

**Study No. T-6295.30; ST69**  
**Oxygen Saturation and pO<sub>2</sub> Levels in Rat Pups Borne**  
**to PFOS Exposed Dams**

Study Location:  
3M Strategic Toxicology Laboratory  
Corporate Toxicology  
3M Medical Department  
3M Center, Building 270-SB-314  
St. Paul, MN 55144

Study Director: Deanna J. Luebker, M.S., Advanced Research Toxicologist  
3M Medical Dept. / Corporate Toxicology  
3M Center, Building 220-2E-02  
Saint Paul, MN 55144  
Ph: 651-737-1374 FAX: 651-733-1773

Study Toxicologist: Andrew M. Seacat, Ph.D., DABT, Toxicology Specialist  
3M Medical Dept. / Corporate Toxicology  
3M Center, Building 220-2E-02  
Saint Paul, MN 55144  
Ph.: 651-575-3161 FAX: 651-733-1773

Sponsored by 3M Specialty Chemicals Division  
3M Center Bldg 236  
St. Paul, MN 55144

### **Study Objective**

This study was the first of a series of exploratory pilot studies designed to investigate the mechanism of perinatal mortality in rats and mice borne to timed-pregnant dams dosed with PFOS during gestation. The hypothesis that PFOS interferes with normal respiration and/or gas exchange in the pups was investigated. In addition, amniotic fluid was analyzed for PFOS levels and specimens were gathered for possible future analysis (i.e., to investigate the ability of PFOS to interfere with the glucose metabolism).

### **Method Summary**

This study was performed in the 3M Strategic Toxicology Laboratory under a defined protocol (1) and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure (2). All in-life procedures were approved by the 3M Laboratory Animal Review Committee (3M LARC) and are detailed in animal usage application 2001-0182 (3).

Under the initial study protocol (1), dams were dosed on days 14-17 of gestation to reach a cumulative dose of 100 mg/kg. The intent was to allow these animals to litter, and to then sacrifice both dams and pups within 24 hours of birth. This dosing regime, however, resulted in very high rates of stillborn pups and early deliveries, greatly complicating the study and preventing the endpoints of interest (oxygen saturation and partial oxygen pressure in blood (pO<sub>2</sub>)) from being examined. In response, the study protocol was amended and a second study was performed. As discussed in the protocol amendment (4), it is thought that an excessive dose of PFOS may have been delivered to the pups and/or that dosing period may have been too late in gestation. Under the amended study protocol, animals were treated via oral gavage with 25 mg PFOS per kg body weight per day for 3 consecutive days (days 12-14 of gestation) to reach a cumulative dose of 75 mg/kg. All animals, including dams, were euthanized via decapitation just prior to delivery. Pups were removed via caesarean-section (c-section), to standardize the time of data collection, and manually stimulated to breath prior to decapitation under a heat lamp set to maintain a temperature of ~ 95 to 98 degrees F. Blood and amniotic fluid (pooled from multiple amniotic sacs using a needle and syringe prior to removing each pup) were collected from each dam for PFOS analysis (see Appendix 1 for details on PFOS analytical method). Liver was also collected from each c-sectioned dam and flash frozen in liquid nitrogen for possible future analysis. Pups were decapitated within 5 minutes being removed from the placental sac. Immediately following decapitation of the pups, blood was drained onto a heparinized weigh boat and pipetted into an I-Stat cartridge for determination of pO<sub>2</sub> and other blood gases by the I-Stat method (described in the study protocol, 1). No oxygen saturation measurements using the pulse oximeter were obtained. One additional dam was received from the vendor. It was dosed with PFOS as described above and allowed to litter. This dam and her litter were then monitored to assess viability after birth using this study design.

## **Results**

### **Initial Study**

In-life observations from the initial study can be found in Table 1. Four control animals and six PFOS treated animals were included in this study. One control and one treated animal were not pregnant. Pregnant control animals gained an average of  $33.0 \pm 7.8$  grams from day 14-17 of gestation while treated pregnant animals gained only an average of  $8.6 \pm 12.2$  grams. All control animals littered on day 21 of gestation and all treated animals littered on either day 20 or 21 of gestation. Control litters appeared normal while treated litters were either borne dead or died shortly after birth (within approximately 10 minutes). Liver and sera were gathered from some dams and pups and flash frozen for possible future analysis. An attempt was made to measure oxygen saturation using a pulse oximeter, and pO<sub>2</sub> levels with an I-Stat blood gas analyzer on those pups borne alive. No useful data was gathered for either parameter. It was decided that the pulse oximeter method was not suitable for use on such small pups and that future blood gas analysis would be limited to the I-Stat method.

### **Amended Study**

In-life observations from the amended study can be found in Table 2. Three control animals and 4 PFOS treated animals were included in this study. All animals were pregnant. Control animals gained an average of  $99.5 \pm 0.7$  grams from day 12-21 of gestation while treated animals gained only an average of  $74.8 \pm 11.4$  grams. Dams were palpitated on gestation day (GD) 19 by Dr. DeWayne Walker (3M Lab Animal Veterinarian) and pups were determined to be too immature to survive if delivered. One control animal was decapitated and c-sectioned on GD20 and pups (n=12) were unable to breath and survive. C-sectioning of the remaining dams was thus postponed on the recommendation of Dr. Walker until early morning (5:30 – 6:30am) on GD21. The remaining 2 control animals were decapitated and c-sectioned on GD21, and litters of 10 and 11 pups were delivered, all appearing normal. Three of the treated dams were also decapitated and c-sectioned and litters of 10-13 pups were delivered. Blood was evident in the uterine horns of each of the PFOS treated dams. Treated pups had difficulty breathing and some died within minutes after birth. It was determined that these treated pups were not as mature as the control pups. The remaining PFOS dam was allowed to litter. She delivered 13 pups (10 on GD22 am, 3 on GD23 am), all borne dead or dying within minutes of birth.

