**Attachments to Letter to C. Auer dated May 4, 2000**
**Perfluoroctane Sulfonate Studies**

Planned Studies

1) Preliminary Study Outline, One Generation Reproduction Study of PFOS in Rats, Pharmacokinetic Analysis, May 3, 2000

2) Preliminary Study Outline, One Generation Reproduction Study of PFOS in Rats, Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats, April 27, 2000

PS-1

**One Generation Reproduction Study of PFOS in Rats – Pharmacokinetic Analysis**
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 objective of the study is to gain additional pharmacokinetc (PK) data on PFOS in pregnant rats and their pups.

**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:** Six: Vehicle Control; 0.1 mg/kg PFOS; 0.4 mg/kg PFOS; 0.8 mg/kg PFOS; and 1.6 mg/kg PFOS.

**Number Per Group:** 36 per group = **150 total**

**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. Compound administration will start 6 weeks prior to mating and continue through day 21 of lactation.
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.

**Day Zero of Gestation Sacrifice and Examination:** On day zero of gestation, 6 pregnant females in each group will be sacrificed by $CO_2$ asphyxiation. A terminal body weight for each dam will be obtained and recorded. Animals will be necropsied and any gross lesions will be described and recorded. Serum and liver samples will be taken from each dam. All specimens 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. Upon receipt, sera will be analyzed for PFOS and livers will be stored for possible future analysis.

**Day 15 of Gestation Sacrifice and Examination:** On day 15 of gestation, 6 pregnant females in each group will be sacrificed by $CO_2$ 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. Dams will be necropsied and any gross lesions will be described and recorded. Serum, liver and milk samples will be taken from each dam. Dam sera, liver, milk and whole pups 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. Upon receipt, sera, milk and whole pups will be analyzed for PFOS and livers will be stored for possible future analysis.

**Pre-Birth Sacrifice and Examination:** Just prior to delivery (morning of day 21 of gestation) 6 pregnant females in each group will be sacrificed by $CO_2$ 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 and liver samples will be taken from each dam and her pups (pup samples pooled by litter). Milk samples will be collected from each dam. All specimens 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. Upon receipt, sera and milk will be analyzed for PFOS and livers will be stored for possible future analysis. *MAY ADD ADDITIONAL DAMS IN SOME DOSE GROUPS SO WHOLE PUP PFOS LEVELS CAN BE EXAMINED OR* MAY SAVE REMAINDER OF EACH PUP AFTER LIVER AND SERA ARE COLLECTED.

**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, 7, 14 and 21.

**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 and liver samples will be taken (individually for each dam and her pups – pup samples pooled by litter). Milk samples will be collected from each dam. All specimens 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. Upon receipt, sera and milk will be analyzed for PFOS and livers will be stored for possible future analysis.

**Lactation Day 7 Sacrifice and Examination:** Six dams in each dose group and all of their pups will be sacrificed by $CO_2$ asphyxiation on lactation day 7. 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 and liver samples will be taken from each dam and her pups (pup samples pooled by sex for each litter). Milk samples will be collected from each dam. All specimens 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. Upon receipt, sera and milk will be analyzed for PFOS and livers will be stored for possible future analysis.

**Lactation Day 14 Sacrifice and Examination:** Six dams in each dose group and all of their pups will be sacrificed by $CO_2$ asphyxiation on lactation day 14. 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 and liver samples will be taken from each dam and her pups (pup samples pooled by sex for each litter). Milk samples will be collected from each dam. All specimens 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. Upon receipt, sera and milk will be analyzed for PFOS and livers will be stored for possible future analysis.

**Lactation Day 21 Sacrifice and Examination:** Six dams in each dose group and all of their pups will be sacrificed by $CO_2$ asphyxiation on lactation day 21. 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 and liver samples will be taken from each dam and her pups (pup samples pooled by sex for each litter). Milk samples will be collected from each dam. All specimens 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. Upon receipt, sera and milk will be analyzed for PFOS and livers will be stored for possible future analysis.

| Table 1 – Scheduled Sacrifices (N dams/group/sacrifice) | | | | | | |
|---|---|---|---|---|---|---|
| **Grp #** | | **1** | **2** | **3** | **4** | **5** |
| **Group Name** | | Vehicle control | 0.1 mg/kg PFOS | 0.4 mg/kg PFOS | 0.8 mg/kg PFOS | 1.6 mg/kg PFOS |
| Sacrifice | Dy 0 of gestation | 6 | 6 | 6 | 6 | 6 |
| | Dy 15 of gestation | | | | | |
| | Pre-Birth (dy -1) | 6 | 6 | 6 | 6 | 6 |
| | Lactation Dy 7 | 6 | 6 | 6 | 6 | 6 |
| | Lactation Dy 14 | 6 | 6 | 6 | 6 | 6 |
| | Lactation Dy 21 | 6 | 6 | 6 | 6 | 6 |
| | **TOTAL N / grp** | 30 | 30 | 30 | 30 | 30 |

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