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**CO-OPERATION ON EXISTING CHEMICALS**

**DRAFT ASSESSMENT OF PERFLUOROOCTANE SULFONATE (PFOS) AND ITS SALTS:  
COMPLETE ASSESSMENT**

**34th Joint Meeting, 5-8 November 2002, to be held at the OECD Headquarters, Paris,  
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*This is a background document to ENV/JM(2002)29: DRAFT ASSESSMENT OF PERFLUOROOCTANE SULFONATE (PFOS) AND ITS SALTS: CONCLUSIONS AND RECOMMENDATIONS.*

*This document was prepared by the US-EPA and the UK Environment Agency and has been endorsed by the Task Force on Existing Chemicals.*

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## Preface

In the margins of the ninth meeting of the Task Force on Existing Chemicals (29-30 May 2000) several Member countries agreed to informally work together to collect information on the environmental and human health hazards of perfluorooctane sulfonate (PFOS) to produce a hazard assessment. The decision followed the announcement by a major US manufacturer – 3M – to globally phase out the manufacture and use of these chemicals beginning in 2001. The US and the UK agreed to lead the activity with the Secretariat assisting by requesting readily available exposure information from Member countries as well as from non-Member countries through IFCS.

An informal meeting was hosted by the US on 26-27 October 2000 (Crystal City, Virginia, US) to:

- review the current status of assessment activities;
- learn about actions being taken in other countries and
- identify planned or ongoing work on this issue.

In preparation for the meeting 3M circulated a draft initial assessment report including robust study summaries of key studies, together with exposure information.

At the 31<sup>st</sup> Joint Meeting (7-10 November 2000), it was agreed that, since this was a matter of sufficient interest to all Member countries, this activity should be undertaken under the existing Chemicals Programme, overseen by the Task Force. As PFOS is not an HPV Chemical, it was not dealt with under the HPV Chemicals Programme.

A draft hazard assessment was posted on the OECD web site for comment in December 2000. The OECD established an electronic discussion group to exchange comments and information. A special session on PFOS and its salts was held on 25 January 2001 in Orlando, USA, as part of the 11<sup>th</sup> SIAM meeting. At this session an overview of the draft hazard assessment was presented.

The draft hazard assessment has been revised twice since December 2000 to incorporate comments that were received, as well as to incorporate newly completed studies. Comments were received from 3M, World Wildlife Fund, Health Canada, Environment Canada, and Australia.

At the 11<sup>th</sup> meeting of the Task Force on Existing Chemicals (27-28 May 2002), the revised hazard assessment was discussed. The Task Force agreed with the conclusions and recommendations of the hazard assessment. The Task Force also agreed that the Secretariat should gather information from governments and BIAC on risk management activities currently undertaken or planned in Member countries on PFOS.

This hazard assessment of perfluorooctane sulfonate (PFOS) and its salts includes all information that was available by July 2002. A quantitative risk assessment was not conducted as this should entail regional exposure information. The hazard information on PFOS should be used with caution in evaluating the potential hazards of other perfluorinated compounds. The perfluorinated compounds represent a very unique chemistry whose toxicological properties are presently not well understood and clearly the presence of different length (perfluorinated) carbon chains and functional groups are likely to influence toxicity. It is not clear at this time whether the hazard concerns of PFOS can be extrapolated to other perfluorinated compounds except under circumstances where the compound may degrade to PFOS.

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## RECOMMENDATIONS OF THE HAZARD ASSESSMENT

Perfluorooctane sulfonate (PFOS) is a candidate for further work.

Sufficient information exists to address hazard classification for all SIDS human health endpoints. PFOS is persistent, bioaccumulative and toxic to mammalian species. There are species differences in the elimination half-life of PFOS; the half-life is 100 days in rats, 200 days in monkeys, and years in humans. The toxicity profile of PFOS is similar among rats and monkeys. Repeated exposure results in hepatotoxicity and mortality; the dose-response curve is very steep for mortality. This occurs in animals of all ages, although the neonate may be more sensitive. In addition, a 2-year bioassay in rats has shown that exposure to PFOS results in hepatocellular adenomas and thyroid follicular cell adenomas; the hepatocellular adenomas do not appear to be related to peroxisome proliferation. Further work to elucidate the species differences in toxicokinetics and in the mode of action of PFOS will increase our ability to predict risk to humans. Epidemiologic studies have shown an association of PFOS exposure and the incidence of bladder cancer; further work is needed to understand this association.

Sufficient information exists to address hazard classification for all SIDS environmental endpoints. PFOS is persistent in the environment and has been shown to bioconcentrate in fish. It has been detected in a number of species of wildlife, including marine mammals. Its persistence, presence in the environment and bioaccumulation potential indicate cause for concern. It appears to be of low to moderate toxicity to aquatic organisms but there is evidence of high acute toxicity to honey bees. No information is available on effects on soil- and sediment-dwelling organisms and the equilibrium partitioning method may not be suitable for predicting PNECs for these compartments. PFOS has been detected in sediment downstream of a production site and in effluents and sludge from sewage treatment plants.

Given the apparent widespread occurrence of PFOS, national or regional exposure information gathering and risk assessment may need to be considered. In addition, data on its toxicity to soil and sediment-dwelling organisms could be generated as a post-SIDS activity. There is currently no information on effects on soil- or sediment-dwelling organisms and PFOS has been detected in sediment and its presence in sewage sludge could lead to soil exposure if spread on agricultural land.

## SUMMARY AND CONCLUSIONS OF THE HAZARD ASSESSMENT

Perfluorooctane sulfonate (PFOS) and its salts are fully fluorinated organic compounds. The number of production sites is not clear, but there is production in the US, Europe and Japan. In recent years (to 2000), approximately 4,500 metric tons of PFOS-related chemicals have been produced annually. The major global producer of PFOS intends to cease production by the end of 2002.

The majority of PFOS-related chemicals are high molecular weight polymers in which PFOS represents a fraction of the total molecular weight. PFOS-related chemicals are used in a variety of products, including as surface-treatments of fabric for soil/stain resistance, coating of paper as part of a sizing agent formulation and in specialised applications such as fire fighting foams.

PFOS has a solubility of approximately 550 mg/l in pure water at 24-25°C. The solubility decreases significantly with increased salt content, for example the potassium salt of PFOS has a solubility in fresh water of 370 mg/L and of 25 mg/l in filtered sea water. Due to the surface-active properties of PFOS, the Log Kow cannot be measured. The potassium salt of PFOS has a low vapour pressure,  $3.31 \times 10^{-4}$  Pa at 20 °C.

## Human Health

In human blood samples, PFOS has been detected in the serum of occupational and general populations. In the U.S., the highest reported blood serum level of PFOS was observed in 1995 in a manufacturing employee in Decatur, Alabama (12.83 ppm). Mean PFOS levels have been dropping in that plant and a plant in Belgium since 1995, the most recent being 1.32 ppm and 0.80 ppm, respectively, in 2000. In the general population, serum collected from blood banks and commercial sources have indicated mean PFOS levels of 30-53 ppb. In individual serum samples obtained from adults and children in various regions of the U.S., mean PFOS levels were approximately 43 ppb.

Several occupational studies have been conducted on volunteers at the 3M plants in Decatur, Alabama and Antwerp, Belgium. Cross-sectional studies, based on the results of a voluntary medical surveillance program for employees at each plant, did not report consistent associations between workers' PFOS levels less than 6 ppm and certain hematology, hormonal, and other clinical chemistry parameters in 1995 and 1997. In 2000 when the analysis included male employees from both plants, mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly ( $p < .05$ ) higher for workers with the highest PFOS serum levels (1.69 – 10.06 ppm). Serum triiodothyronine was significantly higher and thyroid hormone binding ratio was significantly lower in workers with the highest PFOS serum levels ( $p < .05$  for both). The association with T3 also remained significant and positive in multivariable regression analyses adjusted for potential confounders. A longitudinal analysis of these data did not reveal statistically significant associations over time between PFOS and cholesterol, triglycerides, and other lipid and hepatic parameters. Hormones were not included in these analyses. There are several limitations to both the cross-sectional and longitudinal studies, such as the voluntary nature of the medical surveillance, the small number of employees participating across sampling periods, the different labs and analytical techniques used to measure serum PFOS, and the differences in PFOS levels, demographics, and clinical chemistries between employees in the Decatur and Antwerp plants.

In a mortality study, which followed workers for 37 years, mortality risks for most of the cancer types and non-malignant causes were not elevated. However, a statistically significant risk of death from bladder cancer was reported. Three male employees in the cohort died of bladder cancer (0.12 expected), and all of them had been employed at the plant for more than 20 years. All of them had also worked in high exposure jobs for at least 5 years. In order to screen for morbidity outcomes, an "episode of care" analysis was undertaken for employees who had worked at the plant between 1993 and 1998. Many different types of cancer and other non-malignant conditions were examined. Increased risks were not reported for most of the conditions or did not reach statistical significance. However, an increased risk of episodes was reported for neoplasms of the male reproductive system, the overall category of cancers and benign growths, and neoplasms of the gastrointestinal tract. These risk ratios were highest in employees with the highest and longest exposures to fluorochemicals.

Animal studies show that PFOS is well absorbed orally and distributes mainly in the serum and the liver. No further metabolism is expected. Elimination from the body is slow and occurs via the urine and feces. There are species differences in the elimination half-life of PFOS. The elimination half-life is 100 days in adult rats and 200 days in *Cynomolgus* monkeys. In humans, it appears to be quite longer. A recent half-life analysis was conducted on 9 retired 3M chemical workers. PFOS samples were collected over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. The mean half-life for PFOS was 8.67 years (range 2.29 – 21.3 years, SD = 6.12).

PFOS has shown moderate acute toxicity by the oral route with a rat LD50 of 251 mg/kg. A one-hour LC50 of 5.2 mg/l in rats has been reported. PFOS was found to be mildly irritating to the eyes and non-irritating to the skin of rabbits. PFOS has not been shown to be genotoxic in a variety of assay systems.

Numerous repeat-dose oral toxicity studies on PFOS have been conducted in rats and primates. In general, exposure to PFOS results in hepatotoxicity and mortality; the dose-response curve for mortality is very steep for rats and primates. Adverse signs of toxicity observed in 90-day rat studies included increases in liver enzymes, hepatic vacuolization and hepatocellular hypertrophy, gastrointestinal effects, hematological abnormalities, weight loss, convulsions, and death. These effects were reported at doses of 2 mg/kg/day and above. In a dietary 2-year bioassay in Sprague-Dawley rats, hepatotoxicity, characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm. Hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration were found in all the male treated groups (0.5, 2, 5, or 20 ppm). Based on the pathological findings in the liver, the LOAEL was 5 ppm and the NOAEL was 2 ppm in female rats. In males, the LOAEL was 0.5 ppm, and a NOAEL was not established.

Adverse signs of toxicity observed in Rhesus monkey studies included anorexia, emesis, diarrhea, hypoactivity, prostration, convulsions, atrophy of the salivary glands and the pancreas, marked decreases in serum cholesterol, and lipid depletion in the adrenals. The dose range for these effects was reported between 1.5-300 mg/kg/day. No monkeys survived beyond 3 weeks into treatment at 10 mg/kg/day or beyond 7 weeks into treatment at doses as low as 4.5 mg/kg/day. In a 6-month study of Cynomolgus monkeys, low food consumption, excessive salivation, labored breathing, hypoactivity, ataxia, hepatic vacuolization and hepatocellular hypertrophy, significant reductions in serum cholesterol levels, and death were observed at 0.75 mg/kg/day. No effects were observed at doses of 0.15 or 0.03 mg/kg/day. No effects were noted in animals at any dose level following a 52-week recovery period. The average concentration of PFOS in the serum following 26 weeks of treatment was  $11.1 \pm 1.52$ ,  $58.5 \pm 4.67$  and  $160 \pm 23.9$   $\mu\text{g/ml}$  for the females in the 0.03, 0.15 and 0.75 mg/kg/day groups, respectively; for males, the average concentrations were  $15.9 \pm 5.54$ ,  $68.1 \pm 5.75$  and  $194 \pm 8.93$   $\mu\text{g/ml}$  in the 0.03, 0.15 and 0.75 mg/kg/day groups, respectively. After the 52-week recovery period, the serum levels were  $21.4 \pm 2.01$  and  $41.4 \pm 1.15$   $\mu\text{g/ml}$  for the females in the 0.15 and 0.75 mg/kg/day groups, respectively; for males, the average concentrations were  $19.1 \pm 0.805$  and  $41.1 \pm 25.9$   $\mu\text{g/ml}$  in the 0.15 and 0.75 mg/kg/day groups, respectively.

The potential carcinogenicity of PFOS has been examined in a dietary 2-year bioassay in Sprague-Dawley rats. There was a significant increase in the incidence of hepatocellular adenomas in males and females at the highest dose of 20 ppm; the females at 20 ppm also had a significant increase in combined hepatocellular adenomas and carcinomas. In addition, there was a significant increase in thyroid follicular cell adenomas and combined thyroid follicular cell adenomas and carcinomas in the male recovery group at 20 ppm. There was no evidence of peroxisome proliferation in the livers of the treated animals.

Postnatal deaths and other developmental effects were reported at low doses in offspring in a 2-generation reproductive toxicity study in rats. At the two highest doses of 1.6 and 3.2 mg/kg/day, pup survival in the first generation was significantly decreased. All first generation offspring (F1 pups) at the highest dose died within a day after birth while close to 30% of the F1 pups in the 1.6 mg/kg/day dose group died within 4 days after birth. As a result of the pup mortality in the two top dose groups, only the two lowest dose groups, 0.1 and 0.4 mg/kg/day, were continued into the second generation. The NOAEL and LOAEL for the second generation offspring (F2 pups) were 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in pup body weight.

The liver and serum from the F0 and F1 animals was analyzed for PFOS. Qualitatively, the results for the F0 animals indicate that all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of

the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group.

Based on the results of the two-generation reproductive toxicity study, a cross-fostering study was conducted as a means of determining whether the reductions in pup viability were a result of *in utero* exposure to PFOS or as a result of exposure during lactation; thus the potential for a distinction to be made between prenatal and postnatal effects following continuous maternal treatment. Under the limited conditions of the study, the data appear to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse effect on pup viability.

Several mechanistic studies are being conducted to understand the neonatal death (3M Company, 2001c). Preliminary results indicate that reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the death of the offspring.

Developmental effects were also reported in prenatal developmental toxicity studies in the rat and rabbit, although at slightly higher dose levels. Signs of developmental toxicity in the offspring were evident at doses of 5 mg/kg/day and above in rats administered PFOS during gestation. Significant decreases in fetal body weight and significant increases in external and visceral anomalies, delayed ossification, and skeletal variations were observed. A NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for developmental toxicity were indicated. In the same study, evidence of treatment-related signs of maternal toxicity were also observed at doses of 5 mg/kg/day and above and mainly consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough hair coat, and bloody crust, as well as decreases in body weight gains and food consumption. Reductions in the mean terminal body weights minus the gravid uterine weights were also observed at doses  $\geq$  5 mg/kg/day. A NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for maternal toxicity were indicated. In rabbits, significant reductions in fetal body weight and significant increases in delayed ossification were observed in the offspring of pregnant females administered PFOS during gestation at doses of 2.5 mg/kg/day and above. A NOAEL of 1.0 mg/kg/day and a LOAEL of 2.5 mg/kg/day for developmental toxicity were indicated. Maternal toxicity in the does was evident at doses of 1.0 mg/kg/day and above, and consisted of an increase incidence of abortions and scant feces, as well as significant reductions in mean maternal body weight gains and food consumption. A NOAEL of 0.1 mg/kg/day and a LOAEL of 1.0 mg/kg/day for maternal toxicity were indicated.

## Environment

There is currently little information on the life-cycle steps that may lead to release of PFOS to the environment. However, PFOS has been detected in surface water and sediment downstream of a production facility and in wastewater treatment plant effluent, sewage sludge and landfill leachate at a number of cities in the US. Sampling of several wildlife species from a variety of sites across the United States has shown widespread distribution of PFOS and it was detected in the ppb range in the plasma of several species of eagles, wild birds, and fish. PFOS has been detected in marine mammals at a number of locations across the world.

PFOS is persistent in the environment. It does not hydrolyse, photolyse or biodegrade under environmental conditions and is not expected to volatilize, based on an air/water partition coefficient of  $< 2 \times 10^{-6}$  Pa.m<sup>3</sup>/mol. PFOS has been shown to bioconcentrate in the tissues of bluegill sunfish and carp. In bluegill sunfish, BCF (BCFK) values between 1124 and 4013 were determined and PFOS depurated slowly with estimated 50% clearance times of up to 116 days. In carp, BCF values were determined to be between 200 and 1500.



The substance shows moderate acute toxicity to aquatic organisms, the lowest LC<sub>50</sub> for fish is a 96-hour LC<sub>50</sub> of 4.7 mg/l to the fathead minnow *Pimephales promelas* for the lithium salt. For aquatic invertebrates, the lowest EC<sub>50</sub> for freshwater species is a 48-hour EC<sub>50</sub> of 27 mg/l for *Daphnia magna* and for saltwater species, a 96-hour LC<sub>50</sub> value of 3.6 mg/l for the Mysid shrimp *Mysidopsis bahia*. Both tests were conducted on the potassium salt. For algae, the potassium salt gave a 96h NOEC of >3.2 mg/l with *Skeletonema costatum*.

Long-term toxicity data is available for fish and aquatic invertebrates. The lowest NOEC for fish is a 42 day NOEC (survival) of 0.3 mg/l in an early life stage test with *Pimephales promelas* using the potassium salt. The lowest NOEC for aquatic invertebrates is a 35-day NOEC<sub>reproduction</sub> of 0.25 mg/l for *Mysidopsis bahia* using the potassium salt. For freshwater species, there is a 28-day NOEC<sub>reproduction</sub> of 7 mg/l for *Daphnia magna*, also using the potassium salt.

A growth inhibition test has been carried out on PFOS potassium salt with *Lemna gibba* (Duckweed). The test gave a 7-day IC<sub>50</sub> of 108 mg/l for inhibition of frond production and a 7-day NOEC of 15.1 mg/l based on the inhibition of frond production and evidence of sub-lethal effects.

PFOS does not appear to be toxic to sewage sludge microorganisms. In an activated sludge respiration inhibition test, the 3-hour IC<sub>50</sub> value for PFOS (potassium salt) was >905 mg/l (nominal concentration).

No data are available for effects on soil-dwelling or sediment-dwelling species. The use of equilibrium partitioning models to derive a PNEC for these compartments may not be applicable to this anionic surfactant.

PFOS has been tested on two species of bird, the Mallard duck, *Anas platyrhynchos*, and the Northern Bobwhite quail, *Colinus virginianus*. The lowest acute dietary LC<sub>50</sub> value of 220 mg/kg of food was determined in the test with the quail. The lowest NOEC of 37 mg/kg of food for effects on body weight was, in contrast, obtained in the test with the duck.

There are data available from acute oral and contact toxicity tests on the Honey bee (*Apis mellifera*) using PFOS potassium salt. These studies indicate moderate and high orders of toxicity of PFOS to bees when administered via these routes. The acute oral test yielded a 72-hour LD<sub>50</sub> for ingestion of PFOS of 0.40 µg/bee and a 72-hour NOEL of 0.21 µg/bee. The contact test yielded a 96-hour LD<sub>50</sub> of 4.78 µg/bee and a 96-hour NOEL of 1.93 µg/bee.

The results of an amphibian teratogenesis study carried out with *Xenopus laevis* (African clawed frog) show PFOS potassium salt to be acutely toxic to (96-hour LC<sub>50</sub> = 13.8 mg/l), and cause malformations in (96-hour EC<sub>50</sub> = 12.1 mg/l), frog embryos. The minimum concentration that inhibited growth of the embryos was determined to be 7.97 mg/l. A teratogenic index of 1.1 was determined from the ratio of the 96-hour LC<sub>50</sub> to the 96-hour EC<sub>50</sub>, indicating a low potential for PFOS to be a developmental hazard in this species.

## 1.0 Identity

Chemical Name: Perfluorooctane Sulfonate

The perfluorooctane sulfonate anion (PFOS) does not have a specific CAS number. The acid and salts have the following CAS numbers:

acid (1763-23-1)  
ammonium (NH<sub>4</sub><sup>+</sup>) salt (29081-56-9)  
diethanolamine (DEA) salt (70225-14-8)  
potassium (K<sup>+</sup>) salt (2795-39-3)  
lithium (Li<sup>+</sup>) salt (29457-72-5)

Molecular formula: C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>

Structural formula: CF<sub>3</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-S(=O)(=O)O-

Synonyms: 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro;  
1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid;  
1-Octanesulfonic acid, heptadecafluoro-;  
1-Perfluorooctanesulfonic acid;  
Hepatadecafluoro-1-octanesulfonic acid;  
Perfluoro-n-octanesulfonic acid;  
Perfluorooctanesulfonic acid;  
Perfluorooctylsulfonic acid

## 1.1 Physicochemical Properties

Due to the surface-active properties of PFOS and the test protocol itself, PFOS forms three layers in octanol/water and hence, an n-octanol/water (K<sub>ow</sub>) partition coefficient cannot be determined. Consequently, the various physicochemical properties (e.g., bioconcentration factor, soil adsorption coefficient), which can usually be estimated for conventional organic compounds utilizing K<sub>ow</sub> equations, cannot be estimated, and a calculated (estimated) log K<sub>ow</sub> cannot be trusted. Even if the log K<sub>ow</sub> were known, it may not be appropriate for predictive purposes, e.g., bioconcentration. Studies on laboratory rats indicate that PFOS does not bioconcentrate in the lipid fraction. Instead, it tends to bind to certain proteins. In two studies, PFOS was reported to have a mean solubility of 519 mg/L and 570 mg/L in pure water at 24-25°C. Solubility decreases significantly with increased salt content (12.4 mg/L in natural seawater at 22-23°C, and 20.0 mg/L in a 3.5% NaCl solution at 22-24°C (3M Company, 2001a). In a related study, PFOS was reported to have a mean solubility of 56.0 mg/L in pure octanol (3M Company, 2001b). These data suggest that any PFOS discharged to a water source would tend to remain in that medium, unless it is adsorbed onto particulate matter or assimilated by organisms. If PFOS does bind to particulate matter the material would ultimately end up in the sediment. Further study is underway to determine the presence of PFOS in sediments from various locations and the binding potential of PFOS to sediments.

The available physicochemical properties for the potassium salt of PFOS are as follows (3M Report, 1999):

Melting point: >= 400 C  
Boiling point: not calculable  
Vapor pressure: 3.31 E -4 Pa @ 20 C ( 3.27 E-9 atm)

Air/water partition coefficient in pure water: 0 (<2 E-6)

Solubility: pure water: 570 mg/L

Solubility: fresh water: 370 mg/L

Solubility: unfiltered seawater: 12.4 mg/L

Solubility: filtered seawater: 25 mg/L

The specific gravities (water = 1) and pH values (in parentheses) of the PFOS salts are as follows (3M Report, 1999):

DEA, ~ 1.1 (~7)

NH<sub>4</sub><sup>+</sup>, ~ 1.1 (~7)

Li<sup>+</sup>, ~1.1 (6-8)

K<sup>+</sup>, ~0.6 (7-8)

The above water solubility values correspond to the following Henry's Law constants (atm.m<sup>3</sup>/mol), calculated herein, utilizing the vapor pressure of 3.27 E-9 atm given in the 3M Report (1999):

Pure water: 3.05 E-9

Fresh water: 4.7 E-9

Unfiltered seawater: 1.4 E-7

Filtered seawater: 2.4 E-8

As a point of reference, the Henry's Law constant for pure water @ 20 C is 4.34 E-7.

## 2.0 General Information on Exposure

The starting material for PFOS-related chemicals is perfluorooctanesulfonyl fluoride (POSF). POSF is manufactured through a process known as Simons Electro-Chemical Fluorination (ECF) in which an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedstock of 1-octanesulfonyl fluoride. The ECF process replaces the carbon-hydrogen bonds on molecules of the organic feedstock with carbon-fluorine bonds. Perfluorination occurs when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields between 30-45 percent straight chain (normal) POSF, along with a variable mixture of byproducts and impurities. The output of the ECF process is not a pure chemical, but instead a mixture of isomers and homologues including higher and lower straight-chain homologues; branched-chain perfluoroalkyl fluorides of various chain lengths; straight-chain, branched, and cyclic perfluoroalkanes and ethers; and other byproducts (3M Company, 2000b).

According to information available to the U.S. Environmental Protection Agency (USEPA), 3M Company is the dominant producer of POSF. In 1997, 3M reported the manufacture or importation into the United States of approximately 1,848 metric tons of POSF. For 2000, 3M forecasts a volume of 1,820 metric tons manufactured or imported into the United States. After accounting for 3M operations in Antwerp, Belgium, 3M estimates a total global POSF production of 3,665 metric tons for 2000 (3M Company, 2000d). Production of POSF by 3M is expected to decline to zero by the end of 2002 as 3M scales back the production POSF-derived chemicals (3M Company, 2000d).

Since most POSF is incorporated into higher molecular weight polymers, it comprises only a portion of the mole fraction of the entire polymer in the final product. However, it should be noted that the secondary reactions used to produce POSF derivatives do not necessarily produce pure products. Typically, 1-2% of the final product is comprised of unreacted or partially reacted fluorinated starting materials or intermediates that are carried forward into the final product as impurities (3M Company, 2000b).

To date, 3M has not provided information on the total cumulative production volumes of POSF or PFOS-related chemicals since initial commercialization over 40 years ago. Precise production volume information for manufacturers other than 3M outside the United States has also been difficult to obtain. 3M, however, has asserted that it is the dominant global producer of PFOS chemicals, responsible for the large majority of total global production volumes (3M Company, 2000e). The limited production volume information provided by OECD member countries for companies located outside the United States supports this conclusion.

Aside from the United States and Belgium, other OECD Member countries that reportedly have production capacity include Italy and Japan. There may also be some production in non-OECD countries. Following are companies that have been identified in various chemical buyer's guides as offering PFOS-related chemicals for sale (Directory of World Chemical Producers, 2000; ChemSources USA, 2000; OPD Chemical Buyers Directory, 2000). This information has not been corroborated independently, except for Miteni S.p.A. of Italy and Dianippon Ink & Chemicals, Inc. of Japan.

#### OECD Countries

- Miteni S.p.A. (Italy)
- EniChem Synthesis S.p.A. (Italy)
- Dianippon Ink & Chemicals, Inc. (Japan)
- Midori Kaguka Co., Ltd. (Japan)
- Tohkem Products Corporation (Japan)
- Tokyo Kasei Kogyo Company, Ltd. (Japan)
- Fluka Chemical Co, Ltd. (Switzerland)
- BNFL Fluorochemicals Ltd. (United Kingdom)
- Fluorochem Ltd. (United Kingdom)

#### Non-OECD Countries

- Milenia Agro Ciencias S.A. (Brazil)
- Changjiang Chemical Plant (China)
- Indofine Chemical Company, Inc. (India)
- Scientific Industrial Association P & M Ltd. (Russian Federation)

### **2.1 Production and Use of PFOS**

The majority of PFOS-related chemicals are high molecular weight polymers in which PFOS represents a fraction of the total molecular weight. PFOS-related chemicals are used in a variety of products, as shown in the figure following this section. These products can be divided into three main categories of use:

- Surface treatments,
- Paper protection, and
- Performance chemicals.

Each of these categories and the associated 3M production volume is described in more detail below. Note that in many cases the production volumes represent total solid metric tons of fluorochemical-containing compound, not PFOS itself. Less than 91 metric tons of PFOS and its salts are commercialized as finished products (3M, 2000b).

In addition, varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials

or intermediates) are carried forward into final products at concentrations of 1-2% or less as a result of the ECF manufacturing process. These residuals also have the potential to degrade or metabolize to PFOS (3M, 2000b).

### **2.1.1 PFOS-Based Surface Treatment Applications**

PFOS-related chemicals produced for surface treatment applications provide soil, oil, and water resistance to personal apparel and home furnishings. Specific applications in this use category include protection of apparel and leather, fabric/upholstery, and carpet. These applications are undertaken in industrial settings by customers such as textile mills, leather tanneries, finishers, fiber producers, and carpet manufacturers. PFOS-related chemicals are also used in aftermarket treatment of apparel and leather, upholstery, carpet, and automobile interiors by the general public or professional applicators (3M Company, 2000b). In 2000, the global production volume of PFOS-related chemicals for this use category is estimated at approximately 2,160 metric tons (3M Company, 2000b).

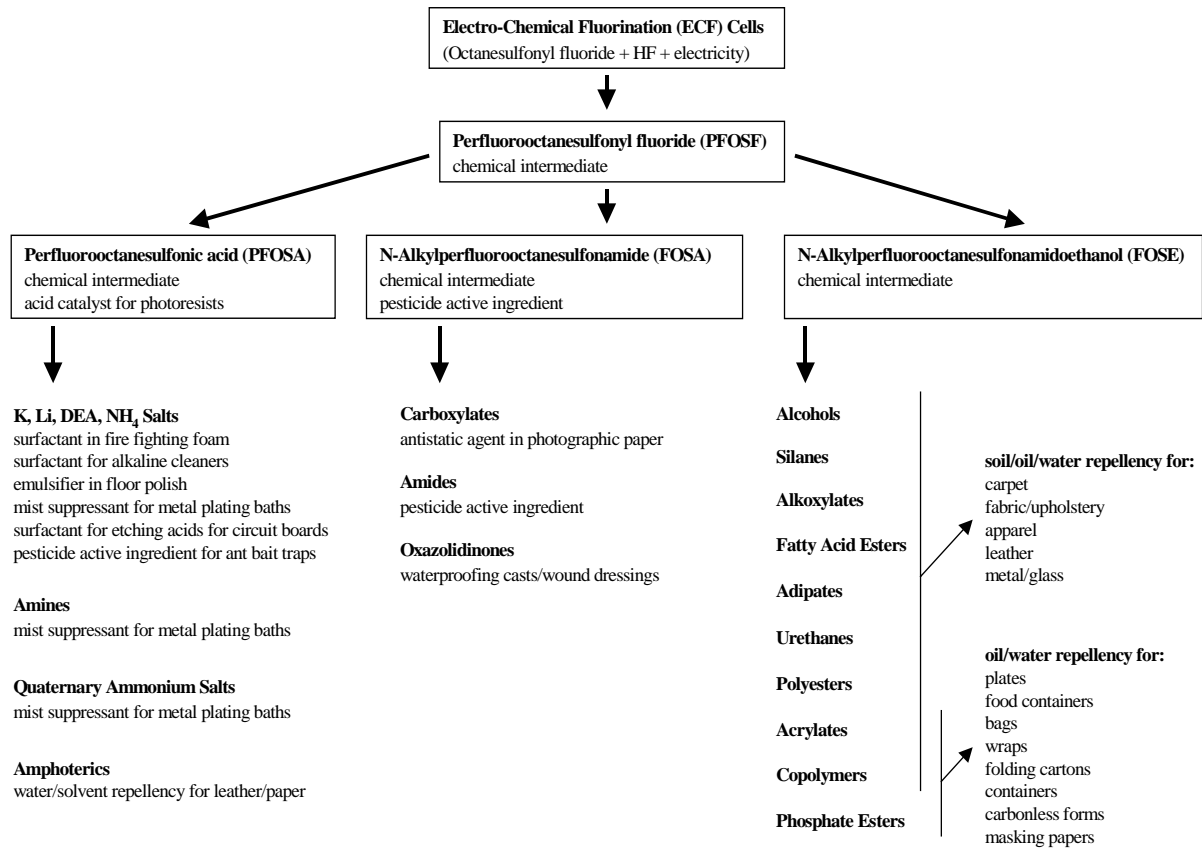
### **2.1.2 PFOS-Based Paper Protection Applications**

PFOS-related chemicals produced for paper protection applications provide grease, oil, and water resistance to paper and paperboard as part of a sizing agent formulation. Specific applications in this use category include food contact applications (plates, food containers, bags, and wraps), as well as non-food contact applications (folding cartons, containers, carbonless forms, and masking papers). The application of sizing agents is undertaken mainly by paper mills and, to some extent, converters who manufacture bags, wraps, and other products from paper and paperboard (3M Company, 2000d). In 2000, the global production volume of PFOS-related chemicals for this use category is estimated at approximately 1,490 metric tons (3M Company, 2000b).

### **2.1.3 PFOS-Based Performance Chemical Applications**

PFOS-related chemicals in the performance chemical category are used in a variety of specialized industrial, commercial, and consumer applications. This category includes various salts of PFOS that are commercialized as finished products. Specific applications in this category include fire fighting foams, mining and oil well surfactants, acid mist suppressants for metal plating and electronic etching baths, photolithography, electronic chemicals, hydraulic fluid additives, alkaline cleaners, floor polishes, photographic film, denture cleaners, shampoos, chemical intermediates, coating additives, carpet spot cleaners, and as an insecticide in bait stations (3M Company, 2000b; Boeing, 2000). In 2000, the global production volume of PFOS chemicals for this use category is estimated at approximately 831 metric tons (3M Company, 2000d). Of this volume, approximately 151 metric tons will be used in fire fighting foams.

**Perfluorooctyl Sulfonates: Major Product Categories**



## 2.2 Environmental Exposure and Fate

PFOS does not hydrolyze, photolyze or biodegrade under environmental conditions and it is persistent in the environment.

### 2.2.1. Volatility

PFOS is not expected to volatilize, based on an assigned air/water partition coefficient of  $< 2 \times 10^{-6}$  Pa.m<sup>3</sup>/mol. This assignment was made by Prof. Donald Mackay based on experimental data generated by 3M. In that study, the Hc was too small to measure experimentally using pure water. The interpretation by Mackay states “[PFOS] is thus essentially non-volatile from aqueous solution. This is probably because of its ionic nature. The simple expedient is to assign it a Kaw of zero, i.e. is a type 2 involatile chemical in our nomenclature.” This determination is in agreement with the Henry’s Law constant of  $3.05 \times 10^{-9}$  calculated herein for pure water. Overall, the conclusion is that PFOS is a substance with very low and possibly negligible volatility.

### 2.2.2 Combustion

To begin to understand the potential degradation pathways for perfluorinated chemicals, the incineration process was thermodynamically modeled. The most pertinent finding of the reported analysis is that the carbon-sulfur bond in the perfluorooctane sulfonate molecule is a fairly weak bond. To validate the models prediction, laboratory combustion studies on a series of perfluoroalkyl compounds, including PFOS and two polymeric product formulations are being conducted. An assessment will be made of combustion by-products through a range of temperatures. These data will be important to determine whether PFOS may enter the atmosphere as a result of incomplete combustion of waste.

### 2.2.3 Photolysis/Oxidation

PFOS does not appear to photolyze (Hatfield, T. 2001). Screening studies on the aqueous photolytic degradation of PFOS, EtFOSE alcohol, MeFOSE alcohol, EtFOSA and MeFOSA as well as a surfactant and foamer product, all appeared to undergo indirect photolysis to FOSA, PFOA, a hydride and olefins; PFOS was not detected (Hatfield, T. 2001, 3M Report No. W2775). One product, an aromatic perfluorooctane sulfonate, did photodegrade to form PFOS (3M Report, 1999).

### 2.2.4 Biodegradation

The biodegradability of PFOS was examined in a MITI-I test (Kurume Laboratory (2002)). No significant degradation of PFOS was observed in 28 days, either as net oxygen demand from degradation of test substance (i.e., ultimate degradation), loss of total organic carbon (TOC; another way to measure ultimate degradation), or loss of parent compound identity (primary degradation). Average percentage biodegradation after 28 days was observed to be 0% by oxygen demand; 6% by removal of total organic carbon; and 3% by liquid chromatography-mass spectrometry (LC-MS; measures primary degradation). These values are indicative of no significant degradation, within the accuracy limits for this test. Validity of the test was confirmed by degradation of the control substance aniline, for which % of theoretical oxygen demand exceeded the pass criterion of 60% at both 7 and 14 days (74% and 85% of theoretical at days 7 and 14, respectively).

## 2.3 Environmental Monitoring

3M’s Multi-City Study reported on PFOS concentrations from water, sludge, sediment, POTW effluent and landfill leachate samples taken in six cities (3M, 2001a). Four of the cities (Decatur AL, Mobile AL,

Columbus GA, Pensacola FL) were supply cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland TN, Port St. Lucie FL) were control cities that do not have significant fluorochemical activities. Across all cities, POTW effluent concentrations ranged from 0.041 to 5.29 ppb. The POTW sludge (dry wt.) range was less than 0.2 ug/kg to 3,120 ppb; the drinking water range was non-detect to 0.063 ppb; the landfill leachate range was non-detect to 53.1 ppb; the surface water range was non-detect to 0.138; the sediment range was non-detect to 1.13 ppb (dry wt.); and the quiet water range was non-detect to 2.93 ppb. The control cities samples generally inhabited the lower end of the above ranges, except for the POTW effluent and sludge findings for Cleveland, which were intermediate in their ranges, and the quiet water samples at Port St. Lucie, which were the highest. 3M states that all the PFOS surface water concentrations are below laboratory-derived NOEC (No Observed Effect Concentrations) for aquatic organisms.

The Multi-City Study also included a market basket sampling of PFOS residue in a total of over 200 samples taken from green beans, apples, pork muscle, cow milk, chicken muscle, chicken eggs, bread, hot dogs, catfish, and ground beef (3M, 2001a). Measurable quantities of PFOS, to 0.852 ng/g, were found in four milk samples and one ground beef sample; one of the four milk samples was from a control city, the balance of the samples with measurable PFOS were from cities with fluorochemical manufacture or use.

Giesy reported the results of a global monitoring survey of PFOS in marine mammals; they were located in Florida, California and Alaskan U.S. coastal waters, the northern Baltic Sea, the Mediterranean Sea, the Arctic (Spitsbergen), and Sable Island in Canada. PFOS was detected in the liver and blood of marine mammals from most locations. The largest liver concentration was 1520 ng/g, wet wt., in bottlenose dolphin from Florida; the largest blood concentration was 475 ng/mL in ringed seal from the northern Baltic Sea.

The plasma of piscivorous (fish-eating) birds was sampled in the late 1980's and early 1990's or obtained from the U.S. Fish and Wildlife Service (date of liver samples was not listed), and these samples were later sent to 3M for analysis of PFOS levels (3M Company, 2000a). Species sampled for PFOS plasma levels included the bald eagle, albatross, and sea eagle. Plasma levels ranged from below the limit of detection (1 ppb) up to a maximum of 1047 ppb, present in a bald eagle. Levels in the livers sampled from six bird species ranged from below the limit of detection up to a maximum of 2055 ppb.

Giesy reported on PFOS in fish and fish-eating water birds. Fish were sampled from the U.S., certain European countries, the North Pacific Ocean and Antarctic locations (Giesy, 2001a). The highest concentration was 923 ng/g, wet wt., in the muscle of fish from a Belgium estuary. The highest concentration of PFOS in U.S. Great Lakes fish was 297 ng/g, wet wt., in the muscle of carp. Muscle tissue of fish from several inland lakes in Michigan U.S. did not contain detectable PFOS, nor did fish from the North Pacific and Antarctic oceans. Fish-eating bird samples were collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, Japanese and Korean coasts (Giesy, 2001b). PFOS concentrations collected from the plasma of bald eagles in the midwestern USA ranged from not quantifiable to 2220 ng/mL, with a mean of 330 ng/mL. The largest PFOS concentration in liver samples from USA birds was 1780 ng/g, wet wt. from a Brandts cormorant. PFOS concentrations in the sera of North Pacific Ocean albatrosses ranged from 3 to 34 ng/mL. PFOS concentrations in blood or livers of birds from the Mediterranean and Baltic Seas were relatively less than those found in U.S. birds.

Giesy reported on PFOS in mink and river otter livers from the U.S. (Giesy, 2001c). PFOS was found in all samples, with the largest concentration of 4800 ng/g, wet wt., found in mink liver. PFOS concentrations in river otter livers from Washington and Oregon states ranged from 34 to 994 ng/g, wet wt.

Giesy reported that PFOS was detected in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast at 51 of 77 sampling locations at a concentration range of <42 to 1225 ng/g, dry wt. (Giesy,



2001d).

Giesy reported on the concentrations of PFOS in surface water, sediments, clams, and fish collected from locations upstream and downstream of the 3M facility at Decatur AL (Giesy, 2001e). Of the three downstream sampling locations, the two closest to the 3M facility had PFOS surface water concentrations significantly greater than the two upstream sites (means of 150ug/L and 82 ug/L, vs. 0.009 (est.) and 0.053 ug/L); the nearest two locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 5930 ug/kg, 1299 ug/kg vs. 0.18(est.) and 0.98). Clam and fish samples were collected at two locations, one upstream and one downstream of the 3M facility. The average fish whole body PFOS concentration for the upstream location was 59.1 ug/kg (wet wt.), while that for the downstream location was 1332 ug/kg. The average PFOS concentration in clams at the upstream location was 15.6(est.) ug/kg; that for the downstream location was 14.1(est.) ug/kg.

Hansen (2002) reported concentrations of PFOS measured from surface water samples taken from the Tennessee River up- and downstream of the outfall from the fluorochemical manufacturing facility at Decatur AL (the 3M facility mentioned above). There were 20 sampling sites above and 20 sites below the outfall location, spaced at approximately 2 mile intervals. Upstream of the facility the average concentration of PFOS was  $32 \pm 11$  ng/L; the downstream concentrations were observed to increase at a point approximately six miles below the outfall; the average PFOS concentration from that point downstream was  $114 \pm 19$  ng/L. The report states that the consistency of the PFOS concentrations within these two regions suggests the absence of either major environmental sinks or additional sources of PFOS in the areas sampled.

The 3M Environmental Lab reported on analyses of PFOS in naive rat livers, rat chow, and fish meal (a component of rat chow). Rat livers from two of three suppliers had endogenous levels of PFOS above the limit of detection (15 ng/g). PFOS levels in the male rats with measurable levels increased with age, while the levels in female rats showed no correlation with age. In the rat chow study, two of the four chows examined had PFOS above the 2-10 ng/g level of quantitation, 18 and 12 ng/g, respectively. The fish meal study analyzed PFOS levels in six types of fishmeal formulated from at least three types of fish. Three of the six types had PFOS levels above the limit of quantitation of 3.5 ng/g; the maximum concentration was 15.7 ng/g.

The 3M Environmental Laboratory also summarized PFOS analyses of avian and aquatic feed matrices. PFOS levels were not detected in the two daphnid feeds, and were below the limits of quantitation in the avian feed. Dry artemia (brine shrimp) cysts used for fish feed had PFOS levels of 9-10 ug/kg.

## **2.4 Human Biomonitoring**

For many years, PFOS has been measured in the serum of workers occupationally exposed to PFOS. It was also recently detected in the serum of the general population, but at much lower levels. The results of the most recent analyses of blood samples are described below and summarized in Table 2.

### **2.4.1 Occupational Exposures**

PFOS serum levels have been measured in workers involved in both the manufacturing of perfluorochemicals and the processing of these compounds into products, such as fire protection and surface protection products. Biomonitoring data are available from manufacturing plants in Alabama and Belgium, a processing facility in Japan, and corporate offices in Minnesota.

*Fluorochemical Manufacturing*

Two 3M plants, located in Decatur, Alabama and Antwerp, Belgium, have produced POSF from electrochemical cell fluorination and then subsequently used POSF to produce various products through polymerization processes. These POSF-based products degrade or metabolize to PFOS. A third manufacturing plant, located in Cottage Grove, Minnesota, has not produced POSF, but has manufactured some POSF-based products. Serum PFOS levels were measured in manufacturing plant employees in 1995 (n = 178), 1997 (n = 149), and 2000 (n = 521) using high performance liquid chromatography/mass spectrometry (Olsen et al, 1999; Olsen et al, 2001d; Olsen et al, 2001e; Olsen et al., 2001f). Employees volunteered to participate for medical surveillance. Arithmetic means and ranges were reported for all surveillance periods. Geometric means have only been reported in 2000. These data are provided in Table 2.

Mean serum PFOS levels have been declining at both plants since 1995. Decatur serum levels have been higher than Antwerp levels during every surveillance period. The PFOS levels in workers across both plants in 1995 ranged from 0.10 ppm to 12.83 ppm. The range of PFOS levels of workers in 1997 was 0.10 - 9.93 ppm. The largest number of employees at each plant participated in the 2000 medical surveillance program (521 out of 840 employees). The mean serum PFOS level at the Decatur plant was 1.32 ppm (range = 0.06 – 10.06 ppm). Most of the employees at both facilities had PFOS serum levels that were <6 ppm across all of the sampling periods.

Except for Y2000, the participation rate of the total number of potentially exposed employees in the medical monitoring program from which these data were derived was not provided. It was reported that the employees were offered a medical monitoring program, but it is not clear how many of them actually volunteered to participate. In addition, age of the employees and duration of employment were not reported for most of the surveillance years, and it is unclear how many of the total number of employees worked at each plant. Therefore, it should be noted that these data only provide a snapshot of the serum levels of participating workers during each sampling period.

In order to address the voluntary nature of the monitoring program at the 3M plants, a random sample of employees from the Decatur plant was chosen to measure seven different fluorochemicals in employees' blood (Olsen et al., 1999). Limited job information, years worked, and building location were collected to provide a better understanding of the distribution of fluorochemical serum levels in employees. The random sample consisted of 187 chemical plant workers (126 randomly chosen workers and 61 volunteers) and 76 film plant workers (60 randomly chosen and 16 volunteers). The levels in these employees, who were sampled in late 1998, were similar to those reported above. Mean PFOS levels were 1.505 ppm (range, 0.091-10.600) for the random sample, 1.259 ppm for the volunteers, and 1.424 ppm for all participants. Geometric means were 0.944 ppm (95% CI 0.787-1.126) for chemical plant employees and 0.136 ppm (95% CI 0.114-0.161) for film plant employees. When compared by job description, workers in the chemical plant had higher PFOS levels than workers in the film plant. PFOS levels in males were positively, although modestly, associated with number of years worked in the chemical plant.

*Fluorochemical Processing*

Biomonitoring data were submitted on workers in Japan processing perfluorinated chemicals into fire protection products and surface products (Burriss et. Al., 1999). In 1999, PFOS, PFOA and PFHS (perfluorohexanesulfonate) were measured in employees' serum at the Sagamihara plant. Serum was drawn from both production employees (n = 32) who regularly handle fluorochemicals and management employees (n = 32) who are not regularly exposed. Serum was also drawn from management employees (n = 30) at the Head Office in Tokyo. The highest PFOS level in 32 production employees was 0.628 ppm, with an arithmetic mean of 0.135 ppm. The highest PFOS level in either of the management groups was

0.0967 ppm, while the average level was approximately 0.04 ppm. The results of biomonitoring performed at the Sagamihara plant indicate lower levels of PFOS than at either of the manufacturing facilities cited above.

Although the exposures would not necessarily be considered “occupational” for either group, PFOS levels in corporate staff/managers at a 3M plant in St. Paul, Minnesota were similar to those reported in the corporate managers in Japan (3M Report, 1999). Thirty-one employees, none of whom had worked in fluorochemical production or research and development, were sampled in 1998. All of the participating employees had measurable levels of PFOS in their serum. The mean PFOS level was 0.047 ppm, with a range of 0.028 to 0.096 ppm. In this group of 3M employees, age was significantly associated with increased serum PFOS. No other data on these workers were provided.

#### **2.4.2 Non-occupational Exposures**

PFOS has been measured in the serum of the general U.S. population and in small numbers of historical samples (dating as far back as 1957) from sources throughout the world. In 1998, PFOS levels in pooled serum from 2 commercial sources in the U.S. ranged from 43- 45 ppb and 26 - 45 ppb (3M Report, 1999). There were approximately 500 donors in the first source and an estimated 200 donors in the second. No other data, such as geographic location or age of the donors, were provided. 3M also analyzed thirty-five lots of individual or pooled human sera samples from U.S. chemical or biological supply companies in 1999 (3M Letter, 1999). These samples yielded an average of 35 ppb PFOS, ranging from 5 to 85 ppb.

Sera pooled from 18 regional blood banks in various geographic regions of the U.S. were analyzed for PFOS in 1998 (3M Report, 1999). There were 68 pools and an estimated 340-680 donors. The overall mean PFOS serum level across the pools was 29.7 ppb. The PFOS levels varied quite a bit depending on the geographic location of the blood bank. The range of the levels across geographic regions was 9 to 56 ppb, while the range of the averages was 14 to 52 ppb.

Pooled sera from blood banks in Belgium (6 pools), the Netherlands (5 pools), and Germany (6 pools) also have been analyzed for PFOS. Each pool had 15-20 donors. The Netherlands had the highest PFOS levels (mean = 53 ppb) and Belgium had the lowest (mean = 17 ppb).

Individual blood samples from 3 different age populations were recently analyzed for PFOS and other fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) (Olsen, et al., 2002(a), 2002(b), 2002(c)). The studies’ participants included adult blood donors, an elderly population participating in a prospective study in Seattle, WA, and children from 23 states participating in a clinical trial. Overall, the PFOS geometric means were similar across all 3 populations (34.9 ppb, 31.0 ppb, and 37.5 ppb, respectively). The geometric means and 95% tolerance limits and their upper bounds were comparable across all 3 studies. However, the upper ranges for the children and adults were much higher than for the elderly population. It is not clear whether this is the result of geographic differences in PFOS levels or some other factor. It should be noted that PFOS and PFOA were highly correlated in all three studies ( $r = .63$ ,  $r = .70$ , and  $r = .75$ ). The details of each study are provided below.