I Stat data from the pups can be found in Table 3. The only parameter that significantly different between control and PFOS treated groups ( $p \leq 0.05$ ) was pH, with controls averaging  $7.14 \pm 0.08$  (N=2) and PFOS treated averaging  $7.07 \pm 0.03$  (N=8). No significant difference in pO<sub>2</sub> levels (mmHg) or O<sub>2</sub> saturation (%) was found between treated and control pups. Control pO<sub>2</sub> levels averaged  $30 \pm 4$  mmHg while levels in PFOS treated pups averaged  $35 \pm 6$  mmHg. Oxygen saturation averaged  $39 \pm 1$  % in controls and  $44 \pm 13$  % in PFOS treated pups. Systemic pO<sub>2</sub> represents the O<sub>2</sub> levels at the exact time the blood is collected and is readily altered by a few breaths (5). Literature values for pO<sub>2</sub> from control pups delivered from c-sectioned decapitated dams are ~ 60 mmHg when taken within 30 seconds of birth and after at least one breath (5). This is equivalent to vaginally born pup PO<sub>2</sub> levels (5). The pO<sub>2</sub> levels found in the current study are substantially lower than those cited in the literature. This may be a result of the

increased time the pups were allowed to breath on their own prior to decapitation (5 minutes as opposed to 30 seconds).

Dam sera and amniotic fluid PFOS levels can be found in Table 4. Control sera and amniotic fluid PFOS levels averaged  $0.017 \pm 0.003$  ug/ml and  $0.011 \pm 0.003$  ug/ml respectively. The ratio of sera to amniotic fluid PFOS levels for controls averaged 1.7:1. PFOS treated sera and amniotic fluid PFOS levels averaged  $68.3 \pm 15.0$  ug/ml and  $16.4 \pm 1.2$  ug/ml respectively. The ratio of sera to amniotic fluid PFOS levels for the PFOS treated dams averaged 4.2:1.

### Conclusions

The low numbers of animals used in this exploratory pilot study prevents definitive conclusions from being drawn. The results of the I Stat analysis suggest, however, that PFOS does not interfere with normal respiration and/or gas exchange in neonatal rat pups borne to PFOS exposed dams. Results of the dam sera and amniotic fluid PFOS analysis suggest that PFOS distributes more readily into the sera than into the amniotic fluid (approximately 4:1 in PFOS treated animals). PFOS was, however, detectable in the amniotic fluid of all animals tested. This shows that neonatal rat pups borne to PFOS treated dams are exposed to PFOS *in utero* via the amniotic fluid. The dose used in the amended study, 25 mg/kg/day on days 12-14 of gestation (cumulative dose of 75 mg/kg/day) produced death in live borne pups within minutes of birth. A dose producing death within the first 6 - 24 hours of live birth is desired to allow time for data collection and observation. It is also thought that a single dose may produce the same effect as multiple daily doses. Thus, a dose response study will be performed to determine if the dosing regime can be limited to a single administration and to find a dose level that will extend life to 6-24 hours following birth.

**List of Tables**

Table 1: Initial Study In-Life Data

Table 2: Amended Study In-Life Data

Table 3: Amended Study I-Stat Data (Pups)

Table 4: Amended Study Maternal Sera and Amniotic Fluid PFOS Levels

| Table 1: Initial Study In-Life Data  |                  |            |                 |      |      |      |      |                    |            |                                                             |
|--------------------------------------|------------------|------------|-----------------|------|------|------|------|--------------------|------------|-------------------------------------------------------------|
|                                      | Dose (GDs 14-17) |            | Body Weight (g) |      |      |      |      |                    |            |                                                             |
| Animal #                             | (mg/kg/day)      | Cumulative | GD14            | GD15 | GD16 | GD17 | GD18 | Change<br>GD 14-17 | Litter day | Comments                                                    |
| 1R05311                              | 0                | 0          | 266             | 272  | 281  | 295  | NA   | 29                 | GD21       | (am) no count, all normal                                   |
| 1R05312                              | 0                | 0          | 322             | 331  | 343  | 364  | NA   | 42                 | GD21       | (am) no count, all normal                                   |
| 1R05316                              | 0                | 0          | 280             | 287  | 295  | 308  | 324  | 28                 | GD21       | (am) 13 pups all normal                                     |
| 1R05317                              | 0                | 0          | 261             | 255  | 258  | 263  | 266  | 2                  | NA         | Not pregnant                                                |
| 1R05313                              | 25               | 100        | 296             | 304  | 307  | 306  | NA   | 10                 | GD20       | (pm) no count, pups borne alive but dying within 10 minutes |
| 1R05314                              | 25               | 100        | 315             | 322  | 330  | 339  | NA   | 24                 | GD20       | (pm) started to litter, performed C-Sect., all pups dead    |
| 1R05315                              | 25               | 100        | 318             | 320  | 318  | 310  | NA   | -8                 | GD20       | 9 pups in am, 1 in pm, all borne dead                       |
| 1R05318                              | 25               | 100        | 314             | 314  | 325  | 316  | 307  | 2                  | GD21       | (am) 2 pups, borne dead                                     |
| 1R05319                              | 25               | 100        | 257             | 249  | 235  | 222  | 213  | -35                | NA         | Not pregnant                                                |
| 1R05320                              | 25               | 100        | 279             | 288  | 293  | 294  | 278  | 15                 | GD21       | (am) 1 pup, borne dead                                      |
| Gestation Day (GD)                   |                  |            |                 |      |      |      |      |                    |            |                                                             |
| Not Applicable or Not Available (NA) |                  |            |                 |      |      |      |      |                    |            |                                                             |

