	0.005	ss (resid)

105370F

Acc Dose mg/kg	[serum] ug/mL
0.00	0.0111
0.04	0.1000
0.06	0.2130
0.14	0.6640
0.28	1.5400
0.58	3.2000

slope	5.66	-0.08	y-int
se (slope)	0.13	0.04	se (y-int)
r-square	1.00	0.06	se (y-est)
F-value	1886.988	4	df
ss (reg)	7.623	0.016	ss (resid)

Dose-Setting Rationale for Six-Month Chronic Oral Study in Cynomolgus Monkeys

Record of August 3, 1998 Conference Call

Participants:

Dr. John Butenhoff, 3M
Dr. Marv Case, 3M
Dr. Jeff Mandel, 3M
Dr. John Moore, IEHR
Dr. Geary Olsen, 3M
Dr. Joe Rodricks, ENVIRON
Dr. Andrew Seacat, 3M

Outcome:

Data from the rangefinder was discussed including the strong relationship between cumulative dose and serum PFOS concentration (avg. of 5.27 +/- 0.61 ppm serum PFOS concentration per mg/kg PFOS dose). Discussion verified July 29, 1998 written rationale for setting doses with one change. Dr. Moore suggested that the low dose be raised from 0.02 mg/kg/day to 0.03 mg/kg/day to keep doses at multiples of 5. The suggestion was accepted. The study will proceed on August 12th, 1998 with the following dose levels:

Control	0.00 mg/kg/day
Low-Dose	0.03 mg/kg/day
Mid-Dose	0.15 mg/kg/day
High-dose	0.75 mg/kg/day

Copies:

Participants
Dr. Larry Zobel
Dr. Bill Weppner
Study File (T-6295.7)