Serum PFOS levels in 645 blood donors (332 males, 313 females), aged 20-69 years, were obtained from 6 American Red Cross blood banks (Olsen, et al., 2002a). The blood banks were located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD. Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex. The only demographic factors known for each donor were age, gender, and location.

The geometric mean serum PFOS level for all locations and gender was 34.9 ppb (95%CI, 33.3-36.5 ppb). The range was < LLOQ (4.3 ppb) to 1656 ppb. Males had significantly higher ( $p < .05$ ) geometric mean PFOS levels than females. The geometric mean for all males was 37.8 ppb (95% CI, 35.5-40.3) and was 31.3 ppb for all females (95% CI, 30.0 – 34.3). Age was not an important predictor of adult serum fluorochemical concentrations. When stratified by geographic location, the highest geometric mean for PFOS was in the samples from Charlotte, NC (51.5 ppb, range: 19.3 – 166.0) and the lowest from Boston (28.0 ppb, range: 4.3 –87.2).

The highest serum PFOS measurement in this sample was 1656 ppb from a male blood donor, 67 years old from Portland. The next highest donor level was 329 ppb from a male donor, 62 years old also from Portland. The next 8 highest serum PFOS values (range 139 – 226 ppb) were measured in 4 females and 4 males representing Charlotte (n=4), Hagerstown (n=2), Los Angeles (n=1) and Minneapolis/St. Paul (n=1). In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 100.0 ppb.

Serum PFOS levels were reported for 238 (118 males and 120 females) elderly volunteers in Seattle participating in a study designed to examine cognitive function in adults aged 65-96 (Olsen, et al., 2002b). Age, gender and number of years' residence in Seattle were the only data available on the participants. Most of the participants were under the age of 85 and had lived in the Seattle area for over 50 years.

The geometric mean of PFOS for all samples was 31.0 ppb (95% CI, 28.8-33.4). The range was 3.4 – 175 ppb. There was no significant ( $p < .05$ ) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ( $p < .05$ ) associated with PFOS in men but not in women. The mean of the 95% tolerance limit for PFOS was 84.1 ppb with an upper 95% confidence limit of 104.0 ppb.

A sample of 599 children, ages 2-12 years old, participating in a study of group A streptococcal infections, was analyzed for PFOS levels (Olsen,et al., 2002c). The samples were collected in 1994-1995 from children residing in 23 states and the District of Columbia. The geometric mean of PFOS for all of the participants was 37.5 ppb (95% CI, 33.3-36.5). The range was 6.7 to 515.0 ppb. Male children had significantly ( $p < .01$ ) higher geometric mean PFOS levels than females: 40.1 ppb and 35.2 ppb, respectively. In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. When stratified by age, the geometric mean tended to rise for each age group from age 2 (28.6 ppb) through age 9 (42.8 ppb) where it was highest, and then started to decrease gradually to 32.8 ppb at 12 years. In simple linear regression analyses, age was not significantly ( $p < .05$ ) associated with PFOS. Although the data were not provided, a graphical presentation of log PFOS levels for each state by gender were similar across the states, however, it is difficult to interpret these data given the limited sample size for each gender/location subgroup.

In another study, Olsen et al. (2001g) obtained samples from 31 cadavers (16 males and 15 females) over an 18-month period from the International Institute for the Advancement of Medicine (IIAM). The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were harvested from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Serum samples were obtained from 5 ml of blood; liver samples consisted of 10 g of tissue. Samples were frozen at IIAM and shipped frozen to 3M for analysis. The samples were extracted using an ion-pairing extraction procedure and were quantitatively assayed using HPLC-ES/MS and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the extraction procedure. The average fortified sample recovery of PFOS from human sera was 89% (SD 21%)

and the average fortified sample recovery of PFOS from human liver was 78% (SD 24%).

Serum PFOS levels that were determined to be less than the limit of quantitation (LOQ) were assigned a value midpoint between zero and the LOQ. The mean serum PFOS level for the 24 serum donors analyzed was 17.7 ng/mL; the geometric mean for serum PFOS was 14.7 ng/mL. Of the 30 PFOS liver samples analyzed, 15 (50%) were determined to be <LOQ. These samples were assigned a value midpoint between zero and the LOQ. The mean liver PFOS level was 18.8 ng/g; the geometric mean for liver PFOS was 15.2 ng/g. Mean PFOS levels for male and female donors were similar for both serum (male = 18.2 ng/mL; female = 17.2 ng/mL) and liver (male = 19.2 ng/g; female = 28.4 ng/g). Although the data were not shown, the report stated that no associations were observed between measured PFOS levels and age. The average PFOS serum and liver data for each of the 23 paired samples (serum and liver from the same individual) showed a good correlation. The mean liver to serum ratio was 1.3:1. Of the 13 male donors with paired samples, the mean liver to serum ratio was again 1.3:1; the mean serum level was 18.2 ng/mL and the mean liver level was 20.8 ng/g. For the 10 female donors with paired samples, the mean liver to serum ratio was 1.3:1 with mean serum levels of 16.9 ng/mL and mean liver levels of 16.3 ng/g, respectively.

**TABLE 2. MEAN PFOS SERUM LEVELS IN HUMAN POPULATIONS**

| <b>Occupational Exposures</b>  |                          |                          |
|--|--------------------------|--------------------------|
| <b>Plant Location</b>  | <b>Mean (ppm)</b>        | <b>Range (ppm)</b>       |
| Decatur, Alabama   |                          |                          |
| 1995 (n = 90)  | 2.44                     | 0.25 - 12.83             |
| 1997 (n = 84)  | 1.96                     | 0.10 - 9.93              |
| 1998 (n = 126)   | 1.51                     | 0.09 - 10.6              |
| 2000 (n = 263)   | 1.32                     | 0.06 - 10.06             |
|  | (GM = 0.91)*             | (33.3 - 36.5)**          |
| Antwerp, Belgium   |                          |                          |
| 1995 (n = 93)  | 1.93                     | 0.10 - 9.93              |
| 1997 (n = 65)  | 1.48                     | 0.1 - 4.8                |
| 2000 (n = 258)   | 0.80                     | 0.04 - 6.24              |
|  | (GM = 0.44)*             | (0.38 - 0.51)**          |
| Building 236 (n = 45)  | 0.182                    | <0.037 - 1.036           |
| Sagamihara, Japan (1999)<br>(processing PFOS) n = 32                                     | 0.135                    | 0.0475 - 0.628           |
| <b>General Population Exposures</b>  |                          |                          |
| <b>Source</b>  | <b>Mean (ppb)</b>        | <b>Range (ppb)</b>       |
| Non-occupational (n = 31)<br>(corporate staff or managers)<br>St. Paul, Minnesota (1998) | 47                       | 28 - 96                  |
| Non-occupational (1999)<br>(plant management, Japan)<br>Sagamihara n = 32<br>Tokyo n=30  | 40.3<br>52.3             | 31.9 - 56.6<br>33 - 96.7 |
| Commercial Sources, U.S. (1998)  |                          |                          |
| Intergen<br>n = ~500 donors  | 44                       | 43 - 44                  |
| Sigma<br>n = ~ 200 donors  | 33                       | 26 - 45                  |
| Other Commercial Sources, U.S.<br>(1999) n = 35 lots                                     | 35                       | 5 - 85                   |
| U.S. Blood Banks (1998)<br>n = ~340-680 donors   | 29.7                     | 9 - 56                   |
| European Blood Banks (1999)  |                          |                          |
| Belgium (6 pooled samples)   | 17                       | 4.9 - 22.2               |
| Netherlands (5 pooled samples)   | 53                       | 39 - 61                  |
| Germany (6 pooled samples)   | 37                       | 32 - 45.6                |
| U.S. Blood Banks (2000)<br>American Red Cross (ages 20-69)<br>n = 645                    | 34.9<br>(geometric mean) | 4.3 - 1656               |
| Samples in U.S.children (ages 2-12)<br>(1995) (n = 599)                                  | 37.5<br>(geometric mean) | 6.7 - 515                |
| Samples in elderly in Seattle, WA<br>(ages 65-96) (1999) (n = 238)                       | 31.0<br>(geometric mean) | 3.4 - 175                |

\*GM is geometric mean; \*\* 95% Confidence Interval of the GM

### **3.0 Human Health Hazards**

#### **3.1 Metabolism and Pharmacokinetics**

##### **3.1.1 Absorption**

PFOS is well absorbed following ingestion. After a single oral dose of PFOS-14C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours (Johnson, Gibson and Ober, 1979a). The digestive tract and contents contained on average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the PFOS-14C dose was absorbed from solution after administration to non-fasted rats.

##### **3.1.2 Distribution**

At 24 to 48 hours after a single oral dose of PFOS-14C (4.2 mg/kg) in rats, approximately 86% of the radioactivity recovered was found in the carcass (Johnson, Gibson and Ober, 1979a). The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ~0.2%. The concentrations of total carbon-14 in red blood cells and plasma were compared. The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-14C, there is no selective retention of carbon-14 in red blood cells.

At 89 days after a single intravenous dose of PFOS-14C (4.2 mg/kg) in male rats, mean tissue C-14 concentrations above one ug equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1 (Johnson, Gibson and Ober, 1979b). Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 ug/g. There was a difference in C-14 content of subcutaneous fat (0.2 ug/g) and abdominal fat (<= 0.08 ug/g). Very little C-14 was found in whole eye (0.16 ug/g) and no detectable C-14 was found in brain. Only liver and plasma contained a substantial percentage of dose at 89 days post dose, 25.21% and 2.81%, respectively. The low levels of radioactivity found for kidney, lung, testes, and spleen are due in part to blood still contained in these organs when homogenized.

There have been several studies conducted to examine the levels of PFOS in the dam, fetus and neonate. These studies are described in detail in sections 3.4 and 3.5. In general, the levels of PFOS are higher in the liver than in the serum of dams, fetuses and neonates, and placental transfer of PFOS has been demonstrated

##### **3.1.3 Elimination**

Urinary excretion is the primary route of elimination for PFOS in the rat. By 89 days after a single intravenous dose of PFOS-14C (4.2 mg/kg) in male rats, mean urinary excretion was 30.2+-1.5% of total C-14 administered (Johnson, Gibson, and Ober, 1979b). Mean cumulative fecal excretion was 12.6+-1.2%. Radioactive content in feces was too low to measure after 64 days.

The half-life for elimination of total PFOS carbon-14 from plasma after a single oral dose (4.2 mg/kg) in male rats is 7.5 days (Johnson, Gibson and Ober, 1979a). This determination was based upon analysis of

plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after the single oral dose.

There is evidence of enterohepatic circulation of PFOS. After 21 days of cholestyramine treatment, the mean percentage of PFOS-14C dose eliminated via feces (75.8 ± 5.0) was 9.5-fold the mean percentage of dose eliminated via feces by control rats (8.0 ± 0.8, Johnson, Gibson and Ober, 1984). After adjustment for the amount of carbon-14 excreted in urine (18% for controls and 5% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 19% for cholestyramine-treated rats and 74% for control rats. After PFOS-14C administration, the mean liver carbon-14 content at 21 days represents 11% and 40% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 21 days of cholestyramine treatment. The authors conclude that the high concentration of PFOS-14C in liver at 2 to 3 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 by nearly 10-fold suggest that there is a considerable enterohepatic circulation of PFOS-14C.

### 3.1.4 Half-life in Humans

In order to determine the half-life of PFOS, 3 retirees who worked in 3M's Chemical Division were followed for five and a half years (3M Company, 2000c). Serum PFOS levels suggested a mean elimination half-life ( $t_{1/2}$ ) of 1,428 days (approximately 4 years). A half-life study on a larger group of retirees ( $n = 27$ ) is currently underway, in which serum samples are drawn every 6 months over a 5-year period. Two interim reports describing the results thus far have been submitted (Burriss et al, 2000; Burriss et al., 2002). The first interim report suggested a serum half-life of 139-640 days. However, there were several limitations to these analyses including: 1) the limited data available and the range of serum PFOS levels measured; 2) serum was analyzed after each collection period with only one measurement per time period on different days using slightly different analytical techniques; and 3) the reference material purity was not determined until after the first 3 samples had been analyzed. An effort was made to minimize experimental error, including systematic and random error in the analytical method, involving 9 of the original 27 subjects. Serum samples were collected from each of the subjects over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. This would allow for statistical evaluation of the precision of the measurement and assure that all systematic error inherent in the assay equally affected each sample used for half-life determination.

Of the 9 retirees included in this analysis, there were 7 males and 2 females, all from the Decatur plant. The average age of the retirees was 61 years, the mean number of years worked at Decatur was 27.7 years, and the average number of months retired was 18.9. Average BMI of this group was 27.9. The mean PFOS value at study initiation was 0.89 ppm (range 0.11 – 3.53 ppm, SD = 1.07).

The mean serum half-life for PFOS was 8.67 years (range 2.29 – 21.3 years, SD = 6.12). Age, BMI, number of years worked or years since retirement were not significant predictors of serum half-lives in multivariable regression analyses. This analysis has attempted to reduce experimental error in the determination of a half-life for PFOS. However, several issues should be noted. First, the effect of continued non-occupational, low-level exposure on the half-life is unknown. Second, PFOS is a metabolic product of other compounds found in the retirees' blood; therefore, PFOS is likely being produced in the body while the study was ongoing. Third, it is not known if there are interactions between PFOS and other fluorochemicals in the body. Fourth, this estimate is much higher than that reported in lab animals. However, it may not be appropriate to directly compare the results of the animal half-life data with these data due to potentially different protein binding sites and affinities. Fifth, systematic error of the analytical method could be as high as ± 20% and still satisfy the data quality criteria.



### 3.2 Acute Toxicity

Four reports of acute studies of PFOS have been submitted, one inhalation toxicity of rats, two oral studies of rats, and one dermal and eye irritation study of rabbits.

In a study to determine the median lethal concentration (LC<sub>50</sub>), Rusch et al. (1979) administered PFOS dust in air to Sprague-Dawley rats, 5/sex/group, levels of 1.89 to 45.97 mg/l PFOS to eight test groups. A Wright dust-feed mechanism with dry air at a flow rate of 12 to 16 liters per minute was used to administer the PFOS dust. Rats were exposed for 1 hour. The test group rats weighed 201-299 g at study initiation. The control group rats weighed 203-263 g at study initiation. The test group rats were exposed to 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09 or 45.97 g/l. The control rats were exposed to dry air at a flow rate of 12 liters per minute. All other protocols were the same as the test group rats. The rats were observed for abnormal signs prior to exposure, at 15-minute intervals during the 1-hour exposure, at removal from the exposure chamber, hourly for four hours after exposure, and daily thereafter for 14 days. Individual body weights were recorded on Day 0 (prior to exposure), Day 1, Day 2, Day 4, Day 7, and Day 14. It is reported that all animals dying spontaneously were necropsied as soon as possible after death. Blood samples were collected on Day 14 from all surviving animals, but analyses were not provided. The LC<sub>50</sub> of 5.2 mg/l and 95% confidence limits of 4.4 and 6.4 mg/l were calculated using the method of Litchfield and Wilcoxon.

The highest dose group, 45.97 mg/l, was not used in the LC<sub>50</sub> calculations and terminated on Day 2. At that point, only 5 animals survived and blood samples were taken at termination. The 13.9 mg/l group was also terminated early (Day 1) because of a mechanical problem during exposure. These animals were also not used in the LC<sub>50</sub> determination.

In the 24.09 mg/l exposure group, all animals died by Day 6. At 7.05 and 6.49 mg/l there was 80% mortality. At 4.88, 2.86, and 1.89 mg/l there was 20%, 10%, and 0% mortality, respectively. The rats in all these groups showed signs of toxicity including emaciation, red material around the nose or other nasal discharge, yellow material around the anogenital region, dry rales or other breathing disturbances, and general poor condition. Abnormal in-life observations were reported to be less frequent in the lower exposure groups.

At necropsy, the most common abnormality was discoloration of the liver and lung. Discoloration of the lung was also observed in control rats and therefore may not be treatment related. Therefore, the most significant treatment related abnormality was varying degrees of discoloration of the liver. Among animals that died prematurely, decreased body weight, discoloration of the lung, and discoloration and distention of the small intestine were also observed.

In a study to determine the acute oral LD<sub>50</sub>, Dean et al. (1978) administered CD rats, 5/sex/group, doses of 0, 100, 215, 464, and 1000 mg/kg PFOS by gavage. The powdered test material was suspended in a 20% acetone/80% corn oil mixture. All dose levels were administered as volumes of 10ml/kg body weight. The rats weighed 172-212 g at the beginning of the study immediately prior to dosing and weights were recorded at Day 7 and Day 14. The rats were observed for abnormal signs during the four hours after exposure, and daily thereafter for 14 days. It is reported that all animals dying spontaneously were grossly necropsied, as well as all rats that survived to the end of the 14 day study.

Acute oral LD<sub>50</sub> values and 95% confidence limits were calculated for males (233 [160-339] mg/kg), females (271 [200-369] mg/kg) and combined male and female rats (251 [199-318] mg/kg).

All rats in the 464 and 1000 mg/kg dose groups died before the end of the study. Three animals in the 215 mg/kg group died prematurely. It appears signs of toxicity most frequently observed included:

hypoactivity, decreased limb tone, and ataxia. At necropsy, observations included: yellow-stained urogenital region, stomach distention and signs of irritation of the glandular mucosa, and lung congestion.

In a second oral study, Gabriel (1978; cited in Dean et al., 1978) administered two groups of Sherman-Wistar albino rats (5 /sex/group) two doses of PFOS by gavage in water. The rats weighed between 200 - 300 grams. It was determined the acute oral LD<sub>50</sub> was greater than 50 mg/kg and less than 1500 mg/kg.

All of the rats administered 1500 mg/kg died before the 14-day observation period ended, with the last rat dead on Day 9. None of the rats administered 50 mg/kg died before the end of the study. It is stated that rats were observed for signs of toxicity and mortality but it is not clear how frequently they were observed and no individual animal data was provided. It is stated that the high dose group rats were “depressed” at 2- 3 hours after dosing and “severely depressed or semi-comatose” by 24 hours; staying in this state until death occurred. No individual pathology data were provided. The author states: “Gross pathologic examination revealed nothing remarkable.”

Biesemeier and Harris, 1974 examined the potential for PFOS to cause skin and eye irritation. Six albino rabbits had their hair clipped from their backs and flanks, and it appears five tenths of one gram (0.5 g; the procedure states either 0.5 g or 0.5 ml and the test material as supplied by 3M was a solid) of the test material was placed on abraded or intact prepared test sites, then covered with gauze patches. After 24 hours and 72 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale. No reference is provided for method or scoring of results. Concentration or total dose of test material was not provided. In all six rabbits, it is reported the primary skin irritation scores were 0; which indicates no reddening or swelling detected.

In the eye irritation study, six albino New Zealand White rabbits were placed in collars so they could not rub their eyes. It appears one tenth of a gram (0.1 g; the procedure states either 0.1 g or 0.1 ml was used and T117 was a solid as supplied by 3M) of the test substance was instilled in one eye, the other eye was left untreated as a control. The concentration or total dose of the test substance were not provided. It is reported that the reaction to the test material was read against a scale of damage to the cornea, iris, and the bulbar and palpebral conjunctivae at 1, 24, 48, and 72 hours after treatment. The scale criteria were not presented or referenced. Each time the eyes were scored, any accumulated discharge or residue of test material was flushed from the eye. There is no reference provided for the method. It appears that scores were maximal at 1 hour and 24 hours after treatment then decreased over the rest of the study (Biesemeier and Harris, 1974).

### 3.3 Mutagenicity

PFOS was tested for its ability to induce mutation in the Ames Salmonella/Microsome Plate Test and in the D4 strain of *Saccharomyces cerevisiae* (Litton Bionetics, Inc., 1979). It was also tested in: (1) a Salmonella – *Escherichia coli*/Mammalian-microsome reverse mutation assay (Mecchi, 1999); (2) an in vitro assay for chromosomal aberrations in human whole blood lymphocytes (Murli, 1999); and (3) an assay for unscheduled DNA synthesis (UDS) in rat liver primary cell cultures (Cifone, 1999). It was negative in all assays in which it was tested. PFOS does not induce reverse mutation at the histidine locus of *S. typhimurium* or at the tryptophan locus of *E. coli* when tested with or without metabolic activation. It does not induce chromosomal aberrations in human lymphocytes when tested with or without metabolic activation and does not induce UDS in primary cultures of rat hepatocytes. PFOS was also tested in an in vivo mouse micronucleus assay (Murli, 1996). PFOS did not induce micronuclei in the bone marrow of Crl:CD-1 BR mice and is negative in the mouse bone marrow micronucleus assay.

T-2247 CoC which is a 50% by weight solution of the diethanolammonium salt of perfluorooctanesulfonate in water was tested for its ability to induce gene mutation in 5 strains of *S.*

typhimurium and also for its ability to induce recombination in *S. cerevisiae* strain D3. (Simmon, 1978). The chemical was negative in all 5 strains of *Salmonella* and in *S. Cerevisiae* D3 when tested with and without metabolic activation. T-2247 CoC was tested with *S. typhimurium* strains TA98 and TA100 in a desiccator assay for the detection of volatile compounds. It was nonmutagenic when tested under these conditions.

### 3.4 Repeated Dose Toxicity

Three 90-day subchronic studies of PFOS have been conducted, two gavage studies in rhesus monkeys and one dietary study in rats. In addition, a four week and a 26 week capsule study in cynomolgus monkeys have been conducted. Both reports were unaudited drafts at the time of this review. In addition, a two-year cancer bioassay is currently being conducted, but was not available at the time of this review.

In the rat subchronic study, Goldenthal et al. (1978b) administered CD rats, 5/sex/group, dietary levels of 0, 30, 100, 300, 1000 or 3000 ppm PFOS (FC-95) for 90 days. The males weighed 196-232 g and the females weighed 165-206 g at study initiation. The dietary levels were equivalent to doses of 0, 2, 6, 18, 60 and 200 mg/kg/day. The rats were observed daily for general clinical signs; body weights and food consumption were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. The rats were sacrificed after 90 days of treatment and a gross necropsy was conducted. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All of the rats in the 300, 1000 and 3000 ppm groups died. Death occurred between days 13-25 and days 18-28 for the males and females, respectively, in the 300 ppm group. At 1000 ppm, death occurred between days 8-14, and at 3000 ppm, the rats died between days 7-8 of treatment. The rats in all groups showed signs of toxicity including emaciation, convulsions following handling, hunched back, red material around the eyes, yellow material around the anogenital region, increased sensitivity to external stimuli, reduced activity and moist red material around the mouth or nose.

Three males and two females in the 100 ppm group died prior to scheduled sacrifice. Two of the males and the two females died during week 5 and the third male died during week 11 of the study. At study termination, mean body weights were reduced by 16.7% and 16.3% in the male and female groups, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for males and females at 100 ppm. After 30 days of treatment, hematologic values were comparable among the control and 100 ppm groups. Clinical chemistry analyses at one month showed a significant increase in mean glucose in males, blood urea nitrogen values in males and females, and creatinine phosphokinase and alkaline phosphatase values for females. After 90 days of treatment at 100 ppm, the two surviving males had significantly reduced erythrocyte, hemoglobin, hematocrit and leukocyte counts; the three surviving females had significantly reduced hemoglobin and reticulocyte counts, as well as slightly lower erythrocyte, hematocrit and leukocyte counts. Two of the surviving females showed slight to moderate increases in plasma glutamic oxalacetic and pyruvic transaminase activities. Urinalysis results were comparable among treated and control groups at 30 and 90 days. Relative liver weight was significantly increased in the males and absolute and relative liver weights were significantly increased in the females. Relative kidney weights were significantly increased in both sexes.

All rats in the 30 ppm group survived until the end of the study. At study termination, mean body weights were reduced by 8.7 and 8% in the males and females, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for the males at 30 ppm. Hematologic values were comparable among the control and 30 ppm group at 30 and 90 days. One female showed a slightly elevated glucose level and one male showed a slightly increased alkaline phosphatase level at 30 days. At 90 days, one male showed moderate increases in glucose, blood urea nitrogen and  $\gamma$ -glutamyl transpeptidase activity. The females had significant increases in absolute and relative liver weights. The males had significant decreases in absolute and relative adrenal weights, absolute thyroid/parathyroid weight and absolute pituitary weight. The biological significance of the changes in male organ weights is unclear since similar changes were not noted in higher dose groups.

At necropsy, treatment related gross lesions were present in all treated groups and included varying degrees of discoloration and/or enlargement of the liver and discoloration of the glandular mucosa of the stomach. Histologic examination also showed lesions in all treated groups. Centrilobular to midzonal cytoplasmic hypertrophy of hepatocytes and focal necrosis was observed in the liver; the incidence and relative severity were greater in the males. In addition, especially among rats in the 300, 1000 and 3000 ppm groups, treatment related histologic lesions were noted in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. In the thymus, this consisted of depletion in the number and size of the lymphoid follicles and in the bone marrow hypocellularity was noted. The spleen was slightly atrophied with a corresponding decrease in the size and number of lymphoid follicles and cells and a similar depletion was noted in the mesenteric lymph nodes. Mucosal hyperkeratosis and/or acanthosis was observed in the forestomach and mucosal hemorrhages were noted in the glandular portion of the stomach. Decreases in the height and thickness of the villi were noted in the small intestine. Atrophy of the skeletal muscle was noted, as well as epidermal hyperkeratosis and/or acanthosis was noted in the skin.

In the first rhesus monkey study, Goldenthal et al. (1979) administered rhesus monkeys, 2/sex/group, doses of 0, 10, 30, 100 or 300 mg/kg/day PFOS (FC-95) in distilled water by gavage. The males weighed 3.05-3.80 kg at study initiation and the females weighed 2.75-4.10 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study. The study was terminated after 20 days due to the death of the monkeys. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All of the treated monkeys died. The monkeys in the 300 mg/kg/day group died between days 2-4, the monkeys in the 100 mg/kg/day group died between days 3-5, the monkeys in the 30 mg/kg/day group died between days 7-10, and the monkeys in the 10 mg/kg/day group died between days 11-20 of treatment. The monkeys from all the groups showed similar signs of toxicity including decreased activity, emesis with some diarrhea, body stiffening, general body trembling, twitching, weakness, convulsions and prostration. At necropsy, several of the monkeys in the 100 and 300 mg/kg/day groups had a yellowish-brown discoloration of the liver; histologic examination showed no microscopic lesions. Congestion, hemorrhage and lipid depletion of the adrenal cortex was noted in all treated groups. No other lesions were noted.

In the second study, Goldenthal et al. (1978a) administered rhesus monkeys, 2/sex/group, doses of 0, 0.5, 1.5 or 4.5 mg/kg/day PFOS (FC-95) in distilled water by gavage for 90 days. The males weighed 2.55-3.55 kg at study initiation and the females weighed 2.7-3.75 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. The monkeys were sacrificed after 90 days of treatment and a gross necropsy was conducted. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All monkeys in the 4.5 mg/kg/day group died or were sacrificed in extremis between week 5 and 7 of the study. Beginning on the first or second day of the study, these monkeys exhibited signs of gastrointestinal tract toxicity including anorexia, emesis, black stool and dehydration. All of the monkeys had decreased activity and just prior to death showed marked to severe rigidity, convulsions, generalized body trembling and prostration. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.7 kg at week 5. After 30 days of treatment, there was a significant reduction in serum cholesterol and a 50% reduction in serum alkaline phosphatase activity. At necropsy, mean organ weights were comparable among the control and treated monkeys. Histologic examination showed several treatment related lesions. All the male and females had marked diffuse lipid depletion in the adrenals. One male and two females had moderate diffuse atrophy of the pancreatic exocrine cells with decreased cell size and loss of zymogen granules. Two males and one female had moderate diffuse atrophy of the serous alveolar cells characterized by decreased cell size and loss of cytoplasmic granules.

All monkeys in the 1.5 mg/kg/day group survived until the end of the study. During the first week of the study, the monkeys had decreased activity. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included black stool, diarrhea, mucous in the stool and bloody stool; at the end of the study, anorexia, dehydration or general body trembling were noted. Although statistical significance was not achieved, the mean body weight of the males dropped from 3.15 kg at the beginning of the study to 2.93 kg at the end of the study, and the mean body weight of the females dropped from 3.22 kg to 2.75 kg. One of the females had very low serum cholesterol and another had a reduction in inorganic phosphate. Necropsy revealed no treatment related lesions.

All monkeys in the 0.5 mg/kg/day group survived until the end of the study. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included diarrhea, soft stools, anorexia and emesis. Occasionally, decreased activity was noted in three of the monkeys. Necropsy revealed no treatment related lesions.

Serum samples from monkeys in the 0, 0.5 and 1.5 mg/kg/day dose group were individually analyzed for PFOS levels; liver samples from the 0, 0.5, 1.5 and 4.5 mg/kg/day dose groups were also analyzed for PFOS levels. Except for the control animals where animal 7355M was used in both the serum and liver control groups, different animals were used for serum and liver determinations. PFOS levels in serum were developed by pyrolysis; precision is estimated to be  $\pm 10 - 25\%$ .

**LEVELS OF PFOS IN SERUM**

| Monkey # | Dosage Group  | PFOS Levels in Serum (ppm) |
|----------|---------------|----------------------------|
| 7355M    | 0             | 40                         |
| 7358M    | 0             | 20                         |
| 7368F    | 0             | 15                         |
| 7460M    | 0.5 mg/kg/day | 150                        |
| 7466F    | 0.5 mg/kg/day | 150                        |
| 7462M    | 1.5 mg/kg/day | 250                        |
| 7500F    | 1.5 mg/kg/day | 275                        |

No explanation is offered for the relatively high serum levels in the controls. Sample 7355M was run on two separate days and gave the same results.

**LEVELS OF PFOS IN LIVER**

| Monkey # | Dosage Level  | PFOS in Liver (mg) | PFOS in Liver (ppm) |
|----------|---------------|--------------------|---------------------|
| 7355M    | 0             | 3000               | 50                  |
| 7368F    | 0             | 1500               | 20                  |
| 7463M    | 0.5 mg/kg/day | 7000               | 100                 |
| 7466F    | 0.5 mg/kg/day | 8000               | 100                 |
| 7462M    | 1.5 mg/kg/day | 45000              | 650                 |
| 7500F    | 1.5 mg/kg/day | 40000              | 600                 |
| 7484M    | 4.5 mg/kg/day | 40000              | 650                 |
| 7502F    | 4.5 mg/kg/day | 80000              | 1000                |

Livers were stored refrigerated for several months prior to analysis. This resulted in the separation of some liquid from the samples. At the time of analysis, only partial samples were taken in which the ratio of solid to liquid was estimated visually. Recovery of PFOS from the liver samples was estimated from experiments where a known amount of PFOS was added to a control liver. Here as in the serum, control values were higher than expected. Because of this, a 0.133 g liver sample was analyzed for total fluorine. The fluorine value calculated as PFOS was equivalent to 11 ppm in the liver.

PFOS values in serum and liver are approximately the same at the 0.5 mg/kg/day level but are substantially higher in the liver at the 1.5 mg/kg/day level. Serum levels were not determined at the 4.5 mg/kg level. That may be because only a single female survived until scheduled sacrifice in the 4.5 mg/kg/day dose group. From this study, there do not appear to be any differences in PFOS in either serum or liver between the sexes.

The values of PFOS in the liver and serum should be viewed with caution. There were a very limited number, one male and one female, of animals per group. Storage of the liver samples at refrigeration temperature for long periods of time was not ideal. Although PFOS is stable, the liver itself obviously underwent some deterioration as evidenced by the separation of a liquid phase. Sample size may also have been inaccurate since the visual estimation of solid to liquid phase is not a precise measurement.

In order to determine the dose range for a six-month study, Thomford et al. (unaudited draft, 1998) conducted a 4-week range-finding study in cynomolgus monkeys. Male and female cynomolgus monkeys were administered doses of 0 (2/sex/group), 0.02 (3/sex/group), or 2.0 mg/kg/day (1/sex/group) PFOS in capsules placed directly into the stomach. The monkeys weighed 2.1- 2.4 kg at study initiation. It appears the monkeys were observed at least daily for general clinical signs and body weights were recorded twice

weekly. Hematological and clinical chemistry analyses were conducted on samples collected before the beginning of the study at day -7(baseline values) and day 29. Additional blood samples for clinical chemistry were collected on study days 2, 7, and 14. Blood samples for serum PFOS concentrations were taken on days 7, 2, 3, 7, 14, and 29. In addition, samples from day -7 and day 29 were analyzed for levels of estradiol, estrone, estriol, thyroid stimulating hormone, triiodothyronine, and thyroxin. The study animals were terminated as scheduled at 30 days. At necropsy a sample of liver was collected from each animal for palmitoyl CoA oxidase activity analyses. Samples of liver, testes, and pancreas were collected for proliferation cell nuclear antigen evaluation. A sample of liver was also collected from each animal for PFOS concentration analysis. The following organs from control and all treated groups were examined microscopically: adrenals, eye, kidney, liver, lung, spleen, pancreas, femoral bone marrow, testes, and thymus.

None of the monkeys died before the study was ended. There were no test-related effects on clinical observations, body weight, food consumption, body temperatures, hematology, or macroscopic or microscopic pathology findings. No test-related effects were noted in the levels of estrone, estriol, thyroid stimulating hormone, and thyroxin. Cell proliferation, as measured by immunohistochemical detection of proliferating cell nuclear antigen, was not increased in the liver, testes, or pancreas of monkeys.

The monkeys in the 2.0 mg/kg/day group both showed estradiol levels that were less than their prestudy values and controls at day 29. Similarly, the monkeys in the 2.0 mg/kg/day group both showed triiodothyronine levels that were less than their prestudy values and controls at day 29. However, pre-treatment, the high-dose monkeys also had triiodothyronine values lower than controls in baseline samples as well. Since the numbers of tested animals are small and baseline levels are variable, it is not clear if these hormone level changes are treatment-related effects.

In the final study, Thomford (2002) administered PFOS to cynomolgus monkeys by oral capsule at doses of 0 (6 monkeys per sex), 0.03 (4 monkeys per sex), 0.15 (6 monkeys per sex), or 0.75 mg/kg/day (6 monkeys per sex) for 26 weeks. Two animals from the control, 0.15 and 0.75 mg/kg/day groups were assigned to a recovery group and were not treated for at least 52 weeks following the last administration of PFOS. Animals were observed twice daily for mortality and moribundity and were examined at least once daily for abnormalities and signs of toxicity; food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during weeks 26 and 52. Body weight data were recorded weekly before the start of treatment, on Days -1 and 1 and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and at specified intervals during treatment and recovery. Blood samples were also taken for hormone determinations. Samples of serum were collected at various time points during the study and sent to 3M for analysis of PFOS levels. The following organs were weighed at scheduled and unscheduled sacrifices; paired organs were weighed separately: adrenal (2), brain, epididymis (2), kidney (2), liver, ovary (2), pancreas, testis (2), and thyroid (2) with parathyroid. The following tissues were collected for histopathology: adrenals (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eyes (2), femur with bone marrow, gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mammary gland, mesenteric lymph node, ovary (2), pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, trachea, urinary bladder, uterus, and vagina. Liver specimens from the 0.15 and 0.75 mg/kg/day recovery animals were collected via biopsy and analyzed for PFOS levels.

Males weighed 3.3-3.4 kg and females weighed 2.8-2.9 kg at the beginning of the study. At the end of 26 weeks of treatment, males weighed 3.7, 3.8, 3.5, and 3.3 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. Females weighed 3.1, 3.1, 3.1 and 2.8 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. The difference between the control and the 0.75 mg/kg/day

female treatment groups was statistically significant. At the end of the recovery period, differences in weight between the control and treated animals were no longer obvious.

Two males from the 0.75 mg/kg/day group did not survive to the scheduled sacrifice. One animal died after dosing on Day 155 (Week 23). Clinical signs noted in this animal included: constricted pupils, pale gums, few, mucoid, liquid and black-colored feces, low food consumption, hypoactivity, labored respiration, dehydration, and recumbent position. In addition, the animal was cold to the touch. An enlarged liver was detected by palpation. Cause of death was determined to be pulmonary necrosis with severe acute inflammation. On day 179, the second male was sacrificed in a moribund condition. Clinical signs noted included low food consumption, excessive salivation, labored respiration, hypoactivity and ataxia. The cause of death was not determined.

Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and males and females in the 0.15 and 0.75 mg/kg/day groups had lower high density lipoprotein cholesterol during treatment. The effect on total cholesterol worsened with time. By day 182, mean total cholesterol for males and females in the high dose group were 68% and 49% lower, respectively, than levels in the control animals. The effect on high density lipoprotein was greater than that seen with cholesterol. On day 182, the mean high density lipoprotein levels were 79% and 62% lower, respectively in males and females from the high dose group than they were in male and female control animals. Males in the high dose group also had lower total bilirubin concentrations and higher serum bile acid concentrations than males in either the control or other treatment groups. The effect on total cholesterol was reversed within 5 weeks of recovery and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of recovery.

Estradiol values were lower in males given 0.75 mg/kg/day on days 62, 91, and 182 but because of variation only the day 182 value was significant. Estrone values were generally higher in the treated females on days 37, 62 and 91 but again because of variation in the data none of these values were significantly different from the controls. Except for males in the 0.15 mg/kg/day group, triiodothyronine values were significantly lower on days 91 and 182 in males and females given 0.15 and 0.75 mg/kg/PFOS per day. There were other instances in which hormone values in treated groups were different from those of controls but these differences were not consistent over time or between sexes, were not clearly dose-related and did not appear to be related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in the study complicates the interpretation of the hormone data.

At terminal sacrifice, females in the 0.75 mg/kg/day dose-group had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to body weight percentages were increased in the high-dose group compared to the controls. "Mottled" livers were observed in two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had a "mottled" and large liver. Three of 4 high-dose males (including those that did not survive to scheduled sacrifice) had centrilobular or diffuse hepatocellular hypertrophy that was also observed in all high-dose females. Centrilobular or diffuse hepatocellular vacuolation occurred in 2 of 4 females and 2 of 4 males in the high-dose group.

No PFOS related lesions were observed either macroscopically or microscopically at recovery sacrifice indicating that the effects seen at terminal sacrifice may be reversible.

Serum and liver specimens collected from test animals were sent to the 3M Laboratory and analyzed for the presence of PFOS. Serum was harvested from blood that was centrifuged within one hour of collection. Liver specimens were flash frozen in liquid nitrogen. Both liver and serum samples were stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Samples were shipped frozen and on dry ice from Covance Laboratories to 3M periodically from August 1998 through March 2000 which



covered the in-life phase of the study. Once received at 3M specimens were stored in freezer at either  $-55^{\circ}\text{C} \pm 10\text{-}20^{\circ}\text{C}$  or  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

During the first 26 weeks of the study a total of 550 serum specimens and 30 liver specimens were collected. Of the serum specimens, 151 were from Group 1, 99 from Group 2, 152 from Group 3 and 148 from Group 4. Eight liver samples were collected from Group 1, 8 from Group 2, 12 from Group 3 and 14 from Group 4. In the recovery Groups, 72 serum and 4 liver samples were collected from Group 1; 72 serum and 4 liver samples from Group 3 and 80 serum and 4 liver samples from Group 4.

Liver and serum samples were extracted using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to extraction. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external standard calibration.

Liver samples were homogenized in water. An aliquot of each liver homogenate and all serum samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the samples and the ion pairs were partitioned into MtBE. The extracts were evaporated until dry on a nitrogen evaporator and then were reconstituted in 1.0 mL of methanol and passed through a 0.2  $\mu\text{m}$  nylon filter.

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of the fluorochemical of interest using HPLC/ES/MS/MS. Molecular ion 499, the primary ion for PFOS ( $\text{C}_8\text{F}_{17}\text{SO}_3^-$ ) analysis, was fragmented to produce ion 99 ( $\text{FSO}_3^-$ ). Ion 99 was monitored for quantitative analysis.

Although low levels of PFOS were often detected in the sera and liver of the control animals, these levels were significantly lower than those found in the low dose test animals. PFOS levels in the sera of test animals increased with dose during treatment from  $21.0 \pm 1.57$  and  $20.4 \pm 2.71$   $\mu\text{g}/\text{ml}$  in the Group 4 males and females respectively at the end of Week 1 to  $194 \pm 8.94$  and  $160 \pm 23.1$   $\mu\text{g}/\text{ml}$  in males and females respectively in Group 4 at the end of Week 27. During recovery, PFOS levels in serum samples decreased over time until they reached  $41.1 \pm 25.9$   $\mu\text{g}/\text{ml}$  in males and  $41.4 \pm 1.15$   $\mu\text{g}/\text{ml}$  in females from Group 4 at 79 weeks post-treatment. Control values were  $< \text{LOQ}$  (the limit of quantitation) at Week 4 in both males and females and  $0.0215 \pm 0.00296$  and  $0.0243 \pm 0.00355$   $\mu\text{g}/\text{ml}$  in males and females respectively at the end of Week 79. The serum values for selected weeks of treatment and recovery are shown in the table below. There were no significant differences between PFOS levels in the sera of treated males and females.

**Average PFOS Concentrations ( $\mu\text{g/ml}$ ) in Serum of Monkeys for  
Selected Weeks During Treatment and Recovery**

|         | Group 1<br>0.0 mg/kg/day |                         | Group 2<br>0.03 mg/kg/day |                      | Group 3<br>0.15 mg/kg/day |                     | Group 4<br>0.75 mg/kg/day |                   |
|---------|--------------------------|-------------------------|---------------------------|----------------------|---------------------------|---------------------|---------------------------|-------------------|
|         | Males                    | Females                 | Males                     | Females              | Males                     | Females             | Males                     | Females           |
| Week 1  | <LOQ                     | <LOQ                    | 0.869<br>$\pm 0.147$      | 0.947 $\pm$<br>0.110 | 4.60 $\pm$ 0.782          | 3.71 $\pm$<br>0.455 | 21.0 $\pm$ 1.57           | 20.4 $\pm$ 2.71   |
| Week 4  | <LOQ                     | <LOQ                    | 3.20 $\pm$ 0.577          | 3.40 $\pm$ 0.291     | 17.8 $\pm$ 1.68           | 16.5 $\pm$ 1.87     | 95.3 $\pm$ 70.4           | 92.7 $\pm$ 39.6   |
| Week 16 | 0.0407 $\pm$<br>0.0110   | 0.0432 $\pm$<br>0.0081  | 11.2 $\pm$<br>2.44        | 10.5 $\pm$ 1.90      | 56.2 $\pm$ 5.84           | 42.1 $\pm$ 4.04     | 189 $\pm$ 15.9            | 162 $\pm$ 19.3    |
| Week 27 | 0.0529 $\pm$<br>0.0145   | 0.0416 $\pm$<br>0.0148  | 15.9 $\pm$ 5.54           | 11.1 $\pm$ 1.52      | 68.1 $\pm$<br>5.75        | 58.5 $\pm$<br>4.67  | 194 $\pm$<br>8.93         | 160 $\pm$<br>23.9 |
| Week 35 | 0.0459 $\pm$<br>0.00303  | 0.0723 $\pm$<br>0.00352 | Not<br>Determined         | Not<br>Determined    | 84.5 $\pm$ 12.0           | 74.7 $\pm$ 9.53     | 181 $\pm$ 19.5            | 171 $\pm$ 10.1    |
| Week 47 | 0.0355 $\pm$<br>0.00221  | 0.0459 $\pm$<br>0.00323 | Not<br>Determined         | Not<br>Determined    | 48.3 $\pm$ 3.69           | 42.6 $\pm$ 6.70     | 124 $\pm$ 25.9            | 98.3 $\pm$ 8.32   |
| Week 57 | 0.0327 $\pm$<br>0.00526  | 0.0445 $\pm$<br>0.00385 | Not<br>Determined         | Not<br>Determined    | 30.2 $\pm$ 2.36           | 32.3 $\pm$ 1.34     | 78.0 $\pm$ 16.3           | 106 $\pm$ 3.84    |
| Week 69 | 0.0406 $\pm$<br>0.00313  | 0.0400 $\pm$<br>0.00301 | Not<br>Determined         | Not<br>Determined    | 26.4 $\pm$ 2.59           | 34.5 $\pm$ 3.46     | 84.0 $\pm$ 52.4           | 75.0 $\pm$ 5.25   |
| Week 79 | 0.0215 $\pm$<br>0.00296  | 0.0243 $\pm$<br>0.00355 | Not<br>Determined         | Not<br>Determined    | 19.1 $\pm$ 0.805          | 21.4 $\pm$ 2.01     | 41.1 $\pm$ 25.9           | 41.4 $\pm$ 1.15   |

LOQ = Lowest Observable Concentration

Liver values behaved in a manner similar to serum values and increased over time. At Week 27 mean PFOS values on an RSD basis were  $22.2 \pm 0.0269$  in Group 1 males and  $16.8 \pm 0.0178$  in females in Group 1;  $27.0 \pm 4.66$  and  $9.73 \pm 2.15$  in males and females in Group 2;  $33.1 \pm 19.5$  in males and  $21.4 \pm 14.9$  in females in Group 3, and  $6.03 \pm 23.9$  in males and  $5.00 \pm 13.6$  in females in Group 4. At Week 79 values in the liver were  $71.0 \pm 33.4$  in males and  $21.4 \pm 10.8$  in females in Group 4. At Week 80, values were  $14.9 \pm 1.38$  in Group 3 males and  $23.5 \pm 4.98$  in Group 3 females.

### 3.5 Carcinogenicity

The chronic toxicity and carcinogenicity of perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) have been studied in rats (3M, 2002). The results of the study show that PFOS is hepatotoxic and carcinogenic, inducing tumors of the liver, and of the thyroid and mammary glands. Based on the liver toxicity, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is 2 ppm in male rats and 5 ppm

in female rats.

In this study, groups of 40-70 male and female Crl:CD (SD)IGS BR rats were given PFOS in the diets at concentrations of 0.5, 2, 5, or 20 ppm for 104 weeks. A control group was given diets containing acetone, the vehicle. A recovery group was given the test material at 20 ppm for 52 weeks and was observed till death. Five animals/sex in the treatment groups were sacrificed during weeks 4, 14 and 53; liver samples were collected for mitochondrial activity, hepatocellular proliferation rate, and determination of palmitoyl-CoA oxidase activity. Serum and liver specimens were collected for analyses of the presence and concentration of PFOS in liver and serum during and at the end of the in-life phase of the study.

There was a significant increased trend in survival that occurred in the males that was due to significant increases in survival in mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control group. None of the other treated groups in the males revealed any significant differences in survival. No significant trend was noted in survival in females. There was a significant decrease in survival in the mid-dose (2.0 ppm) group and not in the mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control.

Males given 20 ppm had significantly lower mean body weights compared to animals in the control group during weeks 9 through 37. Females given 20 ppm had significantly lower body weights compared to animals in the control group during weeks 3 through 101. At week 105, mean body weights of the surviving males and females were not significantly different from the controls. Food consumption for males and females was similar in all treated groups compared to animals given the control material except for the high-dose females which had statistically significantly lower food consumption during weeks 2 through 44.

At the week 14 and week 53 interim sacrifice, absolute and relative liver weights were significantly increased in the males given 20 ppm. In females given 20 ppm, only the liver-to-body weight percentage was significantly increased. Treatment-related histomorphologic changes were seen in the liver in the males given 5 or 20 ppm and in the females given 20 ppm. The changes consisted of hypertrophy of hepatocytes in centrilobular areas in males and females, and midzonal to centrilobular hepatocytic vacuolation. The incidence and severity of the changes tended to be greater in the males. Dietary administration of PFOS for approximately 53 weeks was associated with mildly to moderately lower cholesterol for males and females fed 20 ppm; and mildly higher alanine aminotransferase for males fed 20 ppm. In the unscheduled sacrifices between Weeks 54 and 105, animals given 20 ppm had increased hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment were noted. Increased hepatocellular centrilobular hypertrophy was seen in animals given 5 ppm.

At the terminal sacrifice, the livers of animals given 5 or 20 ppm exhibited a slight increase in macroscopic findings, including enlarged, mottled, diffuse darkened, or focally lightened. Hepatotoxicity, characterized by significant increases ( $P < 0.05$ ) in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm. A significant increase ( $P < 0.05$ ) in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration was found in all the male treated groups (0.5, 2, 5, or 20 ppm); however, this lesion is believed to be due to old age of the animals and is not considered to be treatment-related.

Based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is 2 ppm in male rats and 5 ppm in female rats.

There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant

increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) at weeks 4 and 14, or by bromodeoxyuridine (BrdU) at week 53.

For neoplastic effects, a significant positive trend ( $P=0.0276$ ) was noted in the incidences of hepatocellular adenoma in male rats. This was due to a significant increase ( $P<0.05$ ) in the high-dose group (11.7%, 7/60) over the control (0%, 0/60). A significantly increased incidence ( $P<0.05$ ) was observed for thyroid follicular cell adenoma in the high-dose recovery group (23.1%, 9/39) when compared to the control group (5%, 3/60). There was also a slight increase in the combined thyroid follicular cell adenoma and carcinoma in the high-dose recovery group (25.6%, 10/39) as compared to that of the control group (10%, 6/60); the increase did not reach statistical significance relative to the control but did reach statistical significance relative to the high-dose group (8.5%, 5/59).