| Table 2: Amended Study In-Life Data  |                  |                    |                 |      |      |      |      |      |                 |            |               |                      |                                                                                                                                     |
|--------------------------------------|------------------|--------------------|-----------------|------|------|------|------|------|-----------------|------------|---------------|----------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Animal #                             | Dose (GDs 12-14) |                    | Body Weight (g) |      |      |      |      |      |                 | Litter day | C-section day | Number pups / litter | comments                                                                                                                            |
|                                      | (mg/kg/day)      | Cumulative (mg/kg) | GD12            | GD13 | GD14 | GD19 | GD20 | GD21 | Change GD 12-21 |            |               |                      |                                                                                                                                     |
| 1R06028                              | 0                | 0                  | 241             | 248  | 259  | 320  | 329  | NA   | NA              | NA         | GD20          | 12                   | pups not mature enough to breath & survive                                                                                          |
| 1R06029                              | 0                | 0                  | 280             | 290  | 293  | 350  | 363  | 379  | 99              | NA         | GD21          | 10                   | normal                                                                                                                              |
| 1R06030                              | 0                | 0                  | 287             | 294  | 296  | 352  | 371  | 387  | 100             | NA         | GD21          | 11                   | normal                                                                                                                              |
| 1R06031                              | 25               | 75                 | 255             | 269  | 256  | 311  | 330  | 345  | 90              | NA         | GD21          | 13                   | not as mature as controls, difficulty breathing, some dying before we could decipatate them (within minutes); blood in uterine horn |
| 1R06032                              | 25               | 75                 | 255             | 262  | 254  | 302  | 319  | 332  | 77              | NA         | GD21          | 11                   | not as mature as controls, difficulty breathing, some dying before we could decipatate them (within minutes); blood in uterine horn |
| 1R06033                              | 25               | 75                 | 216             | 227  | 219  | 251  | 266  | 281  | 65              | NA         | GD21          | 10                   | not as mature as controls, difficulty breathing, some dying before we could decipatate them (within minutes); blood in uterine horn |
| 1R06034                              | 25               | 75                 | 232             | 232  | 220  | 268  | 291  | 299  | 67              | GD22 & 23  | NA            | 13                   | 10 on GD22 (am), 3 on GD23 (am), all borne dead or died shortly after birth (within minutes)                                        |
| Gestation Day (GD)                   |                  |                    |                 |      |      |      |      |      |                 |            |               |                      |                                                                                                                                     |
| Not Applicable or Not Available (NA) |                  |                    |                 |      |      |      |      |      |                 |            |               |                      |                                                                                                                                     |

**Table 3: Amended Study I Stat Data (Pups)**

| Animal #<br>(dam)                                                                           | Dose<br>Group | cumulative<br>maternal<br>dose<br>(mg/kg) | pup # * | pH   | p CO2<br>(mmHg) | p O2<br>(mmHg) | BE<br>(mmol/L) | HCO3(m<br>mol/L) | TCO2<br>(mmol/L) | O2 sat<br>(%) | Na<br>(mmol/L) | K<br>(mmol/L) | ICa++<br>(mmol/L) | Hct<br>(%PCV) | Hgb<br>(g/dL) |
|---------------------------------------------------------------------------------------------|---------------|-------------------------------------------|---------|------|-----------------|----------------|----------------|------------------|------------------|---------------|----------------|---------------|-------------------|---------------|---------------|
| 1R06030                                                                                     | Control       | 0                                         | 1-5     | 7.08 | 80.0            | 32             | -8             | 23               | 26               | 38            | 131            | 9             | 0.74              | 39            | 13            |
| 1R06030                                                                                     | Control       | 0                                         | 6-10    | 7.20 | 46.1            | 27             | -9             | 18               | 19               | 39            | 130            | >9            | 0.33              | 29            | 10            |
| Control Average                                                                             |               |                                           |         | 7.14 | 63.1            | 30             | -9             | 21               | 23               | 39            | 131            | 9             | 0.54              | 34            | 12            |
| Control St Dev                                                                              |               |                                           |         | 0.08 | 24.0            | 4              | 1              | 4                | 5                | 1             | 1              | 0             | 0.29              | 7             | 2             |
| 1R06031                                                                                     | PFOS          | 75                                        | 1-5     | 7.07 | 76.5            | 33             | -8             | 22               | 25               | 41            | 128            | >9            | 0.75              | 35            | 12            |
|                                                                                             |               |                                           | 6-8     | 6.99 | 76.1            | 24             | -13            | 18               | 21               | 20            | 128            | 8             | 0.32              | 34            | 12            |
|                                                                                             |               |                                           | 9-11    | 7.05 | 63.7            | 38             | -13            | 17               | 19               | 49            | 129            | >9            | 0.74              | 37            | 13            |
| 1R06032                                                                                     | PFOS          | 75                                        | 1-5     | 7.10 | 70.7            | 34             | -7             | 22               | 24               | 44            | 133            | 7             | 0.30              | 28            | 10            |
|                                                                                             |               |                                           | 6-10    | 7.09 | 51.3            | 36             | -13            | 16               | 17               | 49            | 125            | 8             | 0.37              | 29            | 10            |
| 1R06033                                                                                     | PFOS          | 75                                        | 1-5     | 7.08 | 67.7            | 29             | -9             | 20               | 22               | 33            | 121            | 9             | 0.31              | 20            | 7             |
|                                                                                             |               |                                           | 6-8     | 7.09 | 49.3            | 41             | -14            | 15               | 17               | 58            | 124            | >9            | 0.39              | 35            | 12            |
|                                                                                             |               |                                           | 9-11    | 7.07 | 40.5            | 42             | -16            | 12               | 13               | 58            | 122            | 8             | NA                | 18            | 6             |
| PFOS Average                                                                                |               |                                           |         | 7.07 | 62.0            | 35             | -12            | 18               | 20               | 44            | 126            | 8             | 0.45              | 30            | 10            |
| PFOS St Dev                                                                                 |               |                                           |         | 0.03 | 13.4            | 6              | 3              | 3                | 4                | 13            | 4              | 1             | 0.20              | 7             | 3             |
| T-Test control/treated                                                                      |               |                                           |         | 0.04 | 0.47            | 0.15           | 0.11           | 0.17             | 0.21             | 0.29          | 0.09           | 0.12          | 0.33              | 0.23          | 0.27          |
| * Blood samples pooled from multiple pups per litter for each reading.<br>Not available: NA |               |                                           |         |      |                 |                |                |                  |                  |               |                |               |                   |               |               |



