Summary PFOS Rat Two-Generation Reproduction Study

Study Numbers: 3M **T-6295.9**, Argus 418-008 (in-life), FACT-TOX-012 (analytical).

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

CAS No. 2795-39-3

Study Title: Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats.

Report Date: 10 June 1999 (in-life); 19 April 1999 (analytical).

Study Year and GLP status: 1997-1999 and GLP study

Procedures: Groups of male and female rats (35/sex/group) were administered (daily oral intubation) PFOS at dose levels of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day. Dosing started when the animals were ≈ 70 days old. The male and female rats (F_0 generation) were dosed for 42 days prior to mating. After mating, dosing continued in the mated females throughout gestation. At day 10 of gestation, Caesarean section was done on 10 females/group to check for any effects in early gestation. The remaining 25 females/group were allow to deliver their litters. Dosing of the females continued during the 21-day lactation period. At weaning, 2 males and 2 females per litter were randomly selected for continuation on the study (F_1 generation). Note: Because of post-natal effects at high doses (see below for details) only three groups of F_1 animals continued the study, namely 0, 0.1 and 0.4 mg/kg/day.
Daily dosing began in the F_1 pups the day after weaning and continued throughout their growth to sexual maturity. At 24 days of age the F_1 rats were tested in a passive avoidance paradigm. The animals were also tested in a water-filled M-maze when they were 70 days old. Cohabitation for mating began when the F_1 animals were 90 days old – littermate matings were avoided. Dosing continued in the females through gestation and 21-day lactation after delivery of F_2 pups. The study ended upon weaning of the F_2 pups.

Results: No deaths occurred in any of the F_0 animals and there were no compound related clinical signs. Dose related reduced weight body weights occurred in both male and females at the higher two dose levels (3.2 & 1.6 mg/kg/day). Reduced food consumption correlated with these body weight effects. The 0.4 mg/kg/day males also had a slight, but statistically significant, reduced body weight gain during the pre-mating period. Dosing of PFOS as high as 3.2 mg/kg/day had no effect on estrous cycling; all mating and fertility parameters of the F_0 rats were unaffected. There were no significant differences at any dose level in regard to litter averages for corpora lutea, implanations, viable embryos or nonviable embryos from the day 10 of gestation Caesarean section females.
Upon littering, post-natal survival was reduced at the higher two dose levels (1.6 & 3.2 mg/kg/day). The post-natal survival effect was manifested by increased number of

apparent still-births and increased pup deaths during the first 24 to 72 hours after birth. Necropsy of dead pups revealed many had not nursed – no milk curd in the stomach.

Reflex and physical development (surface righting, pinna unfolding, eye opening, acoustic startle reflex, air right reflex, pupil constriction) was not affected in the 0.1 & 0.4 mg/kg/day F_1 pups. Some of these parameters were slightly delayed in the 1.6 mg/kg/day F_1 pups and they were not evaluated at the high dose (3.2 mg/kg/day) as no pups survived beyond four days post-natal.
Because of adverse effects in the higher two dose levels (1.6 & 3.2 mg/kg/day) F_1 pups, reduced survival and/or reduced growth, the decision was made, in conjunction with the attending laboratory veterinarian, not to continue these two dose levels into the second generation. It had been determined that dose of 1.6 mg/kg/day or higher produced compound toxicity. Continuance of these dose levels would not result in any additional useful information and would subject the animals to unnecessary stress.
Death did not occur post-weaning in the F_1 animals nor were there any compound related clinical signs. The male and female F_1 rats in the 0.4 mg/kg/day group had somewhat reduced body weight and food consumption; at some points in time (weeks of growth) the differences from controls were statistically significant. The appearance of external evidence of sexual development (preputial separation in males & vaginal patency in females) was not affected at either dose level. Also, the passive avoidance and water maze testing did not reveal any differences in regard to learning, short-term retention nor long-term memory. Likewise, none of the mating or reproductive performance measurements (parameters) were adversely affected in the 0.1 & 0.4 mg/kg/day F_1 male and female rats when they were mated and allowed to deliver litters. There were no significant differences in regard to F_2 pup survival or pup weights at weaning.

Conclusions: The results of the study indicated the following NOEL levels.

The F_0 maternal and paternal NOEL is 0.1 mg/kg/day. [higher dose levels had reduced body weight and food consumption]

The F_0 reproductive NOEL is > 3.2 mg/kg/day. [no effect on reproductive performance even at highest dose level]

The F_1 post-natal NOEL is 0.4 mg/kg/day. [higher dose levels had reduced pup viability and growth]

The F_1 maternal and paternal NOEL is 0.1 mg/kg/day. [0.4 mg/kg/day animals had reduced body weight and food consumption]

The F_1 reproductive NOEL is > 0.4 mg/kg/day. [no effect on reproductive performance at either 0.1 or 0.4 mg/kg/day]

The F_2 post-natal NOEL is 0.4 mg/kg/day. [no important toxicological effect on pup survival or growth at either 0.1 or 0.4 mg/kg/day]

Compound Level Samples: At the terminal sacrifice of the F_0 males (after mating was completed), serum and liver samples were collected from 5 males in each of the dose groups. Serum samples were collected from 5 F_0 females in all the dose groups except 3.2 mg/kg/day group at the terminal sacrifice the day after weaning the F_1 litters. Liver samples were collected from the same F_0 females and liver samples were also collected from the weaned F_1 pups of the same dams.
Analysis of the samples for PFOS (3M Analytical Report TOX012) yielded the following mean PFOS concentrations.
Male F_0 Serum: control – 0.024 ppm, 0.1 mg/kg/day – 10.5 ppm, 0.4 mg/kg/day – 45.4 ppm, 1.6 mg/kg/day – 152 ppm, 3.2 mg/kg/day – 273 ppm.
Male F_0 Liver: control – 0.665 ppm, 0.1 mg/kg/day – 84.9 ppm, 0.4 mg/kg/day – 176 ppm, 1.6 mg/kg/day – 323 ppm, 3.2 mg/kg/day – 1357 ppm.
Female F_0 Serum: control – 0.037 ppm, 0.1 mg/kg/day – 5.28 ppm, 0.4 mg/kg/day – 18.9 ppm, 1.6 mg/kg/day – 82.0 ppm.
Female F_0 Liver: control – 0.171 ppm, 0.1 mg/kg/day –14.8 ppm, 0.4 mg/kg/day – 58.0 ppm, 1.6 mg/kg/day – 184 ppm.
Pup F_1 Liver: control – 0.051 ppm, 0.1 mg/kg/day – 6.19 ppm, 0.4 mg/kg/day – 57.6 ppm, 1.6 mg/kg/day – 70.4 ppm.
Note: Serum and liver reflect PFOS concentrations of F_0 dams and F_1 pups at the end of 21-day lactation, i.e. when the samples were collected. Compound levels at parturition when reduced pup survival was observed could differ and those are being measured in a follow-up PK study.

Data Evaluation: A well conducted GLP study following established testing guidelines (OECD 416). Study evaluated reproductive parameters and post-natal pup development including neurological parameters. The study established valid NOEL values.

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Milk Curd Samples: On day 4 of lactation, all litters, as stated in the protocol, were reduced to 8 pups per litter. At this time stomach contents (milk curd) samples, pooled by litter, were collected from 4 or 5 litters from three dose groups – control, low dose (0.1 mg/kg/day) and high dose (3.2 mg/kg/day). A validated analytical method for milk curd matrix has not been developed and these samples have not been analyzed.