In the females, significant positive trends were observed in the incidences of hepatocellular adenoma ( $P=0.0153$ ) and combined hepatocellular adenoma and carcinoma ( $P=0.0057$ ). These cases were due to significant increases in the high-dose group (8.3%, 5/60, and 10%, 6/60) as compared to the control (0%, 0/60). A significant increase ( $P=0.0471$ ) for combined thyroid follicular cell adenoma and carcinoma was observed in the mid-high (5.0 ppm) group (6%, 3/50) as compared to the control group (0%, 0/60). Except for the high-dose group (which showed a slight decrease in incidences of mammary fibroadenoma/adenoma and combined mammary fibroadenoma and carcinoma), increases in mammary tumors were observed in all treatment groups when compared to the controls. Significant increases ( $P<0.05$ ) in mammary fibroadenoma/adenoma (60%, 30/50) and combined mammary fibroadenoma/adenoma and carcinoma (72%, 36/50) were observed in the low-dose (0.5 ppm) group as compared to the respective controls (38.3%, 23/60 and 48.3%, 29/60). The mid-dose (2.0 ppm) group also exhibited a statistically significant ( $P<0.05$ ) increase (64.6%, 31/48) in the incidence of combined mammary fibroadenoma/adenoma/carcinoma over the control group (43.8%, 29/60). Increases in mammary tumors in the mid-high (5 ppm) dose group did not reach statistical significance relative to the control.

The carcinogenicity data of PFOS in rats are summarized in Table 1.

Table 1. Summary of carcinogenicity data of PFOS in rats.

| Tumors                              | Tumor incidence (%) |                   |                   |                  |                  |                     |
|-------------------------------------|---------------------|-------------------|-------------------|------------------|------------------|---------------------|
|                                     | Male                |                   |                   |                  |                  |                     |
| Liver<br>Hepatocellular<br>adenoma* | 0                   | 0.5 ppm           | 2 ppm             | 5 ppm            | 20 ppm           | 20 ppm<br>recovery# |
|                                     | 0<br>(0/60)         | 6.0<br>(3/50)     | 6.0<br>(3/50)     | 2.0<br>(1/50)    | 11.7**<br>(7/60) |                     |
| <b>Thyroid</b>                      |                     |                   |                   |                  |                  |                     |
| Follicular cell<br>adenoma          | 5.0<br>(3/60)       | 10.2<br>(5/49)    | 8.0<br>(4/50)     | 8.2<br>(4/49)    | 6.8<br>(4/59)    | 23.1**<br>(9/39)    |
| Follicular cell<br>carcinoma        | 5.0<br>(3/60)       | 2.0<br>(1/49)     | 2.0<br>(1/50)     | 4.1<br>(2/49)    | 1.7<br>(1/59)    | 2.6<br>(1/39)       |
| Combined                            | 10.0<br>(6/60)      | 12.2<br>(26/49)   | 10.0<br>(5/50)    | 10.2<br>(5/49)   | 8.5<br>(5/59)    | 25.6<br>(10/39)     |
|                                     | Female              |                   |                   |                  |                  |                     |
| <b>Liver</b>                        |                     |                   |                   |                  |                  |                     |
| Hepatocellular<br>adenoma*          | 0<br>(0/60)         | 2.0<br>(1/50)     | 2.0<br>(1/49)     | 2.0<br>(1/50)    |                  | 8.3**<br>(5/60)     |
| Hepatocellular<br>carcinoma         | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 0<br>(1/50)      |                  | 1.7<br>(1/60)       |
| Combined*                           | 0<br>(0/60)         | 2.0<br>(1/50)     | 2.0<br>(1/49)     | 2.0<br>(1/50)    |                  | 10.0**<br>(6/60)    |
| <b>Thyroid</b>                      |                     |                   |                   |                  |                  |                     |
| Follicular cell<br>adenoma          | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 4.0<br>(2/50)    |                  | 1.7<br>(1/60)       |
| Follicular cell<br>carcinoma        | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 2.0<br>(1/50)    |                  | 0<br>(0/60)         |
| Combined                            | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 6.0**<br>(3/50)  |                  | 1.7<br>(1/60)       |
| <b>Mammary</b>                      |                     |                   |                   |                  |                  |                     |
| Fibroadenoma/<br>adenoma            | 38.3<br>(23/60)     | 60.0**<br>(30/50) | 45.8<br>(22/48)   | 52.04<br>(26/50) |                  | 25<br>(15/60)       |
| Carcinoma                           | 18.3<br>(11/60)     | 24.0<br>(12/50)   | 31.2<br>(15/48)   | 22.0<br>(11/50)  |                  | 23.3<br>(14/60)     |
| Combined                            | 48.3<br>(29/60)     | 72.0**<br>(36/50) | 64.6**<br>(31/48) | 58.0<br>(29/50)  |                  | 40.0<br>(24/60)     |

\*Significant positive trend ( $P < 0.03$ ).

\*\* Significantly increased over the control ( $P < 0.05$ ).

# Recovery group; after 52 weeks of treatment.

### Serum and Liver level of PFOS

Under the conditions of the studies, PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals. Detailed specimen data are presented in Table 2 and Table 3.

**Table 2. Summary of PFOS Concentration-Serum (:g/mL)**

| Timepoint            | Sex    | 0 ppm                           | 0.5 ppm                      | 2 ppm                       | 5 ppm                       | 20 ppm                      | Group 6                     |
|----------------------|--------|---------------------------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                      |        |                                 |                              |                             |                             |                             | High Recovery               |
|                      |        | Average $\pm$ SD                | Average $\pm$ SD             | Average $\pm$ SD            | Average $\pm$ SD            | Average $\pm$ SD            | Average $\pm$ SD            |
| Week 0               | Male   | <LOQ <sup>b</sup><br>(n=5)      | 0.907 $\pm$ 0.0619<br>(n=5)  | 4.33 $\pm$ 1.16<br>(n=5)    | 7.57 $\pm$ 2.17<br>(n=5)    | 41.8 $\pm$ 7.92<br>(n=5)    |                             |
|                      | Female | 0.0259 $\pm$ 0.00663<br>(n = 5) | 1.61 $\pm$ 0.207<br>(n = 5)  | 6.62 $\pm$ 0.499<br>(n = 5) | 12.6 $\pm$ 1.73<br>(n = 5)  | 54.0 $\pm$ 7.34<br>(n = 5)  |                             |
| Week 14 <sup>a</sup> | Male   | <LOQ <sup>c</sup><br>(n = 5)    | 4.04 $\pm$ 0.801<br>(n = 5)  | 17.1 $\pm$ 1.22<br>(n = 5)  | 43.9 $\pm$ 4.90<br>(n = 5)  | 148 $\pm$ 13.8<br>(n = 5)   |                             |
|                      | Female | 2.67 $\pm$ 4.58<br>(n = 5)      | 6.96 $\pm$ 0.993<br>(n = 4d) | 27.3 $\pm$ 2.34<br>(n = 5)  | 64.4 $\pm$ 5.48<br>(n = 5)  | 223 $\pm$ 22.4<br>(n = 5)   |                             |
| Week 53              | Male   | 0.0249 $\pm$ 0.0182<br>(n = 5)  |                              |                             |                             | 146 $\pm$ 33.5<br>(n = 4)   |                             |
|                      | Female | 0.395 $\pm$ 0.777<br>(n = 5)    |                              |                             |                             | 220 $\pm$ 44.0<br>(n = 5)   |                             |
| Day 719              | Male   |                                 |                              |                             |                             |                             |                             |
|                      | Female |                                 |                              | 20.2 $\pm$ 13.3<br>(n = 9)  |                             |                             |                             |
| Week 105             | Male   | 0.0118 $\pm$ 0.0104<br>(n = 11) | 1.31 $\pm$ 1.30<br>(n = 10)  | 7.60 $\pm$ 8.60<br>(n = 17) | 22.5 $\pm$ 23.5<br>(n = 25) | 69.3 $\pm$ 57.9<br>(n = 22) |                             |
|                      | Female | 0.0836 $\pm$ 0.134<br>(n = 24)  | 4.35 $\pm$ 2.78<br>(n = 15)  |                             | 75.0 $\pm$ 45.7<br>(n = 15) | 233 $\pm$ 124<br>(n = 25)   |                             |
| Week 106             | Male   |                                 |                              |                             |                             |                             | 2.42 $\pm$ 5.09<br>(n = 10) |
|                      | Female |                                 |                              |                             |                             |                             | 9.51 $\pm$ 8.70             |

<sup>a</sup> Not corrected for purity of the standard material.

<sup>b</sup> LOQ-Limit of Quantitation = 0.00910 pg/mL

LOQ-Limit of Quantitation = 0.0457 pg/mL

C92987F sample spilled during extraction, no sample remaining for analysis. It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated (that the sera data are accurate to  $\pm$ 30%; liver data are accurate to  $\pm$ 50%.

**Table3. Summary of PFOS Concentration-Liver (:g/g)**

| Timepoint | Sex    | Group 1<br>Control<br>Average $\pm$ SD | Group 2<br>Low<br>Average $\pm$ SD | Group 3<br>Mid<br>Average $\pm$ SD | Group 4<br>Mid-High<br>Average $\pm$ SD | Group 5<br>High<br>Average $\pm$ SD    | Group 6<br>High Recovery<br>Average $\pm$ SD |
|-----------|--------|--|------------------------------------|------------------------------------|---|--|--|
| Week 0    | Male   | 0.104 $\pm$ 0.0673<br>(n = 5)          | 11.0 $\pm$ 2.31<br>(n = 5)         | 31.3 $\pm$ 5.84<br>(n = 5)         | 47.6 $\pm$ 12.5<br>(n = 5)              | 282 $\pm$ 45.3 <sup>a</sup><br>(n = 5) |  |
|           | Female | 0.107 $\pm$ 0.0486<br>(n = 5)          | 8.71 $\pm$ 0.552<br>(n = 5)        | 25.0 $\pm$ 6.11<br>(n = 5)         | 83.0 $\pm$ 14.1<br>(n = 5)              | 373 $\pm$ 44.1<br>(n = 5)              |  |
| Week 10   | Male   | 0.459 $\pm$ 0.0573<br>(n = 5)          | 23.8 $\pm$ 3.45<br>(n = 5)         | 74.0 $\pm$ 6.16<br>(n = 5)         | 358 $\pm$ 28.8<br>(n = 5)               | 568 $\pm$ 107<br>(n = 5)               |  |
|           | Female | 12.0 $\pm$ 22.4<br>(n = 5)             | 19.2 $\pm$ 3.77<br>(n = 5)         | 69.2 $\pm$ 3.46<br>(n = 5)         | 370 $\pm$ 22.3<br>(n = 5)               | 635 $\pm$ 49.0<br>(n = 5)              |  |
| Week 53   | Male   | 0635 $\pm$ 1.04<br>(n = 10)            |                                    |                                    |   | 435 $\pm$ 96.9<br>(n = 9)              |  |
|           | Female | 0923 $\pm$ 1.77<br>(n = 10)            |                                    |                                    |   | 560 $\pm$ 180<br>(n = 10)              |  |
| Day 719   | Male   |  |                                    |                                    |   |  |  |
|           | Female |  |                                    | 55.1 $\pm$ 31.5<br>(n = 9)         |   |  |  |
| Week 105  | Male   | 0.114 $\pm$ 0.148<br>(n = 11)          | 7.83 $\pm$ 7.34<br>(n = 10)        | 26.4 $\pm$ 20.4<br>(n = 17)        | 70.5 $\pm$ 63.1<br>(n = 25)             | 189 $\pm$ 141<br>(n = 22)              |  |
|           | Female | 0.185 $\pm$ 0.184<br>(n = 24)          | 12.9 $\pm$ 6.81<br>(n = 15)        |                                    | 131 $\pm$ 61.4<br>(n = 15)              | 381 $\pm$ 176<br>(n = 25)              |  |
| Week 106  | Male   |  |                                    |                                    |   |  | 3.12 $\pm$ 5.97<br>(n = 10)                  |
|           | Female |  |                                    |                                    |   |  | 12.9 $\pm$ 10.4<br>(n = 17)                  |

<sup>a</sup> Not corrected for purity of the standard material.

<sup>b</sup> The values in this mean were tentative

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to  $\pm$ 30%; liver data are accurate to  $\pm$ 50%.

### 3.6 Developmental Toxicity

Three prenatal developmental toxicity studies of PFOS have been conducted, two studies in rats and one study in rabbits. In addition, preliminary results are available for developmental toxicity studies in rats and mice.

The first study administered four groups of 22 time-mated Sprague-Dawley rats 0, 1, 5, and 10 mg/kg/day PFOS in corn oil by gavage on gestation days (GD) 6-15 (Gortner, 1980). Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to a skeletal examination using alizarin red.

Signs of maternal toxicity consisted of significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day. No other signs of maternal toxicity were reported. Under the conditions of the study, a NOAEL of 5 mg/kg/day and a LOAEL of 10 mg/kg/day for maternal toxicity were indicated.

Developmental toxicity evident at 10 mg/kg/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and the mean numbers of viable male, female, and total fetuses, but the differences were not statistically significant. In addition, unusually high incidences of unossified, assymetrical, bipartite, and missing sternbrae were observed in all dose groups; however, these skeletal variations were also observed in control fetuses at the same rate and therefore these effects were not considered to be treatment-related. The most notable sign of developmental toxicity observed in all dose groups consisted of abnormalities of the lens of the eye, which was not seen in controls. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group. All eye abnormalities appeared to be localized to the area of the embryonal lens nucleus, although a variety of morphological appearances were present within that location. According to the authors, this abnormality appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. Under the conditions of the study, a LOAEL for developmental toxicity of 1 mg/kg/day was indicated; a developmental NOAEL could not be established.

In a second prenatal developmental toxicity study, groups of 25 pregnant Sprague-Dawley rats were administered 0, 1, 5, and 10 mg/kg/day PFOS in corn oil by gavage on gestation days (GD) 6-15 (Wetzel, 1983). Sexually mature Sprague-Dawley rats, one per sex per cage, were paired until confirmation of mating or until two weeks had elapsed. Mating was confirmed by daily vaginal examinations for the presence and viability of sperm or the presence of a copulatory plug. The day of confirmation of mating was designated as day 0 of gestation. Doses were adjusted according to the most recently recorded body weight measurements. Dams were observed twice daily for signs of mortality and moribundity and once daily for clinical signs of toxicity. Individual body weights and food consumption were recorded on GD 6, 8, 12, 16, and 20. Animals were sacrificed on GD 20 by CO<sub>2</sub> asphyxiation and the fetuses were delivered by cesarean section on GD 20. A gross necropsy was performed on all dams. The uterus from each female was excised, weighed and examined for the number and placement of implantation sites, number and of live and dead fetuses, number of early and late resorptions, and any abnormalities and then weighed again after the contents were removed. The ovaries were examined for the number of corpora lutea. Each female was examined by gross necropsy. Each fetus was sexed, weighed, and examined externally. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by the Wilson technique, with particular attention to the eyes, palate, and brain. The remaining fetuses were subjected to a skeletal examination that included evaluation of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles using alizarin red; bone alignment and degree of ossification were also evaluated.

Evidence of maternal toxicity, that was observed at the 5 and 10 mg/kg/day dose groups both during and following treatment and considered to be treatment-related, consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough haircoat, and bloody crust. Significant decreases in mean body weight gains during GD 6-8, 6-16, and 0-20 were also observed at the 5 and 10 mg/kg/day dose groups. These reductions were considered to be treatment-related since mean body weight gains were greater than controls during the post-exposure period (GD 16-20). Significant decreases in mean total food consumption were observed on GD 17-20 in the 10 mg/kg/day dose group, and on GD 7-16 and 0-20 in both the 5 and 10 mg/kg/day dose groups. The mean gravid uterine weight in the 10 mg/kg/day dose group was significantly lower when compared with controls. The mean terminal body weights minus the gravid uterine weights were lower in all treated groups, with significant decreases at 5 and 10 mg/kg/day. High-



dose animals also exhibited an increased incidence in gastrointestinal lesions. No significant differences were observed in pregnancy rates, number of corpora lutea, and number and placement of implantation sites among treated and control groups. Two dams in the 10 mg/kg/day dose group were found dead on GD 17. Under the conditions of the study, a NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for maternal toxicity were indicated.

Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although, none of these effects were statistically significantly different from controls. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg/day dose groups. The percent of male fetuses was 52%, 54%, and 60% for 1, 5, and 10 mg/kg/day, respectively, compared to 44% in controls. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups but not in the mid-dose group. Statistically significant increases in the incidences in the number of litters containing fetuses with visceral anomalies, delayed ossification, and skeletal variations were observed in the high dose group of 10 mg/kg/day. These included external and visceral anomalies of the cleft palate, subcutaneous edema, and cryptorchism as well as delays in skeletal ossification of the skull, pectoral girdle, rib cage, vertebral column, pelvic girdle, and limbs. Skeletal variations in the ribs and sternbrae were also observed. Under the conditions of the study, a NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for developmental toxicity were indicated.

The developmental eye abnormalities that were seen in the previous study (Gortner, 1980) were not observed in the 1983 developmental toxicity study even though the study design and doses were the same. Findings of abnormalities in eye development were initially thought to be treatment-related but later determined to be artifacts of sectioning (3M Company, 1999a).

Lau et al. (2001) administered Sprague-Dawley rats and CD-1 mice doses of 0, 1, 5 or 10 mg/kg/day PFOS in 0.5% Tween-20 by gavage beginning on gestation day 2 and continuing until term. Half of the dams were sacrificed on gestation day 21 (rats) or gestation day 17 (mice) and the remaining dams were allowed to deliver. Preliminary results are available. In rats, there was a significant reduction in maternal body weight gain at 5 and 10 mg/kg/day. Maternal serum cholesterol and triglycerides were reduced at 10 mg/kg/day, but liver weights were comparable to control. At 10 mg/kg/day, there was a reduction in fetal body weight and an increase in cleft palate and anasarca. All pups were born alive, but within 4-6 hours after birth all the pups in the 10 mg/kg/day group died, and 95% of the pups in the 5 mg/kg/day group died within 24 hours. In mice, maternal body weight was unaffected and liver weights were significantly increased at 5 and 10 mg/kg/day; serum triglycerides were reduced were elevated at 5 and 10 mg/kg/day. The incidence of fetal mortality was slightly increased at 10 mg/kg./day and mean fetal body weights were comparable to control. However, neonatal body weights were reduced during the first 3 days of life. Additional studies are underway to further elucidate the dose-response relationships and to examine the mechanism for the neonatal death.

Christian et al. (1999a) administered pregnant New Zealand White rabbits, 22 per group, doses of 0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day PFOS in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weights. The does were observed twice daily for viability, and clinical observations were recorded 1 hour prior to and after dosing during the treatment period and once daily during the post-treatment period (i.e. gestation days 20-29). Maternal body weights were recorded on gestation days 0 and 6-29; food consumption was recorded daily throughout the study. On gestation day 29, the does were euthanized; a gross necropsy of the thoracic, abdominal and pelvic viscera was conducted and the number of corpora lutea in each ovary was recorded. The uteri were examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. The fetuses were weighed, sexed and examined for external abnormalities. All fetuses were examined for visceral and skeletal abnormalities and the brain of one-half of the fetuses were free-

hand cross-sectioned and examined *in situ*.

In addition, a satellite study was conducted in which pregnant New Zealand White rabbits were administered the same doses as in the main study. The number of does was 3, 5, 3, 3 and 5 in the control, 0.1, 1.0, 2.5 and 3.75 mg/kg/day groups, respectively. The does were euthanized on gestation day 21, blood samples were collected, and the liver was weighed and sectioned. The fetuses were removed and examined for external abnormalities. Fetuses and placentae were pooled per litter. All samples were sent to the Sponsor (3M) for analysis. At this time, only the liver and serum analyses have been reported (3M Environmental Laboratory, 2001d).

Maternal toxicity was evident at doses of 1.0 mg/kg/day and above. One doe in the 2.5 mg/kg/day group and nine does in the 3.75 mg/kg/day aborted. All abortions occurred on gestation days 22-28 and were considered treatment-related by the study authors. There was a significant increase in the incidence of scant feces in the 3.75 mg/kg/day group. Scant feces were also noted in one and three does in the 1.0 and 2.5 mg/kg/day groups, respectively. Mean maternal body weight gains were significantly reduced in the 3.75 mg/kg/day group on gestation days 10-13, 13-16, 16-19 and 21-24. Mean body weight gains were also calculated for the treatment period (days 7-21), post-treatment period (days 21-29) and duration of the study (days 7-29). There was a significant reduction in mean maternal body weight gain during the treatment period in the 1.0, 2.5 and 3.75 mg/kg/day groups. Mean body weight gain for the entire study period was also significantly reduced in the 2.5 mg/kg/day group. Mean food consumption (g/kg/day) was significantly reduced in the 2.5 mg/kg/day group on gestation days 16-19, 19-21 and 21-24, as well as for the entire study period (days 7-29). Mean food consumption was significantly reduced in the 3.75 mg/kg/day group on gestation days 13-16, 16-19, 19-21 and 21-24, as well as the entire treatment period. The LOAEL for maternal toxicity was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day.

Developmental toxicity was evident at doses of 2.5 mg/kg/day and above. The number of corpora lutea, resorptions, live/dead fetuses, litter size and sex ratio were comparable among treated and control groups. Mean fetal body weight (male, female and sexes combined) was significantly reduced in the 2.5 and 3.75 mg/kg/day groups. There was also a significant reduction in the ossification of the sternum (litter averages) in the 2.5 and 3.75 mg/kg/day groups, and a significant reduction in the ossification of the hyoid (litter averages), metacarpals (litter averages) and pubis (litter and fetal averages) in the 3.75 mg/kg/day group. The LOAEL for developmental toxicity was 2.5 mg/kg/day and the NOAEL was 1.0 mg/kg/day.

In the satellite study of does euthanized on gestation day 21, the liver and serum analyses were reported by 3M Environmental Laboratory (2001d). All serum and liver samples (including those from untreated controls) had detectable levels of PFOS; the values are presented below:

#### Average Concentration of PFOS in Rabbit Liver and Serum by Dose Group

| Dose group<br>(mg/kg/day) | PFOS conc.<br>liver (ug/g) | PFOS conc.<br>serum (ug/ml) |
|---------------------------|----------------------------|-----------------------------|
| 0.0                       | 0.239                      | 0.0690                      |
| 0.1                       | 13.1                       | 2.73                        |
| 1.0                       | 133                        | 23.8                        |
| 2.5                       | 317                        | 45.8                        |
| 3.75                      | 416                        | 88.9                        |

Qualitatively, increasing concentrations of PFOS were found in samples of liver and serum as doses of PFOS increased. The levels of PFOS are much higher in the liver than in the serum.

These values should be viewed with caution. It was stated that because radio-labeled reference material was not available, "it is not possible to verify true recovery of endogenous analyte from tissues." Matrix spike recovery indicates the accuracy of quantitation to be  $\pm 30\%$ . It is also noted that liver concentrations may be biased high. The only conclusion presented in the laboratory report is that "PFOS was observed in the liver and serum of all rabbits dosed with the test article" (3M Environmental Laboratory, 2001d).

### **3.7 Reproductive Toxicity**

A two-generation reproductive toxicity study, designed to test for the toxic effects of PFOS on reproductive function in adult animals and on developmental, learning, and reproductive effects in the offspring, was conducted in Sprague-Dawley rats (Christian et al., 1999b). Five groups of 35 rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day for six weeks prior to and during mating. Treatment in male rats continued until one day before sacrifice (approximately 22 days total); female rats were treated throughout gestation, parturition, and lactation.

#### F0 Generation:

Parental animals (F0) were observed twice daily for clinical signs. Body weights and food consumption values were recorded weekly during the treatment period in male rats; and weekly during mating and then daily during gestation, and on lactation days 1, 4, 7, 10, 14, and at sacrifice in female rats. Each dosage group consisted of two sets of female rats. One set consisted of the first ten female rats with confirmation of mating; this group was dosed until gestation day (GD) 10 and delivered via Caesarean-sectioning. The remaining females comprised the second set which delivered naturally. During the 21-day lactation period, the dams were evaluated for clinical signs during parturition and length of gestation, and then each litter was evaluated at least twice daily for size and pup viability at birth. Pup observations during the 21-day lactation period included physical signs, body weights, nursing behavior, surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air righting reflex. Pupil constriction was evaluated only on lactation day 21. On lactation day 4, litters were randomly culled to four male and four female pups. The remaining pups were sacrificed and necropsied. The F0 male rats were sacrificed and necropsied after the end of dosing at the time of parturition (lactation day 1). The testes, epididymides, prostate, and seminal vesicles were weighed. Evaluations of sperm number, motility, and morphology were not included in the protocol. The F0 generation females that delivered by Caesarean-section were sacrificed on GD 10 and necropsied. Pregnancy status was confirmed, the ovaries were examined for the number and distribution of corpora lutea, implantation sites were determined, and embryos were examined for viability. The F0 generation females that delivered naturally were sacrificed on lactation day (LD) 21 and necropsied. Ovaries were examined as above and the number and distribution of implantation sites was recorded. The liver from each parental rat was removed, weighed and analyzed. Blood samples were collected from 5 male rats that had mated and from 5 female rats on LD 21 for pharmacokinetic analysis; livers from the pups from the litters of these five dams were also collected for analysis.

#### F1 Generation:

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation. Twenty-five F1 generation rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, and 0.4 mg/kg/day beginning on LD 22 and continuing through the day before sacrifice. At 24 days of age, one rat per sex per litter in each dose group was tested in a passive avoidance paradigm. On LD 28, females were evaluated for the age of vaginal patency and on LD 34, male rats were evaluated for the age of preputial separation. One rat per sex per litter were evaluated in a water-filled M-maze on LD 70. Assignment to cohabitation within each dose group began on LD 90. Females with evidence of mating were considered to be at GD 0 and assigned to individual housing for the remainder of the dosing period. The F1 generation male rats

were sacrificed after mating, necropsied and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver naturally. Dams that delivered litters were sacrificed and necropsied on LD 21. All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups.

In the F0 generation male rats, there were no treatment-related clinical signs of toxicity, no mortality, and no effects on mating or on any of the fertility parameters evaluated in any dose group tested. Reported effects included reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dose groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4 and 1.6 mg/kg/day dose groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate were observed in F0 males at 3.2 mg/kg/day; no other organ weight changes were reported. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported.

In the F0 generation female rats, no deaths were reported at any dose level. In dams sacrificed on GD 10 for Caesarean-sectioning, there did not appear to be any effects on estrous cycling, mating and fertility parameters, the numbers of corpora lutea and implantations, or in the number of viable or non-viable embryos. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dose groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and body weight gain and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Reversible delays in reflex and physical development were observed in the F1 generation offspring. The ability to surface right was significantly delayed in the 1.6 and 3.2 mg/kg/day dose groups on LDs 3-10 (delays in the 3.2 mg/kg/day dose group were observed on LD 1, after which there were no surviving pups remaining for further observation). By the end of the observation period, however, all surviving pups in the 1.6 mg/kg/day dose group had the ability to surface right. There were no delays observed in the ability to surface right in dose groups  $\leq 0.4$  mg/kg/day. Similar responses were seen for pinna unfolding and eye opening. Although there were transient delays seen with these signs of physical development across all dose groups, by the end of the observation period responses in pups were similar to controls. The time of development of the acoustic startle reflex and the ability to air right were both significantly reduced in the 1.6 mg/kg/day dose group. No effects on these reflexes were observed in the low dose group of 0.1 mg/kg/day and only a transient delay (on LD 16 only) in the ability to air right was seen in the 0.4 mg/kg/day group. At the end of lactation (LD 21), all live pups in all dose groups (0, 0.1, 0.4, and 1.6 mg/kg/day) had pupil constriction response.

The most significant finding reported in the offspring was that of reduced pup viability at the two highest dose groups. The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. Statistically significant increases in the number of dams with stillborn pups were also observed at 3.2 mg/kg/day. As a result, the viability index was greatly reduced in these dose groups (0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day). The lactation index was also significantly reduced (94.6%) in the 1.6 dose group. In addition, gestation length was significantly reduced in the high-dose group and there also was a significant reduction in the number of implantation sites followed by a concomitant reduction in litter size. Statistically significant reductions in pup body weights were also observed at the two highest dose groups. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead.

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation.

Clinical observations in the F1 generation male rats appeared unremarkable. No treatment-related deaths were reported and no statistically significant differences were reported for any of the following parameters: body weights/body weight gains, average day of preputial separation; values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating or fertility parameters; necroscopic examinations; absolute or relative weights for the right or left testis, seminal vesicles, right epididymis, or prostate; and terminal body weights. The only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels.

Clinical observations for the F1 generation females were likewise unremarkable. Observations at the 0.4 mg/kg/day dose group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation. There were no statistically significant differences reported for any of the following parameters: values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or water maze performance paradigm; mating and fertility parameters; gestation index; pregnancy rates; and necroscopic examinations.

Evidence of treatment-related effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. Body weights were comparable to control levels by LD 14. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7 and 14. Mean body weights on LD21 continued to remain lower than controls, although the difference was not statistically significant (46.5 g in 0.4 mg/kg/day dose group vs. 50 g in controls). Clinical and necroscopic observations of the F2 generation pups were unremarkable. No other toxicologically significant effects were reported.

Under the conditions of the study, the NOAEL and LOAEL for both the F0 generation male and female parents are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in body weight gain and food consumption. The NOAEL for the F1 generation parental males could not be established since treatment-related reductions in absolute food consumption values were reported at the lowest dose tested, 0.1 mg/kg/day. The NOAEL and LOAEL for the F1 generation parental females are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on significant reductions in body weights and food consumption. The NOAEL and LOAEL for the F1 generation offspring are 0.4 mg/kg/day and 1.6 mg/kg/day, respectively, based on significant reductions in the number of implantation sites, litter size, pup viability, pup body weight and survival. The NOAEL and LOAEL for the F2 generation offspring are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on significant reductions in mean pup body weight.

Liver and sera samples were collected from the F0 and F1 animals at terminal sacrifice (after cohabitation for males and on lactation day 21 for females) and analyzed for the presence of PFOS (3M Environmental Laboratory, 1999a). The results for the F0 animals are presented below:

| Dose group<br>(mg/kg/day) | Average PFOS conc.<br>in serum (ug/ml) | Average PFOS conc.<br>in liver (ug/g) |
|---------------------------|--|---------------------------------------|
| 0.0                       | female 0.0307<br>male 0.0244           | female 0.171<br>male 0.665            |
| 0.1                       | female 5.28<br>male 10.5               | female 14.8<br>male 84.9              |

|     |                          |                         |
|-----|--------------------------|-------------------------|
| 0.4 | female 18.9<br>male 45.4 | female 58.0<br>male 176 |
| 1.6 | female 82<br>male 152    | female 184<br>male 323  |
| 3.2 | female NR*<br>male 273   | female NR*<br>male 1360 |

\*samples not received

Qualitatively, the F0 results indicate all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group. The average PFOS concentrations in pooled liver samples from F1 animals shortly after birth were 0.0511, 6.19, 57.6, and 70.4 ug/g in the 0.0, 0.1, 0.4, and 1.6 mg/kg/day dose groups, respectively. These quantitative values for the PFOS concentration in the liver and serum should be viewed with caution. The accuracy of quantitation is  $\pm 30\%$ , the purity of the analytical reference substance is unknown, and there were several uncorrected dilution errors.

Two studies were then conducted to further understand the distribution of PFOS in the dam, fetus and neonate. In the first study, Sprague-Dawley rats were administered oral doses of PFOS (0.1, 0.4, 1.6, and 3.2 mg/kg/day) once daily beginning 42 days prior to cohabitation, and continued through day 14 or day 20 of presumed gestation (3M Environmental Laboratory, 2001e). Serum, urine, and feces specimens were collected from the dams before mating and at GD 7, GD 15 and GD 21; liver specimens were collected on GD21. A total of 54 pooled serum and liver specimens were collected from fetuses on GD 21. The results are shown below:

Average Results for the Analysis of Serum Samples (ug/ml)

|              | 0 mg/kg | 0.1 mg/kg | 0.4 mg/kg | 1.6 mg/kg | 3.2 mg/kg |
|--------------|---------|-----------|-----------|-----------|-----------|
| Day 0        | 0.0723  | 8.89      | 40.7      | 160       | 318       |
| Day 7        | 0.126   | 7.82      | 40.9      | 154       | 105       |
| Day 15       | 0.0926  | 8.80      | 41.4      | 156       | 275       |
| Day 21       | 0.0714  | 4.24      | 26.2      | 136       | 155       |
| Day 21 Fetal | 0.125   | 9.07      | 34.3      | 101       | 165       |

Average Results for the Analysis of Liver Samples

| Dose Group<br>(mg/kg/day) | PFOS Conc. (ug/g)<br>Female Adult | PFOS Conc. (ug/g)<br>Fetal Liver |
|---------------------------|-----------------------------------|----------------------------------|
| 0                         | 0.288                             | 0.169                            |
| 0.1                       | 29.2                              | 7.93                             |
| 0.4                       | 107                               | 30.6                             |
| 1.6                       | 347                               | 86.7                             |
| 3.2                       | 610                               | 230                              |

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the fetuses. PFOS was also observed in the control dams, as well as the control fetuses. As observed in the 2-generation reproductive toxicity study described above, the levels of PFOS were much higher in the liver than in the serum for the dams. The levels of PFOS remained fairly steady in the serum of the dams from GD0 – GD 15, but the levels dropped at GD21. In the GD21 fetuses, the level of PFOS in the serum was generally comparable to the level observed in the dams, whereas the level of PFOS in the fetal livers was well below that seen in the dams.

In the second study, Sprague-Dawley rats were administered oral doses of PFOS (0.1 and 1.6 mg/kg/day), once daily beginning 43 days prior to cohabitation until confirmed evidence of mating (3M Environmental Laboratory, 2001f). Urine and fecal samples were collected from the dams during the following intervals: one day prior to initiation of cohabitation to the following morning, days 6 to 7, 14 to 15, and 20 to 21 of presumed gestation and days 21-22 of lactation. Blood samples were collected from each of the dams on the day cohabitation was initiated (prior to cohabitation), GD 7, 15 and 21, and LD 14 and 22. Day 1 of lactation was defined as the day of birth. On LD 4, litters were culled to five male pups and five female pups per litter, where possible. Sera specimens were collected from pooled litter samples on LD 21. Liver specimens were collected from the dams, and the liver from each pup was collected and pooled per litter. Blood samples were collected and pooled per liter. The results are shown below:

#### Average Results for the Analysis of Serum Samples (ug/ml)

|            | 0 mg/kg | 0.1 mg/kg | 1.6 mg/kg |
|------------|---------|-----------|-----------|
| GD 0       | 0.100   | 9.21      | 161       |
| GD 7       | 0.0796  | 7.24      | 129       |
| GD 15      | 0.0742  | 5.68      | 90.6      |
| GD 21      | <LLQ    | 2.58      | 39.5      |
| LD14       | 0.0542  | 1.63      | 20.6      |
| LD22       | 0.0492  | 0.979     | 14.1      |
| LD 21 Pups | 0.0531  | 1.80      | 27.1      |

#### Average Results for the Analysis of Liver Samples (ug/g)

|     | 0 mg/kg | 0.1 mg/kg | 1.6 mg/kg |
|-----|---------|-----------|-----------|
| Dam | 0.243   | 6.15      | 59.7      |
| Pup | 0.174   | 5.00      | 56.2      |

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the levels in the serum decreased with time. The levels of PFOS were much higher in the liver than in the serum of the dams and the pups. The levels of PFOS were similar in the liver of the dams and pups, while the levels in the serum were slightly higher in the pups than in the dams.

Based on the results of the two-generation reproductive toxicity study in which significant reductions in pup viability were observed at 1.6 and 3.2 mg/kg/day, a cross-fostering study was conducted as a means of determining whether the effects observed in pups were a result of *in utero* exposure to PFOS or as a result of exposure during lactation; thus the potential for a distinction to be made between prenatal and postnatal effects following continuous maternal treatment (Christian et al., 1999c).

In this study, two groups of 25 female Sprague-Dawley rats were administered 0 and 1.6 mg/kg/day PFOS in 0.5% Tween-80 by gavage, beginning 42 days prior to mating to untreated (breeder) males, and

continuing throughout gestation and into day 21 of lactation. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weight. Parental females were observed twice daily for viability and clinical observations were recorded 1 hour prior to and after dosing during the treatment period. Maternal body weights were recorded once during the acclimation period and then daily during the treatment period and at sacrifice; food consumption was also recorded once during the acclimation period and then daily during gestation and on days 1, 4, 7, 10, and 14 of lactation. During parturition, females were continually evaluated for clinical signs and also for duration of gestation, length of parturition, litter sizes, and pup viability at birth. Maternal behavior was recorded daily throughout lactation. All maternal rats were sacrificed by carbon dioxide asphyxiation on day 22 of lactation and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed; any gross lesions were preserved for future analysis. In addition, the number and distribution of implantation sites were recorded. Rats that did not deliver a litter were not included in the cross-fostering procedure and were sacrificed on lactation day 25, examined for gross lesions, and the uteri examined to confirm the presence/absence of implantation sites. Dams with no surviving pups were sacrificed after the last pup was found dead, missing, or presumed cannibalized.

Following completion of parturition, litters were immediately removed from their respective dams and placed with either a control- or PFOS-treated dam for rearing. This cross-fostering procedure resulted in four groups of 12-13 dams or pups as follows: A) control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; B) control dams with litters from control dams, i.e., negative control; C) PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure; and D) PFOS-treated dams with litters from control dams, i.e., post-natal exposure only.

On day 1 of lactation (birth), each pup was individually weighed and each litter was evaluated twice daily during lactation for viability. Pups were observed once daily for clinical signs and gross external physical anomalies. Pup body weights were recorded on days 1, 4, 7, 14, and 21 of lactation, and then at sacrifice. On day 4 of lactation, each cross-fostered litter was culled to 5 males and 5 females. On day 21 of lactation, all pups were sacrificed via decapitation and examined for gross lesions. Pups found dead or sacrificed because of moribundity were examined for gross lesions and for the cause of death or the moribund condition. The lungs, liver, and any gross lesions were collected from selected pups at various timepoints and preserved for possible future analysis. Liver samples were evaluated via electron microscopy. The method of statistical analysis consisted of calculation of averages and percentages; litter values were used where appropriate.

In addition, samples of blood, milk (including the milk-secreting glands), and liver were collected from selected maternal rats and pups (blood and liver samples were pooled per litter) at various timepoints for analysis of PFOS concentration. Only the analysis of the sera samples are available at this time.

All maternal rats survived to schedule sacrifice. Signs of clinical toxicity observed in the dams during the study period (e.g., chromorhinorrhea, scaly tail, abrasion on the head, neck, tail and/or forelimb, missing, broken and/or misaligned incisors, and localized alopecia, among others) were not considered to be treatment-related since they also occurred in the control animals. Mean maternal body weight gains at 1.6 mg/kg/day were reduced compared to controls during premating and continuing throughout gestation. Mean maternal body weights and body weight changes in the treated group were comparable to those seen in control animals during lactation. During the premating period, and on into gestation and lactation, food consumption was reduced in treated animals as compared to controls. Reductions in gestation length, the average number of implantation sites, delivered sizes, and live litter size were observed in treated animals. All pregnant animals delivered live offspring.

Following cross-fostering on LD 1, live litter sizes were comparable between treated and control groups. Pup mortality was observed in two of the cross-fostered groups. On lactation days 2-4, approximately 19%



of the pups in group C were either found dead or presumed cannibalized. Pup mortality was also observed in group A at a rate of 9%. In addition, on day 4 of lactation, the number of live pups, numbers of surviving pups per litter, and live litter sizes were also reduced in these two groups. Pup mortality in groups B and D during lactation days 2-4 were at 1.6% and 1.1%, respectively. Reductions in pup body weights were observed in groups A and C on day 1 of lactation. Pup body weights in group D were comparable to controls during that same period. From lactation day 4-21, pup body weights in groups A, C, and D were reduced when compared to group B (negative control), with the reductions greatest in group C. Two litters in group A and one litter on group C did not nurse. Milk analysis of the stomachs of pups found no milk in the stomachs of 57%, 100%, and 87% of the pups found dead and necropsied in groups A, C, and D, respectively. Sex ratios and the lactation index were comparable among all groups. Signs of clinical toxicity were observed in pups, but were not considered to be treatment-related since they also occurred in group B (negative control) at the same rate.

Electron microscopic examination of the liver revealed an increase in the number of peroxisomes in pups from dams treated with 1.6 mg/kg/day PFOS. No significant differences were observed between group B and the other groups following examination of pup lungs.

PFOS concentrations in the serum of untreated dams ranged from below the limit of detection (0.05 ug/ml) to 5.34 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with untreated dams, were below the limit of detection. Serum PFOS concentrations in the pups from treated dams, fostered with untreated dams, ranged from 47.6 ug/ml to 59.2 ug/ml. PFOS concentrations in the serum of treated dams ranged from 59.2 ug/ml to 157 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with treated dams, ranged from below the limit of detection to 35.7 ug/ml. Serum PFOS concentrations in the pups from treated dams, fostered with treated dams, ranged from 79.5 ug/ml to 96.9 ug/ml. These data indicate that exposure to PFOS can occur both *in utero* and via milk from treated dams (3M Environmental Laboratory, 1999b). The accuracy of quantitation for the analyses was  $\pm 30\%$ .

In conclusion, pups from control dams that were cross-fostered with PFOS-treated dams (post-natal exposure only) had the same low mortality rate (1.1%) as pups from control dams cross-fostered with control dams (1.6%; negative control). Mortality rates in the remaining two groups, however (i.e. control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; and PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure), were much higher at 9.6 % and 19.2%, respectively. Under the limited conditions of the study, the data appear to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse affect on pup viability. Additionally, analysis of PFOS concentration showed that PFOS was observed in the sera of F0 female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera samples taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed via lactation, but not exposed *in utero*.

Several mechanistic studies are being conducted to understand the neonatal death (3M Company, 2001c). Preliminary results indicate that reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the death of the offspring.

### 3.8 Human Hazard

Several occupational studies on the health effects associated with fluorochemical exposure have been conducted. The following studies have all been conducted at the 3M Decatur, Alabama plant where PFOS has been manufactured since 1991, and PFOA since 1998. The studies conducted in 2000 also included the Antwerp, Belgium plant. Only some of the studies examined a possible association between serum

PFOS levels in workers and corresponding health effects. The other studies use exposure categories as surrogates for fluorochemical serum levels in workers.

Cross-sectional data from medical surveillance conducted at the plants were analyzed to determine if there were any associations between PFOS levels and hematology, clinical chemistries, and hormonal parameters (Olsen et. al., 1998, 1999, 2001a). A group of male volunteers working at the Decatur plant in 1995 and 1997 and volunteers from the 2000 medical surveillance, which also included females and Antwerp employees, were analyzed.

In the 1995 and 1997 analyses, there were no consistent associations between PFOS levels in the workers and the hematology and other clinical chemistry parameters when the data from both plants were combined. Mean serum cholesterol levels remained constant or increased with increasing PFOS serum levels depending on the year; however, those employees with the highest PFOS levels had lower mean HDL values. It should be noted that age was significantly associated with higher PFOS levels. In addition, there were no associations between clinical hepatic enzyme tests and serum PFOS levels in workers.

Eleven hormone values were collected from 88 of the employees who participated in the study in 1995. The employees who participated in the hormone portion of the study were significantly different from the non-participants at both locations (younger, higher PFOS levels, smokers, and higher WBC). After adjusting for confounders, there were no significant associations between PFOS and the hormones analyzed, except estradiol; however, it seems that one employee with high PFOS measurements (12.83 ppm) and a large BMI may have influenced these results. Removal of this employee from the analyses resulted in no significant associations with estradiol.

There were several differences between the employees at the two plants, such as body mass index, alcohol consumption, and age. In addition, there were only 61 employees common to both the 1995 and 1997 cohort due to a high rate of turnover at both plants from 1996-1997. Some of the findings were not consistent over the 2 time periods. The participation rate in this voluntary biomonitoring was very low at the Decatur plant (35-40%). In addition, the employees at both sites used APFO as a surfactant (mean PFOA serum levels in 1995 were 1.46 ppm and in 1997 were 1.57 ppm). The number of years that each group worked at the 2 plants (exposure) were not provided in the analyses, although the youngest employees had the lowest levels of serum PFOS. Also, ninety-five percent of the employees across both plants and both time periods had serum PFOS levels less than 6 ppm. These limitations indicate that the results of this study should be interpreted carefully.

The results from the 2000 cross-sectional analysis were analyzed for all employees from both plant locations combined and by plant since the Antwerp and Decatur populations were significantly different from each other in several ways. Antwerp employees as compared to Decatur employees had lower PFOS serum levels (mean 0.96 ppm), were younger, had lower BMIs, worked fewer years, had higher alcohol consumption, higher mean HDL and bilirubin values, lower mean triglyceride, alkaline phosphatase, GGT, AST, and ALT values, and mean thyroid hormone values tended to be higher. The data were also stratified by production status and gender and divided into quartiles of their serum PFOS distribution. However, the PFOS levels are different for each subgroup, making it difficult to compare the results.

Comparisons across quartiles within plant locations did not reveal many differences in thyroid, hematology, or urinalysis values. The only significant ( $p < .05$ ) differences noted were: BUN was significantly higher in male production workers and females in the highest PFOS quartiles at the Antwerp plant, and ALT was significantly higher in the highest quartile in Decatur male production employees.

When the data were analyzed by number of employees who had values above the reference range for hepatic clinical chemistry tests and liver enzyme and bilirubin tests, a higher percentage of male Decatur

production workers were in the highest PFOS quartile (2.31- 10.06 ppm) for ALT, GGT, and total liver panel than the other quartiles. Most notable were the results for ALT where 8% of employees in the lowest PFOS exposure group (Q1) and 28% in the highest exposure group (Q4) had values above the reference range, while the percentages for total liver panel (which includes alkaline phosphatase, AST, ALT, GGT, and total and direct bilirubin) were 18% and 35%, respectively. This trend was not evident in Decatur non-production employees, in Decatur females, or in any of the Antwerp employees. However, it should be noted that each sub-population had a different serum PFOS quartile distribution.

Mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly ( $p < .05$ ) higher in Q4 (mean PFOS level 2.69, range 1.69 – 10.06 ppm) than in Q1 (mean PFOS level 0.27 ppm, range 0.04 – 0.42 ppm) for all male employees at both plants ( $n = 421$ ). It should be noted that the number of Antwerp production employees were evenly distributed among the quartiles while this was not the case for Decatur employees. The highest number of Decatur employees was in Q4. These data were analyzed for employees who had values above the reference range for alkaline phosphatase, AST, ALT, GGT, and total liver panel. For male employees for all of these measures, the levels increased from Q1 to Q4; however, statistical significance was not reported. In Q1, 4% of the employees had values above the reference range for ALT and 6% for GGT, while 12% was reported for Q4 for both of these tests. For total liver panel, 14% of the employees had values above the reference range in Q1 as compared to 23% in Q4. The numbers of female employees with values above the reference range was very small ( $n = 8$ ). In female employees combined for both plants ( $n = 97$ ), alkaline phosphatase and GGT were significantly higher ( $p < .05$ ) and total bilirubin significantly lower in Q4 than in Q1.

Thyroid results for male production employees of both plants indicated that T3 was significantly higher ( $p < .05$ ) and THBR (T3 uptake) was significantly lower ( $p < .05$ ) in Q4 than in Q1. The range of T3 values was very large for females; however, there were no statistically significant differences between the mean values across the quartiles.

Multivariable regression analyses were conducted to adjust for possible confounders that may affect the results of the clinical chemistry tests. The following variables were included: production job (yes or no), plant, age, BMI, cigarettes/day, drinks/day and years worked at the plant.

A positive significant ( $p = .04$ ) association between T3 and PFOS was observed. Plant location was highly significant ( $p < .0001$ ) in the model. BMI, cigarettes/day, alcohol/day were also significant. In the univariate analyses, Antwerp employees had higher mean T3 levels than Decatur employees overall. However, for each plant (individually) T3 values increased by quartile as PFOS serum levels increased, although the differences were not statistically significant. THBR, as well as the other thyroid hormones, were not significant in the regression analyses.

A positive statistically significant ( $p = .04$ ) association between PFOS and cholesterol was observed. When both PFOS and PFOA were included in the model, neither were statistically significant at  $p = .05$ . PFOS was not significantly associated with HDL, but triglycerides were positively associated with PFOS ( $p = .01$ ). No significant associations were observed with PFOS in relation to alkaline phosphatase, GGT, AST or total bilirubin.

A longitudinal analysis of the above data was performed to determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of the Antwerp and Decatur facilities (Olsen, et al., 2001b). The medical surveillance data from 1995, 1997, and 2000 were analyzed using multivariable regression. The plants were analyzed using 3 subcohorts that included those who participated in 2 or more medical exams between 1995 and 2000. When male employees from both plants were combined, no statistically significant ( $p < .05$ ) associations were observed over time between PFOS and cholesterol or triglycerides. In addition, there were no significant

associations between PFOS and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

There were several limitations to the 2000 cross-sectional and longitudinal studies including: 1) serum PFOS levels in these workers have been declining over time, 2) PFOS serum levels were significantly higher at the Decatur plant than at the Antwerp plant, 3) all participants were volunteers, 4) there were several consistent differences in clinical chemistry profiles and demographics between employees of the Decatur and Antwerp plants, 5) PFOA and other perfluorinated chemicals are also present in these plants, 6) plant populations cannot be compared because quartiles are different for each subgroup, and 7) only one measurement at a certain point in time was collected for each clinical chemistry test. In addition, in the longitudinal study only a small number of employees participated in all 3 sampling periods (24%), different labs and analytical techniques for PFOS were used each year, and female employees could not be analyzed because of the small number of participants.