| Table 4: Amended Study Maternal Sera and Amniotic Fluid PFOS Levels |                 |                         |                    |                             |                                |
|---------------------------------------------------------------------|-----------------|-------------------------|--------------------|-----------------------------|--------------------------------|
| Animal #                                                            | Dose Group      | Cumulative Dose (mg/kg) | Serum PFOS (ug/ml) | Amniotic Fluid PFOS (ug/ml) | sera:amniotic fluid PFOS ratio |
| 1R06028                                                             | control         | 0                       | NA                 | NA                          | NA                             |
| 1R06029                                                             | control         | 0                       | 0.019              | 0.009                       | 2.1:1                          |
| 1R06030                                                             | control         | 0                       | 0.015              | 0.013                       | 1.2:1                          |
|                                                                     | Control Average |                         | 0.017              | 0.011                       | 1.65:1                         |
|                                                                     | Control Std Dev |                         | 0.003              | 0.003                       | 0.64                           |
| 1R06031                                                             | PFOS            | 75                      | 56.0               | 16.0                        | 3.5:1                          |
| 1R06032                                                             | PFOS            | 75                      | 64.0               | 17.7                        | 3.6:1                          |
| 1R06033                                                             | PFOS            | 75                      | 85.0               | 15.5                        | 5.5:1                          |
| 1R06034                                                             | PFOS            | 75                      | NA                 | NA                          | NA                             |
|                                                                     | PFOS Average    |                         | 68.3               | 16.4                        | 4.2:1                          |
|                                                                     | PFOS Std Dev    |                         | 15.0               | 1.2                         | 1.13                           |
| Not Available (NA)                                                  |                 |                         |                    |                             |                                |

**Signatures:**

**Prepared By:**

Deanna Luebker 12/31/2001  
Deanna Luebker, MS Date  
Advanced Research Toxicologist  
Study Director

Andrew M. Seacat 1/2/02  
Andrew Seacat, Ph.D. Date  
Toxicology Specialist  
Study Toxicologist

**Reviewed By:**

John W. Butenhoff 2/15/2002  
John Butenhoff, Ph.D. Date  
Corporate Toxicology Management

Dan Hakes 2/20/02  
Dan Hakes Date  
Sponsor Representative

## **References**

1. 3M Medical Department, Corporate Toxicology Protocol for Study No. T-6295.30 DT-69 Oxygen Saturation and pO<sub>2</sub> Levels in Rat Pups Borne to PFOS Exposed Dams, 2001.
2. 3M Medical Department, Corporate Toxicology Strategic Toxicology Laboratory Standard Operating Procedure (TOX SOP) No. 0950 – GLP Program Procedure, 1999.
3. 3M Lab Animal Review Committee Animal Usage Application No. 2001-0182.  
MECHANISTIC RESEARCH IN TIMED PREGNANT RATS AND MICE –  
AMENDMENT TO AUA # 2001-0182, 2001.
4. 3M Medical Department, Corporate Toxicology Amendment #1 for Study No. T-6295.30 DT-69 Oxygen Saturation and pO<sub>2</sub> Levels in Rat Pups Borne to PFOS Exposed Dams, 2001.
5. Vaillancourt C., Berger N., and Boksa P. (1999) Effects of vaginal birth versus Caesarean section birth with general anesthesia on blood gasses and brain energy metabolism in neonatal rats. *Experimental Neurology* 160:142-150.

