

One Generation Reproduction Study of PFOS –
Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats
Draft Study Outline

Study Objectives: This study will serve as a follow-up study to Combined Oral (gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats (Argus Protocol 418-008, 3M study number T-6295.9). The objectives of the study are as follows:

- 1) to determine whether or not co-administration of mevalonic acid or cholesterol can prevent or antagonize the adverse maternal and fetal effects (reduced maternal body weight gain, increased percent stillborn, decreased birth weight and decreased pup survival) observed in the previous study; and
- 2) to refine the no observed effect level (NOEL).

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Female Harlan CD rats.

Dose Groups: Thirteen: Vehicle Control; Mevalonic Acid Control; Cholesterol Control; 0.4 mg/kg PFOS; 0.8 mg/kg PFOS; 1 mg/kg PFOS; 1.2 mg/kg PFOS; 1.6 mg/kg PFOS; 1.6 mg/kg PFOS + mevalonic acid; 1.6 mg/kg PFOS + cholesterol; 2 mg/kg PFOS; 2 mg/kg PFOS + Mevalonic Acid; 2 mg/kg PFOS + cholesterol.

Number Per Group: 22-28 in groups 1-3 & 8-13; 16-20 in groups 4-7. TOTAL: 246-312

Test Article: Compound will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Females only, daily oral intubation, dose volume 5ml/kg. The vehicle (0.5 % Tween 80 in deionized water) will be prepared weekly. PFOS will be prepared daily as a suspension in deionized water with 0.5 % Tween 80. Mevalonic acid will be prepared fresh twice daily in deionized water. Cholesterol will be prepared fresh daily in olive oil. Compound administration will start 6 weeks prior to mating and continue through day 4 postnatal. (see table 1).

Table 1 – Dose Groups & Dosing Schedule					
Dose Groups			Dosing schedule		
			1 st dosing	2 nd dosing ½ hr after 1 st	3 rd dosing 5 hr after 2 nd
#	Name	N			
1	Vehicle Control	30-40	Deionized H ₂ O	0.5 % Tween 80	Deionized H ₂ O
2	Mevalonic Acid Control	22-28	500 mg/kg Mevalonic Acid	0.5 % Tween 80	500 mg/kg Mevalonic Acid
3	Cholesterol Control	22-28	*500 mg/kg Cholesterol	0.5% Tween 80	NA
4	0.4 mg/kg PFOS	16-20	NA	0.4 mg/kg PFOS	NA
5	0.8 mg/kg PFOS	16-20	NA	0.8 mg/kg PFOS	NA
6	1 mg/kg PFOS	16-20	NA	1 mg/kg PFOS	NA
7	1.2 mg/kg PFOS	16-20	NA	1.2 mg/kg PFOS	NA
8	1.6 mg/kg PFOS	22-28	NA	1.6 mg/kg PFOS	NA
9	1.6 mg/kg PFOS + Mevalonic Acid	22-28	500 mg/kg Mevalonic Acid	1.6 mg/kg PFOS	500 mg/kg Mevalonic Acid
10	1.6 mg/kg PFOS + Cholesterol	22-28	*500 mg/kg Cholesterol	1.6 mg/kg PFOS	NA
11	2 mg/kg PFOS	22-28	NA	2 mg/kg PFOS	NA
12	2 mg/kg PFOS + Mevalonic Acid	22-28	500 mg/kg Mevalonic Acid	2 mg/kg PFOS	500 mg/kg Mevalonic Acid
13	2 mg/kg PFOS + Cholesterol	22-28	*500 mg/kg Cholesterol	2 mg/kg PFOS	NA

* Cholesterol dosing may occur before or after PFOS or Tween 80 dose.

Samples of dosing preparations will be collected once during the six weeks prior to mating, once during gestation and once during the postnatal period. A portion of each of these samples will be frozen and shipped to the Sponsor for possible analysis. A backup sample of each will be retained by the testing facility. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily - approximately one hour after dosing and again 4-6 hours later.

Body Weights: Weekly except when females are cohabiting with males during mating.

Food Consumption: Weekly except when females are cohabiting with males during mating.

Mating: After 6 weeks of compound administration, one male (Argus breeder colony) will be co-inhabited with each female. A female will be determined as mated upon the presence of sperm positive vaginal smear. The day of mating shall be considered day 0 of gestation.

Pre-Birth Sacrifice and Examination: Just prior to delivery (morning of day 21 of gestation) 6-8 pregnant females in groups 1-3 and 8-13 will be sacrificed by CO₂ asphyxiation and cesarean-section will be performed. A terminal body weight for each dam and a pooled terminal body weight for each litter will be obtained and recorded. Animals will be necropsied and any gross lesions will be described and recorded.