A retrospective cohort mortality study was performed on Decatur employees to determine whether plant employees had significantly different death experiences from the general population (Mandel and Johnson, 1995). There were 1957 employees who had worked at least one year at the Decatur plant between 1961 and 1991. Seventy-four deaths were recorded, and there were no significantly elevated SMRs for all major causes of death regardless of the comparison population used (Alabama state, US, or Alabama counties). When the data were analyzed by job description (chemical department vs. film plant employees), there was a statistically significant deficit for all causes of death in both job categories.

This study had almost complete follow-up of the cohort and ascertainment of causes of death. However, since the workforce was relatively young, only 74 deaths were reported for 1,951 employees. The number of women in the study was very small (4 deaths). Additionally, PFOS serum levels were not examined in relation to mortality.

An update of this study was recently conducted to follow the cohort through December 31, 1998, thus adding 7 years of follow-up (Alexander, 2001). This cohort consisted of 2083 employees who had worked at the Decatur plant for at least 1 year. There were 145 deaths reported, almost twice that reported in the last cohort. In this study, employees were placed into 3 exposure categories based on their job descriptions: high, low, and non-exposed. Almost half of the employees (47%) were placed in the high exposure group, in which there were 65 deaths. Fourteen percent of the cohort was in the low exposure group (27 deaths), and 39% were in the non-exposed group (53 deaths).

SMRs were calculated using state of Alabama reference data. When the entire cohort was analyzed, SMRs were not elevated for most of the cancer types and for non-malignant causes. SMRs that were above 1 (cancer of the esophagus, liver, breast, urinary organs, bladder, and skin) were also elevated when the cohort was limited to any employee ever employed in a high exposure job (except breast cancer). Only 2 or 3 deaths were reported for each of these cause-specific categories and were not statistically significant, except for bladder cancer.

Workers who were employed in high exposure jobs were 13 times more likely to die of bladder cancer than the general population of Alabama (SMR = 12.77, 95% CI = 2.63 - 37.35). This effect remained when the data were analyzed using county death rates. Three male employees in the cohort died of bladder cancer (0.12 expected). All of these workers had been employed at the Decatur plant for more than 20 years, and all of them had worked in high exposure jobs for at least 5 years (SMR = 24.49). In the previous cohort mortality study, 1 bladder cancer death was reported.

Two deaths were reported for liver cancer. One was in the low exposure group and one in the high exposure group. The SMR for workers who were employed in either high or low exposure jobs was 3.08

(95% CI = 0.37 - 11.10). Five cases of cirrhosis of the liver were reported in this cohort, 2 in the high exposure group, 1 in the low exposure group, and 2 in the non-exposed. The observed did not exceed the expected mortality experience in any of these exposure groups.

Most of the same limitations discussed above for the original cohort study apply to this update. A larger cohort was followed in the update, thus reporting twice as many deaths; however, the cohort is fairly young and the number of deaths is still small (especially for females in all categories). Death certificates were located for 96% of the cohort, but the 6 not obtained could greatly impact the results of the analyses since the number of deaths in most of the sub-cohorts was very small. In addition to fluorochemicals, workers were exposed to other chemicals in the workplace. Biological measurements of fluorochemicals were used to define jobs into exposure categories based on job descriptions; however, there is still a potential for misclassification of exposure.

The high ratio of observed bladder cancer deaths in long-time employees to those expected in the general population is troublesome. Although only 3 deaths were reported in high exposure jobs, the expected number in the general population was 0.23. It seems unlikely that this effect would be due to chance, given the magnitude of the risk estimate (12.77). Many years of follow up without another death from bladder cancer would have to occur before there would no longer be an appreciable risk. Currently, animal studies on PFOS have not reported a higher incidence of bladder cancer tumors. It is unlikely that tobacco smoking could be solely responsible for the excess in bladder cancer mortality, given the 13-fold increase. Smoking status was not determined for this cohort; however, lung cancer and other smoking-related cancers were not elevated. Therefore, it is unlikely that tobacco smoking was any higher in the employees than in the general population.

Given the limitations of this study, it is unclear whether fluorochemicals are responsible for the excess of bladder cancer deaths, or whether other carcinogens may be present in the Decatur plant. At the 3M Cottage Grove facility, where APFO and other fluorochemicals are manufactured, 5 bladder cancer deaths were reported (SMR = 1.31, 95% CI = 0.42 - 3.05). Four of these deaths occurred in employees who did not work primarily in the chemical division and therefore were assumed to have little exposure to APFO. The authors of the Decatur study report that the bladder cancer cases worked mostly in maintenance jobs or at the incinerator and wastewater treatment plant and could have been exposed to many chemicals in addition to fluorochemicals. It is important that follow up of this cohort is continued in order to gain a better understanding of the mortality experience of workers exposed to fluorochemicals.

In order to gain additional insight into the effects of fluorochemical exposure on workers' health, an "episode of care" analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals (Olsen et al., 2001c). An "episode of care" is a series of health care services provided from the start of a particular disease or condition until solution or resolution of that problem. Episodes of care were identified in employees' health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers, procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998. Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M's employee population experience using indirect standardization techniques. A ratio of the chemical plant's observed to expected experience divided by the film plant's observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). 95% confidence intervals were calculated for each RREpC. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected).

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group had 2 ½ times more episodes of care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the reported episodes occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. Episodes of care are reported, not disease incidence; therefore, this parameter cannot be interpreted in any other manner. The data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease. In addition, many of the risk ratios

for episodes of care had very wide confidence intervals. The analysis was limited to 6 years. Also, the utilization of health care services may reflect local medical practice patterns. Individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications. Episodes of care may include the same individual several times. Not all employees were included in the database, such as those on long-term disability. The analysis may be limited by the software used, which may misclassify episodes of care. The software may also assign 2 different diagnoses to the same episode. Certain services, such as lab procedures may not have been reported in the database.

#### 4.0 Hazards to the Environment

The 3M Company have reported ecotoxicity test results on five PFOS salts: potassium, lithium, ammonium, didecyldimethylammonium and DEA (diethanolamine). The majority of testing has however been carried out on the PFOS potassium salt. Only limited data are available for the other salts, but because the salts all dissociate instantaneously at neutral pH to the PFOS ion and the appropriate counterion the potential exists for read-across. For the purposes of this review it is assumed that the toxicity of the PFOS anion will be dominant and that read-across of toxicity from the potassium salt to the other salts is acceptable as a first approximation, except for the didecyldimethylammonium salt. This is because these counterions are unlikely to be toxicologically significant compared with the contribution of the PFOS ion. Published toxicity data relating to, for example, potassium (as chloride) to fish and invertebrates show acute and chronic effect concentrations to be  $\geq 100$  mg/l (Dierickx and Bredael-Rozen, 1996; Biesinger and Christensen, 1972). Toxic concentrations of the PFOS potassium salt reported for fish and invertebrates in the 3M studies are however all well below 100 mg/l, suggesting that the potassium ion was not a major contributor to the overall toxicity of the substance. For the present purpose correction for the molecular weight of the counterions is considered to be unnecessary. Didecyldimethylammonium is an exception due not only to its higher molecular weight, but also because high molecular weight amines are known to have the potential for exhibiting significant toxicity to aquatic species. Only two of the studies presented for review related to the salt of this cation – acute studies with the Fathead minnow (*Pimephales promelas*) and the water flea (*Daphnia magna*). Both were judged to be unacceptable for assessing PFOS toxicity because of the use of an inappropriate method of test medium preparation. The result of the test with the fish did however suggest that the didecyldimethylammonium ion was not a source of significant toxicity in the test medium whilst the result of the test with the water flea suggested that it might have been.

Wherever possible measured exposure concentrations have been used as the basis for expressing the toxicity of the test substance. In some instances the measured concentrations were significantly below nominal. In basing effect concentrations on measured concentrations it is assumed that there is a direct relationship between PFOS concentration and toxicity.

The data summaries in Tables 4 to 15 include columns headed ‘Study Standard’ and ‘Comments’, in addition to study information required under the standard OECD column headings.

‘Study standard’ indicates the overall quality of the reported study taking into account the technical and scientific procedures employed and the information content of the test report. Five standards have been used:

- **Good** – the study fulfils all requirements in terms of method, reporting and interpretation.
- **Acceptable** – the study is of an overall acceptable standard for use in hazard and risk assessment although there are inadequacies in some elements.
- **Questionable** – there are significant inadequacies in elements of the study that raise concerns about its validity. The results should only be considered supportive of other data.
- **Unacceptable** – there are significant inadequacies in elements of the study that invalidate the data.
- **Unknown** – there is insufficient information to evaluate the standard of the study.

The column headed 'Comments' highlights observations on the study procedures and results that are considered relevant to the assessment. Reasons why procedures or results were considered inappropriate are also given.

The tables also contain a column headed 'Study reference number' that refers to the list of studies in Annex 1. A summary of the lowest acceptable effect concentrations for each trophic level is given in Annex 4.

#### **4.1 Effects on Fish, Invertebrates and Aquatic plants (Algae and higher plants)**

Acute (short-term) data were available from a total of 31 studies covering three species of freshwater fish, one species of saltwater fish, a fish acclimated to saltwater, two species of freshwater and three species of saltwater invertebrate, three species of freshwater and one species of saltwater algae and one species of freshwater higher plant. The studies have been reviewed and this has resulted in 21 being considered to be of an acceptable standard for assessing the acute (short-term) toxic hazard of PFOS to fish, invertebrates and aquatic plants.

Sub-chronic/chronic (prolonged/longer-term) data were available from a total of 8 studies covering two species of freshwater fish, one species of fresh and saltwater invertebrate and one species of freshwater unicellular algae. A review of the studies has resulted in seven being considered to be of an acceptable standard for assessing the toxic hazard of PFOS to fish and invertebrates. It should be noted that study number 25 with the Fathead minnow (*Pimephales promelas*) was considered unacceptable because of a lack information in the report. It is probable that this study was the same as that described more fully in report number 14. Study 14 was judged to be acceptable.

In addition, flow-through bioconcentration studies have been conducted in the bluegill sunfish (*Lepomis macrochirus*) and carp (*Cyprinus carpio*).

The acceptable and unacceptable studies are identified in Table 3.



**Table 3. Acceptable and unacceptable studies for determining the acute and sub-chronic/chronic (prolonged/long-term) toxic hazard of PFOS to fish, invertebrates and aquatic plants (algae and higher plants)**

| Endpoint  | Test species                                  | Acceptable study reference numbers | Unacceptable study reference numbers |
|---|---|------------------------------------|--------------------------------------|
| Acute (short-term) toxicity to fish (freshwater)            | <i>Pimephales promelas</i>                    | 1, 16, 28                          | 25, 26, 27                           |
|   | <i>Lepomis macrochirus</i>                    | 20                                 | 25                                   |
|   | <i>Oncorhynchus mykiss</i>                    | 31, 42                             | 25                                   |
| Acute (short-term) toxicity to fish (saltwater)             | <i>Oncorhynchus mykiss</i>                    | 30                                 | -                                    |
|   | <i>Cyprinodon variegatus</i>                  | 43                                 | -                                    |
| Sub-chronic/ chronic toxicity to fish (freshwater)          | <i>Pimephales promelas</i>                    | 8, 14                              | 25                                   |
|   | <i>Lepomis macrochirus</i>                    | 41                                 | -                                    |
| Acute (short-term) toxicity to invertebrates (freshwater)   | <i>Daphnia magna</i>                          | 3, 15, 17, 29, 33                  | 23, 25                               |
|   | <i>Unio complamatus</i>                       | 5                                  | -                                    |
| Acute (short-term) toxicity to invertebrates (saltwater)    | <i>Mysidopsis bahia</i>                       | 4                                  | -                                    |
|   | <i>Crassostrea virginica</i>                  | 7                                  | -                                    |
|   | <i>Artemia</i> sp                             | 32                                 | -                                    |
| Sub-chronic/ chronic toxicity to invertebrates (freshwater) | <i>Daphnia magna</i>                          | 9, 15                              | -                                    |
| Sub-chronic/ chronic toxicity to invertebrates (saltwater)  | <i>Mysidopsis bahia</i>                       | 10                                 | -                                    |
| Toxicity to freshwater unicellular algae                    | <i>Selenastrum capricornutum</i> <sup>1</sup> | 2, 13                              | 24                                   |
|   | <i>Anabaena flos-aquae</i>                    | 36                                 | -                                    |
|   | <i>Navicula pelliculosa</i>                   | 38                                 | -                                    |
| Toxicity to saltwater unicellular algae                     | <i>Skeletonema costatum</i>                   | 39                                 | -                                    |
| Longer-term toxicity to freshwater unicellular algae        | <i>Selenastrum capricornutum</i> <sup>1</sup> | 13                                 | -                                    |
| Toxicity to freshwater higher plants                        | <i>Lemna gibba</i>                            | 37                                 | -                                    |

#### 4.1.1 Fish

##### Acute (short-term) toxicity

The results of the acute (short-term) studies are summarized in Table 4. Three species of freshwater fish have been used for testing - *Pimephales promelas* (Fathead minnow), *Lepomis macrochirus* (Bluegill sunfish) and *Oncorhynchus mykiss* (Rainbow trout). The end point assessed in all the tests was mortality during a 96-hour exposure period.

*P. promelas* was the most susceptible freshwater fish species in acute tests with a lowest 96-hour LC<sub>50</sub> for PFOS lithium salt of 4.7 mg/l<sup>2</sup>. *L. macrochirus*, and *O. mykiss*, were only marginally less susceptible - a 96-hour LC<sub>50</sub> value of 7.8 mg/l was determined with both species for the diethanolamine (DEA) and potassium salt respectively. A second study on *Oncorhynchus mykiss* showed an LC<sub>50</sub> of 22 mg/l again using the potassium salt. A very high 96-hour LL<sub>50</sub> value of approximately 200 mg/l determined for the didecyldimethylammonium salt of PFOS was obtained for *P. promelas* in a test on water accommodated

<sup>1</sup> This species has recently been renamed *Pseudokirchneriella subcapitata*.

<sup>2</sup> There are only three acute toxicity reports for Fathead minnow of acceptable or better quality, so the most sensitive value is used in this assessment.

fractions of an aqueous mixture containing the substance. However, the actual exposure concentrations of PFOS were not determined and could not be estimated in this test.

PFOS also exhibits acute toxicity to fish in saltwater. A 96-hour LC<sub>50</sub> value of 13.7 mg/l has been determined for the potassium salt in a test with *O. mykiss* acclimated to saltwater at a salinity of 30 parts per thousand. The data show saltwater acclimated *O. mykiss* to be of similar susceptibility to PFOS when compared with *O. mykiss* living in freshwater. However, in the absence of measured exposure concentrations, it should be noted that this study might have been conducted in excess of the substance's salt water solubility (2.5 to 20 mg/l, depending on salinity and purity). A further study using a saltwater fish, Sheepshead minnow (*Cyprinodon variegatus*) showed no toxicity up to the water solubility limit, indicated as 15 mg/l in the test. Loss of concentration during the test, probably due to sedimentation, indicates that this value may be above the true solubility, however.

### **Sub-chronic/chronic (prolonged/long-term) toxicity**

The results of the sub-chronic/chronic (prolonged/long-term) studies are summarized in Table 5. Studies have been carried out with two species of freshwater fish – *Pimephales promelas* (Fathead minnow) and *Lepomis macrochirus* (Bluegill sunfish). Tests with *P. promelas* were designed to determine concentrations affecting early life-stages of the fish over exposure periods of up to 42 days. Mortality data for *L. macrochirus* were obtained from a bioconcentration study in which deaths in the treated and control groups of fish were recorded over the 62-day uptake phase of the study.

The lowest definitive no observed effect concentration (NOEC) of 0.3 mg/l was determined for *P. promelas* for the potassium salt of PFOS. This value was applicable to both survival and growth end points. This NOEC is supported by results from a bioconcentration study with *L. macrochirus* that showed no significant mortality at an exposure concentration of 0.086 mg/l over a 62-day uptake phase but 100% mortality at a concentration of 0.87 mg/l after 35 days. A NOEC of 1.0 mg/l reported for effects of PFOS potassium salt on early life-stages of *P. promelas* could not be attributed to a more specific end point because insufficient information was contained in the study report.

### **Bioconcentration**

A flow-through bioconcentration study of PFOS in bluegill sunfish was conducted. Test concentrations of 0, 0.086 and 0.87 mg/L were used. At the 0.086 mg/L level, the fish were exposed for 62 days, while at the 0.87 mg/L level, the fish were exposed for 35 days due to excessive mortality. Fish were collected from the test chambers by random selection at 12 time points during the study. They were euthanized, blotted dry, weighed and measured. They were dissected into edible and nonedible tissue fractions and the fractions were weighed. The head, fins and viscera were considered to be nonedible tissue and the remaining tissue, including skin, was considered to be edible tissue. Whole fish concentrations were calculated from the sum of the edible and nonedible parts. Steady-state BCF values were calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different. The kinetic bioconcentration factor (BCFK), uptake rate and depuration rate were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L using BIOFAC computer software.

In this study, PFOS bioconcentrated in the tissues of bluegill sunfish. Apparent steady-state was not clearly attained for the fish exposed to 0.086 mg /L. Although the tissue residue levels of PFOS were not statistically significantly different on days 49, 56 and 62, the concentration of PFOS appeared to be still increasing up to the last day of exposure. BCFK values for edible, nonedible and whole fish tissues were calculated to be 1124, 4013, and 2796, respectively. PFOS depurated slowly. The BIOFAC estimates for

the time to reach 50% clearance for edible, nonedible, and whole fish tissues were 86, 116, and 112 days, respectively.

Kurume Laboratory (2001) conducted a flow-through bioconcentration study of PFOS in carp (*Cyprinus carpio*). The fish were checked visually and those demonstrating any abnormality were removed. The fish were reared for 8 days in a flow through system following an external disinfection. After rearing, the fish were medicated to eliminate parasites and transferred to an acclimatizing aquarium. After the second external disinfection, they were acclimatized. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 15 days in a flow through system at temperatures of  $25 \pm 2^\circ \text{C}$ . The fish were transferred to test tanks and reared at the same temperature in the flow through system for another 27 days. Temperature of the test water was measured with alcohol thermometer and recorded once a day. Dissolved oxygen concentrations was measured with a dissolved oxygen probe and recorded twice a week. The pH of the test water was measured with a pH meter one a week. Temperature of test water was measured and recorded once a day. Dissolved oxygen in test water was measured and recorded twice a week. During the experimental period, the excreta of carp, dirt on test tanks, were removed once a day. The temperature ranged from 25.0 to 25.4°C; the dissolved oxygen concentration ranged from 7.9 to 8.1 mg/L; and the pH ranged from 7.6 to 7.8.

Forty carp were exposed to two concentrations of PFOS, 2 and 20 ug/L respectively for 58 days in a flow through system. The test water of each level was analyzed once before first analysis of test fish and at the same time as analysis of test fish. Analysis of test fish was performed six times at each level in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. Analysis of control fish was performed before the experimental starting and after the experimental completion. Six fish were taken out at each sampling time and divided into three groups, and then both were analyzed individually. Two fish were employed to measure the lipid content because of insufficient size.

Analysis of PFOS in the test water and carp was performed using high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The test water of each level was analyzed once before first analysis of test fish and at the same time as the analysis of the test fish. The fish were separated into parts; tegument, head, viscera except liver, liver and remaining matter. Steady-state was reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within + 20% of each other. When BCFs were less than 100, it was evaluated that a steady-state had been reached after 28 days.

In this study, PFOS bioconcentrated in the tissues of carp. Test concentrations of 2 and 20 ug/L were used. The fish were exposed for 58 days to makeup for excessive mortality. Bioconcentration factors were calculated to be 720 for 2 ug/L and 200- 1500 for 20 ug/L.

**Table 4. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish**

| Medium     | Species                                     | Protocol                           | Results (mg/l)                                       | Study Standard | Comments   | Study Ref. No. |
|------------|---|------------------------------------|--|----------------|--|----------------|
| Freshwater | <i>Pimephales promelas</i> (Fathead minnow) | OECD 203 & OPPTS 850.1075 (Static) | 96-hour LC <sub>50</sub> = 9.5<br>96-hour NOEC = 3.3 | Good           | Test substance was PFOS potassium salt. Measured exposure concentrations   | 1              |
|            |   | Not noted (Static)                 | 96-hour LC <sub>50</sub> = 4.7 (see comments)        | Acceptable     | Test substance was PFOS lithium salt. Nominal exposure concentrations expressed relative to concentration of test substance (24.5% PFOS Li salt and 74.5% water). Test result divided by 4 to express 96 h LC <sub>50</sub> in terms of PFOS Li salt concentration. Very little information on study protocol; however, result agrees reasonably well with more other studies (1, 25, 26 and 27) | 16             |
|            |   | Not noted (Static)                 | 96-hour LC <sub>50</sub> = 37.6 and 51               | Unknown        | Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of studies cannot be judged from information supplied. However, results are consistent with other studies (1, 16, 26 and 27)  | 25             |
|            |   | Not noted (Static)                 | 96-hour LC <sub>50</sub> = 21                        | Questionable   | Test substance was PFOS ammonium salt. Nominal exposure concentrations Isopropanol also present in test samples. However, results are consistent with other studies (1, 16, 25 and 27)   | 26             |
|            |   | Not noted (Static)                 | 96-hour LC <sub>50</sub> = 25                        | Questionable   | Test substance was PFOS ammonium salt. Nominal exposure concentrations Isopropanol also present in test samples. Results consistent with other studies (1, 16, 25 and 26)  | 27             |

**Table 4 contd. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish**

| Medium     | Species                                       | Protocol   | Results (mg/l)  | Study Standard | Comments   | Study Ref. No. |
|------------|---|--|---|----------------|--|----------------|
| Freshwater | <i>Pimephales promelas</i> (Fathead minnow)   | OECD 203 (Static)  | 96-hour LL <sub>50</sub> = 200*<br>96-hour NOEL = <170*<br>(*calculated assuming 35% of substance in mixture) | Acceptable     | Test substance was a mixture of didecyldimethylammonium salt of PFOS (approximately 35%) and water with up to 5% residual perfluorochemicals. Test media were water-accommodated fractions (WAFs). Nominal exposure concentrations.  | 28             |
|            | <i>Lepomis macrochirus</i> (Bluegill sunfish) | OECD 203 & OPPTS 850.1075 (Static)   | 96-hour LC <sub>50</sub> = 7.8<br>96-hour NOEC = 4.5<br>(see comments)  | Acceptable     | Test substance was PFOS DEA salt. Nominal exposure concentrations expressed relative to concentration of test substance (~25% PFOS DEA salt and 75% water). Results have therefore been divided by 4 to calculate the effect concentrations in terms of PFOS DEA salt concentration based on reported 96-hour LC <sub>50</sub> of 31 mg/l and NOEC of 18 mg/l. | 20             |
|            |   | Not noted (Static)   | 96-hour LC <sub>50</sub> = 68   | Unknown        | Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of study cannot be judged from information supplied.  | 25             |
|            | <i>Oncorhynchus mykiss</i> (Rainbow trout)    | Not noted (Static)   | 96-hour LC <sub>50</sub> = 11   | Unknown        | Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of study cannot be judged from information supplied.  | 25             |
|            |   | Standard procedures for testing acute lethality of liquid effluents (Environment Canada) | 96-hour LC <sub>50</sub> = 7.8  | Acceptable     | Test substance was PFOS potassium salt. Sample purity not characterised Nominal exposure concentrations  | 31             |

**Table 4 contd. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish**

| Medium     | Species   | Protocol   | Results (mg/l)                    | Study Standard | Comments   | Study Ref. No. |
|------------|---|--|-----------------------------------|----------------|--|----------------|
| Freshwater | <i>Oncorhynchus mykiss</i><br>(Rainbow trout)       | OPPTS 850.1075<br>OECD 203<br>Static   | 96-hour LC <sub>50</sub> = 22     | Acceptable     | Test substance was PFOS potassium salt.<br>Sample purity 86.7%; results reported as a.i<br>Based on measured concentrations  | 42             |
| Saltwater  | <i>Cyprinodon variegatus</i><br>(Sheepshead minnow) | OPPTS 850.1075<br>OECD 203<br>Semi-static<br>(24 hour renewal)                           | 96-hour LC <sub>50</sub> >15 mg/l | Acceptable     | Test substance was PFOS potassium salt.<br>Sample purity 86.7%; results reported as a.i<br>Based on mean of measured concentrations<br>Concentration losses during each 24 hour period would suggest some sedimentation indicating that the water solubility may have been exceeded. Final concentrations in each 24 hour period varied between 11 – 16 mg/l   | 43             |
| Saltwater  | <i>Oncorhynchus mykiss</i><br>(Rainbow trout)       | Standard procedures for testing acute lethality of liquid effluents (Environment Canada) | 96-hour LC <sub>50</sub> = 13.7   | Acceptable     | Test substance was PFOS potassium salt.<br>Sample purity not characterised<br>Test fish were acclimated to 30 parts per thousand saltwater<br>Nominal exposure concentrations<br>In the absence of measured exposure concentrations, it should be noted that this study might have been conducted in excess of the substance's salt water solubility (2.5 to 20 mg/l, depending on salinity and purity). | 30             |

**Table 5. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with fish**

| Medium     | Species  | Protocol                                 | Results (mg/l)   | Study Standard | Comments   | Study Ref. No.                         |
|------------|--|--|--|----------------|--|--|
| Freshwater | <i>Pimephales promelas</i><br>(Fathead minnow)   | OECD 210 & OPPTS 850.1400 (Flow-through) | 42-day NOEC <sub>surv.</sub> = 0.30<br>42-day NOEC <sub>growth</sub> = 0.30<br>5-day NOEC <sub>hatch</sub> ≥ 4.6 | Good           | Test substance was PFOS potassium salt. Measured exposure concentrations. Lighting regime not described in summary. Conclusion (post-hatch survival as the most sensitive endpoint): it is possible that growth was also affected at this concentration, but the test design did not enable this to be determined. | 8                                      |
|            |  | Non-standard (Flow-through)              | 30-day NOEC <sub>early life-stages</sub> = 1   | Acceptable     | Test substance was PFOS potassium salt. Measured exposure concentrations. Results agree with later study<br>Acetone present at 43 µl/l   | 14                                     |
|            |  | Not noted (Flow-through)                 | NOEC <sub>early life-stages</sub> = 1  | Unknown        | Test substance was PFOS potassium salt. Standard of study cannot be judged from information supplied. It is however assumed that this study is the same as 14  | 25<br>(Assumed to be same study as 14) |
|            | <i>Lepomis macrochirus</i><br>(Bluegill sunfish) | OECD 305 & OPPTS 850.1730                | 62-day NOEC <sub>mortality</sub> = >0.086, <0.87   | Good           | Bioconcentration study<br>Test substance was PFOS potassium salt. Measured exposure concentrations.  | 41                                     |

#### **4.1.2 Invertebrates**

The results of acute (short-term) and sub-chronic/chronic (prolonged/long-term) studies are summarised in Tables 6 and 7.

##### **Acute (short-term) toxicity to freshwater species**

Two species of freshwater invertebrate have been used for testing - *Daphnia magna* (the 'water flea') and *Unio complamatus* (fresh water mussel). Immobilisation/mortality during 48-hours exposure was assessed in the tests with *D. magna*. Mortality during 96-hours exposure was assessed in the test with *U. complamatus*.

A lowest acceptable 48-hour EC<sub>50</sub> value of 27 mg/l was determined for PFOS potassium salt in tests with the daphnid.<sup>1</sup> A 48-hour EL<sub>50</sub> value of approximately 4.0 mg/l was also determined for the didecyldimethylammonium salt of PFOS in a daphnid test on water accommodated fractions of an aqueous mixture containing the substance. However, the actual exposure concentrations of PFOS were not determined in this test and, as pointed out in the introduction to this section, it is possible that didecyldimethylammonium may have contributed to the toxicity of the test medium. These values compare with a 96-hour LC<sub>50</sub> value determined for mortality of the mussel of 59 mg/l.

##### **Acute (short-term) toxicity to saltwater species**

Three species of saltwater invertebrate have been used for testing - *Mysidopsis bahia* (Mysid shrimp), *Crassostrea virginica* (Eastern oyster) and *Artemia* sp (Brine shrimp). Mortality during 96-hours exposure was assessed in the tests with *M. bahia* and *Artemia* sp. Reduction in shell deposition was assessed over a 96-hour exposure period in the test with *C. virginica*. The test substance was PFOS potassium salt in all cases.

A 96-hour LC<sub>50</sub> value of 3.6 mg/l and an associated NOEC of 1.1 mg/l were determined in the test with the Mysid shrimp. This compares with a 96-hour EC<sub>50</sub> value of >3.0 mg/l for effects on shell deposition in the oyster and a 48-hour LC<sub>50</sub> of 8.9 mg/l for mortality of the Brine shrimp.

##### **Sub-chronic/chronic (prolonged/long-term) toxicity to freshwater species**

Two tests have been carried out on PFOS potassium salt with one species of freshwater invertebrate – the 'water flea', *Daphnia magna*. The end points assessed in the tests were survival, growth and reproduction measured over exposure periods of up to 28 days.

NOECs of 12 and 7 mg/l have been determined for *D. magna* reproduction in 21 and 28-day tests respectively. In the 21-day test the NOECs for survival and growth were also 12 mg/l, indicating that reproduction was no more sensitive than these two other end points.

##### **Sub-chronic/chronic (prolonged/long-term) toxicity to saltwater species**

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<sup>1</sup> For *Daphnia magna* there are five 48-hour acute toxicity studies of acceptable or better quality, although one of these relates to water accommodated fractions. The remaining 48-hour EC<sub>50</sub>s are 27, 58, 61 and 210 mg/l. The latter value is based on nominal exposure concentrations, but since the stock solution was prepared at a concentration that exceeded water solubility, exposure concentrations are likely to be lower than nominal. This leaves just three reports with comparable measures of toxicity, and so the most sensitive value is used in this assessment.



A test has been carried out on PFOS potassium salt with one species of saltwater invertebrate; the Mysid shrimp, *Mysidopsis bahia*. The end points assessed in the test were survival, growth and reproduction measured over an exposure period of 35 days.

The 35-day NOECs determined for survival, growth and reproduction in this test were 0.55, 0.25 and 0.25 mg/l respectively.

**Table 6. Acute (short-term exposure) toxicity data for PFOS determined in tests with invertebrates**

| Medium     | Species                              | Protocol                                    | Results (mg/l)  | Study Standard | Comments   | Study Ref. No. |
|------------|--------------------------------------|---|---|----------------|--|----------------|
| Freshwater | <i>Daphnia magna</i><br>(Water flea) | OECD 202 &<br>OPPTS<br>850.1010<br>(Static) | 48-hour EC <sub>50</sub> = 61<br>48-hour NOEC = 33              | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations  | 3              |
|            |                                      | ASTM 1981 &<br>OECD 1981<br>(Static)        | 48-hour EC <sub>50</sub> = 27                                   | Acceptable     | Test substance was PFOS potassium salt.<br>Nominal exposure concentrations.<br>Result agrees reasonably well with those<br>obtained in studies 3 and 23.   | 15             |
|            |                                      | Not noted<br>(Static)                       | 48-hour EC <sub>50</sub> = 210<br>48-hour NOEC = 100            | Acceptable     | Test substance was PFOS lithium salt.<br>Nominal exposure concentrations.<br>EC <sub>50</sub> is somewhat higher than previous value<br>but stock solution was prepared at a<br>concentration that exceeded water solubility,<br>therefore exposure concentrations are likely<br>to be lower than nominal. | 17             |
|            |                                      | OECD 202<br>(Static)                        | 24-hour EC <sub>50</sub> = >42<br>48-hour EC <sub>50</sub> = 14 | Questionable   | Test substance was PFOS potassium salt.<br>Nominal exposure concentrations.<br>Data questionable in view of the presence of<br>diethylene glycol butyl ether in the test<br>material (as part of the formulation).<br>Result is in reasonable agreement with later<br>study (3).                           | 23             |
|            |                                      | Not noted<br>(Static)                       | 48-hour EC <sub>50</sub> = 49.2                                 | Unknown        | Test substance was PFOS potassium salt.<br>Nominal exposure concentrations.<br>Standard of study cannot be judged from<br>information supplied.  | 25             |

**Table 6 contd. Acute (short-term exposure) and sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with invertebrates**

| Medium    | Species  | Protocol   | Results (mg/l)  | Study Standard | Comments  | Study Ref. No. |
|-----------|--|--|---|----------------|---|----------------|
|           | <i>Daphnia magna</i><br>(Water flea)           | OECD 202<br>(Static)   | 48-hour EL <sub>50</sub> = 4.0<br>48-hour NOEL = 2.2  | Acceptable     | Test substance was a mixture of didecyldimethylammonium salt of PFOS (approximately 35%) and water with up to 5% residual perfluorochemicals.<br>Test media were water-accommodated fractions (WAFs).<br>Nominal exposure concentrations.<br>48-hour EL <sub>50</sub> and NOEL calculated assuming 35% of substance in mixture. | 29             |
|           |  | ISO, 1982  | 48-hour EC <sub>50</sub> = 58                         | Acceptable     | Test substance was PFOS potassium salt.<br>Sample purity not characterised<br>Nominal exposure concentrations.  | 33             |
|           | <i>Unio complamatus</i><br>(Freshwater mussel) | OECD 203,<br>OPPTS<br>850.1075 &<br>ASTM-E-729-<br>88a (Semi-<br>static) | 96-hour LC <sub>50</sub> = 59<br>96-hour NOEC = 20    | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.  | 5              |
| Saltwater | <i>Mysidopsis bahia</i><br>(Mysid shrimp)      | OPPTS<br>850.1035<br>(Static)  | 96-hour LC <sub>50</sub> = 3.6<br>96-hour NOEC = 1.1  | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.  | 7              |
|           | <i>Crassostrea virginica</i> (Eastern oyster)  | OPPTS<br>850.1025<br>(Static)  | 96-hour EC <sub>50</sub> = >3.0<br>96-hour NOEC = 1.9 | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.<br>Solubility in seawater should have been used to set highest test concentration.   | 4              |
|           | <i>Artemia</i> sp.<br>(Brine shrimp)           | Draft ISO,<br>1981   | 48-hour LC <sub>50</sub> = 8.9                        | Acceptable     | Test substance was PFOS potassium salt.<br>Sample purity not characterised<br>Nominal exposure concentrations.  | 32             |

Table 7. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with invertebrates

| Medium     | Species                                   | Protocol  | Results (mg/l)   | Study Standard | Comments  | Study Ref. No. |
|------------|---|---|--|----------------|---|----------------|
| Freshwater | <i>Daphnia magna</i><br>(Water flea)      | OECD 211,<br>OPPTS<br>850.1300 &<br>ASTM 1193-<br>87E (Semi-<br>static) | 21-day NOEC <sub>repro</sub> = 12<br>21-day NOEC <sub>surv</sub> = 12<br>21-day NOEC <sub>growth</sub> = 12          | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.<br>No effects on reproduction at highest<br>concentration that had no effect on mortality. | 9              |
|            |   | ASTM 1981 &<br>OECD 1981<br>(Semi-static)                               | 21-day EC <sub>50repro</sub> = 12<br>28-day NOEC <sub>repro</sub> = 7<br>28-day EC <sub>50repro</sub> = 11           | Acceptable     | Test substance was PFOS potassium salt.<br>Nominal exposure concentrations.<br>Result agrees well with that obtained in study<br>9.                                     | 15             |
| Saltwater  | <i>Mysidopsis bahia</i><br>(Mysid shrimp) | OPPTS<br>850.1350<br>(Flow-<br>through)                                 | 35-day NOEC <sub>repro</sub> = 0.25<br>35-day NOEC <sub>surv</sub> = 0.55<br>35-day NOEC <sub>growth</sub> =<br>0.25 | Good           | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.  | 10             |

### 4.1.3 Aquatic plants

The results of all the short- and longer-term studies are summarised in Tables 8 and 9 (algae) and 10 (higher plants). Note: The species *Selenastrum capricornutum* has been renamed *Pseudokirchneriella subcapitata*. For transparency, the name used in the test report has been retained in the discussion below.

#### Freshwater algae – Short-term

Three species of freshwater unicellular algae have been used for testing - *Selenastrum capricornutum*, *Anabaena flos-aquae* and *Navicula pelliculosa*. The end point assessed in the tests was growth measured in terms of cell density, growth rate and/or the area under the growth curve over 96-hours. Only studies that assessed toxicity by reference to effects on growth rate are considered here for the purposes of determining the toxicity of PFOS to algae. The results of study number 13 are therefore excluded because they are only expressed relative to cell numbers and cell dry weight. These results could be considered were the EC<sub>50</sub> values to be recalculated in respect of growth rate, but since algae were relatively insensitive compared with fish, this is not considered a high priority.

The lowest 96-hour EC<sub>50</sub> value for effects on growth rate of 71 mg/l was determined for *S. capricornutum*. However there are some uncertainties over the validity of this result given that exposure concentrations were not measured and diethylene glycol butyl ether was present in the test material (as part of the formulation). A 96-hour EC<sub>50</sub> value of 126 mg/l was obtained for the same species using a test protocol that was judged to meet all the criteria for acceptability of the data. The 96-hour NOECs associated with these two tests were 35 and 44 mg/l respectively. The tests with the two other species yielded 96-hour EC<sub>50</sub> values of 176 mg/l (*A. flos-aquae*) and 305 mg/l (*N. pelliculosa*) and respective NOECs of 94 and 206 mg/l.

#### Saltwater algae – Short-term

A 96-hour growth inhibition test has been carried out on PFOS potassium salt with *Skeletonema costatum*. The test was unable to determine a definitive 96-hour EC<sub>50</sub> value because no effects were determined at the highest dissolved PFOS concentration that could be attained under the test conditions (3.2 mg/l).

#### Freshwater algae – Longer-term

One species of freshwater unicellular algae has been tested - *Selenastrum capricornutum* (study reference no. 13). The test assessed effects on growth rate expressed relative to cell density over 14 days. The results from the test are not discussed further here for two reasons. Firstly, the results are expressed relative to cell density (see comments in respect of short-term tests). Secondly, there are concerns that the 14-day test period and absence of test medium renewal may have resulted in a decline in exposure concentrations over time (the test media were not analysed for PFOS concentration). The latter is suggested by comparison of the 14-day EC<sub>50</sub> value of 95 mg/l with the 96-h EC<sub>50</sub> value of 82 mg/l determined in the same test.

#### Freshwater higher plants

A growth inhibition test has been carried out on PFOS potassium salt with *Lemna gibba* (Duckweed). The test yielded a 7-day IC<sub>50</sub> of 108 mg/l for inhibition of frond production and a 7-day NOEC of 15.1 mg/l based on the inhibition of frond production and evidence of sub-lethal effects.

**Table 8. Acute (short-term exposure) toxicity data for PFOS determined in tests with algae**

| Medium     | Species                          | Protocol  | Results (mg/l)   | Study Standard | Comments   | Study Ref. No. |
|------------|----------------------------------|---|--|----------------|--|----------------|
| Freshwater | <i>Selenastrum capricornutum</i> | OECD 201, OPPTS 850.5400 & ASTM 1218-90E (Static)     | 96-hour $EC_{50}(\text{cell density}) = 71$<br>96-hour $E_bC_{50}(\text{area under the curve}) = 71$<br>96-hour $E_rC_{50}(\text{growth rate}) = 126$<br>96-hour $NOEC(\text{growth rate, cell density, area under the growth curve}) = 44$<br>72-hour $EC_{50}(\text{cell density}) = 70$<br>72-hour $E_bC_{50}(\text{area under the curve}) = 74$<br>72-hour $E_rC_{50}(\text{growth rate}) = 120$<br>72-hour $NOEC(\text{growth rate, cell density, area under the growth curve}) = 70$ | Good           | Test substance was PFOS potassium salt. Measured exposure concentrations.  | 2              |
|            |                                  | OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23 (Static) | 96-hour $EC_{50}(\text{cell density}) = 82$<br>96-hour $EC_{10}(\text{cell density}) = 10$   | Acceptable     | Test substance was PFOS potassium salt. Result not expressed relative to growth rate. Nominal exposure concentrations. Result agrees with those obtained in studies 2 and 24.                          | 13             |
|            |                                  | OECD 201 (Static)                                     | 96-hour $EC_{50}(\text{growth rate}) = 71$<br>96-hour $NOEC(\text{growth rate}) = 35$  | Questionable   | Test substance was PFOS potassium salt. Nominal exposure concentrations. Data questionable in view of the presence of diethylene glycol butyl ether in the test material (as part of the formulation). | 24             |

|           |                             |                   |  |      |  |    |
|-----------|-----------------------------|-------------------|--|------|--|----|
|           | <i>Anabaena flos-aquae</i>  | OPPTS<br>850.5400 | 96-hour $EC_{50(\text{growth rate})} = 176$<br>96-hour $NOEC_{(\text{growth rate})} = 94$    | Good | Test substance was PFOS potassium salt.<br>Measured exposure concentrations. | 36 |
|           | <i>Navicula pelliculosa</i> | OPPTS<br>850.5400 | 96-hour $EC_{50(\text{growth rate})} = 305$<br>96-hour $NOEC_{(\text{growth rate})} = 206$   | Good | Test substance was PFOS potassium salt.<br>Measured exposure concentrations. | 38 |
| Saltwater | <i>Skeletonema costatum</i> | OPPTS<br>850.5400 | 96-hour $EC_{50(\text{growth rate})} = >3.2$<br>96-hour $NOEC_{(\text{growth rate})} = >3.2$ | Good | Test substance was PFOS potassium salt.<br>Measured exposure concentrations. | 39 |

**Table 9. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with freshwater algae**

| Species                          | Protocol  | Results (mg/l)  | Study Standard | Comments   | Study Ref. No. |
|----------------------------------|---|---|----------------|--|----------------|
| <i>Selenastrum capricornutum</i> | OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23 (Static) | 14-day EC <sub>50</sub> cell density = 95<br>14-day NOEC <sub>cell density</sub> = <26<br>14-day EC <sub>10</sub> cell density = 16 | Acceptable     | Test substance was PFOS potassium salt. Result not expressed relative to growth rate. No analysis of exposure but result agrees with those obtained in studies 2 and 24. | 13             |

**Table 10. Acute (short-term exposure) toxicity data for PFOS determined in tests with higher plants**

| Medium     | Species                          | Protocol       | Result (mg/l)                | Study Standard | Comments  | Study Ref. No. |
|------------|----------------------------------|----------------|------------------------------|----------------|---|----------------|
| Freshwater | <i>Lemna gibba</i> G3 (Duckweed) | OPPTS 850.4400 | 7-day IC <sub>50</sub> = 108 | Good           | Test substance was PFOS potassium salt. Measured exposure concentrations. | 37             |



## 4.2 Effects on Other Aquatic Organisms

### 4.2.1 Amphibians

Data summarised in Table 11 were obtained in an embryo teratogenesis assay carried out on PFOS potassium salt with *Xenopus laevis* (African clawed frog). Exposure of the embryos for 96 hours resulted in an LC<sub>50</sub> for mortality of 13.8 mg/l and an EC<sub>50</sub> for malformations of 12.1 mg/l. The minimum concentration that inhibited growth was 7.97 mg/l, and the teratogenic index was calculated, as the ratio of the 96-hour LC<sub>50</sub> to the 96-hour EC<sub>50</sub>, to be 1.1. The latter value indicates that PFOS has a low potential to be a developmental hazard in this species.

**Table 11. Toxicity data for PFOS determined in an embryo teratogenesis assay with the amphibian, *Xenopus laevis***

| Medium     | Species  | Protocol         | Result (mg/l)  | Study Standard | Comments  | Study Ref. No. |
|------------|--|------------------|--|----------------|---|----------------|
| Freshwater | <i>Xenopus laevis</i><br>(African clawed frog) | ASTM<br>E1439-91 | 96-hour LC <sub>50</sub> = 13.8<br>96-hour EC <sub>50(malformations)</sub> = 12.1<br>Minimum concentration to inhibit growth = 7.97<br>Teratogenic index 1.1 | Acceptable     | Test substance was PFOS potassium salt.<br>Measured exposure concentrations.<br>In-life phases were not subject to GLP. | 40             |

#### 4.2.2 Sediment Dwelling Invertebrates

No data are available for effects on sediment dwelling invertebrates. It is possible that a predicted no effect concentration (PNEC) for sediment-dwelling invertebrates could be determined by applying equilibrium partitioning models to the data for water column organisms. However, as discussed in Annex 3, this is unlikely to be feasible at the present time.

#### 4.2.3 Bacteria

Data summarised in Table 12 were obtained from 2 reports describing Microtox® studies (study report reference numbers 18 and 22). The Microtox® procedure assesses light output inhibition from the luminescent marine bacterium *Photobacterium phosphoreum*, following exposure to a toxicant over a short exposure period (30 minutes in the case of these two studies). Both studies are of an unacceptable standard for assessing the hazard of PFOS (lithium salt) to bacteria because of uncertainty over the true exposure concentrations and in one case because of the presence of diethylene glycol in the test sample. There are also more general concerns over the relevance of the test system and test species for determining effects for this particular group of organisms. No toxic effects were observed in either test at nominal concentrations (>250 mg/l) that were significantly in excess of the solubility of PFOS salts in saline medium (2.5 to 20 mg/l, depending on purity/salinity).

**Table 12. Toxicity data for PFOS determined in Microtox tests**

| Species                                       | Protocol                    | Results (mg/l)   | Study Standard | Comments   | Study Ref. No. |
|---|-----------------------------|--|----------------|--|----------------|
| <i>Photobacterium phosphoreum</i> (Microtox®) | Microbics Microtox® "BASIC" | 15-minute EC <sub>50</sub> = >250<br>30-minute EC <sub>50</sub> = >250 | Unacceptable   | Test substance was PFOS lithium salt. Nominal exposure concentrations. Initial stock solution prepared at concentration that was likely to exceed solubility in saline water (2.5 to 20 mg/l, depending on purity/salinity) It is therefore possible that actual exposure concentrations were much lower than nominal. Result indicates probable absence of toxicity at limit of solubility. | 18             |
|   |                             | 30-minute EC <sub>50</sub> = >280                                      | Unacceptable   | Test substance was PFOS potassium salt. Nominal exposure concentrations. Data questionable in view of use of diethylene glycol to aid dosing. Test concentrations likely to be in excess of solubility in saline medium (2.5 to 20 mg/l, depending on purity/salinity). Result is consistent with that obtained in study 18 indicating probable absence of toxicity at limit of solubility.  | 22             |

### 4.2.3 Activated Sludge Microorganisms

Data summarised in Table 13 were obtained from 3 reports describing activated sludge respiration inhibition studies (study report reference numbers 6, 19 and 21). Only study reference number 9 was considered to be of an acceptable standard for assessing the hazard of PFOS to activated sludge. Studies 19 and 21 had significant inadequacies in test procedures related to test conditions, duration of the exposure and/or absence of a reference substance.

The 3-hour  $IC_{50}$  value for PFOS (potassium salt) determined in the acceptable study was >905 mg/l (nominal concentration).

**Table 13. Toxicity data for PFOS determined in Activated Sludge Respiration Inhibition tests**

| Test system      | Protocol  | Results (mg/l)   | Study Standard | Comments  | Study Ref. No. |
|------------------|-----------|--|----------------|---|----------------|
| Activated Sludge | OECD 209  | 3-hour IC <sub>50</sub> = >905 (Nom.)                              | Good           | Test substance was PFOS potassium salt. Nominal exposure concentrations of 0.9 – 905 mg/l tested. Highest test concentration gave the maximum inhibition of 38.8%.  | 6              |
|                  | OECD 209  | 3-hour IC <sub>50</sub> = >245 (as a nom. 24.5% solution in water) | Unacceptable   | Test substance was PFOS potassium salt. Nominal exposure concentrations. Test temperature not controlled and fell well below requirements of standard guidelines. Respiration rates extrapolated to 20°C. Oxygenation was inadequate during the first 30 minutes of the 3-hour test. No inhibitory effect observed. | 19             |
|                  | Not noted | 7-minute IC <sub>50</sub> = >250 (as a nom. 25% solution in water) | Unacceptable   | Test substance was PFOS DEA salt. Nominal exposure concentrations. Exposure period inadequate by current standards. No reference substance used. No inhibitory effect observed.   | 21             |

### **4.3 Effects on Terrestrial Organisms**

#### **4.3.1 Soil-dwelling Invertebrates**

No data are available for effects on soil dwelling invertebrates. It is however possible that a predicted no effect concentration (PNEC) for soil-dwelling invertebrates could be determined by applying equilibrium partitioning models to the data for water column organisms. However, as discussed in Annex 3, this is unlikely to be feasible at the present time. It is noted that an earthworm acute toxicity and uptake study is in the planning stages, and a final report should be available in 2002.

#### **4.3.2 Terrestrial Plants**

No data are available for effects on terrestrial plants. It is however possible that a predicted no effect concentration (PNEC) for terrestrial plants could be determined by applying equilibrium partitioning models to the data for aquatic plants. However, as discussed in Annex 3, this is unlikely to be feasible at the present time. It is noted that a terrestrial plant toxicity and uptake study should be available in 2002.

#### **4.3.3 Birds**

Data summarised in Table 14 were from 2 reports describing dietary acute studies with the Mallard duck, *Anas platyrhynchos*, and the Northern Bobwhite quail, *Colinus virginianus* (study report reference numbers 11 and 12). Birds in both tests were exposed to PFOS potassium salt in their diets for 5 days and observations were made after 3 or 17 days. Both studies are considered to be of an acceptable standard for assessing the acute toxic hazard of PFOS to birds via dietary exposure.