Appendix 1

**3M Corporate Toxicology**  
**Analytical Laboratory Bldg 236**

**To:** Deanna Luebker  
Andrew Seacat

**From:** David J. Ehresman

**Compound:** Oxygen Saturation and pO<sub>2</sub> Levels in Rat Pups  
Borne to PFOS Exposed Dams  
3M Medical Department Study Number T-6295.30  
3M Strategic Toxicology Lab Folder Number DT-69

**Date** December 10, 2001

Pregnant female rats were exposed to PFOS to evaluate the pup fetal lung maturity in an attempt to determine rat pup oxygen levels at birth. Part of this study involved determining the concentration of PFOS in the female rat serum and in the amniotic fluid post exposure to correlate with the Oxygen Saturation levels of the exposed and control pups.

Samples of the rat serum and amniotic fluid from both exposed and control animals were delivered to the Strategic Toxicology Laboratory for analysis by LC-MS. The amniotic fluid samples were collected at the time of Caesarean section delivery for both the control and dosed animals.

Control animals were pregnant females rats with the same approximate gestational status. Control amniotic fluid and serum were collected from them for analysis.

**Analytical Method:**

PFOS levels were determined from both the rat serum and amniotic fluid using a single extraction in ethyl acetate. The pH of the tested solution (100 uL; serum or amniotic fluid) was adjusted to an approximate pH of 3.0 with the addition of 400 uL of a 0.2 N formic acid buffer solution (pH = 3.0). Internal standard (PFHS) was added prior to the extraction and the mixture was shaken on a mechanical shaker for 1 hour. The tubes were then centrifuged at 3000 rpm's for 5 minutes and the ethyl acetate transferred to a clean polypropylene tube. The ethyl acetate was dried to dryness using a gentle stream of nitrogen gas on the N-Evap drier. The resultant residue was re-dissolved in 250 uL of 25% acetonitrile and 75% 10 mM ammonium acetate buffer. This mixture was then filtered through a 0.2 micron syringe filter and placed in a polypropylene injection vial. 25 uL was injected on to the LC-MS system.

## Appendix 1

The liquid chromatograph used was the Finnigan MAT Spectra system P4000. The column used was a Keystone Fluorophase (150 x 2.1 mm) speciality column featuring a selective, fluorinated, branched-chain alkyl packing. The flow rate was maintained at a constant 0.4 ml/min. A gradient flow of acetonitrile was used to elute the compounds of interest starting with 15% acetonitrile and ending with a concentration of 70 % acetonitrile over a six and one half minute gradient program. There was a constant methanol concentration of 10% methanol with the balance of the mobile phase being 10 mM ammonium acetate buffer. The initial buffer concentrations were returned to starting 6.5 minutes into the run and reached at 8.0 minutes. Column equilibration period was then 2.5 additional minutes before the next injection for a cycle time of 10.5 minutes per injection. All compounds of interest eluted within the first 5 minutes of the gradient run.

The Finnigan TSQ system was operated in the MS/MS mode using the Electro Spray source in the negative ionization mode with a constant source potential of 3.0 kV applied. The reactant gas in the Q2 region of the triple quad system was argon. The method involved the transition of the following mass ions:

| <u>Compound</u>  | <u>Base Peak (Q1 mass)</u> | <u>MS/MS Ion</u> | <u>Q2 Offset Voltage</u> |
|------------------|----------------------------|------------------|--------------------------|
| Int. Std. (PFHS) | 399.0                      | 80               | 60 volts                 |
| PFOS             | 499.0                      | 80               | 60 volts                 |

The capillary heater was held at a constant 300 degrees C. All quantitative calculations were based on the MS/MS ion ratios between the compound of interest (PFOS) and the Internal Standard (PFHS).