Serum/plasma and liver samples will be taken from each dam and her pups (pup samples pooled by litter) and milk will be collected from each dam. Liver and serum will be analyzed for LDL, HDL, total cholesterol, and triglycerides. Plasma mevalonic acid levels will be measured. Milk will be analyzed for total cholesterol. Additional liver and sera samples will be frozen (-20°C) and shipped on dry ice to Kris J. Hansen, Ph.D., 3M Environmental Technology and Safety Services, 935 Bush Avenue, Building 2-3E-09, St. Paul, MN 55133-3331 for PFOS analysis. A portion of each liver sample will be flash frozen in liquid nitrogen and shipped on dry ice to the Sponsor for possible future analysis (i.e., liver adenine nucleotides & HMG-CoA reductase inhibition). Additional liver tissue will be collected and fixed in gluteraldehyde for possible future analysis by electron microscopy.

Female Parturition: Remaining rats will be allowed to deliver their litters.

Pup Examinations: Pups will be counted, sexed, weighed and observed for moribundity/mortality on postnatal days 0, 1, 2, 3 and 4.

Rats Found Dead or Moribund: Rats that die or are sacrificed because of moribund condition or abortion will be examined for cause of death or moribund condition on the day the observation is made. Aborted fetuses and/or in-utero pups will be examined to the extent possible. When possible, the following specimens will be collected and analysis performed:

Rats will be necropsied and any gross lesions will be described and recorded. Serum/plasma and liver samples will be taken (individually for each dam and her pups – pup samples pooled by litter). Liver and serum will be analyzed for LDL, HDL, total cholesterol and triglycerides. Plasma mevalonic acid levels will be measured. Milk will be collected from each dam and analyzed for total cholesterol. Additional liver and sera samples will be frozen (-20°C) and shipped on dry ice to Kris J. Hansen, Ph.D., 3M Environmental Technology and Safety Services, 935 Bush Avenue, Building 2-3E-09, St. Paul, MN 55133-3331 for PFOS analysis. A portion of each liver sample will be flash frozen in liquid nitrogen and shipped on dry ice to the Sponsor for possible future analysis (i.e., liver adenine nucleotides & HMG-CoA reductase inhibition). Additional liver tissue will be collected and fixed in gluteraldehyde for possible future analysis by electron microscopy. Myocardial tissue will be collected for possible histology and electron microscopy.

Day 4 Postnatal Sacrifice and Examination: Sixteen to 20 dams in each dose group and all of their pups will be sacrificed by CO₂ asphyxiation on day four postnatal. A terminal body weight for each dam and a pooled terminal body weight for each litter will be obtained and recorded. Animals will be necropsied and any gross lesions will be described and recorded. Serum/plasma and liver samples will be taken from each dam and her pups (pup samples pooled by sex for each litter) and milk samples will be collected from each dam. Liver and serum will be analyzed for LDL, HDL, total cholesterol and triglycerides. Plasma mevalonic acid levels will be measured. Milk will be collected from each dam and analyzed for total cholesterol. Additional liver and serum samples will be frozen (-20°C) and shipped on dry ice to Kris J. Hansen, Ph.D.,

3M Environmental Technology and Safety Services, 935 Bush Avenue, Building 2-3E-09, St. Paul, MN 55133-3331 for PFOS analysis. A portion of each liver sample will be flash frozen in liquid nitrogen and shipped on dry ice to the Sponsor for possible future analysis (i.e., liver adenine nucleotides & HMG-CoA reductase inhibition). Additional liver tissue will be collected and fixed in gluteraldehyde for possible future analysis by electron microscopy. Myocardial tissue will be collected from each dam and four of her pups (2 males and 2 females) for possible histology and electron microscopy.

Table 2 - Scheduled Sacrifices (N dams/group/Sacrifice)

Grp #		1	2	3	4	5	6	7	8	9	10	11	12	13
Group Name		Vehicle control	Mevalonic acid control	Cholesterol Control	0.4 mg/kg PFOS	0.8 mg/kg PFOS	1 mg/kg PFOS	1.2 mg/kg PFOS	1.6 mg/kg PFOS	1.6 mg/kg PFOS + mevalonic acid	1.6 mg/kg PFOS + cholesterol	2 mg/kg PFOS	2 mg/kg PFOS + mevalonic acid	2 mg/kg PFOS + cholesterol
Sacrifice	Pre-Birth (dy -1)	6-8	6-8	6-8					6-8	6-8	6-8	6-8	6-8	6-8
	Dy 4 Postnatal	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20	16-20
	TOTAL N / grp	22-28	22-28	22-28	16-20	16-20	16-20	16-20	22-28	22-28	22-28	22-28	22-28	22-28

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