The lowest acute dietary LC<sub>50</sub> value of 220 mg/kg of food was determined in the test with the quail. This value was approximately half that obtained in the test with the duck. The lowest NOEC of 37 mg/kg of food for effects on body weight was, in contrast, obtained in the test with the duck. Once again this value was approximately half that obtained with the other species – the quail.

It is noted that avian reproductive data will be submitted for review in 2002.

**Table 14. Acute dietary toxicity data for PFOS determined in tests with birds**

| <b>Test species</b>                                     | <b>Protocol</b>                         | <b>Results (mg/kg of food)</b>  | <b>Study Standard</b> | <b>Comments</b>   | <b>Study Ref. No.</b> |
|---|---|---|-----------------------|---|-----------------------|
| <i>Anas platyrhynchos</i><br>(Mallard duck)             | OECD 205, OPPTS 850.2200 & FIFRA E 71-2 | LC <sub>50</sub> = 628<br>NOEC <sub>mortality</sub> = 146<br>NOEC <sub>body weight</sub> = 37 | Good                  | Test substance was PFOS potassium salt. Measured exposure concentration in food. 5 days exposure followed by 3 or 17 days observation | 11                    |
| <i>Colinus virginianus</i><br>(Northern Bobwhite quail) | OECD 205, OPPTS 850.2200 & FIFRA E 71-2 | LC <sub>50</sub> = 220<br>NOEC <sub>mortality</sub> = 73<br>NOEC <sub>body weight</sub> = 73  | Good                  | Test substance was PFOS potassium salt. Measured exposure concentration in food. 5 days exposure followed by 3 or 17 days observation | 12                    |



#### 4.3.4 Bees

Data summarised in Table 15 were obtained in acute oral and contact toxicity tests carried out with the Honey bee (*Apis mellifera*) on PFOS potassium salt. The studies fully meet the required standard for acceptability of the data.

The acute oral test yielded a 72-hour LD<sub>50</sub> for ingestion of PFOS of 0.40 µg/bee and a 72-hour NOEL of 0.21µg/bee. The contact test yielded a 96-hour LD<sub>50</sub> of 4.78 µg/bee and a 96-hour NOEL of 1.93 µg/bee. The respective LD<sub>50</sub> values indicate moderate and high orders of toxicity of PFOS to bees when administered via these routes.

**Table 15. Acute oral and contact toxicity data for PFOS determined in tests with Honey bees**

| <b>Test species</b>                  | <b>Protocol</b>   | <b>Results (<math>\mu\text{g}/\text{bee}</math>)</b>   | <b>Study Standard</b> | <b>Comments</b>   | <b>Study Ref. No.</b> |
|--------------------------------------|---|--|-----------------------|---|-----------------------|
| <i>Apis mellifera</i><br>(Honey bee) | OECD 213,<br>EPPO 170<br>(Oral)                               | 72-hour $\text{LD}_{50}$ = 0.40<br>72-hour NOEL = 0.21 | Good                  | Test substance was PFOS potassium salt.<br>Nominal exposure following feeding on 50% w/v<br>sucrose.  | 34                    |
| <i>Apis mellifera</i><br>(Honey bee) | OECD 214,<br>EPPO 170,<br>OPPTS 850.3020<br>(draft) (Contact) | 96-hour $\text{LD}_{50}$ = 4.78<br>96-hour NOEL = 1.93 | Good                  | Test substance was PFOS potassium salt.<br>Nominal exposure following direct application of test<br>substance dissolved in acetone to thorax. | 35                    |

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## Annex 1. Ecological Studies

The following lists of study reports were contained on a 3M Fluorochemical EPA Submissions CDs dated 12<sup>th</sup> July 2000, 28<sup>th</sup> June 2001, and February 2002

12<sup>th</sup> July 2000

1. 96-Hour Static Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
2. 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)
3. 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)
4. 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
5. 96-Hour Static Acute Toxicity Test with the Freshwater Mussel (*Unio complamatus*)
6. Activated Sludge, Respiration Inhibition Test
7. 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
8. Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
9. Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)
10. Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
11. Dietary LC<sub>50</sub> Study with the Mallard
12. Dietary LC<sub>50</sub> Study with the Northern Bobwhite
13. Multi-Phase Exposure / Recovery Algal Assay Test
14. The Effects of Continuous Aqueous Exposure to 14C-78.02 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (*Pimephales promelas* / Summary of histopathological examinations of Fathead Minnow (*Pimephales promelas*) exposed to 78.02 for 30 Days
15. Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)
16. *Pimephales promelas* 96-hour Toxicity Test Data Summary. Sample FC-94-X (Li salt of PFOS)
17. 48-HR Acute Toxicity to Daphnia, *Daphnia magna*. FC-94-X (Li salt of PFOS)
18. Microbics Microtox Toxicity Test. Sample : FC-94-X (Li salt of PFOS)
19. Evaluation of FC-94-X by OECD Activated Sludge Respiration Inhibition Test #209 Review of OECD 209 and BOD/COD Test Results for FC-94- X, test data sheets (Li salt of PFOS)
20. 96-Hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)
21. Acute Toxicity to Activated Sludge (FC-99, DEA salt of PFOS)
22. Microtox data for FM-3820 (28% PFOS)
23. Acute Toxicity to *Daphnia magna* for FM-3820 (28% PFOS)
24. Toxicity to Algae (*Selenastrum capricornutum*) for FC-3820 (28% PFOS)
25. Final Comprehensive Report : FC-95
26. Data from Fathead Minnow Study on FC-93 (25% NH<sub>4</sub> salt of PFOS in IPA and water), 3M Environmental Lab, Aug. 2, 1974.
27. Data from Fathead Minnow Study on FC-93 (25% NH<sub>4</sub> salt of PFOS in IPA and water), 3M Environmental Lab, Oct. 19, 1974.
28. Acute toxicity of P3025 developmental material to Fathead minnow (*Pimephales promelas*).
29. Acute toxicity of P3025 developmental material to *Daphnia magna*.

28<sup>th</sup> June 2001

30. Acute toxicity of PFOS to Rainbow trout in saltwater
31. Acute toxicity of PFOS to Rainbow trout in freshwater
32. Acute toxicity of PFOS to *Artemia* sp.
33. Acute toxicity of PFOS to *Daphnia magna*
34. Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee
35. Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee
36. PFOS: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*)
37. PFOS: A 7-day toxicity test with Duckweed (*Lemna gibba* G3)
38. PFOS: A 96-hour toxicity test with freshwater diatom (*Navicula pelliculosa*)

39. PFOS: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)
40. PFOS: A frog embryo teratogenesis assay – *Xenopus* (FETAX)
41. Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill (*Lepomis macrochirus*)

Xx February 2002

42. Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) in freshwater
43. Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*) in saltwater

**Annex 2. Robust summaries of key ecotoxicology studies**

| Study reference number | Title   |
|------------------------|---|
| 1                      | 96-Hour Static Acute Toxicity Test with the Fathead Minnow ( <i>Pimephales promelas</i> )   |
| 2                      | 96-Hour Toxicity Test with the Freshwater Alga ( <i>Selenastrum capricornutum</i> )   |
| 3                      | 48-Hour Static Acute Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> )   |
| 4                      | 96-Hour Shell Deposition Test with the Eastern Oyster ( <i>Crassostrea virginica</i> )  |
| 5                      | 96-Hour Static Acute Toxicity Test with the Freshwater Mussel ( <i>Unio complamatus</i> )   |
| 6                      | Activated Sludge, Respiration Inhibition Test   |
| 7                      | 96-Hour Static Acute Toxicity Test with the Saltwater Mysid ( <i>Mysidopsis bahia</i> )   |
| 8                      | Early Life-Stage Toxicity Test with the Fathead Minnow ( <i>Pimephales promelas</i> )   |
| 9                      | Semi-Static Life-Cycle Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> )   |
| 10                     | Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid ( <i>Mysidopsis bahia</i> )  |
| 11                     | Dietary LC <sub>50</sub> Study with the Mallard   |
| 12                     | Dietary LC <sub>50</sub> Study with the Northern Bobwhite   |
| 13                     | Multi-Phase Exposure / Recovery Algal Assay Test  |
| 14                     | The Effects of Continuous Aqueous Exposure to 14C-78.02 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow ( <i>Pimephales promelas</i> ) / Summary of histopathological examinations of Fathead Minnow ( <i>Pimephales promelas</i> ) exposed to 78.02 for 30 Days |
| 15                     | Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)   |
| 16                     | <i>Pimephales promelas</i> 96-hour Toxicity Test Data Summary. Sample FC-94-X (Li salt of PFOS)   |
| 17                     | 48-HR Acute Toxicity to Daphnia, <i>Daphnia magna</i> . FC-94-X (Li salt of PFOS)   |
| 20                     | 96-Hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)   |
| 23                     | Acute Toxicity to <i>Daphnia magna</i> for FM-3820 (28% PFOS)   |
| 26                     | Data from Fathead Minnow Study on FC-93 (25% NH <sub>4</sub> salt of PFOS in IPA and water), 3M Environmental Lab, Aug. 2, 1974.  |
| 27                     | Data from Fathead Minnow Study on FC-93 (25% NH <sub>4</sub> salt of PFOS in IPA and water), 3M Environmental Lab, Oct. 19, 1974.   |
| 28                     | Acute toxicity of P3025 developmental material to Fathead minnow ( <i>Pimephales promelas</i> ).  |
| 29                     | Acute toxicity of P3025 developmental material to <i>Daphnia magna</i> .  |
| 30                     | Acute toxicity of PFOS to Rainbow trout in saltwater  |
| 31                     | Acute toxicity of PFOS to Rainbow trout in freshwater   |
| 32                     | Acute toxicity of PFOS to <i>Artemia</i> sp.  |
| 33                     | Acute toxicity of PFOS to <i>Daphnia magna</i>  |
| 34                     | Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee  |
| 35                     | Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee   |
| 36                     | PFOS: A 96-hour toxicity test with the freshwater alga ( <i>Anabaena flos-aquae</i> )   |
| 37                     | PFOS: A 7-day toxicity test with Duckweed ( <i>Lemna gibba</i> G3)  |
| 38                     | PFOS: A 96-hour toxicity test with freshwater diatom ( <i>Navicula pelliculosa</i> )  |
| 39                     | PFOS: A 96-hour toxicity test with the marine diatom ( <i>Skeletonema costatum</i> )  |
| 40                     | PFOS: A frog embryo teratogenesis assay – <i>Xenopus</i> (FETAX)  |
| 41                     | Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill ( <i>Lepomis macrochirus</i> )  |
| 42                     | Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in freshwater   |
| 43                     | Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ) in saltwater   |
| 44                     | Bioconcentration test of Salt (Na, K, Li) of perfluoroalkyl (C=4-12) sulfonic acid [This test was performed using Perfluorooctane sulfonic acid, potassium salt (Test substance number K-1520)] in carp   |

**Robust Study Report Reference No. 1 – 96-Hour Static Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OECD 203 and OPPTS 850.1075

**Type:** Static acute

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Pimephales promelas*

**Supplier:** In-house cultures, Wildlife International, Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured at 0, 48, 96-hours

**Exposure period:** 96-hours

**Statistical methods:** LC<sub>50</sub> values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Test fish age:** Approximately 126 days old

**Length and weight:** 35 (30-38) mm, 0.36 (0.21-0.49) g

**Loading:** 0.24 g fish/L

**Pretreatment:** None

**Test Conditions**

**Dilution water:** 0.45 µm filtered well water

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**Hardness:** 131 (128-136) mg/L as CaCO<sub>3</sub>

**Alkalinity:** 177 (176-178) mg/L as CaCO<sub>3</sub>

**pH:** 8.3

**TOC:** < 1.0 mg/L

**Conductivity:** 311 (310-315) µmhos/cm

**Stock and test solution preparation:** Primary stock prepared in dilution water at 27 mg/L and mixed for ~22 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the four additional test concentrations.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.6 cm.

**Number of replicates:** two

**Number of fish per replicate:** ten

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

7.8 – 8.8 mg/L (control exposure)

7.7 – 9.0 mg/L (28 mg/L exposure)  
**pH range** (0 – 96 hours)  
 8.3 – 8.6 (control exposure)  
 8.4 – 8.5 (28 mg/L exposure)  
**Test temperature range** (0 – 96 hours)  
 20.4 – 22.1°C (control exposure)  
 21.3 – 22.3 °C (28 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

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**Nominal concentrations:** Negative control, 3.6, 5.9, 9.9, 16, 27 mg/L

**Measured concentrations:** <LOQ, 3.3, 5.6, 9.5, 17, 28 mg/L

**Element value:** (95% confidence interval is given in brackets)

24-hour LC<sub>50</sub> = > 28 mg/L (C.I. not calculable)

48-hour LC<sub>50</sub> = > 28 mg/L (C.I. not calculable)

72-hour LC<sub>50</sub> = 27 (22 – 41) mg/L

96-hour LC<sub>50</sub> = 9.5 (8.0 – 11) mg/L

All element values based on mean measured concentrations

**Statistical evaluation of mortality:** Confidence limits for 24 and 48-hours could not be calculated due to lack of mortality. The 72-hour LC<sub>50</sub> value is questionable because a concentration-effect relationship was not demonstrated over a reasonable range of percent dead. The 24 and 48-hour LC<sub>50</sub> values were determined by visual interpretation. Probit was used to calculate the 72-hour LC<sub>50</sub> and Moving Average for the 96-hour LC<sub>50</sub>.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.9. Samples collected at test initiation had measured values from 85.3 to 117% of nominal. Measured values for samples taken at 48 hours ranged from 86.3 to 101% of nominal. Measured values for samples taken at 96 hours ranged from 87.6 to 98.3% of nominal.

### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L | Mean Measured Concentration mg/L | Percent of Nominal |
|----------------------------------|--|----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                             | -                  |
| 3.6                              | 3.16, 3.53, 3.08, 3.22, 3.46, 3.13                                   | 3.3                              | 92                 |
| 5.9                              | 6.05, 5.07, 5.48, 5.89, 5.70, 5.55                                   | 5.6                              | 95                 |
| 9.9                              | 8.99, 9.47, 9.88, 9.33, 9.70, 9.52                                   | 9.5                              | 96                 |
| 16                               | 18.2, 19.3, 15.0, 15.6, 14.8, 16.2                                   | 17                               | 106                |
| 27                               | 28.5, 28.5, 27.0, 27.8, 26.8, 26.6                                   | 28                               | 104                |

**Biological observations after 96-hours:** Fish in the negative control and the 3.3 mg/L exposure concentration appeared normal. Some or all of the surviving fish were observed to be swimming erratically (4/16 in 5.6 mg/L exposure, 10/10 in 9.5 mg/L, 4/4 in 17 mg/L) at test termination.

**Cumulative percent mortality:**

| Mean Measured Test Concentration mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|---------------------------------------|----------|----------|----------|----------|
| Neg. Control                          | 0        | 0        | 0        | 0        |
| 3.3                                   | 0        | 0        | 0        | 0        |
| 5.6                                   | 0        | 0        | 0        | 20       |
| 9.5                                   | 0        | 0        | 0        | 50       |
| 17                                    | 0        | 0        | 15       | 80       |
| 28                                    | 0        | 0        | 50       | 100      |

**Lowest concentration causing 100% mortality:** 28 mg/L

**Mortality of controls:** None

**CONCLUSIONS**

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The potassium perfluorooctanesulfonate 96-hour LC<sub>50</sub> for fathead minnow was determined to be 9.5 mg/L with a 95% confidence interval of 8.0 –11 mg/L. The 96-hour no mortality and no effects concentration was 3.3 mg/L.

Submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

**DATA QUALITY**

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**Reliability:** Klimisch ranking = 1

**REFERENCES**

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

**OTHER**

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 2 – 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonicacid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OECD 201, OPPTS 850.5400, ASTM 1218-90E

Test: Static acute

GLP: Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Selenastrum capricornutum*

**Source:** Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured at 0, 72, 96-hours

**Element basis:** Reported three ways: number of cells/ml, area under the growth curve and growth rate

**Exposure period:** 96-hours

**Start date:** 4/12/99

**End date:** 4/16/99

**Analytical monitoring:** Test concentrations measured at 0, 72 and 96-hours.

**Test organisms laboratory culture:** Algae cultures had been actively growing in freshwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

**Test Conditions:**

**Test temperature range:** 23.6- 25.8°C

**Growth medium:** ASTM Standard Guide1218-90E, 1990

| Compound   | Nominal concentration | Units |
|--|-----------------------|-------|
| MgCl <sub>2</sub> 6H <sub>2</sub> O                | 12.16                 | mg/l  |
| CaCl <sub>2</sub> 2H <sub>2</sub> O                | 4.40                  | mg/l  |
| H <sub>3</sub> BO <sub>3</sub>                     | 0.1856                | mg/l  |
| MnCl <sub>2</sub> 4H <sub>2</sub> O                | 0.416                 | mg/l  |
| ZnCl <sub>2</sub>                                  | 3.28                  | µg/l  |
| FeCl <sub>2</sub> 6H <sub>2</sub> O                | 0.1598                | mg/l  |
| CoCl <sub>2</sub> 6H <sub>2</sub> O)               | 1.428                 | µg/l  |
| Na <sub>2</sub> MoO <sub>4</sub> 2H <sub>2</sub> O | 7.26                  | µg/l  |
| CuCl <sub>2</sub> 2H <sub>2</sub> O                | 0.012                 | µg/l  |
| Na <sub>2</sub> EDTA2H <sub>2</sub> O              | 0.300                 | mg/l  |
| NaNO <sub>3</sub>                                  | 25.50                 | mg/l  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O                | 14.70                 | mg/l  |
| K <sub>2</sub> HPO <sub>4</sub>                    | 1.044                 | mg/l  |

|                    |      |      |
|--------------------|------|------|
| NaHCO <sub>3</sub> | 15.0 | mg/l |
|--------------------|------|------|

**Dilution water source:** Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to  $7.5 \pm 0.1$  using 10% HCl and the medium was sterilized by filtration (0.22 $\mu$ m) prior to use.

**Stock and test solution preparation:** A primary stock solution was prepared in algal medium at a concentration of 183 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with algal medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

**Exposure vessels:** Sterile 250 mL polycarbonate Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

**Agitation:** Shaken continuously at 100 rpm

**Number of replicates:** three

**Initial algal cell loading:**  $1.0 \times 10^4$  cells/mL

**Number of concentrations:** six plus a negative control plus an abiotic control at the highest concentration tested

**Water chemistry:**

**pH range** (0- 96 hours)

7.5 - 8.1 (control exposure)

7.4 - 7.5 (179 mg/L exposure)

**Test temperature range** (0- 96 hours)

23.6 - 25.8°C

**Light levels** (0- 96 hours)

3870 - 4610 lux from continuous cool-white fluorescent lighting

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

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**Nominal concentrations:** Negative control, 5.7, 11, 23, 46, 91, 183 mg/L plus 183 mg/L abiotic control.

**Measured concentrations:** <LOQ, 5.5, 11, 21, 44, 86, 179, 169 mg/L

**Element value:** (95% confidence interval is given in brackets)

24-hour EC<sub>50</sub> (cell density) = 163 (74 - 191) mg/L

24-hour EbC<sub>50</sub> (area under curve) = 122 (19 - 176) mg/L

24-hour EC<sub>50</sub> (growth rate) = 136 (30 - 204) mg/L

48-hour EC<sub>50</sub> (cell density) = 81 (72 - 90) mg/L

48-hour EbC<sub>50</sub> (area under curve) = 84 (67 - 146) mg/L

48-hour ErC<sub>50</sub> (growth rate) = 142 (107 - 185) mg/L

72-hour EC<sub>10</sub> (cell density) = 37 (<O - 64) mg/L

72-hour EbC<sub>10</sub> (area under curve) = 46 (<O - 56) mg/L

72-hour ErC<sub>10</sub> (growth rate) = 53 (23 - 64) mg/L

72-hour EC<sub>50</sub> (cell density) = 70 (44 - 78) mg/L

72-hour EbC<sub>50</sub> (area under curve) = 74 (55 - 82) mg/L

72-hour ErC<sub>50</sub> (growth rate) = 120 (103 - 132) mg/L

72-hour EC<sub>90</sub> (cell density) = 153 (130 - 165) mg/L

72-hour EbC<sub>90</sub> (area under curve) = 165 (145 - 176) mg/L

72-hour ErC<sub>90</sub> (growth rate) = >179 mg/L (C.I. not calculable)

96-hour EC<sub>10</sub> (cell density) = 49 (43 - 50) mg/L

96-hour EbC<sub>10</sub> (area under curve) = 49 (40 - 50) mg/L

96-hour ErC<sub>10</sub> (growth rate) = 59 (54 - 63) mg/L



96-hour EC<sub>50</sub> (cell density) = 71 (66 - 73) mg/L  
 96-hour EbC<sub>50</sub> (area under curve) = 71 (67- 74) mg/L  
 96-hour ErC<sub>50</sub> (growth rate) = 126 (115 - 138) mg/L  
 96-hour EC<sub>90</sub> (cell density) = 137 (105 - 153) mg/L  
 96-hour EbC<sub>90</sub> (area under curve) = 145 (125 - 155) mg/L  
 96-hour ErC<sub>90</sub> (growth rate) = >179 mg/L (C.I. not calculable)  
 72-hour NOEC (growth rate, cell density, area under the curve) = 44 mg/L  
 96-hour NOEC (growth rate, cell density, area under the curve) = 44 mg/L

All element values based on mean measured concentrations

**Statistical methods:** Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC<sub>10</sub>, EC<sub>50</sub>, and EC<sub>90</sub> values and 95% confidence limits at 72 and 96 hours. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilks's test and Bartlett's test, respectively. The treatment groups were then compared to the control using Dunnett's test. Results of the statistical analyses were used to determine the NOEC values.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.1. Samples collected at test initiation had measured values from 82.0 to 98.1% of nominal. Measured values for samples taken at 72 hours ranged from 91.7 to 105% of nominal. Measured values for samples taken at 96 hours ranged from 90.3 to 102% of nominal. For the abiotic controls, measured values for samples taken at 72-hours ranged from 81.9 to 105% of nominal and for samples taken at 96-hours, 90.3 to 103% of nominal.

#### Summary of analytical chemistry data:

| Nominal test concentration, mg/l | Measured values at 0, 72, and 96-hours respectively, mg/l | Mean measured concentration, mg/l | Percent nominal |
|----------------------------------|---|-----------------------------------|-----------------|
| Negative control                 | All <LOQ  | <LOQ                              | -               |
| 5.7                              | 4.73, 6.04, 5.84  | 5.5                               | 96              |
| 11                               | 10.7, 11.2, 12.1  | 11                                | 100             |
| 23                               | 19.8, 23.1, 20.7  | 21                                | 91              |
| 46                               | 42.7, 41.9, 46.3  | 44                                | 96              |
| 91                               | 83.3, 86.0, 88.3  | 86                                | 95              |
| 183                              | 179, 186, 172   | 179                               | 98              |
| 183 (abiotic)                    | Not analyzed, 150, 188                                    | 169                               | 92              |

**Control response:** satisfactory

**Biological observations after 96-hours:**

| Mean measured concentration, mg/l | Mean number of cells per ml | Percent Inhibition via Density | Percent Inhibition via Area Under the Curve | Percent Inhibition via Growth Rate |
|-----------------------------------|-----------------------------|--------------------------------|---|------------------------------------|
| Negative control                  | 2,740,00                    | -                              | -   | -                                  |
| 5.5                               | 3,040,000                   | -11                            | -8.5  | -1.9                               |
| 11                                | 2,880,000                   | -5.1                           | -3.3  | -0.84                              |
| 21                                | 3,240,000                   | -18                            | -13   | -3.0                               |
| 44                                | 3,080,000                   | -12                            | -5.3  | -2.0                               |
| 86                                | 626,667                     | 77                             | 75  | 27                                 |
| 179                               | 33,667                      | 99                             | 98  | 79                                 |

**Observations:** After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the negative control or any test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative control, although a few cells appeared enlarged in the 86 and 179 mg/L treatment groups.

**Reversibility of Growth Inhibition:** The 179 mg/L treatment group was maximally inhibited after 96-hours. Aliquots of the test solution were diluted with algal medium and cultured for five days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

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## CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC<sub>50</sub> and 95% confidence interval for *Selenastrum capficornutum* was determined using three calculation methods. By cell density, it was 71 (66 - 73) mg/L, by area under the growth curve it was 71 (67 - 74) mg/L and by growth rate 126 (115 - 138) mg/L. The 96-hour NOEC was determined by Dunnett's procedure ( $p < 0.05$ ) to be 44 mg/L using all three methods. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

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## DATA QUALITY

**Reliability:** Klimisch ranking 1

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## REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

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## OTHER

**Last changed:** 5/3/00

## Robust Study Report Reference No. 3 - 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

### TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

### METHOD

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**Method:** OECD 202 and OPPTS 850.1010

**Test type:** Static acute

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Daphnia magna*

**Analytical monitoring:** PFOS measured at 0, 24, 48-hours

**Statistical methods:** EC<sub>50</sub> values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Test daphnid source:** Obtained from cultures maintained by Wildlife International Ltd., Easton, MD. Identification of the original brood stock was verified by the Academy of Natural Sciences, Philadelphia, PA., USA

**Test daphnid age at study initiation:** < 24-hours

#### Test conditions

**Dilution water:** 0.45 µm filtered well water

#### Dilution water chemistry (during the 4-week period immediately preceding the test):

**Hardness:** 132 (128-136) mg/L as CaCO<sub>3</sub>

**Alkalinity:** 178 (176-178) mg/L as CaCO<sub>3</sub>

**pH:** 8.3 (8.2-8.3)

**TOC:** < 1.0 mg/L

**Conductivity:** 313 (310-315) µmhos/cm

**Ca/Mg ratio:** 35/13.5

**Na/K ratio:** 21.3/6.62

**Lighting:** Colortone® 50 fluorescent lights, intensity approximately 359 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

**Stock and test solutions preparation:** A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for ~19.5 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

**Exposure vessels:** 250 mL plastic beakers containing 240 mL of test solution. The approximate depth of test solution was 6.4 cm.

**Number of replicates:** two

**Number of daphnids per replicate:** ten

**Number of concentrations:** five plus a negative control

#### Water chemistry during the study:

**Dissolved oxygen range (0 – 48 hours):**

8.6 – 8.9 mg/L (control exposure)

8.6 – 9.1 mg/L (91 mg/L exposure)  
**pH range** (0 – 48 hours)

8.2 – 8.5 (control exposure)  
 8.5 – 8.6 (91 mg/L exposure)

**Test temperature range** (0 – 48 hours)

19.5 – 20.2°C (control exposure)  
 19.3 – 20.1°C (91 mg/L exposure)

**Element basis:** mortality and immobilization

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

**Nominal concentrations:** Negative control, 12, 20, 33, 55, 91 mg/L

**Measured concentrations:** <LOQ, 11, 20, 33, 56, 91 mg/L

**Element value:** (95% confidence interval is given in brackets)

24-hour EC<sub>10</sub> = 82 (81-83) mg/L  
 24-hour EC<sub>50</sub> = >91 mg/L (C.I. not calculable)  
 24-hour EC<sub>90</sub> = >91 mg/L (C.I. not calculable)  
 48-hour EC<sub>10</sub> = 53 (<11->91) mg/L  
 48-hour EC<sub>50</sub> = 61 (33-91) mg/L  
 48-hour EC<sub>90</sub> = 63 (<11->91) mg/L

All element values based on mean measured concentrations

**Statistical evaluation:** The EC<sub>50</sub> values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan. The EC<sub>10</sub> and EC<sub>90</sub> values were calculated when possible using the Bruce-Versteeg method because there were less than two concentrations with partial mortality or immobility.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.58 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 96.2. Samples collected at test initiation had measured values from 85.5 to 112% of nominal. Measured values for samples taken at 24 hours ranged from 92.2 to 115% of nominal. Measured values for samples taken at 48 hours ranged from 91.6 to 106% of nominal.

### Summary of analytical chemistry data:

| Nominal Test Concentration mg/L | Measured duplicated values at 0, 24, and 48-hours respectively, mg/L | Mean Measured Concentration mg/L | Percent Of Nominal |
|---------------------------------|--|----------------------------------|--------------------|
| Negative Control                | All < LOQ  | <LOQ                             | -                  |
| 12                              | 10.5, 10.6, 11.5, 12.5, 10.9, 12.0                                   | 11                               | 92                 |
| 20                              | 17.2, 18.1, 22.8, 21.6, 21.4, 18.8                                   | 20                               | 100                |
| 33                              | 30.2, 34.1, 34.0, 36.1, 31.3, 34.0                                   | 33                               | 100                |
| 55                              | 50.5, 49.9, 57.0, 63.0, 56.8, 56.4                                   | 56                               | 102                |
| 91                              | 87.6, 102, 90.1, 84.4, 88.7, 92.4                                    | 91                               | 100                |

**Biological observations after 48-hours:** Daphnids in the negative control, the 11 and the 20 mg/L

treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. Five percent mortality was observed at 48-hours in the negative control. The effects noted in this study were mortality; no immobilization was noted at any test concentration.

**Cumulative percent mortality:**

| Mean Measured Test Concentration mg/L | 24-hours | 48-hours |
|---------------------------------------|----------|----------|
| Negative Control                      | 0        | 5        |
| 11                                    | 0        | 0        |
| 20                                    | 0        | 0        |
| 33                                    | 0        | 0        |
| 56                                    | 0        | 35       |
| 91                                    | 35       | 100      |

**Control response:** satisfactory

**CONCLUSIONS**

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The potassium perfluorooctanesulfonate 48-hour EC<sub>50</sub> for *Daphnia magna* was determined to be 61 mg/L with a 95% confidence interval of 33-91 mg/L. The 48-hour no immobilization and no observed effect concentration was 33 mg/L.

Submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

**DATA QUALITY**

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**Reliability:** Klimisch ranking 1

**REFERENCES**

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This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

**OTHER**

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 4 - 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.1025

**Type:** Static acute

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Crassostrea virginica*

**Supplier:** P. Cummins Oyster Company, Inc., Baltimore, MD, USA

**Shell grinding:** Prior to test initiation, recently deposited shell at the rounded (ventral) end was removed using a small electric grinder. Care was taken to remove the shell rim uniformly to produce a smooth, rounded, blunt profile.

**Analytical monitoring:** PFOS measured at 0,48,96-hours

**Exposure period:** 96-hours

**Statistical methods:** Shell growth inhibition was calculated for each treatment group as the percent reduction in shell growth relative to mean shell growth in the negative control. The EC<sub>50</sub> value was estimated by visual inspection of shell growth inhibition data. The shell growth data was evaluated for normality and homogeneity of variances using the Chi-Square test and Bartlett's test, respectively. Dunnett's test was used to identify treatment groups that had a statistically significant ( $\leq 0.05$ ) reduction in shell growth as compared to the control.

**Test oyster age:** unknown

**Length:** 33.8 (27.8-41.5) mm,

**Pretreatment:** None

**Test conditions:**

**Dilution water:** Natural seawater diluted to a salinity of 20‰ with well water

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**salinity:** 21 (20-21)‰

**pH:** 8.1(8.0-8.2)

**TOC:** < 1.0 mg/L

**Stock and test solution preparation:** Primary stock prepared in dilution water at 9.1mg/L and mixed for ~24 hours prior to use. After mixing, primary stock solution appeared clear and colorless with some white particulate material suspended throughout the solution. It was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless. Due to the relatively low solubility of PFOS in natural seawater, the highest concentration attainable with this matrix is approximately 3.3mg/L.

**Concentrations dosing rate:** Once

**Exposure vessels:** 52L polyethylene aquaria containing approximately 40L of test solution; water depth approximately 21 cm. Each chamber was continuously stirred to circulate the supplemental

algae diet using an electric paddle mixer.

**Feeding:** Algal cells (*Thalassiosira pseudonana*, *Skeletonema sp.*, *Chaetoceros sp.*, and *Isochrysis sp.*) were provided to supplement naturally occurring algae and to maximize oyster growth rates during the test.

**Number of replicates:** one

**Number of oysters per replicate:** twenty

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0- 96 hours):**

6.6 - 7.6mg/L (control exposure)

6.1 - 7.7mg/L (3.0 mg/L exposure).

**pH range (0- 96 hours):**

7.6 - 8.1 (control exposure)

7.6 - 8.1 (3.0 mg/L exposure)

**Test temperature range (0- 96 hours):**

22.2 - 22.3 °C (control exposure)

21.8 - 22.7 °C (3.0 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

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**Nominal concentrations:** Negative control, 1.2, 2.0, 3.3, 5.5, 9.1 mg/L

**Measured concentrations:** <LOQ, 0.36, 0.40, 1.3, 1.9, 3.0 mg/L

**Element value:** (95% confidence interval is given in brackets)

96-hour EC<sub>50</sub> = >3.0 mg/L (C.I. not calculable)

96-hour NOEC = 1.9 mg/L

All element values based on mean measured concentrations

### Statistical evaluation of shell growth:

EC<sub>50</sub> values could not be calculated due to insufficient shell growth inhibition at the highest attainable concentration.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. Samples collected at test initiation had measured values from 28 to 46% of nominal. Measured values for samples taken at 48-hours ranged from 15 to 41 % of nominal. Measured values for samples taken at 96-hours ranged from <LOQ to 52% of nominal.

### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/l | Measured Duplicate Values at 0, 48 and 96-hours, Respectively, mg/L | Mean Measured Concentration, mg/l | Percent of Nominal |
|----------------------------------|---|-----------------------------------|--------------------|
| Negative Control                 | All <LOQ  | <LOQ                              | -                  |
| 1.2                              | 0.331, 0.353, 0.341, 0.429, <LOQ, <LOQ                              | 0.36                              | 30                 |
| 2.0                              | 0/622, 0.633, 0.299,  | 0.40                              | 20                 |

|     |  |     |    |
|-----|--|-----|----|
|     | 0.313, 0.249, 0.257                          |     |    |
| 3.3 | 1.36, 1.15, 0.924,<br>0.878, 1.58, 1.72      | 1.3 | 39 |
| 5.5 | 2.42, 2.53, 2.02,<br>2.24, 1.45, 0.970       | 1.9 | 35 |
| 9.1 | 3.39, 3.44, 3.01*,<br>3.74, 3.57, 1.99, 2.19 | 3.0 | 33 |

\*3 replicates analyzed at time 0

**Biological observations after 96-hours:** Oysters in the negative control and all PFOS treatment groups appeared normal and healthy throughout the exposure period.

**Shell deposition and shell growth Inhibition at test termination:**

| Mean Measured Concentration, mg/l | Shell Deposition Mean $\pm$ SD, mm | Percent Inhibition in Shell Growth |
|-----------------------------------|------------------------------------|------------------------------------|
| Negative Control                  | 2.67 $\pm$ 0.824                   | -                                  |
| 0.36                              | 2.50 $\pm$ 0.933                   | 6.4                                |
| 0.40                              | 2.40 $\pm$ 0.820                   | 10                                 |
| 1.3                               | 2.51 $\pm$ 0.919                   | 6.0                                |
| 1.9                               | 2.13 $\pm$ 0.804                   | 20                                 |
| 3.0                               | 1.91 $\pm$ 0.591                   | 28                                 |

**Mortality of controls:** None

CONCLUSIONS

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The potassium perfluorooctanesulfonate 96-hour EC<sub>50</sub> for the Eastern Oyster was determined to be > 3.0 mg/L, the highest concentration tested and the practical limit of solubility in unfiltered seawater. The 96-hour no effect concentration was 1.9 mg/L.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

DATA QUALITY

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**Reliability:** Klimisch ranking = 1

REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

OTHER

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**Last changed:** 5/3100



## Robust Study Report Reference No. 5 - 96-Hour Static Acute Toxicity Test with the Freshwater Mussel (*Unio complamatus*)

### TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

### METHOD

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**Method:** The study was conducted using a protocol based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Guidelines, OPPTS Number 850.1075 ; OECD 203: *Fish, Acute Toxicity Test*; and ASTM Standard E729-88a, *Standard Guide for Conducting Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*.

**Test type:** Semi-static Renewal

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Unio complamatus*

**Analytical monitoring:** Test substance concentrations measured by LCMS at 0, 48, 96-hours

**Statistical methods:** LC<sub>50</sub> values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Test organism source:** Obtained from Carolina Biological Supply Company, Burlington, North Carolina, USA. Carolina collected from the wild.

**Test organism age at study initiation:** Unknown

#### Test Conditions

**Dilution water:** 0.45 µm filtered well water

#### Dilution water chemistry (during the 4-week period immediately preceding the test):

**Hardness:** 126 (120-132) mg/L as CaCO<sub>3</sub>

**Alkalinity:** 174 (170-178) mg/L as CaCO<sub>3</sub>

**pH:** 8.3 (8.1-8.5)

**TOC:** < 1.0 mg/L

**Conductivity:** 321 (310-330) µmhos/cm

**Ca/Mg ratio:** 35/13.5

**Na/K ratio:** 21.3/6.62

**Lighting:** Colortone® 50 fluorescent lights, intensity approximately 369 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

**Stock and test solutions preparation:** A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for approximately 24 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

**Exposure vessels:** 25 liter polyethylene aquaria containing approximately 20 L of test solution. The approximate depth of test solution was 23.2 cm.

**Number of replicates:** two

**Number of test organisms per replicate:** ten

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

5.8 – 8.5 mg/L (control exposure)

5.0 – 8.6 mg/L (79 mg/L exposure)

**pH range (0 – 96 hours):**

8.0 – 8.4 (control exposure)

7.9 – 8.5 (79 mg/L exposure)

**Test temperature range (0 – 96 hours):**

21.4– 21.8°C (control exposure)

21.8 – 23.7 °C (79 mg/L exposure)

**Element Basis:** Mortality. Mussels with open shells and not responding to gentle prodding were considered dead. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated.

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

**Nominal concentrations:** <LOQ, 5.7, 11, 23, 46, 91 mg/L

**Measured concentrations:** <LOQ, 5.3, 12, 20, 41, 79 mg/L

**Element value:** (95% confidence interval is given in brackets)

96-hour LC<sub>50</sub> = 59 mg/L (51-68 mg/L)

**Statistical evaluation:** The LC<sub>50</sub> values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7%. Samples collected at test initiation had measured values from 73.7% to 96.0% of nominal. Measured values for samples taken at 48 hours ranged from 81.2 to 98.9% of nominal. Measured values for samples taken at 96 hours ranged from 88.5 to 130% of nominal.

**Summary of analytical chemistry data:**

| Nominal Test Concentration , mg/L | Measured duplicated values at 0, 48, and 96-hours respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|-----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                  | All < LOQ  | <LOQ                              | -                  |
| 5.7                               | 5.47, 4.93, 5.18, 5.70, 5.24, 5.26                                   | 5.3                               | 93                 |
| 11                                | 11.4, 10.1, 11.2, 10.5, 10.9, 15.4                                   | 12                                | 109                |
| 23                                | 19.0, 16.8, 18.7, 18.7, 22.9, 22.4                                   | 20                                | 87                 |
| 46                                | 37.2, 40.6, 37.1, 39.5, 48.2, 40.5                                   | 41                                | 89                 |
| 91                                | 69.0, 74.7, 81.3, 77.6, 88.2, 85.7                                   | 79                                | 87                 |

**Biological observations after 96-hours:** Mussels in the negative control, the 5.3, 12 and the 20 mg/L treatments appeared healthy and normal throughout the test with no mortality or overt clinical signs of toxicity. Five percent mortality was observed at 96-hours in the 41 mg/L treatment and 90% mortality was observed in the 79 mg/L treatment. No abnormal behavior was noted in these concentrations.

**Cumulative percent mortality:**

| Mean Measured Test Concentration mg/L | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
|---------------------------------------|----------|----------|----------|----------|
| Negative Control                      | 0        | 0        | 0        | 0        |
| 5.3                                   | 0        | 0        | 0        | 0        |
| 12                                    | 0        | 0        | 0        | 0        |
| 20                                    | 0        | 0        | 0        | 0        |
| 41                                    | 0        | 0        | 0        | 5        |
| 79                                    | 30       | 40       | 50       | 90       |

**Control response:** Satisfactory

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**CONCLUSIONS**


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The potassium perfluorooctanesulfonate 96-hour LC<sub>50</sub> for the Freshwater Mussel, *Unio complamatus* was determined to be 59 mg/L with a 95% confidence interval of 51-68 mg/L. The 96-hour no mortality concentration was 20 mg/L.

**Submitter:** 3M Corporation, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

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**DATA QUALITY**


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**Reliability:** Klimisch ranking 1.

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**REFERENCES**


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This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

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**OTHER**


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**Last changed:** 5/3/00

Robust Study Report Reference No. 6 – Activated Sludge, Respiration Inhibition Test

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OECD 209

**Test type:** Static acute

**GLP:** Yes

**Year Completed:** Study completed 1999. Report completed 2000

**Analytical monitoring:** Dissolved oxygen concentrations.

**Statistical methods:** Probit analysis using the computer software of C.E. Stephan.

**Test organism source:** Activated sludge collected from the Prospect Bay Wastewater Treatment Facility, Grasonville, Maryland, USA.

**Test conditions**

**Dilution water:** NANOpure®

**Synthetic Sewage:**

1 liter municipal water

16.0 g peptone

11.0 g meat extract

3.0 g urea

0.7 g NaCl

0.4 g CaCl<sub>2</sub> 2H<sub>2</sub>O

0.2 g MgSO<sub>4</sub> 7H<sub>2</sub>O

2.8 g K<sub>2</sub>HPO<sub>4</sub>

**Reference and test solution preparation:** A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL of 1N NaOH, diluted to 30 mL with NANOpure® water, then brought to the point of incipient precipitation with 1 NH<sub>2</sub>SO<sub>4</sub>, and diluted to 1 L with NANOpure4® water. The pH of the reference solution was measured to be 7.18. PFOS was added directly to test vessels rather than volumetric addition of a stock solution. This method was deemed appropriate based on the observed solubility of the test substance in water.

**Test vessels:** Mixtures were prepared and aerated in 500 mL Erlenmeyer flasks and then transferred into 300 mL Biochemical Oxygen Demand (BOD) bottles

**Number of concentrations:** 7 plus 3 reference controls and 2 Blank controls

**Temperature:** 19-21°C

**Total Suspended Solids and pH for sludge on day of testing:** 4380 mg/L and 7.87 respectively.

**Element Basis:** Respiration inhibition as determined by dissolved oxygen concentration.

**Method Remarks:** Stock solutions of PFOS that were prepared at nominal concentration of approximately 500 and 1 000 mg/L in NANOpure® water contained test material that was not in solution after 20-minutes of sonication. Therefore, direct weight addition was employed to administer PFOS to the test system.

Test mixtures were prepared at 15-minute intervals and aerated until the contact time of the test substance with the activated sludge was three hours. After 3-hours of contact time, dissolved oxygen was measured over a period of up to 10-minutes.

## RESULTS

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**Nominal concentrations:** Two blank controls, three reference substance controls, 0.90, 2.7, 9.0, 27, 90, 271. 905 mg/L test material solutions.

**Statistical Analyses:** EC<sub>50</sub> values were calculated for the reference material by probit analysis using the computer software of C.E. Stephan. An EC<sub>50</sub> value could not be calculated for the test substance.

**Analytical Methodology:** Analysis of DO concentrations in all test solutions were performed at Wildlife International Ltd. using a YSI Model 50B Dissolved Oxygen Meter. Dissolved oxygen readings were recorded every 10 seconds for 10 minutes or until the dissolved oxygen dropped below 1.0 mg/L

### Respiration Rates and Percent Inhibitions

| Treatment                  | Respiration Rate<br>mg O <sub>2</sub> /L/hour | Percent Inhibition |
|----------------------------|---|--------------------|
| Control 1                  | 39.6  | NA                 |
| Control 2                  | 41.1  | NA                 |
| 3,5-dichlorophenol 3mg/L   | 31.1  | 22.9               |
| 3,5-dichlorophenol 15 mg/L | 14.6  | 63.8               |
| 3,5-dichlorophenol 50 mg/L | 5.1   | 87.4               |
| Test substance 0.90 mg/L   | 38.9  | 3.6                |
| Test substance 2.7 mg/L    | 35.1  | 13.0               |
| Test substance 9.0 mg/L    | 33.5  | 17.0               |
| Test substance 27 mg/L     | 37.9  | 6.1                |
| Test substance 90 mg/L     | 32.7  | 19.0               |
| Test substance 271 mg/L    | 28.1  | 30.4               |
| Test substance 905 mg/L    | 24.7  | 38.8               |

**Control response:** satisfactory

## CONCLUSIONS

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The test substance exhibited a maximum inhibitory effect of 38% upon respiration at a nominal test substance concentration of 905 mg/L. The EC<sub>50</sub> (respiration inhibition) is therefore greater than the solubility of the test substance.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

## DATA QUALITY

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**Reliability:** Klimisch ranking 1.

## REFERENCES

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This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

ENV/JM/RD(2002)17

OTHER

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 7 - 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, 1H-NMR, 19F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.1035

**Type:** Static acute

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Mysidopsis bahia*

**Supplier:** In-house cultures, Wildlife International, Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured at 0, 48, 96-hours

**Exposure period:** 96-hours

**Statistical methods:** LC<sub>50</sub> values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Test fish age:** < 24-hours old

**Pretreatment:** None

**Test Conditions:**

**Dilution water:** Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

Dilution water chemistry (during the 4-week period immediately preceding the test):

**Salinity:** 20 (20-20) ‰

**pH:** 8.2 (8.1-8.2)

**TOC:** < 1.0 mg/L

**Stock and test solution preparation:** Primary stock prepared at 8.2 mg/L and mixed for ~22 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** 2L polyethylene aquaria containing approximately 1000mL of test solution; water depth approximately 6.6 cm.

**Number of replicates:** two

**Number of mysids per replicate:** ten

**Number of concentrations:** five plus a negative control

**Feeding:** Live brine shrimp nauplii daily

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

6.8 – 7.4 mg/L (control exposure)

6.8 – 7.3 mg/L (5.4 mg/L exposure)

**pH range (0 – 96 hours):**

8.1 – 8.2 (control exposure)

8.1 – 8.2 (5.4 mg/L exposure)

**Test temperature range (0 – 96 hours):**

24.2 – 25.4°C (control exposure)

23.8 – 24.5°C (5.4 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean**RESULTS****Nominal concentrations:** Negative control, 1.1, 1.8, 3.0, 4.9, 8.2 mg/L**Measured concentrations:** <LOQ, 0.57, 1.1, 1.9, 3.0, 5.4 mg/L**Element value:** (95% confidence interval is given in brackets)24-hour LC<sub>50</sub> = > 5.4 mg/L (CI not calculable)48-hour LC<sub>50</sub> = > 5.4 mg/L C.I. not calculable)72-hour LC<sub>50</sub> = 4.4 (3.6-6.2) mg/L96-hour LC<sub>50</sub> = 3.6 (3.0-4.6) mg/L

All element values based on mean measured concentrations

**Statistical evaluation of mortality:** LC<sub>50</sub> values could not be calculated for 24 and 48-hours of exposure due to the lack on an adequate concentration-response pattern. The probit method was used to evaluate mortality at 72 and 96 hours.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctane sulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.4. Samples collected at test initiation had measured values from 52.4 to 70.7% of nominal. Measured values for samples taken at 48 hours ranged from 43.5 to 71.0% of nominal. Measured values for samples taken at 96 hours ranged from 35.5 to 71.1% of nominal.

**Summary of analytical chemistry data:**

| Nominal Test Concentration, mg/L | Measured duplicate values at 0, 48, and 96-hours respectively, mg/L | Mean Measured Concentration mg/L | Percent of Nominal |
|----------------------------------|---|----------------------------------|--------------------|
| Negative Control                 | All < LOQ   | <LOQ                             | -                  |
| 1.1                              | 0.575, 0.622, 0.605, 0.640, 0.391, 0.580                            | 0.57                             | 52                 |
| 1.8                              | 1.12, 1.19, 1.10, 1.09, 1.04, 1.13                                  | 1.1                              | 61                 |
| 3.0                              | 1.92, 1.99, 1.92, 1.91, 1.79, 1.91                                  | 1.9                              | 63                 |
| 4.9                              | 3.05, 2.66, 2.96, 3.35, 3.11, 3.11                                  | 3.0                              | 61                 |
| 8.2                              | 5.82, 5.78, 3.58, 5.85, 5.22, 5.86                                  | 5.4                              | 66                 |

**Biological observations after 96-hours:** Mysids in the negative control, and the 0.57 and 1.1 mg/L (mean measured concentrations) treatment groups appeared normal and healthy during the test.

**Cumulative percent mortality:**

| Mean Measured Test Concentration, mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|--|----------|----------|----------|----------|
|  |          |          |          |          |



|                  |    |    |    |    |
|------------------|----|----|----|----|
| Negative Control | 0  | 0  | 0  | 0  |
| 0.57             | 0  | 0  | 0  | 0  |
| 1.1              | 0  | 0  | 0  | 0  |
| 1.9              | 0  | 0  | 5  | 10 |
| 3.0              | 5  | 15 | 30 | 40 |
| 5.4              | 15 | 45 | 60 | 75 |

**Mortality of controls:** None

#### CONCLUSIONS

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The potassium perfluorooctanesulfonate 96-hour LC<sub>50</sub> for saltwater mysids was determined to be 3.6 mg/L with a 95% confidence interval of 3.0 – 4.6 mg/L. The 96-hour no mortality and NOEC concentration was 1.1 mg/L.