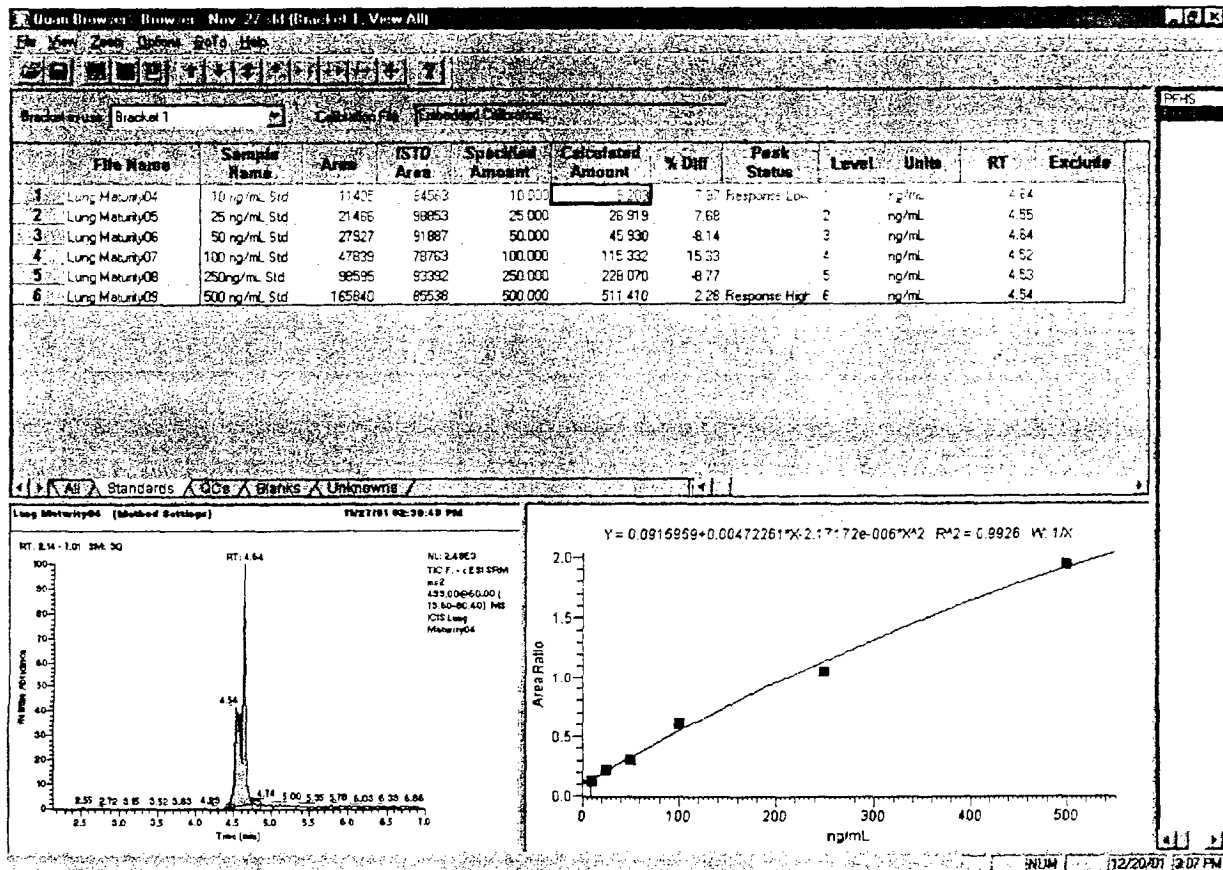
## Study Results:

| Animal Number | Status       | Serum PFOS Level | Amniotic Fluid Level |
|---------------|--------------|------------------|----------------------|
| 6029          | Control      | 19 ng/mL         | 9 ng/mL              |
| 6030          | Control      | 15 ng/mL         | 13 ng/mL             |
| 6031          | Dosed Animal | 56.0 ug/mL       | 16.0 ug/mL           |
| 6032          | Dosed Animal | 64.0 ug/mL       | 17.7 ug/mL           |
| 6033          | Dosed Animal | 85.0 ug/mL       | 15.5 ug/mL           |

Due to the high concentration of PFOS in the serum a 1:500 dilution was required to bring the concentration down to be within the quantitation limits of the established standard curve for the dosed animals. The dosed animal amniotic fluid was diluted 1:100 before the concentration was within the acceptable curve limitations. The routine standard curves were established using 6 points between 10 ng/mL and 500 ng/mL.

