Summary PFOS Rat Cross-Fostering

Study Numbers: 3M **T-6295.13**, Argus 418-014

Compound & Lot: PFOS (Perfluorooctanesulfonate) – Lot 217, 98.4% pure (SMD Analytical Request 53030)

C.A.S. Registry Number: 275-39-3

Study Title: Oral (Gavage) Cross-Fostering Study of PFOS in Rats

Report Date: 23 July 1999 (in-life); 11 February 2000 (serum analysis)

Study Year and GLP status: 1998 – 2000 and GLP study

Procedures: Groups of female rats (25/group) were administered Potassium Perfluorooctanesulfonate (PFOS) by daily oral intubation at dose levels of 0 or 1.6 mg/kg/day. Dosing started when the females were approximately 70 days old. The female rats were dosed for 42 days prior to mating to untreated males. After mating, dosing continued in the females throughout gestation and the 21 days of lactation.

At parturition, the control and treated groups were subdivided into additional groups for cross-fostering of litters and for obtaining pharmacokinetic samples. For cross-fostering, the litters were transferred to another dam which had delivered – no dam was allowed to raise its own litter. The four subgroups were as follows: control dams with litters from control dams (negative control); treated dams with litters from treated dams (positive control); control dams with litters from treated dams; and, treated dams with litters from control dams. On day of lactation 4, litters were culled to 10, with equal numbers per sex where possible.

On lactation day one (day of birth) lung and liver samples were collected from selected pups of control and treated litters not used for cross-fostering. These tissue samples were fixed for electron microscopic evaluation.

In addition to the cross-fostered litters, eight control dams and litters and two exposed dams and litters were assigned to pharmacokinetic sample collection on day 14 of lactation. Samples of milk, serum, liver and milk secreting glands were obtained from the dams. Pup serum and liver samples were obtained and pooled from the litters corresponding to the dams. All samples were frozen and sent to the 3M Environmental Analytical Laboratory for possible future analysis.

On days of lactation 21 and 22, serum and liver samples were obtained at term from dams and litters. Litter samples were pooled. All samples were frozen and shipped to the 3M Environmental Laboratory for possible future analysis.

Results: Mean body weights were reduced in the treated (1.6 mg/kg/day) females during the pre-mating and gestation periods. These body weight effects correlated with reduced food consumption. Reduced pup survival occurred in two of the four sub-groups as shown by the following data. Control dams X Control pups had 3/191 (1.6%) pup deaths (single death in three litters). Treated dams X Treated pups had 34/177 (19.2%) pup deaths (deaths in 8 of 12 litters). Control dams X Treated pups had 16/166 (9.6%) pup deaths (deaths in 10 of 11 litters). Treated dams X Control pups had 2/181 (1.1%) pup deaths (single death in two litters). These data indicate that reduced pup survival was a result of pup exposure *in utero* and not a result of effects on the dam from exposure.

Electron microscopy of the day-old rat pups' lungs showed no compound-related changes on Type II pneumocytes nor on the lamellar bodies or lung surfactant. Electron microscopy of the liver did reveal a compound related effect in the day-old rat pups. The liver of the pups from treated dams had increased number (2 to 3 X) of peroxisomes. Peroxisome proliferation is a known PFOS liver effect in rats. This finding indicates compound-related liver changes in the new-born rat pup delivered from dams treated with PFOS at 1.6 mg/kg/day.

Only the analysis of serum samples taken during the study is complete. On lactation day 14, control dams (n=8) and their litters (n=8) demonstrated low background levels of PFOS, with seven of the eight pairs (dams and litters) having serum concentrations of PFOS below the lower limit of quantitation (< 0.05 ppm). The eighth control pair (dam and litter) measured 0.08 ppm for the dam and 0.1 ppm for the pooled pup serum. The two exposed dams had serum PFOS levels of 98 and 218 ppm. For the dam with the 98 ppm value, the pooled pup serum was at 89 ppm PFOS. There was insufficient sample volume to determine the serum PFOS concentration from the pups associated with the dam having the 218 ppm serum PFOS.

From serum samples taken on days 21 and 22 of lactation, it is evident that exposure of pups can occur after birth via the milk. Pooled serum from pups in litters born from untreated dams and cross-fostered with treated dams (only exposed via milk) had 34 ppm PFOS on average (n = 4) compared to their treated foster-dam serum values, which averaged 72 ppm. Serum from litters born to treated dams and cross-fostered to untreated dams (only exposed *in utero*) had 54 ppm PFOS on average (n = 6) compared to their untreated foster-dam serum PFOS values, which averaged 2.54 ppm. The single-digit ppm PFOS levels observed in these untreated foster-dams is most likely from exposure to contaminated pup urine and feces, since all untreated dams which fostered pups from untreated dams had serum PFOS values at or below the lower limit of quantitation of 0.05 ppm. Litters from treated dams which were cross-fostered to other treated dams (exposed *in utero* and via milk) had the highest serum PFOS values, averaging 88 ppm (n = 6). In this latter case, the pooled litter serum PFOS concentrations were in the range of foster-dam serum values (88 ppm on average for litters vs. 98 ppm on average for their repective foster-dams). It should be noted that these samples are from the end of the lactation period (days 21 and 22), and that pups have likely started on feed around lactation days 14 and 15. Their only exposure would be *in utero* or via milk from

treated dams. These analyses clearly demonstrate that exposure occurs both *in utero* and via milk.

Conclusions: Reduced pup survival observed in the PFOS rat reproduction study (see T-6295.9) was a result of pup exposure *in utero* and not a result of effects on the dam from exposure. In-utero exposure of PFOS (dam treated at 1.6 mg/kg/day) can result in liver effects (increased peroxisomes) being present in the new-born pup. The production of pulmonary surfactant by pups does not appear to affected by exposure to PFOS *in utero*. Pups can be exposed to significant levels of PFOS from *in utero* exposure and via milk from exposed dams.

NOTE: Although samples of liver, urine, feces, milk, and milk-producing glands were taken for possible analysis of PFOS, these samples have not been analyzed. Two additional pharmacokinetic studies were undertaken to better characterize disposition of PFOS during pregnancy and lactation. The first study examines the disposition of body burden after pregnancy and through gestation. The second study examines the disposition through pregnancy and lactation. In-life is complete on these studies; however, analysis is not complete. Reports are expected by August 31, 2000. In addition, a mechanistic / pharmacokinetic study is planned to start in the second half of 2000. The objectives of this study are: 1) to better understand the possible mechanism of toxicity to the pups; 2) more precisely define the no-effect level for reduced viability; and, 3) obtain additional pharmacokinetic data. These three additional studies will enhance our complete understanding of the potential perinatal effects of PFOS as they relate to serum and tissue PFOS concentrations and potential mechanistic endpoints.