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

#### DATA QUALITY

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**Reliability:** Klimisch ranking = 1

#### REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

#### OTHER

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 8 - Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OECD 210, OPPTS 850.1400

**Type:** Flow-through chronic

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Pimephales promelas*

**Supplier:** In-house cultures, Wildlife International, Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured on days 0, 4, 7, 14, 21, 28, 35, 42, and 47

**Exposure period:** 47 days

**Statistical methods:** Discrete-variable data were analyzed using 2 x 2 contingency tables to identify treatment groups that showed a statistically significant difference ( $p \leq 0.05$ ) from the negative control group. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means.

**Test fish age:** eggs < 24-hours old at test initiation

**Pretreatment:** None

**Test Conditions**

**Dilution water:** 0.45  $\mu$ m filtered well water

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**Hardness:** 126 (124-128) mg/L as CaCO<sub>3</sub>

**Alkalinity:** 172 (170-172) mg/L as CaCO<sub>3</sub>

**pH:** 8.2 (8.2-8.3)

**TOC:** < 1.0 mg/L

**Conductivity:** 321 (315-330)  $\mu$ mhos/cm

**Stock and test solution preparation:** Primary stock prepared in dilution water at 88.4 mg/L and mixed until all test substance dissolved prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 44.2, 22.1, 11.0, 5.52, and 2.76 mg/L. Stock solutions were prepared every three to four days during the test. The six stocks were injected into the diluter mixing chambers (at a rate of 6.0 mL/minute) where they were mixed with dilution water (at a rate of 116 mL/minute) to achieve the desired test concentrations.

**Flow through rate:** Approximately six volume additions of test water every 24-hours

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** 9L glass aquaria filled with approximately 7 L of test solution with a depth of approximately 17 cm. Embryo incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425  $\mu$ m nylon screen mesh attached to the bottom with

silicone sealant. The cups were suspended in the water column of each 9L glass aquarium and attached to a rocker arm with a reciprocating motion of approximately 2 rpm.

**Number of replicates:** four

**Number of fish per replicate:** twenty

**Number of concentrations:** six plus a negative control

**Feeding:** Live brine shrimp nauplii. Fed 3 times per day during the first 7 days post-hatch. On days 8 through 40 post-hatch, fed 3 times daily on weekdays and 2 times daily on weekends. Not fed for at least 48 hours prior to the termination of test to allow for gut clearance prior to weight measurements.

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 47 days):**

7.6 – 8.2 mg/L (control exposure)

7.6 – 8.2 mg/L (1.2 mg/L exposure)

**pH range (0 – 47 days):**

8.0 – 8.4 (control exposure)

8.0 – 8.4 (1.2 mg/L exposure)

**Test temperature range (0 – 47 days):**

24.4 – 24.7°C (control exposure)

24.3 – 24.7°C (1.2 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

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**Nominal concentrations:** Negative control, 0.14, 0.29, 0.57, 1.1, 2.3, 4.6 mg/L

**Measured concentrations:** <LOQ, 0.15, 0.30, 0.60, 1.2, 2.4, 4.6 mg/L

**Element value:** 5-day hatchability NOEC = 4.6 mg/L  
42-day post-hatch survival NOEC = 0.30 mg/L  
42-day post-hatch growth NOEC = 0.30 mg/L  
42-day post-hatch survival LOEC = 0.60 mg/L

All element values based on mean measured concentrations

**Statistical evaluation of mortality:** The statistical difference for growth at concentrations equal to and higher than 0.60 mg/L was not evaluated due to a significant effect on survival. No statistically significant difference between the negative control and the highest concentration tested was seen for hatchability.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctane sulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 102. Samples collected at pre-test ranged from 91.4 to 105% of nominal. Samples at test initiation had measured values from 95.5 to 114% of nominal. Measured values for samples taken at test termination ranged from 95.2 to 111% of nominal.

### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured duplicate values at 0, 4, 7, 14, 21, 28, 35, 42, and 47 Days respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | -                                 | -                  |

|      |  |      |     |
|------|--|------|-----|
| 0.14 | 0.147, 0.160, 0.141, 0.140, 0.144, 0.148, 0.134, 0.135, 0.153, 0.143, 0.160, 0.158, 0.179, 0.173, 0.157, 0.160, 0.147, 0.155 | 0.15 | 107 |
| 0.29 | 0.287, 0.277, 0.270, 0.289, 0.292, 0.296, 0.269, 0.266, 0.307, 0.315, 0.343, 0.341, 0.311, 0.325, 0.319, 0.313, 0.296, 0.276 | 0.30 | 103 |
| 0.57 | 0.571, 0.576, 0.619, 0.659, 0.597, 0.642, 0.539, 0.535, 0.608, 0.580, 0.639, 0.617, 0.646, 0.644, 0.575, 0.576, 0.545, 0.543 | 0.60 | 105 |
| 1.1  | 1.14, 1.13, 1.21, 1.25, 1.13, 1.23, 1.03, 1.10, 1.19, 1.24, 1.30, 1.31, 1.30, 1.31, 1.14, 1.19, 1.13, 1.09                   | 1.2  | 109 |
| 2.3  | 2.21, 2.27, 2.52, 2.46, 2.43, 2.38, fish all dead at Day 7   | 2.4  | 104 |
| 4.6  | 4.56, 4.40, 4.79, 4.79, 4.46, 4.76, fish all dead at Day 7   | 4.6  | 100 |

### Biological Observations

**Hatching success and time to hatch:** All viable fathead minnow embryos hatched on Day 4 or 5. There were no apparent differences between the time to hatch in the negative control and the PFOS treatment groups.

**Survival:** All fish surviving to test termination appeared normal with no overt signs of sublethal toxicity. Fish which did not survive generally appeared to be swimming erratically prior to death.

**Growth:** Fish exposed to PFOS at concentrations of 0.15 or 0.30 mg/L for 42 days post-hatch showed no statistically significant reduction in total length, wet weight or dry weight in comparison to the negative control.

### Hatchability

| Mean Measured Concentration mg/L | Number of Eggs Exposed | Number Hatched, Day 3 | Number Hatched, Day 4 | Number Hatched, Day 5 | Total Number Hatched | Percent Hatching Success |
|----------------------------------|------------------------|-----------------------|-----------------------|-----------------------|----------------------|--------------------------|
| Negative Control                 | 80                     | 0                     | 20                    | 54                    | 74                   | 93                       |
| 0.15                             | 80                     | 0                     | 18                    | 58                    | 76                   | 95                       |
| 0.3                              | 80                     | 0                     | 14                    | 58                    | 72                   | 90                       |
| 0.6                              | 80                     | 0                     | 28                    | 48                    | 76                   | 95                       |
| 1.2                              | 80                     | 0                     | 25                    | 49                    | 74                   | 93                       |
| 2.4                              | 80                     | 0                     | 16                    | 59                    | 75                   | 94                       |
| 4.6                              | 80                     | 0                     | 14                    | 60                    | 74                   | 93                       |

### Larval Survival

| Mean Measured Concentration, mg/L | Percent Survival, Day 42 |
|-----------------------------------|--------------------------|
| Negative Control                  | 88                       |
| 0.15                              | 79                       |
| 0.3                               | 81                       |
| 0.6                               | 66                       |
| 1.2                               | 5.4                      |
| 2.4                               | 0                        |
| 4.6                               | 0                        |

**Growth**

| Mean Measured Concentration, mg/L | Number of Surviving Larvae | Total Length Mean + SD, mm | Wet Weight Mean + SD, mg | Dry Weight Mean + SD, mg |
|-----------------------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| Negative Control                  | 65                         | 26.5 + 0.721               | 158 + 9.10               | 32.5 + 1.20              |
| 0.15                              | 60                         | 26.6 + 0.208               | 160 + 3.10               | 33.3 + 0.900             |
| 0.30                              | 58                         | 26.6 + 0.813               | 167 + 11.9               | 34.2 + 2.70              |
| 0.60                              | 50                         | 26.5 + 0.399               | 166 + 11.3               | 33.5 + 2.70              |
| 1.2                               | 4                          | 26.7 + 2.02                | 185 + 33.8               | 35.4 + 6.66              |
| 2.4                               | 0                          | -                          | -                        | -                        |
| 4.6                               | 0                          | -                          | -                        | -                        |

**CONCLUSIONS**

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Fathead minnows exposed to potassium perfluorooctanesulfonate at concentrations  $\leq 0.30$  mg/L for 42 days post-hatch showed no statistically significant reductions in time to hatch, hatching success, survival or growth. The most sensitive endpoint in this study was post-hatch survival.

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

**DATA QUALITY**

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**Reliability:** Klimisch ranking = 1

**REFERENCES**

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

**OTHER**

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 9 - Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)**TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.1300, OECD Guideline 211, and ASTM Standard E 1193-87.

**Type:** Semi-Static Life-Cycle Toxicity

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Daphnia magna*

**Supplier:** In-house cultures, Wildlife International, Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured on days 0, 2, 11, 14, 18, and 21.

**Exposure period:** 21 days

**Statistical methods:** Survival data was evaluated on first-generation daphnids, the number of live young and the length and dry weight of the surviving first-generation daphnids. Survival data were analyzed using Fisher's exact test. Reproduction and growth (length and dry weight) data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test was used to identify treatment groups that were statistically significant in comparison to the negative control ( $p \leq 0.05$ ). All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

**Test organism age:** < 24-hours old at test initiation

**Pretreatment:** None

**Test Conditions**

**Dilution water:** 0.45 µm filtered well water passed through a UV sterilizer to remove microorganisms and fine particles

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**Hardness:** 124 (120-128) mg/L as CaCO<sub>3</sub>

**Alkalinity:** 169 (164-172) mg/L as CaCO<sub>3</sub>

**pH:** 8.2 (8.0-8.3)

**TOC:** < 1.0 mg/L

**Conductivity:** 329 (315-340) µmhos/cm

**Ca/Mg ratio:** 35/13.5

**Na/K ratio:** 21.3/6.62

**Stock and test solution preparation:** Primary stock solution was prepared in dilution water at 46 mg/L. It was stirred until all test substance was dissolved prior to use. After mixing, the primary stock solution was proportionally diluted with UV sterilized dilution water to prepare five additional stock solutions at nominal concentrations of 1.4, 2.9, 5.7, 11, and 23 mg/L. All test solutions appeared clear and colorless.

**Renewal rate:** Every Monday, Wednesday and Friday.

**Exposure vessels:** 250-mL plastic beakers containing approximately 200 mL test solution. The depth was approximately 5 cm.

**Number of replicates:** 10

**Number of test organisms per replicate:** 1

**Number of concentrations:** 6 plus a negative control

**Feeding:** Each test chamber was fed 0.3 mL of YCT (a mixture of yeast, Cerophyll®, and trout chow at 1800 mg TSS/L) and 0.60 mL of *Selenastrum capricornutum* ( $3.5 \times 10^7$  cells/mL) once daily.

**Lighting:** Colortone® 50 fluorescent lights. Intensity ranged from 329 - 383 lux at the water surface. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

**Water chemistry of new and old solutions during the study:**

**Dissolved oxygen range (0 – 21 days):**

8.3 – 8.9 mg/L (negative control exposure)

8.3 – 9.0 mg/L (12 mg/L exposure)

8.4 – 8.9 mg/L\* (48 mg/L exposure)

**pH range (0 – 21 days):**

8.1 – 8.4 (negative control exposure)

8.2 – 8.5 (12 mg/L exposure)

8.4 – 8.5\* (48 mg/L exposure)

**Test temperature range (0 – 21 days):**

19.4 – 20.1°C (negative control exposure)

19.4 – 20.1°C (12 mg/L exposure)

19.4 – 19.5 °C\* (48 mg/L exposure)

\* (Measurements discontinued at Day 3 due to 100% mortality.)

**Element basis:** Survival, reproduction and growth. Effect concentrations based on survival.

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

**Nominal concentrations:** Negative control, 1.4, 2.9, 5.7, 11, 23, 46 mg/L

**Measured concentrations:** <LOQ, 1.5, 2.9, 5.6, 12, 24, 48 mg/L

**Element value:** 21-day NOEC = 12 mg/L

21-day LOEC = 24 mg/L

21-day MATC = 17 mg/L

2nd generation acute survival NOEC = 12 mg/L

All element values based on mean measured concentrations.

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for the test substance was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.458 mg/L in this study. The mean procedural recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Measured values of new samples ranged from 94 to 121% of nominal. Measured values from the old solutions ranged from 90 to 108% of nominal values. PFOS was stable throughout the renewal periods.

### Summary of analytical chemistry data:

| Nominal Test Concentration mg/L | Measured duplicate values at 0, 2, 11, 14, 18, and 21 Days respectively, mg/L | Mean Measured Concentration mg/L | Percent of Nominal |
|---------------------------------|---|----------------------------------|--------------------|
|---------------------------------|---|----------------------------------|--------------------|

|                  |  |     |     |
|------------------|--|-----|-----|
| Negative Control | All < LOQ  | -   | -   |
| 1.4              | 1.78, 1.72, 1.58, 1.56, 1.38, 1.47, 1.36, 1.32, 1.38, 1.43, 1.50, 1.45                               | 1.5 | 107 |
| 2.9              | 3.20, 3.05, 3.01, 3.07, 2.75, 2.77, 2.85, 2.71, 2.79, 2.81, 2.81, 2.82                               | 2.9 | 100 |
| 5.7              | 5.97, 5.87, 5.65, 5.72, 5.63, 5.59, 5.36, 5.39, 5.58, 5.75, 5.24, 5.37                               | 5.6 | 98  |
| 11               | 11.5, 11.5, 11.6, 11.8, 11.3, 11.3, 11.2, 11.6, 11.8, 11.6, 11.5, 11.3                               | 12  | 109 |
| 23               | 24.2, 23.1, 24.0, 24.6, 22.8, 22.5, 23.6, 23.1, 24.8, 25.0, all daphnids dead after 18-days exposure | 24  | 104 |
| 46               | 47.3, 48.0, 49.1, 49.4, all daphnids dead after 2-days exposure                                      | 48  | 104 |

NOTE: Mean measured concentrations were determined from new (renewal solutions) and corresponding old solutions during each week of the test. Days 0, 11, and 18 are “new” and days 2, 24, and 21 are “old”.

### Biological Observations

**Survival:** All surviving first generation daphnids appeared normal at test termination. Survival in the 24 and 48 mg/L treatments was statistically significantly different from the negative control group.

**Reproduction:** Daphnids in the control and treatment groups  $\leq 12$  mg/L started producing neonates on Day 9. The Bonferroni t-test showed that reproduction was not significantly reduced in any treatment group  $\leq 12$  mg/L ( $p > 0.05$ ). The 24 and 48 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant effect on survival.

**Growth:** The Bonferroni t-test showed that mean length and dry weight in the treatment groups  $\leq 12$  mg/L were not significantly reduced in comparison to the negative control ( $p > 0.05$ ).

**Second Generation Acute Exposure:** After 48-hours of exposure, survival in the negative control was 95%. Survival in the 1.5, 2.9, 5.6, 12, and 24 mg/L treatment groups was 100, 100, 100, 90, and 0% respectively. Survival in the 24 mg/L treatment group was significantly different from the negative control ( $p \leq 0.05$ ).

### Summary of Percent Mortality

| Mean Measured Concentration, mg/L | Day 7 | Day 14 | Day 21 |
|-----------------------------------|-------|--------|--------|
| Negative Control                  | 0     | 0      | 0      |
| 1.5                               | 0     | 0      | 10     |
| 2.9                               | 0     | 0      | 10     |
| 5.6                               | 0     | 0      | 10     |
| 12                                | 0     | 10     | 10     |
| 24                                | 70    | 90     | 100    |
| 48                                | 100   | 100    | 100    |

### Second Generation Mortality

| Mean Measured Concentrations, mg/L | Total Number Exposed | Number Alive after 48-hours | Cumulative Percent Dead |
|------------------------------------|----------------------|-----------------------------|-------------------------|
| Negative Control                   | 20                   | 19                          | 5                       |
| 1.5                                | 20                   | 20                          | 0                       |
| 2.9                                | 20                   | 20                          | 0                       |



|     |    |    |     |
|-----|----|----|-----|
| 5.6 | 20 | 20 | 0   |
| 12  | 20 | 18 | 10  |
| 24  | 8  | 0  | 100 |

### Summary of Length and Dry Weight of Surviving Individually-Exposed First-Generation Daphnids

| Mean Measured Concentration, mg/L | Number of Surviving Daphnids | Total Length, Mean + SD, mm | Dry Weight, Mean + SD, mg |
|-----------------------------------|------------------------------|-----------------------------|---------------------------|
| Negative Control                  | 10                           | 4.65 + 0.111                | 0.695 + 0.100             |
| 1.5                               | 9                            | 4.66 + 0.118                | 0.669 + 0.0623            |
| 2.9                               | 9                            | 4.62 + 0.100                | 0.724 + 0.110             |
| 5.6                               | 9                            | 4.61 + 0.124                | 0.727 + 0.0665            |
| 12                                | 9                            | 4.59 + 0.102                | 0.723 + 0.0661            |
| 24                                | 0                            | --                          | --                        |
| 48                                | 0                            | --                          | --                        |

### Reproduction

| Mean Measured Concentration, mg/L | Number of Surviving Daphnids | Mean Live Young/ Surviving Adult Daphnid (+SD) | First Day of Reproduction | Total Number of Dead / Immobile Neonates | Total Number of Aborted Eggs |
|-----------------------------------|------------------------------|--|---------------------------|--|------------------------------|
| Negative Control                  | 10                           | 122 + 19.2                                     | 9                         | 0  | 0                            |
| 1.5                               | 9                            | 142 + 24.7                                     | 9                         | 0  | 0                            |
| 2.9                               | 9                            | 136 + 17.9                                     | 9                         | 0  | 0                            |
| 5.6                               | 9                            | 132 + 19.5                                     | 9                         | 0  | 0                            |
| 12                                | 9                            | 119 + 26.5                                     | 9                         | 1  | 0                            |
| 24                                | 0                            | --   | 11                        | 10                                       | 0                            |
| 48                                | 0                            | --   | None                      | --                                       | --                           |

### CONCLUSIONS

There were no adverse effects on survival, reproduction or growth of *Daphnia magna* exposed to the test substance at concentration  $\leq 12$  mg/L for 21 days. *Daphnia magna* exposed to 24 and 48 mg/L had significantly reduced survival.

**Author and/or submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

### DATA QUALITY

**Reliability:** Klimisch ranking 1.

### REFERENCES

This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

### OTHER

**Last changed:** 5/3/00

**Robust Study Report Reference No. 10 - Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.1350

**Type:** Flow-through chronic

**GLP:** Yes

**Year completed:** Study completed 1999. Report completed 2000

**Species:** *Mysidopsis bahia*

**Supplier:** In-house cultures, Wildlife International, Ltd., Easton, MD, USA

**Analytical monitoring:** PFOS measured on days 0, 7, 14, 21, 28, and 35

**Exposure period:** 35 days

**Statistical methods:** Survival data was evaluated (prior to pairing and after pairing) using 2 x 2 contingency tables to identify treatment groups that showed a statistically significant difference ( $p \leq 0.05$ ) from the negative control group. All continuous-variable data (reproduction and growth) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

**Test mysids age:** < 24-hours old at test initiation

**Pretreatment:** None

**Test Conditions:** Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**Salinity:** 20 (20-20) ‰

**TOC:** < 1.0 mg/L

**Stock and test solution preparation:** Primary stock prepared at 0.0895 mg/L and mixed for approximately 24 hours prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 0.0447, 0.0224, 0.0112, 0.00559, and 0.00280 mg/L. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water (at a rate of 150 mL/minute) to achieve the desired test concentrations.

**Flow through rate:** Approximately eleven volume additions of test water every 24 hours

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** Prior to pairing, mysids placed in glass beakers with nylon mesh screen attached to two holes on opposite sides. After reaching sexual maturity, pairs placed in glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. Both pre-pairing and post-pairing exposure vessels were placed in 9L glass aquaria filled with approximately 5 L of test solution. The depth was approximately 6.2 cm prior to pairing and 5.5 cm after pairing.

The test chambers for the second generation exposure were 2L beakers with 1L of test solution which was dipped out of a test chamber from the appropriate treatment group.

**Number of replicates:** four

**Number of concentrations:** six plus a negative control

**Number of fish per replicate:** Fifteen juveniles before pairing, 5 pairs (10 adults) when possible after pairing.

**Feeding:** Fed live brine shrimp nauplii 3 or four times per day. Not fed the last day of the test.

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 35 days):**

6.0 – 6.4 mg/L (control exposure)

5.9 – 6.3 mg/L (1.3 mg/L exposure)

**pH range (0 – 35 days):**

8.2 – 8.4 (control exposure)

8.3 – 8.4 (1.3 mg/L exposure)

**Test temperature range (0 – 35 days):**

24.5 – 25.2°C (control exposure)

24.4 – 25.1°C (1.3 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

**Nominal concentrations:** Negative control, 0.086, 0.17, 0.34, 0.69, 1.4, 2.7 mg/L

**Measured concentrations:** <LOQ, 0.057, 0.12, 0.25, 0.55, 1.3, 2.6 mg/L

**Element value:** 20-day survival (pre-pairing) NOEC = 0.55 mg/L

35-day (post-pairing) survival NOEC = 0.55 mg/L

35-day reproduction NOEC = 0.25 mg/L

35-day growth NOEC = 0.25 mg/L

35-day reprod & growth LOEC = 0.55 mg/L

2nd generation acute survival NOEC = 0.55 mg/L (highest concentration tested)

All element values based on mean measured concentrations

**Analytical methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 92.8. Samples collected at pretest ranged from 57.4 to 99.3% of nominal. Samples at test initiation had measured values from 67.1 to 103% of nominal. Measured values for samples taken at test termination ranged from 59.8 to 90.0% of nominal.

### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured duplicate values at 0, 7, 14, 21, 28, 35, Days respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | -                                 | -                  |
| 0.086                            | 0.0694, 0.0578, 0.0478, 0.0619, 0.0606, 0.0614, 0.0554,                    | 0.057                             | 66                 |

|      |  |      |    |
|------|--|------|----|
|      | 0.0509, 0.0515, 0.0569, 0.0580, 0.0514   |      |    |
| 0.17 | 0.125, 0.114, 0.0778, 0.125, 0.124, 0.127, 0.0970, 0.112, 0.122, 0.128, 0.124, 0.119 | 0.12 | 71 |
| 0.34 | 0.289, 0.286, 0.231, 0.197, 0.276, 0.253, 0.227, 0.212, 0.262, 0.271, 0.278, 0.251   | 0.25 | 74 |
| 0.69 | 0.562, 0.659, 0.581, 0.450, 0.543, 0.542, 0.516, 0.528, 0.529, 0.544, 0.556, 0.583   | 0.55 | 80 |
| 1.4  | 1.23, 1.32, 1.13, 1.20, 1.35, 1.27, 1.23, 1.15, 1.39, 1.39, 1.26, 1.20               | 1.3  | 93 |
| 2.7  | 2.56, 2.79, 2.58, 2.30, 2.54, 2.69, all mysids dead after 14-days exposure           | 2.6  | 96 |

### Biological observations

**Survival:** All surviving mysids appeared normal. Survival in the 1.3 and 2.6 mg/L treatments were statistically significantly different from the negative control group.

**Reproduction:** The day of first brood release in this study was Day 22. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg/L treatment group when compared to the negative control ( $p < 0.05$ ). The 1.3 and 2.6 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

**Growth:** Mysids exposed to PFOS at concentrations  $< 0.25$  mg/L showed no statistically significant reductions in length or dry weight ( $p < 0.05$ ).

**Second Generation Acute Exposure:** Survival in all PFOS treatment groups was  $> 95\%$  and was not statistically different from the controls. All surviving mysids in the second-generation exposure appeared normal with no overt signs of toxicity.

### Percent Survival

| Mean Measured Concentration, mg/L | Juvenile Pre-Pairing Survival, Day 20 | Adult Post- Pairing Survival, Day35 |
|-----------------------------------|---------------------------------------|-------------------------------------|
| Negative Control                  | 78                                    | 92                                  |
| 0.057                             | 92                                    | 96                                  |
| 0.12                              | 75                                    | 90                                  |
| 0.25                              | 82                                    | 97                                  |
| 0.55                              | 83                                    | 95                                  |
| 1.3                               | 32                                    | 57                                  |
| 2.6                               | 0                                     | -                                   |

### Second Generation Survival

| Mean Measured Concentrations, mg/L | Total Number Exposed | Number Alive after 96-hours | Percent Survival |
|------------------------------------|----------------------|-----------------------------|------------------|
| Negative Control                   | 71                   | 68                          | 96               |
| 0.057                              | 65                   | 63                          | 97               |
| 0.12                               | 83                   | 79                          | 95               |
| 0.25                               | 62                   | 59                          | 95               |
| 0.55                               | 13                   | 13                          | 100              |

**Adult Mysid Growth**

| Mean Measured Concentration, mg/L | Number of Surviving Mysids/Number Exposed | Total Length, Mean $\pm$ SD, mm | Dry Weight, Mean $\pm$ SD, mg |
|-----------------------------------|---|---------------------------------|-------------------------------|
| Negative Control                  | 36/39                                     | 6.43 $\pm$ 0.0634               | 0.634 $\pm$ 0.0510            |
| 0.057                             | 44/46                                     | 6.43 $\pm$ 0.0729               | 0.599 $\pm$ 0.0276            |
| 0.12                              | 36/40                                     | 6.56 $\pm$ 0.105                | 0.641 $\pm$ 0.0241            |
| 0.25                              | 36/37                                     | 6.40 $\pm$ 0.0548               | 0.622 $\pm$ 0.0227            |
| 0.55                              | 35/37                                     | 6.14 $\pm$ 0.0794               | 0.562 $\pm$ 0.00624           |
| 1.3                               | 8/14                                      | 5.85 $\pm$ 0.178                | 0.436 $\pm$ 0.0441            |

**Reproduction**

| Mean Measured Concentration, mg/L | Replicate | Number of Reproductive Days | Number of Young | Mean Number of Young/ Reproductive Day | Overall Mean $\pm$ SD |
|-----------------------------------|-----------|-----------------------------|-----------------|--|-----------------------|
| Negative control                  | A         | 70                          | 18              | 0.257                                  | 0.315 $\pm$ 0.0925    |
|                                   | B         | 53                          | 14              | 0.264                                  |                       |
|                                   | C         | 70                          | 20              | 0.286                                  |                       |
|                                   | D         | 42                          | 19              | 0.452                                  |                       |
| 0.57                              | A         | 60                          | 17              | 0.283                                  | 0.261 $\pm$ 0.0873    |
|                                   | B         | 70                          | 14              | 0.200                                  |                       |
|                                   | C         | 70                          | 13              | 0.186                                  |                       |
|                                   | D         | 56                          | 21              | 0.375                                  |                       |
| 0.12                              | A         | 70                          | 21              | 0.300                                  | 0.361 $\pm$ 0.101     |
|                                   | B         | 46                          | 22              | 0.478                                  |                       |
|                                   | C         | 54                          | 22              | 0.407                                  |                       |
|                                   | D         | 70                          | 18              | 0.257                                  |                       |
| 0.25                              | A         | 70                          | 19              | 0.271                                  | 0.252 $\pm$ 0.0723    |
|                                   | B         | 56                          | 12              | 0.214                                  |                       |
|                                   | C         | 61                          | 21              | 0.344                                  |                       |
|                                   | D         | 56                          | 10              | 0.179                                  |                       |
| 0.55                              | A         | 54                          | 3               | 0.0556                                 | 0.0559 $\pm$ 0.0376   |
|                                   | B         | 56                          | 6               | 0.107                                  |                       |
|                                   | C         | 70                          | 3               | 0.0429                                 |                       |
|                                   | D         | 56                          | 1               | 0.0179                                 |                       |
| 1.3                               | A         | 22                          | 0               | -                                      | -                     |
|                                   | B         | 14                          | 0               | -                                      |                       |
|                                   | C         | 0                           | 0               | -                                      |                       |
|                                   | D         | 11                          | 0               | -                                      |                       |

**CONCLUSIONS**

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp exposed to potassium perfluorooctanesulfonate at concentrations  $\leq$  0.25 mg/L for 35 days. Reproduction, length and dry weight were the most sensitive biological endpoints in this study. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

ENV/JM/RD(2002)17

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

**DATA QUALITY**

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**Reliability:** Klimisch ranking = 1

**REFERENCES**

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

**OTHER**

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**Last changed:** 7/9/01

**Robust Study Report Reference No. 11 – Dietary LC<sub>50</sub> Study with the Mallard duck****TEST SUBSTANCE**

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

**METHOD**

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**Method:** OPPTS 850.2200, OECD 206, and FIFRA Subdivision E. Section 71-2

**Type:** Dietary acute

**GLP:** Yes

**Year completed:** 2000

**Species:** *Anas platyrhynchos*

**Supplier:** Whistling Wings, Inc., Hanover, IL, USA

**Analytical monitoring:** PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.

**Test phases:** Acclimation - 9 days  
Exposure - 5 days  
Post-exposure observation - 3 or 17 days

**Statistical methods:** LC<sub>50</sub> values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.

**Test bird age:** 10 days

**Pretreatment:** None

**Test conditions:**

**Housing and environmental conditions:** Indoors In batteries of thermostatically controlled brooding pens. Floor space of each pen measured approximately 62 x 90 cm. Ceiling height was approximately 25.5 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

**Identification:** Each group of birds Identified by pen number and test concentration. Individuals identified by wing bands.

**Number of replicates:** Six for controls, two for each treatment group

**Number of ducks per replicate:** five

**Number of concentrations:** Eight plus a negative control

**Feed and water:** Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

**Game Bird Ration**

| Ingredients                | Percent |
|----------------------------|---------|
| Fine Corn Meal             | 44.83   |
| Soy Bean Meal, 48% Protein | 30.65   |
| Wheat Midds                | 6.50    |
| Protein Base               | 6.00    |

|  |      |
|--|------|
| Agway Special, 60% Protein             | 4.00 |
| Alfalfa Meal, 20% Protein              | 3.00 |
| Dried Whey                             | 2.50 |
| Ground Limestone                       | 0.90 |
| Eastman CalPhos                        | 0.60 |
| Methionine Premix + Liquid             | 0.35 |
| Vitamin and Mineral Premix (see below) | 0.32 |
| GL Form (Fermatco) <sup>1</sup>        | 0.25 |
| Salt iodized                           | 0.10 |

<sup>1</sup>Fermentation by-products (source of unidentified growth factors)

### Vitamin and Mineral Premix

| Vitamin or Mineral                                  | Amount Per Ton   |
|---|------------------|
| Vitamin D <sub>3</sub>                              | 2,000,000 I.C.U. |
| Vitamin A   | 7,000,000 I.U.   |
| Riboflavin  | 6 g              |
| Niacin  | 40 g             |
| Pantothenic Acid                                    | 10 g             |
| Vitamin B <sub>12</sub>                             | 8 mg             |
| Folic Acid  | 600 mg           |
| Biotin  | 64 mg            |
| Pyridoxine  | 1.2 g            |
| Thiamine  | 1.2 g            |
| Vitamin E   | 20,000 I.U.      |
| Vitamin K (Menadione dimethylpyrimidinol bisulfite) | 5.8 g            |
| Manganese   | 102 g            |
| Zinc  | 47 g             |
| Copper  | 6.8 g            |
| Iodine  | 1.5 g            |
| Iron  | 51 g             |
| Selenium  | 182 g            |

**Prophylaxis:** None

**Brooding compartment mean temperature:** 38 ± 2°C

**Ambient room mean temperature:** 25.2 ± 0.7°C

**Average relative humidity:** 53 ± 18%

**Photoperiod:** Sixteen hours light per day

**Lighting:** fluorescent lights which closely approximate noon-day sunlight; average approximately 207 lux.

**Test diet preparation:** Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

**Diet sampling:** Homogeneity of the test substance in the diet evaluated by collecting six samples from the 9.1 ppm and six from the lowest and highest concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification



samples for these concentrations. Two verification samples from the remaining concentrations and one from the control were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

## RESULTS

**Nominal concentrations:** Negative control, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm

**Measured concentrations:** <LOQ, 9.8, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm

**Element value:** Dietary LC<sub>50</sub> = 628 (448 - 958) ppm

No mortality concentration 146 ppm

NOEC (body weight gain) 36.6 ppm

All element values based on nominal concentrations

**Analytical Methodology:** Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102 - 108% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 94 - 130% of nominal.

### Summary of analytical chemistry data

#### Homogeneity in Avian diet

| Nominal Test Concentration, ppm | Measured Values at Day 0, ppm      | Mean Measured Concentration, ppm | Percent of Nominal |
|---------------------------------|------------------------------------|----------------------------------|--------------------|
| 9.1                             | 9.52, 9.70, 9.79, 8.09, 10.9, 10.5 | 9.8                              | 108                |
| 18.3                            | 18.5, 23.4, 18.3, 17.3, 19.4, 19.9 | 19.6                             | 107                |
| 1171                            | 1239, 1221, 1118, 1301, 1163, 1133 | 1196                             | 102                |

#### Verification in avian diet

| Nominal Test Concentration, ppm | Measured Duplicate Concentrations at Day 0, ppm | Mean Measured Concentration, ppm | Percent of Nominal |
|---------------------------------|---|----------------------------------|--------------------|
| Negative Control                | < LOQ   | -                                | -                  |
| 36.6                            | 46.7, 34.6                                      | 40.2                             | 110                |
| 73.2                            | 77.8, 71.2                                      | 74.5                             | 102                |
| 146                             | 176, 172  | 174                              | 119                |
| 293-                            | 274, 307  | 291                              | 99                 |
| 586                             | 550, 523  | 537                              | 92                 |

#### Ambient stability in avian diet

| Day 0 Mean Measured Concentration, ppm | Measured Duplicate Concentrations at Day 5, ppm | Mean Measured Concentration, ppm | Mean Percent of Day 0 |
|--|---|----------------------------------|-----------------------|
| Negative Control                       | < LOQ   | -                                |                       |

|      |            |      |      |
|------|------------|------|------|
| 9.8  | 12.3, 11.0 | 11.7 | 119  |
| 19.5 | 18.2, 19.7 | 19.0 | 97   |
| 40.2 | 47.9, 56.8 | 52.4 | 130  |
| 74.5 | 77.6, 77.9 | 77.8 | 104  |
| 174  | 167, 160   | 164  | 94.3 |
| 291  | 297, 293   | 295  | 101  |
| 537  | 552, 530   | 541  | 101  |
| 1196 | 1150, 1122 | 1136 | 95   |

### Biological observations

**Survival and clinical observations:** No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. The first deaths occurred on day 4 in the 1171 ppm treatment. Mortality occurred through Day 8 in all dose groups  $\geq 293$  ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations  $\leq 146$  ppm. Birds at all concentrations  $\geq 293$  ppm displayed signs of toxicity including reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy and lower limb weakness. Birds at the 1171 ppm level also displayed prostrate posture, depression and convulsions through Day 8. Recovery with normal appearance and behavior was noted from Day 9 through test termination

**Body weight gain:** When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations  $\leq 36.6$  ppm. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups. There was a statistically significant ( $p < 0.05$ ) reduction in weight gain at the 9.1 ppm level for the Day 0-5 and Day 5-8 periods. However, differences from the control group at the 9.1 ppm level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm level, and were not dose responsive. Therefore, these differences were not considered treatment related. Marked, treatment-related, concentration responsive effects on body weight was noted in concentrations  $> 73.2$  ppm for Days 0-5; Day 5-8 post-exposure weight gain continued to be reduced at concentrations  $\geq 293$  ppm.

**Feed Consumption:** When compared to the control group, there was a marked reduction in feed consumption in the treatment groups  $\geq 293$  ppm throughout the study.

**Gross Necropsy:** All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, empty crops, and empty gastrointestinal tracts. These necropsy findings were considered to be treatment related.

### Percent Cumulative Mortality

| Nominal Concentration, ppm | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8* |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|--------|
| Negative Control           | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 9.1                        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 18.3                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 36.6                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 73.2                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 146                        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 293                        | 0     | 0     | 0     | 0     | 0     | 0     | 10    | 20     |
| 586                        | 0     | 0     | 0     | 0     | 10    | 20    | 30    | 30     |
| 1171                       | 0     | 0     | 0     | 20    | 60    | 80    | 90    | 90     |

\*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

**Bodyweight (grams)**

| Nominal Concentration, ppm | Exposure Period         |                                 | Recovery Period                 |                                  |                                   |  |                         |
|----------------------------|-------------------------|---------------------------------|---------------------------------|----------------------------------|-----------------------------------|--|-------------------------|
|                            | Mean Body Weight, Day 0 | Mean Body Weight Change Day 0-5 | Mean Body Weight Change Day 5-8 | Mean Body Weight Change Day 8-15 | Mean Body Weight Change Day 15-22 | Mean Total Body Weight Change Day 8-22 | Mean Body Weight Day 22 |
| Negative Control           | 135                     | 144                             | 101                             | 230                              | 183                               | 413                                    | 823                     |
| 9.1                        | 119                     | 108                             | 91                              | 230                              | 198                               | 427                                    | 773                     |
| 18.3                       | 146                     | 131                             | 100                             | 241                              | 186                               | 427                                    | 811                     |
| 36.6                       | 147                     | 128                             | 100                             | 243                              | 208                               | 451                                    | 828                     |
| 73.2                       | 143                     | 117                             | 82                              | 216                              | 203                               | 418                                    | 782                     |
| 146                        | 143                     | 100                             | 89                              | 232                              | 124                               | 356                                    | 688                     |
| 293                        | 129                     | 32                              | 57                              | 256                              | 234                               | 490                                    | 701                     |
| 586                        | 144                     | -6                              | 36                              | 221                              | 219                               | 439                                    | 613                     |
| 1171                       | 147                     | -37                             | 1531                            | 198                              | 251                               | 449                                    | 634                     |

**Mean average feed consumption**

| Nominal Concentration, ppm | Grams food/bird/day Days 0-5 | Grams food/bird/day Days 6-8 | Grams food/bird/day Days 8-15 | Grams food/bird/day Days 15-22 |
|----------------------------|------------------------------|------------------------------|-------------------------------|--------------------------------|
| Negative Control           | 92                           | 125                          | 171                           | 180                            |
| 9.1                        | 73                           | 117                          | 172                           | 198                            |
| 18.3                       | 91                           | 132                          | 186                           | 204                            |
| 36.6                       | 94                           | 125                          | 165                           | 179                            |
| 73.2                       | 77                           | 101                          | 148                           | 173                            |
| 146                        | 105                          | 159                          | 159                           | 164                            |
| 293                        | 44                           | 63                           | 109                           | 132                            |
| 586                        | 36                           | 55                           | 114                           | 143                            |
| 1171                       | 22                           | 25                           | 106                           | 154                            |

**Gross pathological observations from Birds that died in study**

| Finding                                 | 293ppm N = 2 | 586ppm N = 3 | 1171ppm N = 9 |
|---|--------------|--------------|---------------|
| Crop empty                              | 0            | 2            | 7             |
| Emaciated                               | 1            | 1            | 4             |
| G.I.Tract, primarily empty              | 1            | 1            | 1             |
| Gizzard contents bile stained           | 0            | 2            | 4             |
| Gizzard, empty                          | 0            | 0            | 1             |
| Intestinal contents, black and tar-like | 0            | 0            | 1             |
| Keel prominent                          | 0            | 0            | 2             |
| Kidneys, pale                           | 0            | 0            | 1             |
| Loss of muscle mass                     | 2            | 2            | 5             |
| Spleen, grey                            | 0            | 0            | 1             |
| Spleen, small and pale                  | 1            | 1            | 2             |
| Spleen, pale                            | 0            | 2            | 3             |
| Thin                                    | 1            | 1            | 4             |

## CONCLUSIONS

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The dietary LC<sub>50</sub> value for Mallard Duck exposed to PFOS was determined to be 628 ppm with a 95% confidence interval of 448 to 958 ppm. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm. Based upon reductions in body weight gain at the 73.2 ppm test concentration, the no observed effect concentration was 36.6 ppm.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

## DATA QUALITY

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**Reliability:** Klimisch ranking 1

## REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

## OTHER

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**Last changed:** 5/1/00

**Robust Study Report Reference No. 12 – Dietary LC<sub>50</sub> Study with the Northern Bobwhite quail****TEST SUBSTANCE**

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks field:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

**METHOD**

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**Method:** OPPTS 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2

**Type:** Dietary acute

**GLP:** Yes

**Year completed:** 1999 (Test), 2000 (Report)

**Species:** *Colinus virginianus*

**Supplier:** Wildlife International Ltd. Production Flock, Easton, Maryland, USA

**Analytical monitoring:** Test substance concentration in standards and samples were determined by reversed-phase HPLC and mass spectroscopy. PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.

**Test phases:** Acclimation – 10 days  
Exposure – 5 days  
Post-exposure observation – 3 or 17 days

**Statistical methods:** LC<sub>50</sub> values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.

**Test bird age:** 10 days

**Pretreatment:** None

**Test conditions:**

**Housing and environmental conditions:** Indoors in batteries of thermostatically controlled brooding pens. Each pen's floor space measured approximately 72 x 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

**Identification:** Each group of birds Identified by pen number and test concentration. Individuals Identified by leg bands.

**Number of replicates:** Six for controls, two for each treatment group

**Number of bobwhite per replicate:** five

**Number of concentrations:** seven plus a negative control

**Feed and water:** Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

**Game Bird Ration**

| Ingredients                | Percent |
|----------------------------|---------|
| Fine Corn Meal             | 44.83   |
| Soy Bean Meal, 48% Protein | 30.65   |
| Wheat Midds                | 6.50    |

|  |      |
|--|------|
| Protein Base                           | 6.00 |
| Agway Special, 60% Protein             | 4.00 |
| Alfalfa Meal, 20% Protein              | 3.00 |
| Dried Whey                             | 2.50 |
| Ground Limestone                       | 0.90 |
| Eastman CalPhos                        | 0.60 |
| Methionine Premix + Liquid             | 0.35 |
| Vitamin and Mineral Premix (see below) | 0.32 |
| GL Form (Fermatco) <sup>1</sup>        | 0.25 |
| Salt iodized                           | 0.10 |

<sup>1</sup>Fermentation by-products (source of unidentified growth factors)

### Vitamin and Mineral Premix

| Vitamin or Mineral                                  | Amount Per Ton   |
|---|------------------|
| Vitamin D <sub>3</sub>                              | 2,000,000 I.C.U. |
| Vitamin A   | 7,000,000 I.U.   |
| Riboflavin  | 6 g              |
| Niacin  | 40 g             |
| Pantothenic Acid                                    | 10 g             |
| Vitamin B <sub>12</sub>                             | 8 mg             |
| Folic Acid  | 600 mg           |
| Biotin  | 64 mg            |
| Pyridoxine  | 1.2 g            |
| Thiamine  | 1.2 g            |
| Vitamin E   | 20,000 I.U.      |
| Vitamin K (Menadione dimethylpyrimidinol bisulfite) | 5.8 g            |
| Manganese   | 102 g            |
| Zinc  | 47 g             |
| Copper  | 6.8 g            |
| Iodine  | 1.5 g            |
| Iron  | 51 g             |
| Selenium  | 182 g            |

**Prophylaxis:** None

**Brooding compartment mean temperature:**  $38 \pm 2^{\circ}\text{C}$

**Ambient room mean temperature:**  $27.3 \pm 1.2^{\circ}\text{C}$ -

**Average relative humidity:**  $31 \pm 14\%$

**Photoperiod:** Sixteen hours light per day

**Lighting:** fluorescent lights which closely approximate noon-day sunlight; average of approximately 139 lux of illumination

**Test diet preparation:** Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

**Diet sampling:** Homogeneity of the test substance in the diet evaluated by collecting six samples from the, 18.3 ppm concentration and six from the 1171 ppm concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the

verification samples for these concentrations. Verification samples of the other treatment groups (two samples from each) and the control (one sample) were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

## RESULTS

**Nominal concentrations:** Negative control, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm

**Measured concentrations:** <LOQ, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm

**Element value:** Dietary LC<sub>50</sub> = 220 (164 - 289) ppm  
No mortality concentration = 73.2 ppm  
NOEC (body weight gain) = 73.2 ppm

All element values based on nominal concentrations

**Analytical Methodology:** Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102-107% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 101 – 122% of nominal.

### Summary of analytical chemistry data

#### Homogeneity in avian diet

| Nominal Test Concentration, ppm | Measured Values at Day 0, ppm      | Mean Measured Concentration, ppm | Percent of Nominal |
|---------------------------------|------------------------------------|----------------------------------|--------------------|
| 18.3                            | 18.5, 23.4, 18.3, 17.3, 19.4, 19.9 | 19.5                             | 107                |
| 1171                            | 1239, 1221, 1118, 1301, 1163, 1133 | 1196                             | 102                |

#### Verification in avian diet

| Nominal Test Concentration, ppm | Measured Duplicate Concentrations at Day 0, ppm | Mean Measured Concentration, ppm | Percent of Nominal |
|---------------------------------|---|----------------------------------|--------------------|
| Negative Control                | < LOQ   | -                                | -                  |
| 36.6                            | 46.7, 34.6                                      | 40.2                             | 110                |
| 73.2                            | 77.8, 71.2                                      | 74.5                             | 102                |
| 146                             | 176, 172  | 174                              | 119                |
| 293                             | 274, 307  | 291                              | 99                 |
| 586                             | 550, 523  | 537                              | 92                 |

**Ambient stability in avian diet**

| Day 0 Mean Measured Concentration, ppm | Measured Duplicate Concentrations at Day 5, ppm | Mean Measured Concentration, ppm | Mean Percent of Day 0 |
|--|---|----------------------------------|-----------------------|
| Negative Control                       | < LOQ   | -                                |                       |
| 19.5                                   | 19.2, 19.9                                      | 19.6                             | 101                   |
| 40.2                                   | 44.4, 53.8                                      | 49.1                             | 122                   |
| 74.5                                   | 76.4, 77.9                                      | 77.2                             | 104                   |
| 174                                    | 177, 174  | 176                              | 101                   |
| 291                                    | 318, 315  | 317                              | 109                   |
| 537                                    | 560, 665  | 613                              | 114                   |
| 1196                                   | 1260, 1187                                      | 1224                             | 102                   |

**Biological observations**

**Mortalities and clinical observations:** One incidental mortality occurred in the control group as a result of a broken leg on the morning of Day 5. It was subsequently euthanized on Day 6. Two other birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were observed to be normal in appearance and behavior throughout the test.

The first treatment-related mortalities occurred on Day 3 in the 586 and 1171 ppm treatment groups. Mortality occurred through Day 8 in all dose groups  $\geq 146$  ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations  $\leq 73.2$  ppm.

There was 11 % mortality in the 146 ppm treatment group, and two additional birds displayed clinical signs of toxicity (wing droop). All other birds in this test group displayed normal appearance and behavior for the duration of the test. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 80% mortality (occurring on Days 5, 6, and 7) for birds in the 293 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 100% mortality (occurring from Day 3 through Day 7) for birds in the 586 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

There was 100% mortality (occurring from Day 2 (noted on Day 3 for Day 2 afternoon) through Day 4) for birds in the 1171 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, and lower limb rigidity.

**Body weight gain:** When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations  $\leq 73.2$  ppm. During Days 0-5 statistically significant reductions in body weight gain or body weight loss occurred in the 146, 293, and 586 ppm treatment groups. Body weight effects could not be determined for test



organisms in the 1171 ppm group due to total mortality.

**Feed Consumption:** No apparent treatment related effects were noted for feed consumption for birds in concentrations  $\leq 146$  ppm. Reduced feed consumption was noted for birds in treatment groups  $\geq 293$  ppm from Days 0-5. No treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period were observed.

**Gross Necropsy:** All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. These necropsy findings were considered to be treatment related. The single bird euthanized from the 293 ppm treatment was found to have treatment related necropsy findings. Necropsy results for all other birds euthanized on Day 8 and Day 22 were unremarkable.

### % Cumulative Mortality

| Nominal Concentration, ppm | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8* |
|----------------------------|-------|-------|-------|-------|-------|-------|-------|--------|
| Negative Control           | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 18.3                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 36.6                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 73.2                       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |
| 146                        | 0     | 0     | 10**  | 0     | 0     | 0     | 10    | 10     |
| 293                        | 0     | 0     | 0     | 0     | 20    | 40    | 80    | 80     |
| 586                        | 0     | 0     | 10    | 20    | 50    | 80    | 100   | 100    |
| 1171                       | 0     | 0     | 30    | 100   | 100   | 100   | 100   | 100    |

\*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

\*\*Bird euthanized on day 3 after sustaining a broken leg.

### Body Weight (grams)

| Nominal Concentration (ppm) | Exposure Period         |                                 | Recovery Period                 |                                  |                                   |   |                          |
|-----------------------------|-------------------------|---------------------------------|---------------------------------|----------------------------------|-----------------------------------|---|--------------------------|
|                             | Mean Body Weight, Day 0 | Mean Body Weight Change Day 0-5 | Mean Body Weight Change Day 5-8 | Mean Body Weight Change Day 8-15 | Mean Body Weight Change Day 15-22 | Mean Total Body Weight Change, Day 8-22 | Mean Body Weight, Day 22 |
| Negative Control            | 20                      | +10                             | +8                              | +23                              | +22                               | 45                                      | 82                       |
| 18.3                        | 21                      | +11                             | +9                              | +24                              | +23                               | 47                                      | 87                       |
| 36.6                        | 20                      | +11                             | +8                              | +26                              | +24                               | 50                                      | 89                       |
| 73.2                        | 20                      | +9                              | +7                              | +24                              | +20                               | 44                                      | 79                       |
| 146                         | 20                      | +7*                             | +6**                            | +24                              | +21                               | 45                                      | 79                       |
| 293                         | 20                      | -2**                            | -1**                            | +14                              | +20                               | 34                                      | 55                       |
| 586                         | 20                      | -4**                            | -                               | -                                | -                                 | --                                      | -                        |
| 1171                        | 20                      | -                               | -                               | -                                | -                                 | -                                       | -                        |

Note: numbers may not add manually due to rounding. Values for 293 ppm treatment group are impacted by the fact that only one bird remained in that group after day 8.