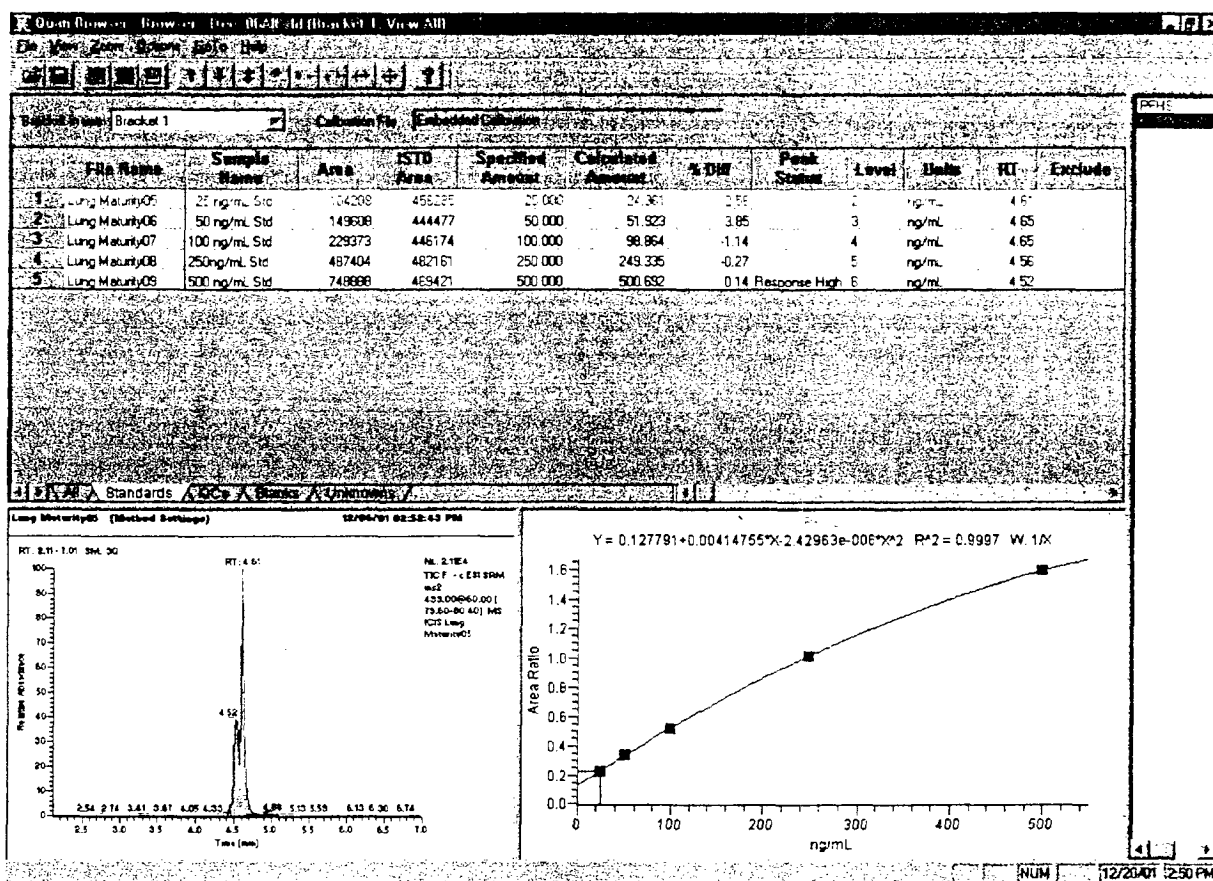
## Appendix 1

The R squared on the curve used for the calculated results was 0.9926 for the straight serum and amniotic fluid results run on the control animals and the 1:100 dilution of the dosed animal amniotic fluid extracts. The 1:500 dilution of the dosed rat serum curve had an R squared value of 0.9997.



Straight serum, diluted serum, and control dams amniotic fluid standard curve graph from original Finnigan Xcalibur program.

# Appendix 1



**3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY**  
**Protocol for Study No. T-6295.30 DT-69**  
**Oxygen Saturation and pO2 Levels in Rat Pups Borne to PFOS Exposed Dams**

***Study Objective:***

This study is the first of a series designed to investigate the mechanism of perinatal mortality in rats and mice borne to timed-pregnant dams dosed with PFOS during gestation. The hypothesis that will be tested in this study is that PFOS interferes with normal respiration and/or gas exchange in the pups. In addition, specimens will be gathered to investigate the ability of PFOS to interfere with the glucose metabolism.

***Research Client:*** 3M Specialty Chemicals Division  
3M Center, Building 236  
Saint Paul MN 55133-3220

***Sponsor:*** 3M Specialty Chemicals Division  
3M Center, Building 236  
Saint Paul MN 55133-3220

***Study Location:*** 3M Strategic Toxicology Laboratory  
3M Center, Building 270-SB314  
Saint Paul, MN 55144-1000

***Study Director:*** Deanna J. Luebker, M.S.  
Advanced Research Toxicologist  
3M Medical Dept. / Corporate Toxicology  
3M Center Building 220-2E-02  
Saint Paul, MN 55144-1000  
Ph.: 651-737-1374, FAX: 651-733-1773

***Study Toxicologist:*** Andrew M. Seacat Ph.D.  
Toxicology Specialist  
3M Medical Dept. / Corporate Toxicology  
3M Center Building 220-2E-02  
Saint Paul, MN 55144-1000  
Ph.: 651-575-3161, FAX: 651-733-1773

***Proposed Study Timeline:***

**In-Life Start Date:** September 24, 2001

**In-Life End Date:** October 2, 2001

***Regulatory Compliance:***

This study will be performed in the 3M Strategic Toxicology Laboratory in the "spirit of GLP". A defined protocol will be in place and the study will be classified as "Class B" as explained in TOX SOP 0905, Strategic Toxicology Lab GLP Program Procedure. All in-



dosing and 5 ml dosing solution / kg body weight will be administered to each rat in the PFOS treatment group. Control rats will receive 5ml/kg 2% Tween 80 on days 16-19 of gestation.

**TABLE 1: Dosing Regime**

| Dose Groups                              | N | Dosing days                                | Cumulative Dose (mg/kg) | Sacrifice dates                                               |
|------------------------------------------|---|--------------------------------------------|-------------------------|---------------------------------------------------------------|
| A. Control<br>(2% Tween 80)              | 4 | A.1: 9/24 - 9/27/01<br>A.2: 9/25 - 9/28/01 | 0                       | A.1: N = 2 on Monday 10/1/01<br>A.2: N = 2 on Tuesday 10/2/01 |
| B. PFOS treatment<br>(25 mg/kg/day PFOS) | 4 | B.1: 9/24 - 9/27/01<br>B.2: 9/25 - 9/28/01 | 100 mg/kg               | B.1: N = 2 on Monday 10/1/01<br>B.2: N = 2 on Tuesday 10/2/01 |

***Observation of Animals:***

**Clinical Observations:**

Each animal will be observed daily (excluding weekends) for mortality and morbidity and notable findings will be recorded.

**Body Weights:**

Dams will be weighed immediately prior to each dosing. Terminal body weights will be recorded on dams and litters.

***Sample Collection:***

***Number & Frequency***

Animals will be sacrificed within 24 hours of delivery. Dams will be euthanized via CO<sub>2</sub> asphyxiation and pups via decapitation. Animals have been ordered such that four will litter Sunday night, September 30<sup>th</sup>. The remaining four will litter Monday night, October 1<sup>st</sup>. The animals that litter/are borne on Sunday night will be sacrificed on Monday and the animals that litter/are borne Monday night will be sacrificed on Tuesday, within 24 hour of their birth. It is estimated that 10 to 14 pups will be born per litter. It is presumed that the dams will cannibalize pups that are stillborn or that die during the night. Eight of the remaining pups (if available) per litter will be used in the study. Three of the eight pups from each litter will be identified (marked) as time-course pups. The oxygen saturation in these pups will be monitored at several recorded time points throughout the day using the oximeter method (described below) to determine changes in oxygen saturation over time. The other pups on the study will be monitored for oxygen saturation by the oximeter method, then immediately sacrificed to obtain blood for partial oxygen pressure in blood (pO<sub>2</sub>) determination by the I-Stat method (described below). Near the end of the study, the time-course pups will be sacrificed to obtain blood for pO<sub>2</sub> determination by the I-Stat method. Any remaining pups may be sacrificed to obtain serum and tissues for possible future analysis.