\*Statistically different from the control group at  $p < 0.05$  (Dunnett's t-test)

\*\*Statistically different from the control group at  $p < 0.01$  (Dunnett's t-test)

(-) = no data available due to mortality

### Mean average feed consumption

| Nominal Concentration, ppm | Grams Feed/Bird/Day Days 0-5 | Grams Feed/Bird/Day Days 6-8 | Grams Feed/Bird/Day Days 8-15 | Grams Feed/Bird/Day Days 15-22 |
|----------------------------|------------------------------|------------------------------|-------------------------------|--------------------------------|
| Negative Control           | 9                            | 10                           | 9                             | 13                             |
| 18.3                       | 9                            | 11                           | 10                            | 12                             |
| 36.6                       | 8                            | 12                           | 14                            | 15                             |
| 73.2                       | 10                           | 13                           | 13                            | 15                             |
| 146                        | 9                            | 10                           | 11                            | 14                             |
| 293                        | 5                            | 9                            | 8                             | -                              |
| 586                        | 6                            | 19                           | -                             | -                              |
| 1171                       | 4                            | -                            | -                             | -                              |

(-) = No data available due to mortality.

### Gross Pathological Observations from Birds that Died In Study

| Finding                                     | Male, Female, and Undetermined (ppm) |              |              |               |                |
|---|--------------------------------------|--------------|--------------|---------------|----------------|
|   | Control<br>N = 1                     | 146<br>N = 2 | 293<br>N = 8 | 586<br>N = 10 | 1171<br>N = 10 |
| Abdominal cavity, some autolysis            | 0                                    | 0            | 2            | 2             | 4              |
| Abdominal cavity, autolysis throughout      | 0                                    | 0            | 0            | 1             | 1              |
| Crop, empty                                 | 0                                    | 0            | 2            | 5             | 2              |
| Emaciated                                   | 0                                    | 0            | 2            | 5             | 8              |
| Fractured leg                               | 1                                    | 1            | 0            | 0             | 0              |
| G.I. Tract, empty                           | 0                                    | 0            | 1            | 1             | 0              |
| Gizzard contents bile stained               | 0                                    | 0            | 2            | 5             | 1              |
| Heart, anterior portion mottled white color | 0                                    | 0            | 1            | 0             | 0              |
| Heart, pale .                               | 0                                    | 0            | 0            | 2             | 1              |
| Intestinal contents tar-like                | 0                                    | 0            | 0            | 2             | 0              |
| Keel, prominent                             | 0                                    | 0            | 1            | 3             | 10             |
| Kidneys, pale                               | 0                                    | 0            | 0            | 2             | 0              |
| Liver, pale and mottled                     | 0                                    | 1            | 0            | 0             | 0              |
| Loss of muscle mass                         | 0                                    | 0            | 4            | 7             | 9              |
| Muscular-skeletal, pale                     | 0                                    | 1            | 0            | 0             | 0              |
| Small in stature                            | 0                                    | 0            | 3            | 0             | 0              |
| Spleen, black                               | 0                                    | 0            | 0            | 1             | 0              |
| Spleen, dark                                | 0                                    | 0            | 0            | 0             | 2              |
| Spleen, grey                                | 0                                    | 0            | 0            | 1             | 0              |
| Spleen, grey-brown                          | 0                                    | 0            | 0            | 0             | 1              |
| Spleen, pale                                | 0                                    | 0            | 1            | 0             | 1              |
| Spleen, small                               | 0                                    | 0            | 0            | 0             | 1              |
| Spleen, small and pale                      | 0                                    | 0            | 0            | 3             | 0              |
| Thin  | 0                                    | 0            | 0            | 4             | 2              |
| Not remarkable                              | 0                                    | 0            | 1            | 0             | 0              |

## CONCLUSIONS

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The dietary LC<sub>50</sub> value for Northern Bobwhite exposed to perfluorooctanesulfonate was determined to be 220 ppm with a 95% confidence Interval of 164 to 289 ppm. The slope of the concentration response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm test concentration, the no observed effect concentration was 73.2 ppm.

**Author and/or submitter:** 3M Corporation, Environmental Laboratory, P.O. Box 33331, St.-Paul, Minnesota, 55133, USA

## DATA QUALITY

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**Reliability:** Klimisch ranking = 1.

## REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M company.

## OTHER

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**Last changed:** 5/3/00

**Robust Report Reference No. 13 - Multi-Phase Exposure / Recovery Algal Assay Test****TEST SUBSTANCE**

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks field:** The test substance is a white powder (3M Lot 583) of uncharacterized purity. The following summary is abbreviated due to the fact that this study has been superseded by a more recent test.

**METHOD**

---

**Method:** Test protocol utilized was modified after those described by USEPA - 600/9-78-018, 1978; ASTM-E-35.23, 1981; OECD 201,1979. ASTM STP #667

**Type:** Semi-chronic

**GLP:** No

**Year completed:** 1981

**Species:** *Selenastrum capricornutum*

**RESULTS**

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**EC<sub>50</sub> Values, mg/L (95% confidence interval is given in brackets)**

| Exposure (contact) Days | Cell-Dry Weight | Cell-Count           |
|-------------------------|-----------------|----------------------|
| 4                       | 115 (18 - 65)   | 82 (No Conf. Limits) |
| 7                       | 122 (41- 366)   | 99 (19 - 398)        |
| 10                      | 128 (46 - 372)  | 98 (16 - 431)        |
| 14                      | 146 (33 - 350)  | 95 (12 - 455)        |

**Remarks:** The statistical program used was questionable.

**DATA QUALITY**

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**Reliability:** Klimisch ranking = 2. This study satisfied criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not properly characterized.

**REFERENCES**

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This study was conducted by the 3M Company, Environmental Laboratory, 1981.

**OTHER**

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

**Last changed:** 5/3/00

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**Robust Study Report Reference No. 14 - The Effects of Continuous Aqueous Exposure to 14C-78.02**

**on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (*Pimephales promelas* / Summary of histopathological examinations of Fathead Minnow (*Pimephales promelas*) exposed to 78.02 for 30 Days**

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TEST SUBSTANCE

---

**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as 14C-78.02, PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was radiolabeled. Sample purity was not characterized.

The following summary is abbreviated due to the fact that this study has been superceded by a more recent test.

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METHOD

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**Method:** Method was developed by E G & G, Bionomic and closely followed those presented in the "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish", U.S. EPA, 1972.

**Type:** Flow-through chronic

**GLP:** No

**Year completed:** 1978

**Species:** *Pimephales promelas*

---

RESULTS

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30-Day NOEC: 1 mg/L

30-Day LOEC: 1.9 mg/L

30-Day MATC: >1 mg/L and <1.9 mg/L

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DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied criteria for quality testing at the time performed, but the analytical methodology was questionable.

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REFERENCES

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This study was conducted at E G & G, Bionomics, Aquatic Toxicology Laboratory in Wareham, Massachusetts at the request of the 3M Company.

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OTHER

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**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

**Last changed:** 5/3/00

**Robust Study Report Reference No. 15 - Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)**

TEST SUBSTANCE

---

**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks field:** The test substance is a white powder of uncharacterized purity.

The following summary is abbreviated due to the fact that this study has been superceded by a more recent test.

METHOD

---

**Methods:** ASTM, 1981, Proposed Standard Practice for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*; OECD, 1981, *Daphnia* sp., 14-day Reproduction Test.

**Type:** Acute static and Chronic renewal

**GLP:** No

**Year completed:** 1984

**Species:** *Daphnia magna*

RESULTS (95% confidence interval is given in brackets)

---

48-hour EC<sub>50</sub> (immobilization): 27 (25-28) mg/L  
14-day EC<sub>50</sub> (reproduction)\*: 14.7(12-18) mg/L  
21-day EC<sub>50</sub> (reproduction)\*: 12.4(11-14) mg/L  
28-day EC<sub>50</sub> (reproduction)\*: 11.4(10-13) mg/L  
28-day NOEC\*\*:  
14, 21 and 28-day MATC: 7 mg/L  
11.2 mg/L

\*Cumulative (Young/Adult)

\*\*This concentration applies to no observed effects on Cumulative results of Young/Adult and Broods/Adult and on the total number of Young/Adult.

DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

REFERENCES

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This study was conducted by the 3M Company, Environmental Laboratory, 1984.

OTHER

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

**Last changed:** 5/3/00

**Robust Study Report Reference No. 16 - *Pimephales promelas* 96-hour Toxicity Test Data Summary. Sample FC-94-X (Li salt of PFOS)**

TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate, Lithium salt; may also be referred to as PFOS Li salt, FC-94, or FC-94-X. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, lithium salt, CAS # 29457-72-5)

**Remarks:** Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

---

**Method:** Not noted.

**Type:** Static acute

**GLP:** No

**Year completed:** 1994

**Species:** *Pimephales promelas*

**Supplier:** Aquatic Biosystems Inc., Fort Collins, CO, USA

**Analytical monitoring:** pH and DO content

**Exposure period:** 96-hours

**Statistical methods:** LC<sub>50</sub> values calculated by Trimmed Spearman - Karber.

**Test fish age:** 79 days.

**Length and weight:** Average length = 2.1 + 0.3cm Average weight = 0.069 ± 0.03 g

**Loading:** 0.69 g fish / L

**Pretreatment:** None

**Test Conditions**

**Dilution water:** Carbon filtered well water

**Dilution water chemistry:**

**pH:** 8.4

**DO:** 8.1 mg/L

**Stock and test solution preparation:** A primary stock solution was prepared in dilution water to yield a test sample concentration of 400 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water to make 1 L of solution per concentration.

**Stability of the test chemical solutions:** Not noted.

**Exposure vessels:** 2 L glass beakers

**Number of replicates:** two.

**Number of fish per replicate:** ten

**Number of concentrations:** six plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

6.0-7.2 mg/L (control exposure)

4.8-7.9 mg/L (56.0 mg/L exposure)

**pH range (0 – 96 hours):**

8.0-8.4 (control exposure)

8.0-8.4 (56.0 mg/L exposure)

**Test temperature range (0 – 96 hours):**

19.2-19.5°C

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## RESULTS

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**Nominal concentrations:** Negative control, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0 mg/L

**Element value** (95% confidence interval is given in brackets):

96-hour LC<sub>50</sub> = 19 mg/L (16-24)

**Mortality of controls:** None

**Remarks:** Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

## CONCLUSIONS

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The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 96-hour LC<sub>50</sub> for fathead minnow of 19 mg/L. This value must be divided by 4 in order to express the result in terms of the concentration of PFOS.

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

## DATA QUALITY

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**Reliability:** Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity.

## REFERENCES

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This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 3/25/94.

## OTHER

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**Last changed:** 7/9/01



**Robust Study Report Reference No. 17 - 48-hour Acute Toxicity to Daphnia, *Daphnia magna*. FC-94-X (Li salt of PFOS)****TEST SUBSTANCE**

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**Identity:** Perfluorooctanesulfonate, Lithium salt; may also be referred to as PFOS Li salt, FC-94, or FC-94-X. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, lithium salt, CAS # 29457-72-5)

**Remarks:** The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

**METHOD**

---

**Method:** Not noted.

**Test type:** Static acute

**GLP:** No

**Year completed:** 1994

**Species:** *Daphnia magna*

**Analytical monitoring:** pH and DO content

**Statistical methods:** EC<sub>50</sub> values calculated using Trimmed Spearman-Kärber method

**Test daphnid source:** Obtained from U.S. EPA-NETAC, Duluth, Minnesota, USA.

**Test daphnid age at study initiation:** < 24-hours

**Test Conditions**

**Dilution water:** Carbon-filtered well water

**Dilution water chemistry:**

**pH:** 8.4

**DO:** 8.6 mg/L

**Stock and test solutions preparation:** A primary stock solution was prepared in dilution water to yield a test sample concentration of 1000 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water to make 50 mL of solution per concentration.

**Exposure vessels:** 100 mL glass beakers containing 50 mL of test solution.

**Number of replicates:** 4

**Number of daphnids per replicate:** 5

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen at test termination:**

7.0 mg/L (control exposure)

7.8 mg/L (1000 mg/L exposure)

**pH at test termination:**

8.6 (control exposure)

8.6 (1000 mg/L exposure)

**Test temperature range (0 – 48 hours)** 20.1-21.0 C

**Element basis:** mortality and immobilization

**RESULTS**

---

**Nominal concentrations:** Negative control, 100, 180, 320, 560, 1000 mg/L

**Element value:** (95% confidence interval is given in brackets)

24-hour EC<sub>50</sub> = 330 (290-370) mg/L

48-hour EC<sub>50</sub> = 210 (190-230) mg/L

48-hour NOEC = 100 mg/L

**Statistical Evaluation:** The EC<sub>50</sub> values and 95% confidence intervals were calculated using the Trimmed Spearman-Kärber method with trim set to 0%.

**Mortality of controls:** None

**Remarks:** Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

## CONCLUSIONS

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The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 48-hour EC<sub>50</sub> for *Daphnia magna* of 210 mg/L. This value must be divided by 4 in order to express the result in terms of the concentration of PFOS.

Submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

## DATA QUALITY

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**Reliability:** Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity.

## REFERENCES

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This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 2/10/94.

## OTHER

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**Last changed:** 7/9/01

**Robust Study Report Reference No. 20 - 96-hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)**

TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate, DEA salt; may also be referred to as PFOS DEA salt, FC-99, or 3M Sample No. 2. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1), CAS # 70225-14-8)

**Remarks:** Test sample is a mixture of the test substance in water (approximately 25% test substance and 75% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

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**Method:** Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. Standard Methods.

**Type:** Static acute

**GLP:** Yes

**Year completed:** 1979

**Species:** *Lepomis macrochirus*

**Supplier:** Osage Catfisheries, Inc. in Osage Beach, Missouri, USA.

**Analytical monitoring:** pH and DO / ammonia content

**Exposure period:** 96-hours

**Statistical methods:** Probit analysis.

**Test fish age:** Not noted.

**Length and weight:** Average length =  $28.6 \pm 2.17$  mm.  
Average weight =  $0.60 \pm 0.15$  g

**Loading:** 0.2 g fish / L

**Pretreatment:** None

Test Conditions

**Dilution water:** Laboratory well water

**Dilution water chemistry:**

**Dissolved oxygen:** 9.3 mg/L

**Hardness:** 255 mg/L as CaCO<sub>3</sub>

**Alkalinity:** 368 mg/L as CaCO<sub>3</sub>

**pH:** 7.8

**Conductivity:** 50 µmhos/cm

**Stock and test solution preparation:** Primary stock prepared in deionized water at a concentration of 150 mg/mL. The test concentrations were prepared by transferring appropriate aliquots of the stock standard directly to the test chambers. The test solutions were noted to foam when stirring in toxicant aliquots. Test concentrations were prepared based on total sample, not on percent concentration of the test substance in the test sample.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Not noted

**Exposure vessels:** 40 liter glass aquaria containing 30L of test solution.

**Number of replicates:** one

**Number of fish per replicate:** ten

**Number of concentrations:** six plus a negative control

**Water chemistry during the study:****Dissolved oxygen range (0 – 96 hours):**

6.0 - 8.4 mg/L (control exposure)

5.8 - 8.3 mg/L (18 mg/L exposure)

**pH range (0 – 96 hours):**

8.2 - 8.3 (control exposure)

8.3 - 8.3 (18 mg/L exposure)

**Test temperature:** Temperature held constant at 22°C through use of a water bath for test vessels.**RESULTS**

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**Nominal concentrations:** Negative control, 18, 37, 75, 160, 320, 650 mg/L**Element value:** (95% confidence interval is given in brackets)24-hour LC<sub>50</sub> = 460 (370-580) mg/L48-hour LC<sub>50</sub> = 370 (290-470) mg/L96-hour LC<sub>50</sub> = 31 (22-43) mg/L

96-hour NOEC = 18 mg/L (C.I. not calculated)

All element values based on nominal concentrations

**Statistical evaluation of mortality:**Probit analysis was used to calculate LC<sub>50</sub> values and the corresponding confidence limits.**Quality Check for Test Organism Health:**The bluegill sunfish were challenged with a reference compound, Antimycin A. The observed 96-hour LC<sub>50</sub> and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition.**Cumulative percent mortality:**

| Nominal Test Concentration mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|---------------------------------|----------|----------|----------|----------|
| Neg. Control                    | 0        | 0        | 0        | 0        |
| 18                              | 0        | 0        | 0        | 0        |
| 37                              | 0        | 0        | 10       | 80       |
| 75                              | 0        | 0        | 30       | 90       |
| 160                             | 0        | 0        | 70       | 100      |
| 320                             | 0        | 20       | 100      | 100      |
| 650                             | 100      | 100      | 100      | 100      |

**CONCLUSIONS**

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The test sample 96-hour LC<sub>50</sub> for bluegill sunfish was determined to be 31 mg/L with a 95% confidence interval of 22-43 mg/L. The 96-hour no observed effect concentration was 18 mg/L. These values must be divided by 4 in order to express the results in terms of the concentration of PFOS.**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.**DATA QUALITY**

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**Reliability:** Klimisch ranking 2. This study, while well conducted, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. There were

also gaps in the measurement of water quality criteria for a number of the concentrations at given time intervals.

REFERENCES

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This study was conducted by Analytical BioChemistry Laboratories, Inc. of Columbia, Missouri on behalf of the 3M Company.

OTHER

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**Last changed:** 5/2/00

**Robust Study Report Reference No. 23 – Acute toxicity to Aquatic Invertebrates (e.g. Daphnia)**

TEST SUBSTANCE

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**Identity:** Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95 or as part of the mixed product FM-3820 (see Remarks). (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test sample is FM-3820, a mixture of the test substance in diethylene glycol butyl ether and water (approximately 24-28% test substance in diethylene glycol butyl ether and water). Calculations were made to adjust test values using the upper limit concentration of the test substance (28%) in the test sample and no adjustment was made for the presence of the diethylene glycol butyl ether or water when noted below. These calculations assumed that all toxicity was due to the presence of the Perfluorooctanesulfonate substance.

METHOD

---

**Method:** OECD 202

**Test type:** Static acute

**GLP:** Yes

**Year completed:** 1991

**Species:** *Daphnia magna*

**Analytical monitoring:** DO, pH, Conductivity, and temperature were monitored daily.

**Statistical methods:** EC<sub>50</sub> values calculated, when possible by standard statistical techniques (Stephan, 1983)

**Test daphnid source:** Obtained from cultures maintained by EnviroSystems Division, Resource Analysts, Inc., Hampton, NH, USA.

**Test daphnid age at study initiation:** < 24-hours

**Test Conditions**

**Dilution water:** Well water from wells at EnviroSystems in Hampton, New Hampshire.

**Dilution water chemistry:**

**pH:** 7.8\*

**Conductivity:** 1200 µmhos/cm\*

**TOC:** <2.0 mg/L

\*Values measured at time of test.

**Lighting:** Cool white fluorescent lights, intensity 23 µE/s/m<sup>2</sup>. Photoperiod of 16-hours light, 8-hours dark. No transition period noted.

**Stock and test solutions preparation:** A primary stock solution was prepared in dilution water at 1000 mg/L. The primary stock was proportionally diluted with dilution water to prepare the five test concentrations.

**Exposure vessels:** 250 mL plastic beakers containing 200 mL of test solution. The approximate depth of test solution was 6 cm.

**Number of replicates:** Four

**Number of daphnids per replicate:** Five

**Number of concentrations:** Five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 48 hours):**

8.2 – 8.5 mg/L (control exposure)

8.1 – 8.5 mg/L (150 mg/L exposure)

**pH range** (0 – 48 hours):

7.8 – 8.6 (control exposure)

7.8 – 8.6 (150 mg/L exposure)

**Test temperature range** (0 – 48 hours):

20.8 – 21.0°C (control exposure)

20.7 – 20.9°C (150 mg/L exposure)

**Conductivity range** (0 – 48 hours):

1200 – 1300 µmhos/cm (control exposure)

1200 – 1300 µmhos/cm (150 mg/L exposure)

**Element basis:** mortality**RESULTS**

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**Nominal concentrations:** Negative control, 25, 40, 60, 100, 150 mg/L**Element values:** 24-hour EC<sub>50</sub> = >150 mg/L (C.I. not calculable)48-hour EC<sub>50</sub> = 49 (43-56) mg/L**Perfluorooctanesulfonate concentration adjusted element value:**24-hour EC<sub>50</sub> = >42 mg/L48-hour EC<sub>50</sub> = 14 mg/L

All element values based on nominal concentrations

**Biological observations:** Ninety five percent survival occurred in the control exposure. The number of surviving organisms and the occurrence of sub-lethal effects and immobilization or other sublethal effects were determined visually and recorded initially and after 24 and 48 hours.**Cumulative percent mortality:**

| Nominal Test Concentration mg/L | 24-hours | 48-hours |
|---------------------------------|----------|----------|
| Neg. Control                    | 0        | 5        |
| 25                              | 0        | 0        |
| 40                              | 5        | 25       |
| 60                              | 0        | 25       |
| 100                             | 0        | 100      |
| 150                             | 20       | 100      |

**Control response:** Satisfactory**CONCLUSIONS**

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The test substance 48-hour EC<sub>50</sub> for *Daphnia magna* was determined to be 49 mg/L with a 95% confidence interval of 43-56 mg/L. If you assume all toxicity of the mixture is due to the Perfluorooctanesulfonate, the adjusted 48-hour EC<sub>50</sub> value is 14 mg/L (49 mg/L x 0.28).

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331 St. Paul, Minnesota 55133, USA.**DATA QUALITY**

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**Reliability:** Klimisch ranking = 3. The study lacks analytical measurement of test substance

concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data are for a mixture and toxicity cannot be positively attributed to PFOS as the diethylene glycol butyl ether could also contribute to the toxicity. The basic water quality parameters (hardness, alkalinity and calcium/magnesium ratio) were not included in the final report.

#### REFERENCES

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This study was conducted at EnviroSystems Division, Resource Analysts, Incorporated, Hampton, NH, USA at the request of the 3M Company.

#### OTHER

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**Last changed:** 5/3/00



**Robust Study Report Reference No. 26 – Acute toxicity to Fish****TEST SUBSTANCE**

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**Identity:** Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH<sub>4</sub><sup>+</sup> salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt, CAS # 29081-56-9)

**Remarks:** Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

**METHOD**

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**Method:** Not noted.

**Type:** Static acute

**GLP:** No

**Year completed:** 1974

**Species:** *Pimephales promelas*

**Supplier:** Not noted.

**Analytical monitoring:** pH and DO content

**Exposure period:** 96-hours

**Statistical methods:** Plotted LC<sub>50</sub>

**Test fish age:** Not noted.

**Length and weight:** Average length = 2 inches, Average weight = 1.5 g

**Loading:** Not noted.

**Pretreatment:** Not noted

**Test Conditions**

**Dilution water:** carbon filtered city of St. Paul, MN water

**Dilution water chemistry:** Not noted.

**Stock and test solution preparation:** Not noted.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Not noted.

**Exposure vessels:** Not noted.

**Number of replicates:** One.

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

5.0-5.9 mg/L (control exposure)

4.2-5.0 mg/L (100 mg/L exposure)

**pH range (0 – 96 hours):**

7.0-7.1 (control exposure)

7.0-7.2 7.0-7.1 (100 mg/L exposure)

**Test temperature range (0 – 96 hours):**

21 – 22°C (70-72°F)

**RESULTS**

---

**Nominal concentrations:** Negative control, 10, 25, 50, 75, 100 mg/L

**Element value:** 96-hour  $LC_{50} = 85$  mg/L (C.I. not determined)

**Mortality of controls:** None

**Remarks:** 95% confidence limits were not calculated for this material. Additionally, testing was conducted on the mixture of the test substance in 20% isopropanol and 55% water. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvent or what portion of the toxicity can be contributed to the Perfluorooctanesulfonate, ammonium salt.

#### CONCLUSIONS

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The test sample containing 25% Perfluorooctanesulfonate, ammonium salt exhibited a 96-hour  $LC_{50}$  for fathead minnow of 85 mg/L.

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

#### DATA QUALITY

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**Reliability:** Klimisch ranking 3.

#### REFERENCES

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This study was conducted by the 3M Company, Environmental Laboratory, 7/29/74 to 8/2/74.

#### OTHER

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 27 – Acute toxicity to Fish****TEST SUBSTANCE**

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**Identity:** Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH<sub>4</sub><sup>+</sup> salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt, CAS # 29081-56-9)

**Remarks:** Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

**METHOD**

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**Method:** Not noted.

**Type:** Static acute

**GLP:** No

**Year completed:** 1974

**Species:** *Pimephales promelas*

**Supplier:** Not noted.

**Analytical monitoring:** pH and DO content

**Exposure period:** 96-hours

**Statistical methods:** Plotted LC<sub>50</sub>.

**Test fish age:** Not noted.

**Length and weight:** Average length = 2 inches, Average weight = 1.5 g

**Loading:** Not noted.

**Pretreatment:** Not noted

**Test Conditions**

**Dilution water:** Carbon filtered city of St. Paul, MN water

**Dilution water chemistry:** Not noted.

**Stock and test solution preparation:** Not noted.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Not noted.

**Exposure vessels:** Not noted.

**Number of replicates:** One.

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 96 hours):**

4.5-5.7 mg/L (control exposure)

3.8-5.0 mg/L (125 mg/L exposure)

Not recorded at highest conc. (150 mg/L) due to 100% mortality.

**pH range (0 – 96 hours):**

7.0-7.0 (control exposure)

7.0-7.0 (125 mg/L exposure)

Not recorded at highest conc. (150 mg/L) due to 100% mortality.

**Test temperature range (0 – 96 hours):**

20 – 21°C (69-70 °F)

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## RESULTS

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**Nominal concentrations:** Negative control, 50, 75, 100, 125, 150 mg/L

**Element value:** 96-hour LC<sub>50</sub> = 100 mg/L (C.I. not determined)

**Mortality of controls:** None

**Remarks:** 95% confidence limits were not calculated for this material. Additionally, testing was conducted on the mixture of the test substance in 20% isopropanol and 55% water. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvent or what portion of the toxicity can be contributed to the Perfluorooctanesulfonate, ammonium salt.

## CONCLUSIONS

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The test sample containing 25% Perfluorooctanesulfonate, ammonium salt exhibited a 96-hour LC<sub>50</sub> for fathead minnow of 100 mg/L

**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA

## DATA QUALITY

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**Reliability:** Klimisch ranking 3.

## REFERENCES

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This study was conducted by the 3M Company, Environmental Laboratory, 10/15/74 to 10/19/74.

## OTHER

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**Last changed:** 5/3/00

**Robust Study Report Reference No. 28 – Acute toxicity of P3025 Developmental Material to Fathead minnow (*Pimephales promelas*)****TEST SUBSTANCE**

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**Identity:** Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

**Remarks:** The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

**METHOD**

---

**Method:** OECD 203

**Type:** Static acute

**GLP:** No

**Year completed:** 1996

**Species:** *Pimephales promelas*

**Supplier:** Not noted.

**Analytical monitoring:** DO, pH, temperature, and conductivity were monitored daily.

**Exposure period:** 96-hours

**Statistical methods:** LL<sub>50</sub> values calculated using the Trimmed Spearman-Kärber method. The NOEL was calculated using Fisher's Exact tests.

**Test fish age:** Not given.

**Length and weight:** Average length = 11.3 mm, Average weight = 7.8 mg

**Loading:** 0.26 g/L

**Pretreatment:** None

**Test Conditions**

**Dilution water:** Dechlorinated City of Duluth, MN tap water. Water was aerated for 24-hours prior to use in the test.

**Dilution water chemistry:**

**Hardness:** 48 mg/L as CaCO<sub>3</sub>

**pH:** 8.08

**Lighting:** Cool-white fluorescent bulbs. Photoperiod of 16-hours light, 8-hours dark used. No transition period noted.

**Stock and test solution preparation:** Water accommodated fractions. Test solutions were prepared individually for each test replicate concentration by mass addition of vigorously shaken test substance in 4 L of dilution water. The solutions were vigorously stirred for 21-hours (vortex 1/2 to 1/3 solution depth). The aqueous phase was siphoned from the vessel at mid-depth.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Not noted.

**Exposure vessels:** 4-L glass jars containing 3-L of test solution. The jars were sealed with Teflon-lined lids fitted with stoppers to accommodate oxygen flushing of headspace.

**Number of replicates:** two

**Number of fish per replicate:** ten

**Number of concentrations:** three plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range:** (0 – 96 hours):

9.1 – 14.6 mg/L (control exposure)

8.7 – 18.2 mg/L (700 mg/L exposure)

**pH range:** (0 – 96 hours):

7.80 – 8.08 (control exposure)

7.78 – 7.99 (700 mg/L exposure)

**Test temperature range** (0 – 96 hours): 20.8 – 20.9°C

**Conductivity range** (0 – 96 hours):

128 – 142 µmhos/cm (control exposure)

118 – 154 µmhos/cm (700 mg/L exposure)

**Remarks:** Oxygen was added to the headspace in the jars before sealing initially and at each observation period. The dissolved oxygen concentrations were super-saturated in the test vessels, particularly in the 700 mg/L exposure concentration.

## RESULTS

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**Nominal loading concentrations:** Negative control, 400, 700, 1,000 mg/L.

**Element value:** (95% confidence interval is given in brackets)

24-hour LL<sub>50</sub> = 618 (568 - 673) mg/L

48-hour LL<sub>50</sub> = 607 (554 - 664) mg/L

72-hour LL<sub>50</sub> = 595 (551 - 643) mg/L

96-hour LL<sub>50</sub> = 562 (523 - 604) mg/L

96-hour NOEL = <490 mg/L

All element values based on nominal concentrations.

**Biological observations after 96-hours:** No mortality or abnormal behavior observed in the negative control during the test. Mortality was observed in the remaining exposure concentrations. Surfacing was observed in half of the fish at the 700 mg/L exposure concentration at 24-hours, and 2 fish were quiescent at 96-hours. No abnormal behavior was observed in the 400 mg/L exposure concentration.

**Cumulative percent mortality:**

| Nominal Loading Test Concentration, mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|--|----------|----------|----------|----------|
| Neg. Control                             | 0        | 0        | 0        | 0        |
| 490                                      | 10       | 15       | 15       | 25       |
| 700                                      | 75       | 75       | 80       | 90       |
| 1,000                                    | 100      | 100      | 100      | 100      |

**Lowest concentration causing 100% mortality:** 1,000 mg/L

**Mortality of controls:** None

**Remarks:** Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

## CONCLUSIONS

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The test sample 96-hour LL<sub>50</sub> for fathead minnow was determined to be 562 mg/L with a 95% confidence interval of 523 – 604 mg/L. The 96-hour no observed effects level (NOEL) was <490 mg/L.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133, USA

#### DATA QUALITY

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**Reliability:** Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone. Also, supersaturation of the test solutions with oxygen could also have contributed to the toxicity.

#### REFERENCES

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This study was conducted at ASci Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

#### OTHER

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**Last changed:** 5/24/00

**Robust Study Report Reference No. 29 – Acute toxicity of P3025 Developmental Material *Daphnia magna***

TEST SUBSTANCE

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**Identity:** Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

**Remarks:** The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

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**Method:** OECD 202

**Test type:** Static acute

**GLP:** No

**Year Completed:** 1996

**Species:** *Daphnia magna*

**Analytical monitoring:** DO, pH, temperature and conductivity were monitored daily.

**Statistical methods:** EL<sub>50</sub> values calculated using Trimmed Spearman-Kärber method. NOEL value calculated using Steel's Many-One Rank test.

**Test daphnid source:** Obtained from cultures maintained by ASci Corporation, Duluth, MN.

**Test daphnid age at study initiation:** < 24-hours

**Test Conditions:**

**Dilution water:** Dechlorinated City of Duluth, MN tap water. Water was aerated for 24-hours prior to use in the test.

**Dilution water chemistry:**

**Hardness:** 44 mg/L as CaCO<sub>3</sub>

**pH:** 8.04

**Lighting:** Cool-white fluorescent bulbs. Photoperiod of 16-hours light, 8-hours dark. No transition period noted.

**Stock and test solutions preparation:** Water-accommodated fractions. Test solutions were prepared individually for each concentration by mass addition of vigorously shaken test substance in 1 L of dilution water. The solutions were vigorously stirred for 23-hours (vortex 1/2 to 1/3 solution depth). The aqueous phase was siphoned from the vessel at mid-depth after settling for 1-hour.

**Exposure vessels:** 250 mL borosilicate glass beakers containing 200 mL of test solution. The solutions were kept covered during the test.

**Number of replicates:** Four

**Number of daphnids per replicate:** Five

**Number of concentrations:** Five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range (0 – 48 hours):**

8.6 – 9.1 mg/L (control exposure)

8.0 – 8.8 mg/L (50 mg/L exposure)



**pH range** (0 – 48 hours):

8.04 – 8.11 (control exposure)

7.92 – 8.00 (50 mg/L exposure)

**Test temperature range** (0 – 48 hours):

20.9 – 21.0°C

**Conductivity range** (0 – 48 hours):

142 – 155 µmhos/cm (control exposure)

120 – 124 µmhos/cm (50 mg/L exposure)

**Element basis:** mortality and immobilization

## RESULTS

**Nominal loading concentrations:** Negative control, 3.13, 6.25, 12.5, 25, 50 mg/L**Element value:** (95% confidence interval is given in brackets)24-hour EL<sub>50</sub> = 27.0 (18.7-39.0) mg/L48-hour EL<sub>50</sub> = 11.3 (9.6-13.2) mg/L

48-hour NOEL = 6.25 mg/L

All element values based on nominal concentrations.

**Statistical Evaluation:** The EL<sub>50</sub> values and 95% confidence intervals were calculated by the Trimmed Spearman-Kärber method. The NOEL was calculated using Steel's Many-One Rank test using the TOXSTAT statistical software Version 3.2, University of Wyoming.**Biological observations:** Daphnids in the negative control, and the 3.13 and 6.25 mg/L treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. The effects noted in this study were mortality; no immobilization was noted at any test concentration. The number of surviving organisms were determined visually and recorded initially and after 24 and 48 hours.**Cumulative percent mortality:**

| Nominal Loading Test Concentration mg/L | 24-hours | 48-hours |
|---|----------|----------|
| Negative Control                        | 0        | 0        |
| 3.13                                    | 0        | 0        |
| 6.25                                    | 0        | 0        |
| 12.5                                    | 5        | 70       |
| 25                                      | 50       | 95       |
| 50                                      | 75       | 100      |

**Control response:** satisfactory**Remarks:** Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

## CONCLUSIONS

The test substance 48-hour EL<sub>50</sub> for *Daphnia magna* was determined to be 11.3 mg/L with a 95% confidence interval of 9.6-13.2 mg/L. The 48-hour no observed effect level (NOEL) was 6.25 mg/L.**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN, 55133, USA.

DATA QUALITY

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**Reliability:** Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone.

REFERENCES

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This study was conducted at ASci Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

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**Last changed:** 5/24/00

**Robust Study Report Reference No. 30 - Acute toxicity of PFOS to Rainbow trout in saltwater****TEST SUBSTANCE**

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder of uncharacterized purity.

**METHOD**

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**Method:** Standard procedures for Testing Acute Lethality of Liquid Effluents (Environment Canada, 1980)

**Type:** Acute static, renewal after 48-hours

**GLP:** No

**Year completed:** 1985

**Species:** Rainbow Trout, *Salmo gairdneri*

**Fish source:** Rainbow Springs, Thamesford

**Fish age at test initiation:** not noted

**Fish acclimation to salt water:** fish gradually acclimated to increasing salinity; held at 30 parts per thousand salinity 8 days prior to test initiation.

**Exposure period:** 96-hours

**Analytical monitoring:** Dissolved oxygen, pH, conductivity

**Statistical methods:** Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC<sub>50</sub>.

**Test conditions:**

**Dilution water:** Mississauga dechlorinated tap water amended with calcium, magnesium, sodium, and chloride to obtain 30 parts per thousand salinity

**Dilution water chemistry (initial):**

**pH:** 7.4 - 8.0

**D.O.:** 8.6 - 9.0 mg/L

**Conductivity:** > 20,000 µmhos/cm

**Stock solution preparation:** 1000 mg/L

**Exposure vessels:** Not noted; solution volume 35 L

**Number of replicates:** 2 tests - run 4 days apart, not replicated

**Number of organisms/vessel:** 6

**Loading:** 0.75 g/L

**Number of concentrations:** 4 plus a blank control

**Water chemistry during the studies:****Dissolved oxygen ranges**

8.1 - 10.3 (control)

8.8 - 10.1 (30 mg/L)

**pH ranges**

7.6 - 8.2 (control)

7.3 - 8.0 (30 mg/L)

**Test temperature (0 - 48 hours):** 15°C

**Photoperiod:** 12-hours light, 12-hours dark

**Element basis:** mortality

## RESULTS

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**Nominal concentrations:** 5, 10, 20, 30 mg/L

**Element values (95% confidence interval) calculated per replicate:**

96-hour LC<sub>50</sub> = 13.7 (10.7 – 17.7) mg/L

96-hour LC<sub>50</sub> = 13.7 (10.7 – 17.8) mg/L

**Mortality of controls:** 17% (1/6 in both studies)

## DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

## REFERENCES

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This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

## OTHER

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed:** 7/19/01

**Robust Study Report Reference No. 31 - Acute toxicity of PFOS to Rainbow trout in freshwater****TEST SUBSTANCE**

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder of uncharacterized purity.

**METHOD**

---

**Method:** Standard procedures for Testing Acute Lethality of Liquid Effluents (Environment Canada, 1980)

**Type:** Acute, renewal after 48-hours

**GLP:** No

**Year completed:** 1985

**Species:** Rainbow Trout

**Fish source:** Rainbow Springs, Thamesford

**Fish age at test initiation:** not noted

**Exposure period:** 96-hours

**Analytical monitoring:** Dissolved oxygen, pH, conductivity

**Statistical methods:** Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC<sub>50</sub>.

**Test conditions:**

**Dilution water:** Mississauga dechlorinated tap water

**Dilution water chemistry (initial):**

**pH:** 7.5 - 8.5

**D.O.:** 9.0 - 10.4 mg/L

**Stock solution preparation:** 1000 mg/L; noted as cloudy

**Exposure vessels:** Not noted; solution volume 35 L

**Number of replicates:** 2 tests - run one week apart, not replicated

**Number of organisms/vessel:** 6

**Loading:** 0.72 g/L

**Number of concentrations:** 5 plus a blank control, 4 plus a blank control

**Water chemistry during the studies:**

**Dissolved oxygen ranges**

8.2 - 10.4 (control)

8.0 - 9.5 (30 mg/L)

**pH ranges**

7.4 - 8.3 (control)

7.5 - 8.7 (30 mg/L)

**Conductivity range:**

270 - 380 µmhos/cm

**Test temperature (0 - 48 hours):** 15°C

**Element basis:** mortality

**RESULTS**

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**Element values (95% confidence interval) calculated per replicate:**

96-hour LC<sub>50</sub> = 7.8 (6.2 - 9.8) mg/L

ENV/JM/RD(2002)17

96-hour LC<sub>50</sub> = 9.9 (7.5 – 13.4) mg/L

**Mortality of controls:** None

#### DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

#### REFERENCES

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This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

#### OTHER

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed:** 7/19/01

**Robust Study Report Reference No. 32 - Acute toxicity of PFOS to *Artemia* sp.**TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder of uncharacterized purity.

METHOD

---

**Method:** Draft International Standards Organization (Vanhaecke and Persoone, 1981)

**Type:** Acute static

**GLP:** No

**Year completed:** 1985

**Species:** *Artemia* sp.

**Artemia source:** Salt lake Brine Shrimp Inc.

**Artemia age at test initiation:** naupuli < 24 hours old.

**Exposure period:** 48-hours

**Analytical monitoring:** Dissolved oxygen, pH, conductivity

**Statistical methods:** Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC<sub>50</sub>.

**Test conditions:**

**Dilution water:** 30 parts per thousand NaCl solution

**Dilution water chemistry (initial):**

**pH:** 8.0 – 8.2

**D.O.:** > 6 mg/L

**Stock solution preparation:** 1000 mg/L; noted as cloudy

**Exposure vessels:** Not noted; solution volume 10 mL

**Number of replicates:** 3

**Number of organisms/replicate:** 10

**Number of concentrations:** 6 plus a blank control

**Water chemistry during the study:**

**Dissolved oxygen ranges (test and control):**

> 6.0 mg/L

**pH (test and control)**

8.0 – 8.2

**Test temperature range (0 – 48 hours):** 21 - 21°C

**Element basis:** mortality

RESULTS

---

**Nominal concentrations:** 1, 2, 3, 5, 10, 20 mg/L

**Element values (95% confidence interval) calculated per replicate:**

48-hour EC<sub>50</sub> = 9.4 (7.4 – 12.1) mg/L

48-hour EC<sub>50</sub> = 9.4 (7.3 – 12.2) mg/L

48-hour EC<sub>50</sub> = 8.9 (6.7 – 11.9) mg/L

**Mortality of controls:** None

## DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

## REFERENCES

---

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

## OTHER

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed:** 6/12/01



**Robust Study Report Reference No. 33 - Acute toxicity of PFOS to *Daphnia magna***TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder of uncharacterized purity.

The following summary is abbreviated due to the fact that this study has been superseded by a more recent test.

METHOD

---

**Method:** International Standards Organization (1982)

**Type:** Acute static

**GLP:** No

**Year completed:** 1985

**Species:** *Daphnia magna*

RESULTS

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**Nominal concentrations:** 10, 20, 30, 50, 100 mg/L

**Number of replicates:** 2

**Element values (95% confidence interval) calculated per replicate**

48-hour EC<sub>50</sub> = 58 (46 – 72) mg/L

48-hour EC<sub>50</sub> = 67 (48 – 92) mg/L

DATA QUALITY

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**Reliability:** Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

REFERENCES

---

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed:** 5/26/01

**Robust Study Report Reference No. 34 - Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee**

TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks field:** The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20°C prior to testing. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OECD Guideline 213, Eppo Guideline 170

**Test type:** Acute Oral

**GLP:** Yes

**Year Completed:** 2001

**Species:** *Apis mellifera L.*

**Analytical monitoring:** None – nominal concentrations

**Test honey bee source:** Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.

**Test honey bee age at study initiation:** Young adult

**Test honey bee type:** Worker honey bees, free of acarine, nosema and amoeba.

**Varroacide treatment:** None within the 4 weeks prior to test initiation.

**Test conditions**

**Humidity:** 65% + 5%

**Temperature:** 25 + 2°C

**Lighting:** Conducted in darkness

**Stock and test solutions preparation:**

**Test substance:** Initial stock solution prepared in analytical grade acetone to a final concentration of 47.8 µg PFOS/µL (nominal concentration). Final test concentrations prepared from dilutions of this solution with 50% w/v sucrose. Resulting acetone concentration was 5%.

**Reference toxicant:** Primary stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Secondary stock solutions were made by diluting the primary stock solution in deionized water containing 1 g/L Triton X-100. Final test concentrations prepared from dilutions of these solutions with 50% w/v sucrose.

**Stability of the test chemical solution:** A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.

**Exposure vessels:** Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.

**Feeding:** During the first four hours of the test, bees provided with 50% w/v aqueous sucrose solutions containing the appropriate PFOS dose. After 4-hours, dosed sucrose removed, and bees provided with 50% w/v aqueous sucrose solutions, continuously available through the end

of the exposure period.

**Number of replicates:** Three

**Number of bees per replicate:** Ten

**Negative control:** 50% w/v sucrose

**Solvent control:** 50% w/v sucrose plus 5% acetone

**Reference substance:** Dimethoate

**Reference substance control:** Triton X-100

**Number of concentrations:** five plus a negative and a solvent control

**Dose administration:** The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each group of 10 bees was offered 0.2 mL of a given test concentration or control solution. The dose was measured into a small, pre-weighed, glass feeder within the cage using a variable volume pipette. This volume of solution is equivalent to 20 µL per bee.

**Dose frequency:** Once, for 4 hours of exposure

**Dose calculation:** Feeders were weighed after removal from the cages to determine the dose consumed per bee.

**Element basis:** Mortality

## RESULTS

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**Nominal concentrations:** Negative control (sucrose only), acetone + sucrose control, 0.205, 0.450, 0.991, 2.17, 4.78 µg/bee

Element value and 95% confidence interval:

24-hour LD<sub>50</sub> = 0.72 (0.60 – 0.85) µg/bee

48-hour LD<sub>50</sub> = 0.46 (0.32 – 0.55) µg/bee

72-hour LD<sub>50</sub> = 0.40 (0.33 – 0.48) µg/bee

72-hour NOEL = 0.21 µg/bee

All element values based on nominal concentrations

**Statistical Evaluation:** Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test (p<0.05)

**Biological observations:** There was significant mortality at all doses above a mean intake of 0.21 µg/bee with a steep dose response between mean intakes of 0.45 and 2.2 µg/bee.

### Cumulative percent mortality:

| Nominal Test Conc.,<br>µg/bee | 4-hours | 24-hours | 48-hours | 72-hours |
|-------------------------------|---------|----------|----------|----------|
| Negative Control              | 0       | 0        | 0        | 0        |
| Solvent Control               | 0       | 3.3      | 3.3      | 3.3      |
| 0.205                         | 0       | 0        | 6.7      | 10       |
| 0.450                         | 0       | 20       | 50       | 60       |
| 0.991                         | 0       | 70       | 93       | 97       |
| 2.17                          | 6.7     | 100      | 100      | 100      |
| 4.78                          | 30      | 100      | 100      | 100      |

### Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

| Nominal Test Conc.,<br>µg/bee | 4-hours | 24-hours | 48-hours | 72-hours |
|-------------------------------|---------|----------|----------|----------|
|-------------------------------|---------|----------|----------|----------|

|                  |        |         |         |         |
|------------------|--------|---------|---------|---------|
| Negative Control | 0      | 0       | 0       | 0       |
| Solvent Control  | 0      | 0       | 0       | 0       |
| 0.205            | 0      | 0       | 0       | 0       |
| 0.450            | 0      | 3.3 (S) | 3.3 (K) | 3.3 (K) |
| 0.991            | 0      | 0       | 0       | 0       |
| 2.17             | 0      | 0       | 0       | 0       |
| 4.78             | 10 (K) | 0       | 0       | 0       |

**Control response:** satisfactory

**Reference toxicant response:** satisfactory – dimethoate 72-hour LD<sub>50</sub> = 0.11 µg/bee

#### CONCLUSIONS

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The potassium perfluorooctanesulfonate 72-hour oral LD<sub>50</sub> for the honey bee was determined to be 0.40 µg/bee with a 95% confidence interval of 0.33 – 0.48. The 72-hour no observed effect level was 0.21 µg/bee. The dose response was steep between a mean uptake of 0.45 and 2.2 µg/bee.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

#### DATA QUALITY

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**Reliability:** Klimisch ranking 1

#### REFERENCES

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This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

#### OTHER

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**Last changed:** 5/1/01

**Robust Study Report Reference No. 35 - Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee**TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks field:** The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20°C prior to testing. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** USEPA OPPTS 850.3020 (draft), OECD Guideline 214, EPPO Guideline 170

**Test type:** Acute Contact

**GLP:** Yes

**Year Completed:** 2001

**Species:** *Apis mellifera* L.

**Analytical monitoring:** None – nominal concentrations

**Test honey bee source:** Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.

**Test honey bee age at study initiation:** Young adult

**Test honey bee type:** Worker honey bees, free of acarine, nosema and amoeba.

**Varroacide treatment:** None within the 4 weeks prior to test initiation.

**Test conditions**

**Humidity:** 65% + 5%

**Temperature:** 25 + 2°C

**Lighting:** Conducted in darkness

**Stock and test solutions preparation:**

**Test substance:** Stock solution prepared in analytical grade acetone to a final concentration of 90.4 µg PFOS/µL (nominal concentration). Final test concentrations prepared in acetone from dilutions of this solution. **Reference toxicant:** Stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Final test concentrations prepared from dilutions of this solution.

**Stability of the test chemical solution:** A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.

**Exposure vessels:** Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.

**Feeding:** 50% w/v aqueous sucrose solution, continuously available

**Number of replicates:** Three

**Number of bees per replicate:** Ten

**Negative control:** Undosed

**Solvent control:** Acetone

**Reference substance:** Dimethoate

**Reference substance control:** Triton X-100

**Number of concentrations:** five plus a negative and a solvent control

**Dose administration:** The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each bee was dosed on the thorax with a 1 µL drop of a given test item concentration or 1µL acetone before being placed into the test chamber.