***Method of Collection/Measurement***

Oxygen saturation will be measured with a pulse oximeter (Ohmeda), and pO<sub>2</sub> will be measured with an I-stat blood gas analyzer (Abbot Labs). Oxygen saturation and pulse rate will be measured on each pup using an Ohmeda Biox 3740 Pulse

**Signatures:**

Deanna J. Luebker 9/19/2001  
Deanna J. Luebker, M.S. Date  
Advanced Research Toxicologist  
Study Director

Andrew M. Seacat 9/19/01  
Andrew M. Seacat, Ph.D. Date  
Toxicology Specialist  
Study Toxicologist

Daniel A. Hakes 9/20/01  
Dan Hakes Date  
Sponsor Representative

**3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY**  
**Amendment #1 for Study No. T-6295.30 DT-69**  
**Oxygen Saturation and pO2 Levels in Rat Pups Borne to PFOS Exposed Dams**

**Sponsor:** 3M Specialty Chemicals Division  
**Study Location:** 3M Strategic Alternative Toxicology Laboratory  
**Study Director:** Deanna J. Luebker, M.S.

***Background:***

The initial study performed under this protocol resulted in very high rates of stillborn pups and early deliveries. This greatly complicated the study, preventing the endpoints of interest from being examined. It is believed that these complications were a result of a dose being delivered to the pups that was in excess of what was necessary to achieve the desired decrease in pup viability. As was stated in the original study protocol, "studies conducted by the EPA NHEERL have demonstrated that a cumulative dose of roughly 100 mg/kg will reduce pup viability by approximately 50% within the first 5 days of lactation". In the EPA studies, however, Tween 20 was used for a vehicle rather than Tween 80, as has been used in the majority of PFOS oral dosing studies. A greater amount of PFOS is presumed to be absorbed when using Tween 80 as compared to Tween 20. Thus, we believe a cumulative dose of 100 mg/kg with Tween 80 is in excess of what is necessary in these studies. In addition, the EPA has demonstrated that the later in gestation dosing occurs, the greater the rate of pup mortality. The period of dosing used under the original study protocol, days 14-17 of gestation, may have been too late to administer such a high dose of PFOS.

***Objective:***

The purpose of this amendment is to adjust the dosing regime used under the original protocol to prevent *in utero* death and early deliveries. In addition, amniotic fluid will be collected and analyzed for PFOS and caesarean-sections (c-sections) will be performed rather than allowing the dams to deliver. The purpose of the c-sections is to standardize the time of data collection. A follow-up study will thus be performed under the original study protocol with the following modifications:

1. **Page 1. Study Objective:**  
In addition to the originally stated objective, amniotic fluid will be collected from c-sectioned rats to measure PFOS levels.
2. **Page 1. Proposed Study Timeline:**  
The in-life start date for the follow-up study will be November 7, 2001 and the in-life end date will be November 14, 2001.
3. **Page 2. Animal Number:**  
Six animals will be used in the follow-up study.
4. **Page 2-3. Dose and Dosing Procedures:**

All six animals will arrive at 3M on day 11 of gestation. Three rats will be treated via oral gavage with 25 mg/kg PFOS per kg body weight per day for 3 consecutive days (days 12-14 of gestation) to reach a cumulative dose of 75 mg/kg. PFOS will be prepared as described in the original protocol. The remaining three rats will serve as controls and receive 5ml/kg body weight per day of 2% Tween 80 on days 12-14 of gestation. Any additional rats received from the vendor will be dosed with PFOS as described above and allowed to litter. Pups and dams will be monitored through day 5 of lactation to assess the dosing regime or sacrificed to collect tissues for additional analyses.

5. **Page 3. Sample Collections, Number and Frequency**

All animals, including dams, will be euthanized via decapitation on gestation day 19. Pups will be removed via c-sectioning.

In addition to that described in the original protocol, amniotic fluid will be collected from each dam following decapitation.

Liver and sera may be collected from some, not all, of the dams and pups.

6. **Page 4. Sample Handling**

Amniotic fluid will temporarily be stored at  $-70^{\circ}\text{C}$  in the Strategic Toxicology Lab, building 270. For PFOS analysis, the amniotic fluid will be shipped on dry ice to Dave Ehersman, building 236. Liver and sera will be handled as described in the original protocol.

7. **Page 4. Analysis**

In addition to the analysis outlined in the original protocol, PFOS level will be compared between PFOS treated and control dose groups and analyzed for statistical differences.

**Signatures:**

Deanna J. Luebker 10/29/01  
Deanna J. Luebker, M.S. Date  
Advanced Research Toxicologist  
Study Director

Andrew M. Seacat 10/29/01  
Andrew M. Seacat, Ph.D. Date  
Toxicology Specialist  
Study Toxicologist

Daniel A. Hakes 11/6/01  
Dan Hakes Date  
Sponsor Representative