**Dose frequency:** Once

**Element basis:** Mortality

## RESULTS

**Nominal concentrations:** Negative control, acetone control (1.0 µg/bee), 1.93, 4.24, 9.30, 20.5, 45, µg PFOS/bee

Element value and 95% confidence interval:

24-hour LD<sub>50</sub> = 38.9 (28.2 – 71.2) µg/bee

48-hour LD<sub>50</sub> = 10.4 (8.2 – 13.0) µg/bee

72-hour LD<sub>50</sub> = 6.0 (4.7 – 7.6) µg/bee

96-hour LD<sub>50</sub> = 4.78 (3.8 – 5.8) µg/bee

96-hour NOEL = 1.93 µg/bee

All element values based on nominal concentrations

**Statistical Evaluation:** Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test (p<0.05)

**Biological observations:** There was significant mortality at all doses above 1.93 µg/bee with a steep dose response between 4.24 and 9.30 µg/bee

### Cumulative percent mortality:

| Nominal Test Conc.,<br>µg/bee | 4-hours | 24-hours | 48-hours | 72-hours | 96-hours |
|-------------------------------|---------|----------|----------|----------|----------|
| Negative Control              | 0       | 0        | 3.3      | 3.3      | 3.3      |
| Solvent Control               | 3.3     | 3.3      | 3.3      | 3.3      | 3.3      |
| 1.93                          | 0       | 6.7      | 6.7      | 13       | 13       |
| 4.24                          | 0       | 0        | 13       | 37       | 37       |
| 9.30                          | 0       | 6.7      | 40       | 63       | 90       |
| 20.5                          | 0       | 40       | 93       | 97       | 100      |
| 45.0                          | 0       | 50       | 93       | 100      | 100      |

### Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

| Nominal Test Conc.,<br>µg/bee | 4-hours | 24-hours | 48-hours | 72-hours | 96-hours |
|-------------------------------|---------|----------|----------|----------|----------|
| Negative Control              | 0       | 0        | 0        | 0        | 0        |
| Solvent Control               | 0       | 0        | 0        | 0        | 0        |
| 1.93                          | 3.3 (K) | 0        | 0        | 0        | 0        |
| 4.24                          | 0       | 0        | 0        | 0        | 0        |
| 9.30                          | 0       | 0        | 0        | 0        | 3.3 (K)  |
| 20.5                          | 0       | 3.3 (K)  | 0        | 3.3 (K)  | 0        |
| 45.0                          | 0       | 3.3 (K)  | 3.3 (K)  | 0        | 0        |

**Control response:** satisfactory

**Reference toxicant response:** satisfactory – dimethoate 96-hour LD<sub>50</sub> = 0.19 µg/bee

#### CONCLUSIONS

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The potassium perfluorooctanesulfonate 96-hour contact LD<sub>50</sub> for the honey bee was determined to be 4.78 µg/bee with a 95% confidence interval of 3.8 – 5.8. The 96-hour no observed effect level was 1.93 µg/bee. The dose response was steep between 4.24 and 9.30 µg/bee.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

#### DATA QUALITY

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**Reliability:** Klimisch ranking 1

#### REFERENCES

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This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

#### OTHER

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**Last changed:** 5/1/01

**Robust Study Report Reference No. 36 - PFOS: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*)**

## TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

## METHOD

**Method:** OPPTS 850.5400

**Test:** Acute static

**GLP:** Yes

**Year completed:** 2001

**Species:** *Anabaena flos-aquae*

**Source:** Originally from UTEX – The Culture Collection of Algae at the university of Texas at Austin, and maintained in culture medium at Wildlife International Ltd., Easton, MD

**Analytical monitoring:** PFOS measured at 0, 72, 96-hours

**Element basis:** Reported three ways: number of cells/ml, area under the growth curve and growth rate

**Exposure period:** 96-hours

**Start date:** 1/28/00

**End date:** 6/5/00

**Test organisms laboratory culture:** Algae cultures had been actively growing in algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water. Solutions were then diluted in purified well water to prepare final growth media.

**Test Conditions:****Freshwater Algal medium**

| Compound  | Nominal Concentration | Units |
|---|-----------------------|-------|
| MgCl <sub>2</sub> .6H <sub>2</sub> O                | 12.16                 | mg/L  |
| CaCl <sub>2</sub> .2H <sub>2</sub> O                | 4.40                  | mg/L  |
| H <sub>3</sub> BO <sub>3</sub>                      | 0.1856                | mg/L  |
| MnCl <sub>2</sub> .4H <sub>2</sub> O                | 0.416                 | mg/L  |
| ZnCl <sub>2</sub>                                   | 3.28                  | µg/L  |
| FeCl <sub>3</sub> .6H <sub>2</sub> O                | 0.1598                | mg/L  |
| CoCl <sub>2</sub> .6H <sub>2</sub> O                | 1.428                 | µg/L  |
| Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O | 7.26                  | µg/L  |
| CuCl <sub>2</sub> .2H <sub>2</sub> O                | 0.012                 | µg/L  |
| Na <sub>2</sub> EDTA.2H <sub>2</sub> O              | 0.300                 | mg/L  |
| NaNO <sub>3</sub>                                   | 25.5                  | mg/L  |
| MgSO <sub>4</sub> .7H <sub>2</sub> O                | 14.7                  | mg/L  |
| K <sub>2</sub> HPO <sub>4</sub>                     | 1.044                 | mg/L  |
| NaHCO <sub>3</sub>                                  | 15.0                  | mg/L  |

**Dilution water source:** The pH of the medium was adjusted to 7.5 ± 0.1 and it was sterilized by



filtration (0.22µm) prior to use.

**Test solution preparations:** Individual test solutions were prepared in algal medium at each of the six nominal concentrations. The solutions were stirred with magnetic stir plates for approximately 18 hours. The final test solutions appeared clear and colorless.

**Exposure vessels:** Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

**Agitation:** Shaken continuously at 100 rpm

**Number of replicates:** six (including 3 for analysis of exposure concentration).

**Initial algal cell loading:**  $1.0 \times 10^4$  cells/mL

**Cell counts method:** hemacytometer and microscope

**Number of concentrations:** six plus a negative control plus an abiotic control at the highest concentration tested

**Water chemistry: pH range (0 – 96 hours)**

7.4 – 7.6 (control exposure)

7.4 – 7.4 (329 mg/L exposure)

**Test temperature range (0 – 96 hours)**

22.8 – 23.8°C

**Light levels: (0 – 96 hours)**

1990 – 2310 lux from cool-white fluorescent lighting

**Photoperiod:** 24-hours light

**Method of calculating mean measured concentrations:** arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

## RESULTS

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**Nominal concentrations:** Negative control, 37.9, 58.6, 88.8, 139, 216, 331 mg/L plus 331 abiotic replicate

**Measured concentrations:** <LOQ, 37.9, 63.9, 93.8, 143, 235, 329 mg/L; abiotic replicate = 349 mg/L

### Element values (95% confidence interval):

24-hour EC<sub>50</sub> (cell density) = 105 mg/L (C.I. not calculable)

24-hour EbC<sub>50</sub> (area under curve) = 90 (40 - 150) mg/L

24-hour ErC<sub>50</sub> (growth rate) = 94 (33 - 145) mg/L

48-hour EC<sub>50</sub> (cell density) = 117 mg/L (C.I. not calculable)

48-hour EbC<sub>50</sub> (area under curve) = 103 mg/L (C.I. not calculable)

48-hour ErC<sub>50</sub> (growth rate) = 128 mg/L (C.I. not calculable)

72-hour EC<sub>10</sub> (cell density) = 43 (34 – 84) mg/L

72-hour EbC<sub>10</sub> (area under curve) = <38 mg/L (C.I. not calculable)

72-hour ErC<sub>10</sub> (growth rate) = 82 (49 - 116) mg/L

72-hour EC<sub>50</sub> (cell density) = 120 (92 - 139) mg/L

72-hour EbC<sub>50</sub> (area under curve) = 116 (49 - 142) mg/L

72-hour ErC<sub>50</sub> (growth rate) = 174 (146 - 208) mg/L

72-hour EC<sub>90</sub> (cell density) = 224 (193 - 275) mg/L

72-hour EbC<sub>90</sub> (area under curve) = 204 (134 - 226) mg/L

72-hour ErC<sub>90</sub> (growth rate) = 275 (162 - 330) mg/L

96-hour EC<sub>10</sub> (cell density) = 82 (29 - 123) mg/L

96-hour EbC<sub>10</sub> (area under curve) = 56 (26 - 107) mg/L

96-hour ErC<sub>10</sub> (growth rate) = 109 (84 - 125) mg/L

96-hour EC<sub>50</sub> (cell density) = 131 (106 - 142) mg/L

96-hour EbC<sub>50</sub> (area under curve) = 124 (104 - 138) mg/L

96-hour ErC<sub>50</sub> (growth rate) = 176 (169 - 181) mg/L

96-hour EC<sub>90</sub> (cell density) = 213 (203 - 219) mg/L

96-hour EbC<sub>90</sub> (area under curve) = 209 (197 - 218) mg/L  
 96-hour ErC<sub>90</sub> (growth rate) = 225 (220 - 235) mg/L  
 72-hour NOAEC (cell density, area under curve): 37.9 mg/L  
 72-hour NOAEC (growth rate): 93.8 mg/L  
 96-hour NOAEC (cell density, growth rate): 93.8 mg/L  
 96-hour NOAEC (area under curve): 63.9 mg/L

#### All element values based on mean measured concentrations

**Statistical methods:** Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. The EC<sub>10</sub>, EC<sub>50</sub>, and EC<sub>90</sub> values and 95% confidence limits were calculated by linear interpolation with treatment response and exposure concentration data using TOXSTAT Version 3.5. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality and homogeneity of variances using the Shapiro-Wilk’s test and Levene’s test, respectively. Where the data were normally distributed with equal variances, the treatment groups were compared to the control using Dunnett’s test. In the one instance where data were not normally distributed, the non-parametric Kruskal-Wallis test was used. Results of the statistical analyses were used to determine the NOAEC values.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.80 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 103%. Samples collected at test initiation had measured values from 100 to 112% of nominal. The measured values for the samples taken at 72-hours were 99.0 - 110% of nominal. The measured values for the samples taken at 96-hours were 99.0 - 109% of nominal. For the abiotic replicate, the measured value for the sample taken at 72-hours was 103% of nominal and for the sample taken at 96-hours, 107% of nominal.

#### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured Values at 0, 72, and 96-hours, Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              | -                  |
| 37.9                             | 37.9, 38.2, 37.6   | 37.9                              | 100                |
| 58.6                             | 65.6, 62.7, 63.4   | 63.9                              | 109                |
| 88.8                             | 94.3, 97.4, 89.8   | 93.8                              | 106                |
| 139                              | 142, 146, 142  | 143                               | 103                |
| 216                              | 230, 238, 236  | 235                               | 109                |
| 331                              | 331, 328, 329  | 329                               | 99.4               |
| 331 (abiotic)                    | Not analyzed, 342, 356                                     | 349                               | 105                |

#### Biological observations after 96-hours:

| Mean Measured Concentration, mg/L | Mean Number of Cells per mL | Percent Inhibition via Density | Percent Inhibition via Area Under the Curve | Percent Inhibition via Growth Rate |
|-----------------------------------|-----------------------------|--------------------------------|---|------------------------------------|
| Negative Control                  | 569,167                     | -                              | -   | -                                  |
| 37.9                              | 605,000                     | -6.3                           | 3.0   | -1.3                               |

|      |         |      |      |       |
|------|---------|------|------|-------|
| 63.9 | 585,833 | -2.9 | 13   | -0.97 |
| 93.8 | 492,500 | 13   | 24*  | 3.6   |
| 143  | 228,833 | 60*  | 66*  | 22*   |
| 235  | 2,333   | 100* | 99*  | 100*  |
| 329  | 8,500   | 99*  | 100* | 96*   |

\*Indicates a significant difference from the negative control using the appropriate statistical test ( $p < 0.05$ )

**Control response:** satisfactory

**Observations:** After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any treatment group. In addition, there were no noticeable changes in cell morphology when compared to the negative control.

**Reversibility of Growth Inhibition:** Aliquots of the 235 and 329 mg/L test solutions were diluted with algal medium and cultured for nine days after the exposure phase of the study concluded. Based on the increase in growth observed by Day 9 of the recovery phase, the effect on algal growth was algistatic at a concentration of 235 mg/L. However, no algal cells were detected during the recovery phase in the 329 mg/L treatment, indicating that PFOS was algicidal at that concentration.

## CONCLUSIONS

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The potassium perfluorooctanesulfonate 96-hour  $EC_{50}$  and 95% confidence interval for *Anabaena flos-aquae* was determined using three calculation methods. By cell density, it was 131 (106 – 142) mg/L, by area under the growth curve it was 124 (104 – 138) mg/L and by growth rate 176 (169 – 181) mg/L. The 96-hour NOAEC values were determined to be 63.9 mg/L using the area under the growth curve, and 93.8 mg/L with the cell density and growth rate calculation method. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. PFOS was determined to be algistatic at a concentration of 235 mg/L and algicidal at 329 mg/L.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

## DATA QUALITY

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**Reliability:** Klimisch ranking = 1

## REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

## OTHER

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**Last changed:** 7/19/01

**Robust Study Report Reference No. 37 - PFOS: A 7-day toxicity test with Duckweed (*Lemna gibba* G3)**

## TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample obtained from 3M production lot number 217. The test substance is a white powder. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

## METHOD

**Method:** OPPTS 850.4400

**Test:** Static acute

**GLP:** Yes

**Year completed:** 2001

**Species:** *Lemna gibba* G3

**Source:** Originally from The United States Department of Agriculture. Maintained in culture medium at Wildlife International Ltd., Easton, MD

**Analytical monitoring:** Test concentrations measured at 0, 3, 5, and 7-days

**Element basis:** Number of fronds

**Exposure period:** 7-days

**Start date:** 3/3/00

**End date:** 3/10/00

**Test organisms laboratory culture:** Duckweed cultures had been actively growing in freshwater medium (20X AAP) for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

**Test Conditions:**

**Test temperature range:** 24.2 – 25.2°C

**Light levels:** 5000 + 750 lux from continuous warm-white fluorescent lighting

**Growth medium:** USEPA OPPTS 850.4400 20X AAP, 1996

| Compound  | Nominal Concentration | Units |
|---|-----------------------|-------|
| MgCl <sub>2</sub> ·6H <sub>2</sub> O                | 243.2                 | mg/L  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O                | 88.0                  | mg/L  |
| H <sub>3</sub> BO <sub>3</sub>                      | 3.712                 | mg/L  |
| MnCl <sub>2</sub> ·4H <sub>2</sub> O                | 8.32                  | mg/L  |
| ZnCl <sub>2</sub>                                   | 65.6                  | µg/L  |
| FeCl <sub>3</sub> ·6H <sub>2</sub> O                | 3.196                 | mg/L  |
| CoCl <sub>2</sub> ·6H <sub>2</sub> O                | 28.56                 | µg/L  |
| Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O | 145.2                 | µg/L  |
| CuCl <sub>2</sub> ·2H <sub>2</sub> O                | 0.240                 | µg/L  |
| Na <sub>2</sub> EDTA·2H <sub>2</sub> O              | 6.00                  | mg/L  |
| NaNO <sub>3</sub>                                   | 510                   | mg/L  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O                | 294                   | mg/L  |
| K <sub>2</sub> HPO <sub>4</sub>                     | 20.88                 | mg/L  |
| NaHCO <sub>3</sub>                                  | 300                   | mg/L  |

The pH of the medium was adjusted to  $7.5 \pm 0.1$  using 10% HCl.

**Dilution water source:** Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to  $7.5 \pm 0.1$  using 10% HCl and the medium was sterilized by filtration (0.22  $\mu\text{m}$ ) prior to use.

**Stock and test solution preparation:** A primary stock solution was prepared in duckweed medium at a concentration of 351 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with duckweed medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

**Exposure vessels:** 250 mL plastic beakers containing 100 mL test solution, each covered with a disposable petri dish lid.

**Agitation:** None

**Number of replicates:** three plus 2 additional replicates for analytical sampling on Days 3 and 5

**Initial loading:** 5 plants/replicate, 15 fronds/replicate

**Number of concentrations:** six plus a negative control plus abiotic controls at the highest concentration tested

**Water chemistry: pH range (0 – 96 hours)**

7.9 – 8.9 (control exposure)

8.4 – 8.7 (230 mg/L exposure)

**Method of calculating mean measured concentrations:** arithmetic mean obtained using results obtained at Days 0, 3, 5, and 7.

## RESULTS

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**Nominal concentrations:** Negative control, 11, 22, 43.9, 87.9, 176, and 351 mg/L plus 351 mg/L abiotic control.

**Measured concentrations:** <LOQ, 7.74, 15.1, 31.9, 62.5, 147, 230 mg/L; abiotic control = 231 mg/L

### Element value and 95% confidence interval (based on frond number):

3-day IC<sub>10</sub>: 101 mg/L (C.I. not calculable)

3-day IC<sub>50</sub>: > 230 mg/L (C.I. not calculable)

3-day IC<sub>90</sub>: > 230 mg/L (C.I. not calculable)

5-day IC<sub>10</sub>: 30.7 mg/L (13.3 – 142 mg/L)

5-day IC<sub>50</sub>: 182 mg/L (89.1 – 240 mg/L)

5-day IC<sub>90</sub>: > 230 mg/L (C.I. not calculable)

7-day IC<sub>10</sub>: 22.1 mg/L (13.3 – 26.0 mg/L)

7-day IC<sub>50</sub>: 108 mg/L (45.7 – 144 mg/L)

7-day IC<sub>90</sub>: > 230 mg/L (C.I. not calculable)

7-day NOAEC (number of fronds): 15.1 mg/L

All element values based on mean measured concentrations

**Statistical methods:** Mean plant and frond numbers, percent inhibition values and the percentages of necrotic, chlorotic and dead fronds were calculated using “Microsoft Excel Version 5.0”, while statistical analyses were conducted using “TOXSTAT Version 3.5”. Percent inhibition values were calculated for each treatment group as the percent reduction in mean frond number relative to mean frond number in the control replicates. The IC<sub>10</sub>, IC<sub>50</sub>, and IC<sub>90</sub> values and 95% confidence intervals were determined, when possible, using linear interpolation with frond number and exposure concentration data. The percentages of dead, chlorotic and necrotic fronds also were calculated relative to the total number of fronds in each test chamber. The frond number data was evaluated for normality and homogeneity of variances ( $p = 0.05$ )

using the Shapiro-Wilks' and Levene's tests, respectively. The data were normally distributed and the variances were homogeneous, thus statistically significant differences between the control and treatment groups were identified using ANOVA and Dunnett's test. Results of the statistical analyses, as well as an evaluation of the concentration-response pattern and other observations of effects were used in the determination of the no -observed-adverse-effect-concentration (NOAEC).

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were centrifuged as necessary prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Samples collected at test initiation had measured values from 64.2 to 82.6% of nominal. Measured values for samples taken at Day 3 ranged from 67.3 to 83.3% of nominal. Measured values for samples taken at Day 5 ranged from 65.4 to 85.4% of nominal. Samples collected at test termination (Day 7) ranged from 63.9 to 83.8% of nominal. For the abiotic controls, measured values for samples taken at Day 3, Day 5, and Day 7 ranged from 64.2 – 66.9% of nominal.

#### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured Values at Days 0, 3, 5, and 7, Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 11                               | 7.57, 8.35, 7.47, 7.55                                     | 7.74                              | 70                 |
| 22                               | 15.2, 15.4, 14.6, 15.2                                     | 15.1                              | 69                 |
| 43.9                             | 32.2, 31.9, 31.8, 31.7                                     | 31.9                              | 73                 |
| 87.9                             | 63.5, 63.1, 61.5, 61.8                                     | 62.5                              | 71                 |
| 176                              | 145, 146, 150, 147   | 147                               | 84                 |
| 351                              | 226, 237, 232, 224   | 230                               | 66                 |
| 351 (abiotic)                    | not analyzed, 225, 235, 232                                | 231                               | 66                 |

#### Biological observations after 7-Days:

##### Counts

| Mean Measured Concentration, mg/L | Mean Number of Plants | Mean Number of Fronds | Percent Inhibition via Frond Number |
|-----------------------------------|-----------------------|-----------------------|-------------------------------------|
| Negative Control                  | 19                    | 197                   | -                                   |
| 7.74                              | 18                    | 177                   | 10                                  |
| 15.1                              | 20                    | 219                   | -11                                 |
| 31.9                              | 14                    | 151*                  | 24                                  |
| 62.5                              | 11                    | 134*                  | 32                                  |
| 147                               | 15                    | 69*                   | 65                                  |
| 230                               | 17                    | 37*                   | 81                                  |

\*Statistically significant difference ( $p < 0.05$ ) from the negative control using ANOVA and Dunnett's Test.

##### Effects

| Mean Measured Concentration, mg/L | Mean Dead Fronds, % | Mean Chlorotic Fronds, % | Mean Necrotic Fronds, % |
|-----------------------------------|---------------------|--------------------------|-------------------------|
| Negative Control                  | 0                   | 0                        | 0                       |

|      |     |     |      |
|------|-----|-----|------|
| 7.74 | 0   | 0   | 0    |
| 15.1 | 0   | 1.1 | 0    |
| 31.9 | 0   | 0   | 0.23 |
| 62.5 | 0   | 0.9 | 0.61 |
| 147  | 1.0 | 11  | 4.5  |
| 230  | 3.8 | 9.4 | 19   |

**Control response:** satisfactory. Plants appeared healthy and exhibited normal growth throughout the test with the exception of one necrotic frond observed on Day 3 and Day 5 of the test.

**Observations:** Duckweed exposed to 147 and 230 mg PFOS/L exhibited a dose-responsive increase in the incidence of dead, chlorotic or necrotic fronds during the test. By Day 7, all treatment groups > 31.9 gm/L showed evidence of sublethal effects, including root destruction and/or a cupping of the plant downward on the water surface.

#### CONCLUSIONS

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The potassium perfluorooctanesulfonate 7-Day IC<sub>50</sub> and 95% confidence interval for duckweed was determined to be 108 (45.7 – 144) mg/L. The 7-Day NOAEC, based on the inhibition of frond production and evidence of sub-lethal effects, was 15.1 mg/L.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

#### DATA QUALITY

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**Reliability:** Klimisch ranking = 1

#### REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

#### OTHER

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**Last changed:** 7/19/01

**Robust Study Report Reference No. 38 - PFOS: A 96-hour toxicity test with freshwater diatom (*Navicula pelliculosa*)**TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample obtained from 3M production lot number 217. The test substance is a white powder. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.5400

**Test:** Acute static

**GLP:** Yes

**Year completed:** 2001

**Species:** *Navicula pelliculosa*

**Source:** Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD

**Analytical monitoring:** PFOS measured at 0, 72, 96-hours

**Element basis:** Reported three ways: number of cells/ml, area under the growth curve and growth rate

**Exposure period:** 96-hours

**Start date:** 2/25/00

**End date:** 2/29/00

**Test organisms laboratory culture:** Algae cultures had been actively growing in freshwater algal culture medium with silica and selenium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

**Test Conditions:****Growth medium**

| Compound  | Nominal Concentration | Units |
|---|-----------------------|-------|
| MgCl <sub>2</sub> ·6H <sub>2</sub> O                | 12.16                 | mg/L  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O                | 4.40                  | mg/L  |
| H <sub>3</sub> BO <sub>3</sub>                      | 0.1856                | mg/L  |
| MnCl <sub>2</sub> ·4H <sub>2</sub> O                | 0.416                 | mg/L  |
| ZnCl <sub>2</sub>                                   | 3.28                  | µg/L  |
| FeCl <sub>3</sub> ·6H <sub>2</sub> O                | 0.1598                | mg/L  |
| CoCl <sub>2</sub> ·6H <sub>2</sub> O                | 1.428                 | µg/L  |
| Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O | 7.26                  | µg/L  |
| CuCl <sub>2</sub> ·2H <sub>2</sub> O                | 0.012                 | µg/L  |
| Na <sub>2</sub> EDTA·2H <sub>2</sub> O              | 0.300                 | mg/L  |
| NaNO <sub>3</sub>                                   | 25.50                 | mg/L  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O                | 14.70                 | mg/L  |
| K <sub>2</sub> HPO <sub>4</sub>                     | 1.044                 | mg/L  |
| NaHCO <sub>3</sub>                                  | 15.0                  | mg/L  |
| Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O | 20.0                  | mg/L  |
| Na <sub>2</sub> SeO <sub>3</sub> ·5H <sub>2</sub> O | 0.010                 | mg/L  |



**Dilution water source:** Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 + 0.1 using 10% HCl and 0.1 N NaOH. The medium was sterilized by filtration (0.22µm) prior to use.

**Test solution preparation:** A primary stock solution was not prepared for this study. Individual test solutions were prepared in algal medium at each of the seven nominal concentrations. The individual test solutions were stirred with a magnetic stir plate for approximately 24 hours. All final test solutions appeared clear and colorless.

**Exposure vessels:** Sterile 250 mL plastic Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

**Agitation:** Shaken continuously at ~100 rpm

**Number of replicates:** three.

**Initial algal cell loading:**  $1.0 \times 10^4$  cells/mL

**Number of concentrations:** seven plus a negative control plus an abiotic control at the highest concentration tested

**Water chemistry: pH range (0 – 96 hours)**

7.5 – 8.6 (control exposure)

7.5 – 7.7 (335 mg/L exposure)

**Test temperature range (0 – 96 hours)**

23.1 – 24.6°C

**Light levels: (0 – 96 hours)**

3910 – 4510 lux from continuous cool-white fluorescent lighting

**Method of calculating mean measured concentrations:** arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

## RESULTS

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**Nominal concentrations:** Negative control, 61.5, 81.3, 110, 147, 198, 264, 347 mg/L plus 347 mg/L abiotic control.

**Measured concentrations:** <LOQ, 62.3, 83.2, 111, 150, 206, 266, 335 mg/L; abiotic control = 339 mg/L

### Element value (95% confidence interval):

24-hour EC<sub>50</sub> (cell density) = 281 (214 - 312) mg/L

24-hour EbC<sub>50</sub> (area under curve) = 262 (205 - 308) mg/L

24-hour ErC<sub>50</sub> (growth rate) = 279 (212 - 306) mg/L

48-hour EC<sub>50</sub> (cell density) = 261 (219 - 306) mg/L

48-hour EbC<sub>50</sub> (area under curve) = 259 (227 - 303) mg/L

48-hour ErC<sub>50</sub> (growth rate) = 294 (271 - 307) mg/L

72-hour EC<sub>10</sub> (cell density) = <62.3 (C.I. not calculable) mg/L

72-hour EbC<sub>10</sub> (area under curve) = <62.3 (C.I. not calculable) mg/L

72-hour ErC<sub>10</sub> (growth rate) = 221 (190 - 252) mg/L

72-hour EC<sub>50</sub> (cell density) = 242 (200 - 276) mg/L

72-hour EbC<sub>50</sub> (area under curve) = 246 (210 - 277) mg/L

72-hour ErC<sub>50</sub> (growth rate) = 295 (288 - 305) mg/L

72-hour EC<sub>90</sub> (cell density) = 317 (306 - 326) mg/L

72-hour EbC<sub>90</sub> (area under curve) = 318 (307 - 325) mg/L

72-hour ErC<sub>90</sub> (growth rate) = 335 (323 - 335) mg/L

96-hour EC<sub>10</sub> (cell density) = <62.3 (C.I. not calculable) mg/L

96-hour EbC<sub>10</sub> (area under curve) = <62.3 (C.I. not calculable) mg/L

96-hour ErC<sub>10</sub> (growth rate) = 243 (209 - 295) mg/L

96-hour EC<sub>50</sub> (cell density) = 263 (217 - 299) mg/L

96-hour EbC<sub>50</sub> (area under curve) = 252 (220 - 285) mg/L  
 96-hour ErC<sub>50</sub> (growth rate) = 305 (295 - 316) mg/L  
 96-hour EC<sub>90</sub> (cell density) = 322 (310 - 328) mg/L  
 96-hour EbC<sub>90</sub> (area under curve) = 319 (308 - 326) mg/L  
 96-hour ErC<sub>90</sub> (growth rate) = >335 mg/L (C.I. not calculable)  
 72-hour NOAEC (cell density, area under the curve): <62.3 mg/L  
 72-hour NOAEC (growth rate): 206 mg/L  
 96-hour NOAEC (cell density): 150 mg/L  
 96-hour NOAEC (area under the curve): <62.3 mg/L  
 96-hour NOAEC (growth rate): 206 mg/L

All element values based on mean measured concentrations

**Statistical methods:** Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC<sub>10</sub>, EC<sub>50</sub>, and EC<sub>90</sub> values and 95% confidence limits. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilkes’s test and Levene’s test, respectively. The treatment groups were then compared to the control using Dunnett’s test. Results of the statistical analyses were used to determine the NOAEC values.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 106% of nominal. Measured values for samples taken at 72 hours ranged from 98.5 to 106% of nominal. Measured values for samples taken at 96 hours ranged from 94.8 to 101% of nominal. For the abiotic controls, the measured value for the sample taken at 72 hours was 98.2% of nominal and for the sample taken at 96 hours, 96.8% of nominal.

**Summary of analytical chemistry data:**

| Nominal Test Concentration, mg/L | Measured Values at 0, 72, and 96-hours, Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 61.5                             | 62.3, 63.6, 61.1   | 62.3                              | 101                |
| 81.3                             | 83.8, 84.7, 81.0   | 83.2                              | 102                |
| 110                              | 109, 113, 110  | 111                               | 101                |
| 147                              | 147, 154, 149  | 150                               | 102                |
| 198                              | 209, 209, 199  | 206                               | 104                |
| 264                              | 271, 268, 258  | 266                               | 101                |
| 347                              | 334, 342, 329  | 335                               | 96.5               |
| 347 (abiotic)                    | Not analyzed, 341, 336                                     | 339                               | 97.7               |

**Biological observations after 96-hours:**

| Mean Measured Concentration, mg/L | Mean Number of Cells per mL | Percent Inhibition via Density | Percent Inhibition via Area Under the Curve | Percent Inhibition via Growth Rate |
|-----------------------------------|-----------------------------|--------------------------------|---|------------------------------------|
| Negative Control                  | 2,726,667                   | -                              | -   | -                                  |
| 62.3                              | 2,366,667                   | 13                             | 24*   | 2.5                                |
| 83.2                              | 2,366,667                   | 13                             | 22*   | 2.7                                |
| 111                               | 2,373,333                   | 13                             | 24*   | 2.5                                |
| 150                               | 2,473,333                   | 9.3                            | 16  | 1.7                                |
| 206                               | 2,093,333                   | 23*                            | 24*   | 4.7                                |
| 266                               | 1,330,000                   | 51*                            | 58*   | 13*                                |
| 335                               | 35,333                      | 99*                            | 99*   | 79*                                |

\*Indicates a significant difference from the negative control using Dunnett's test ( $p < 0.05$ )

**Control response:** satisfactory

**Observations:** After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative control, although at 72 and 96 hours of exposure a few cells in the 335 mg/L treatment group appeared small in comparison to the control.

**Reversibility of Growth Inhibition:** The 335 mg/L treatment group was maximally inhibited after 96-hours. The treatment group was diluted to a concentration of the test substance that would not inhibit growth and exposed for 7 days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

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**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour  $EC_{50}$  and 95% confidence interval for *Navicula pelliculosa* was determined using three calculation methods. By cell density, it was 263 (217 - 299) mg/L, by area under the growth curve it was 252 (220 - 285) mg/L and by growth rate 305 (295 - 316) mg/L. The 96-hour NOAEC was determined by Dunnett's procedure ( $p < 0.05$ ) to be 150 mg/L using cell density, <62.3 mg/L when using area under the curve and 206 mg/L by growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

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**DATA QUALITY**

**Reliability:** Klimisch ranking = 1

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**REFERENCES**

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

ENV/JM/RD(2002)17

OTHER

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**Last changed:** 6/19/01

**Robust Study Report Reference No. 39 - PFOS: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)**

TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** OPPTS 850.5400

**Test:** Acute static

**GLP:** Yes

**Year completed:** 2001

**Species:** *Skeletonema costatum*

**Source:** Originally from The Culture Collection of Algae and Protozoa, Dunstaffnage marine Laboratory at Oban Argyll, Scotland, and maintained in culture medium at Wildlife International Ltd., Easton, MD

**Analytical monitoring:** PFOS measured at 0, 72, 96-hours

**Element basis:** Reported three ways: number of cells/ml, area under the growth curve and growth rate

**Exposure period:** 96-hours

**Start date:** 5/19/00

**End date:** 5/23/00

**Analytical monitoring:** Test concentrations measured at 0, 72, and 96-hours.

**Test organisms laboratory culture:** Algae cultures had been actively growing in saltwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water. Solutions were then diluted in artificial saltwater to prepare final growth media.

**Test Conditions:**

**Algal saltwater medium**

| Compound  | Nominal Concentration | Units |
|---|-----------------------|-------|
| FeCl <sub>3</sub> ·6H <sub>2</sub> O                | 0.72                  | mg/L  |
| MnCl <sub>2</sub> ·4H <sub>2</sub> O                | 2.16                  | mg/L  |
| ZnSO <sub>4</sub> ·7H <sub>2</sub> O                | 0.675                 | mg/L  |
| CuSO <sub>4</sub> ·5H <sub>2</sub> O                | 2.36                  | µg/L  |
| CoCl <sub>2</sub> ·6H <sub>2</sub> O                | 6.06                  | µg/L  |
| H <sub>3</sub> BO <sub>3</sub>                      | 17.1                  | mg/L  |
| Na <sub>2</sub> EDTA·2H <sub>2</sub> O              | 15.0                  | mg/L  |
| K <sub>3</sub> PO <sub>4</sub>                      | 3.0                   | mg/L  |
| NaNO <sub>3</sub>                                   | 50.0                  | mg/L  |
| Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O | 20.0                  | mg/L  |
| Thiamine Hydrochloride                              | 0.25                  | mg/L  |
| Biotin  | 0.05                  | µg/L  |
| B12   | 0.5                   | µg/L  |

**Dilution water source:** The stock nutrient solutions were prepared by adding the appropriate volumes reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The algal medium was prepared by adding appropriate volumes of the stock nutrient solutions to artificial saltwater at 30 ppt salinity. The pH of the medium was 8.1 and it was sterilized by filtration (0.22µm) prior to use.

**Test solution preparation:** A single test solution (3.46 mg/L) was prepared for this study in algal saltwater medium. The solution was sonicated for approximately 30 minutes and was stirred with a magnetic stir plate for approximately 43 hours. The final test solution appeared clear and colorless.

**Exposure vessels:** Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

**Agitation:** Shaken continuously at 100 rpm

**Number of replicates:** six.

**Initial algal cell loading:**  $7.7 \times 10^4$  cells/mL

**Number of concentrations:** one plus a negative control plus an abiotic control at the highest concentration tested

**Water chemistry: pH range (0 – 96 hours)**

8.0 – 8.4 (control exposure)

8.0 – 8.4 (3.20 mg/L exposure)

**Test temperature range (0 – 96 hours)**

20.2 – 21.4°C

**Light levels: (0 – 96 hours)**

3880 – 4710 lux from cool-white fluorescent lighting

**Photoperiod:** 14-hours light and 10 hours dark

**Method of calculating mean measured concentrations:** arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

## RESULTS

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**Nominal concentrations:** Negative control, 3.46 mg/L plus 3.46 mg/L abiotic control. This is apparently the highest concentration of PFOS attainable in this saltwater algal media.

**Measured concentrations:** <LOQ, 3.20 mg/L; abiotic control = 3.18 mg/L

**Element value (95% confidence interval):**

72 and 96-hour EC<sub>10</sub> via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

24, 48, 72, and 96-hour EC<sub>50</sub> via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72 and 96-hour EC<sub>90</sub> via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72-hour NOAEC (cell density, area under the curve, growth rate): 3.20 mg/L

96-hour NOAEC (cell density, area under the curve, growth rate): 3.20 mg/L

**All element values based on mean measured concentrations**

**Statistical methods:** Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. The EC<sub>10</sub>, EC<sub>50</sub>, and EC<sub>90</sub> values and 95% confidence limits could not be calculated using statistical methods. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality using the Shapiro-Wilk’s test and for equality of variance using an F-test. The treatment groups were then compared to the control using ANOVA and a 2-sample t-test.

Results of the statistical analyses were used to determine the NOAEC values.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). The 72 and 96-hour samples were centrifuged approximately 10 minutes at approximately 2000 rpm prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.480 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 88.5% of nominal. The measured value for the sample taken at 72 hours was 92.2% of nominal. The measured value for the sample taken at 96 hours was 91.2% of nominal. For the abiotic control, the measured value for the sample taken at 72 hours was 97.1% of nominal and for the sample taken at 96 hours, 86.8% of nominal.

**Summary of analytical chemistry data:**

| Nominal Test Concentration, mg/L | Measured Values at 0, 72, and 96-hours, Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 3.46                             | 3.26, 3.19, 3.15   | 3.20                              | 92.5               |
| 3.46 (abiotic)                   | Not analyzed, 3.36, 3.00                                   | 3.18                              | 91.9               |

**Biological observations after 96-hours:**

| Mean Measured Concentration, mg/L | Mean Number of Cells per mL | Percent Inhibition via Density | Percent Inhibition via Area Under the Curve | Percent Inhibition via Growth Rate |
|-----------------------------------|-----------------------------|--------------------------------|---|------------------------------------|
| Negative Control                  | 2,481,667                   | -                              | -   | -                                  |
| 3.20                              | 2,601,667                   | -4.8                           | -7.3  | -1.3                               |

**Control response:** satisfactory

**Observations:** After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the treatment group. However there were signs of adherence to the test chamber in the negative control group. There were no noticeable changes in cell morphology when compared to the negative control.

**Reversibility of Growth Inhibition:** After 96-hours of exposure, there was no significant inhibition of growth in the highest concentration tested (3.20 mg/L). Therefore, a recovery phase was not conducted.

**CONCLUSIONS**

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A single concentration of potassium perfluorooctanesulfonate was evaluated for toxicity to *Skeletonema costatum*. This mean measured concentration, 3.20 mg/L, was the highest concentration attainable in this algal media. The 96-hour EC<sub>50</sub> and 95% confidence interval for *Skeletonema costatum*, as determined by cell density, area under the growth curve, and by growth rate was found to be > 3.20 mg/L. The 96-hour NOAEC was determined by ANOVA and a 2-sample t-test to be 3.20 mg/L calculated using cell density, area under the curve and growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls.

ENV/JM/RD(2002)17

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

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**Reliability:** Klimisch ranking = 1

REFERENCES

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This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

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**Last changed:** 7/19/01



**Robust Study Report Reference No. 40 - PFOS: A frog embryo teratogenesis assay – *Xenopus* (FETAX)**TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS, U2723 or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample obtained from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-HMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD

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**Method:** ASTM E1439-91

**Test type:** Static renewal

**GLP:** In-life phase – no; stock solution preparation and measurement of test concentrations - yes

**Year completed:** 2001

**Number of studies:** 3

|                    | <b>Study 1</b> | <b>Study 2</b> | <b>Study 3</b> |
|--------------------|----------------|----------------|----------------|
| <b>Start date:</b> | 5/15/00        | 5/22/00        | 5/22/00        |
| <b>End date:</b>   | 5/19/00        | 5/26/00        | 5/26/00        |

(Study 2 and 3 set up concurrently with common stock solutions)

**Analytical monitoring:** PFOS measured at 0 and 96-hours

**Species:** *Xenopus laevis*

**Source:** Breeding colonies at the University of Maryland Wye Research and Education Center (UMD/WREC), Queenstown, Maryland.

**Test organisms laboratory culture:** Mating pairs were bred in the dark in 23.5 + 0.5°C UMD/WREC non-chlorinated well water at ~ 70 day intervals by injecting 400 and 800 I. U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4 -6 hours after injecting HCG; egg deposition occurred 9-12 hours following HCG injection.

**Age at test initiation:** Embryos; normal stage 8 blastula to normal stage 11 gastrula

**Loading:** 25 embryos/10 mL

**Pretreatment:** Embryos de-jelled in a 2% L -cysteine solution, then rinsed and re-suspended in FETAX solution prior to introduction to test chambers.

**Element basis:** mortality, malformations (via the atlas of Bantle et al., 1991), growth

**Exposure period:** 96-hours

**Test Conditions (all 3 studies):**

Dilution water: ASTM (1998) FETAX solution

**Test temperature:** 24.0 + 0.2°C

**Light levels:** 60-85 foot candle fluorescent lights

**Photoperiod:** 12-hour light: 12-hour dark

**Stock and test solution preparation:** A primary stock solution was prepared in FETAX medium (supplied by UM-WREC) by Wildlife International, Ltd. at 48 mg PFOS/L. The primary stock solution was mixed by sonication and stirring. After mixing, the primary stock solution was proportionally diluted with FETAX medium to prepare the six test concentrations. The six test concentration solutions were delivered to UM-WREC prior to the start of each study.

**Reference substance:** 6-aminonicotinamide

**Stock and reference substance solution preparation:** as outlined in the ASTM (1998) protocol

**Exposure vessels:** Covered 60 mm glass Petri dishes containing 10 mL test solution

**Number of replicates:** controls – 4, treatments – 2

**Number of embryos per replicate:** 25

**Number of concentrations:** six plus a negative control plus an abiotic control at the highest concentration tested, plus two reference substance concentrations.

**Renewal frequency:** every 24 hours

**Stability of the test chemical solutions:** Extremely stable

**Water chemistry during all 3 studies:**

**pH range** (0 – 96 hours)

7.1 – 7.7 (control exposure)

7.0 – 7.6 (24 mg/L nominal exposure)

**Dissolved oxygen range** (0-96 hours)

7.3 – 8.4 mg/L (control exposure)

7.0 – 8.5 mg/L (24 mg/L nominal exposure)

**Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

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**Nominal PFOS concentrations:** Negative control, 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg/L plus 24.0 mg/L abiotic control.

**Nominal 6-aminonicotinamide concentrations:** 5.5 and 2500 mg/L

**Mean measured PFOS concentrations:**

**Study 1:** <LOQ, 2.00, 2.83, 4.73, 7.90, 14.7, 24.6 mg/L;

abiotic control = 23.7 mg/L

**Study 2:** < LOQ, 1.91, 3.04, 4.82, 7.97, 13.3, 23.1 mg/L;

abiotic control = 23.9 mg/L

**Study 3:** < LOQ, 1.93, 3.27, 5.25, 8.26, 14.0, 23.9 mg/L;

abiotic control = 24.1 mg/L

### PFOS element values and 95% confidence intervals, mg/L

| Study Number | 96-Hr LC <sub>50</sub> | 96-Hr EC <sub>50</sub> | Minimum conc. to Inhibit Growth (MCIG) | Teratogenic Index (TI) |
|--------------|------------------------|------------------------|--|------------------------|
| 1            | 13.8 (12.4 - 15.3)     | 12.1 (10.0 – 14.6)     | Not calculable                         | 1.1                    |
| 2            | 17.6 (15.5 – 20.0)     | 17.6 (13.5 – 22.9)     | 7.97                                   | 1.0                    |
| 3            | 15.3 (13.1 – 17.8)     | 16.8 (12.4 – 22.8)     | 8.26                                   | 0.9                    |

### All element values based on mean measured concentrations

**Statistical methods:** The Trimmed Spearman-Kärber statistical procedure was used to determine the 96-hour LC<sub>50</sub> for mortality and 96-hour EC<sub>50</sub> for malformations. The MCIG was determined by Bonferroni's T-Test. All statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used. The teratogenic index (TI) was calculated by dividing the LC<sub>50</sub> by the EC<sub>50</sub>.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd., Easton, MD using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the

basis of individual isomeric components. The LOQ (limit of quantitation) was 0.240 mg/L in these studies. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.2%. Samples collected at test initiation had measured values from 112 to 141% of nominal in the first study, and in the second and third studies, from 95.8 to 117% of nominal. Measured values for samples taken at 96-hours ranged from 54.7 to 98.6% of nominal in the first study and 80.7 to 112% of nominal in the second and third studies. The samples from the abiotic 24.0 mg/L treatment group was comparable to samples from the 24.0 mg/L treatment group with the embryos present.

### Summary of analytical chemistry data:

#### Study 1

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 1.82                             | 2.58, 1.42   | 2.00                              | 110                |
| 3.07                             | 3.94, 1.72   | 2.83                              | 92.2               |
| 5.19                             | 6.62, 2.84   | 4.73                              | 91.1               |
| 8.64                             | 10.7, 5.09   | 7.90                              | 91.4               |
| 14.4                             | 18.5, 10.8   | 14.7                              | 102                |
| 24.0                             | 26.9, 22.3   | 24.6                              | 103                |
| 24.0 (abiotic)                   | not analyzed, 23.7                                   | 23.7                              | 98.6               |

#### Study 2

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 1.82                             | 1.77, 2.04   | 1.91                              | 105                |
| 3.07                             | 3.59, 2.49   | 3.04                              | 99.0               |
| 5.19                             | 5.45, 4.18   | 4.82                              | 92.9               |
| 8.64                             | 8.43, 7.51   | 7.97                              | 92.2               |
| 14.4                             | 14.5, 12.1   | 13.3                              | 92.4               |
| 24.0                             | 23.0, 23.1   | 23.1                              | 96.3               |
| 24.0 (abiotic)                   | not analyzed, 23.9                                   | 23.9                              | 99.6               |

#### Study 3

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              |                    |
| 1.82                             | 1.77, 2.08   | 1.93                              | 106                |
| 3.07                             | 3.59, 2.94   | 3.27                              | 107                |
| 5.19                             | 5.45, 5.05   | 5.25                              | 101                |
| 8.64                             | 8.43, 8.09   | 8.26                              | 95.6               |
| 14.4                             | 14.5, 13.5   | 14.0                              | 97.2               |
| 24.0                             | 23.0, 24.7   | 23.9                              | 99.6               |
| 24.0 (abiotic)                   | not analyzed, 24.1                                   | 24.1                              | 100                |

**Biological observations after 96-hours:****Mortality and Malformations**

| Nominal Concentration*, mg/L | Test 1            |                       | Test 2            |                       | Test 3            |                       |
|------------------------------|-------------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|
|                              | Percent Mortality | Percent Malformations | Percent Mortality | Percent Malformations | Percent Mortality | Percent Malformations |
| Negative Control             | 1.0               | 4.0                   | 1.0               | 4.0                   | 0                 | 2.0                   |
| 1.82                         | 2.0               | 8.2                   | 0                 | 8.0                   | 0                 | 6.0                   |
| 3.07                         | 4.0               | 15                    | 10                | 4.4                   | 0                 | 4.0                   |
| 5.19                         | 10                | 22                    | 8.0               | 11                    | 0                 | 6.0                   |
| 8.64                         | 12                | 25                    | 10                | 20                    | 14                | 14                    |
| 14.4                         | 38                | 65                    | 30                | 37                    | 44                | 39                    |
| 24.0                         | 100               | -                     | 70                | 67                    | 78                | 73                    |

\*Nominal concentrations used for ease of comparison table

**Malformations:** The most common types of malformations noted were improper gut coiling, edema, notochord abnormalities and facial abnormalities.

**Growth – Mean length (mm) after 96-hours Exposure**

| Nominal Concentration*, mg/L | Test 1              | Test 2 | Test 3 |
|------------------------------|---------------------|--------|--------|
| Negative Control             | 8.59                | 8.88   | 9.47   |
| 1.82                         | 8.29                | 8.45   | 9.10   |
| 3.07                         | 8.80                | 8.57   | 9.28   |
| 5.19                         | 8.51                | 8.72   | 9.28   |
| 8.64                         | 8.71                | 7.93** | 8.51** |
| 14.4                         | 8.08                | 7.51** | 8.11** |
| 24.0                         | - (total mortality) | 7.39** | 7.80** |

\*Nominal concentrations used for ease of comparison table \*\*Significantly different at alpha = 0.05 (Bonferroni T-Test)

**Control response:** satisfactory.

**Reference substance response:** satisfactory at low concentration (5.5 mg/L). Did not meet ASTM (1998) criteria for high concentration (2,500 mg/L). However, results obtained at high concentration were consistent and not at variance with previous experience in this testing laboratory.

**Observations:** Majority of embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest test concentrations.

**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour LC<sub>50</sub> range for FETAX was determined to be 13.8 – 17.6 mg/L. The 96-hour EC<sub>50</sub> range was 12.1– 17.6 mg/L. The range for Minimum Concentration to Inhibit Growth (MCIG) was 7.97 to >14.7 mg/L. The Teratogenic Index (TI) was found to be 0.9 – 1.1. This TI range indicates that potassium perfluorooctanesulfonate has a low potential to be a developmental hazard.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

#### DATA QUALITY

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**Reliability:** Klimisch ranking = 2

Although these were well-conducted studies, the in-life phases were not conducted in accordance with Good Laboratory Practices.

#### REFERENCES

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These studies were conducted at the University of Maryland Wye Research and Education Center (UM-WREC) in Queenstown, Maryland and at Wildlife International Ltd., Easton, MD at the request of the 3M Company. Lab Request number U2723

#### OTHER

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**Last changed:** 7/19/01

**Robust Study Report Reference No. 41 - Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill (*Lepomis macrochirus*)**

TEST SUBSTANCE

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**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analyses techniques.

METHOD:

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**Method/guideline followed:** US EPA OPPTS 850.1730 and OECD 305

**Type:** Flow-through exposure with flow-through depuration phase.

**GLP (Y/N):** Yes

**Year:** 2001

**Species:** Bluegill (*Lepomis macrochirus*)

**Supplier:** Osage Catfisheries, Inc., Osage Beach, Missouri

**Length and weight at test termination:** Mean length = 62 mm, range 56-66 mm Mean weight = 2.70 g, range 2.03 – 3.32 g

**Loading:** 0.48 g fish/L/day (based on initial loading of 90 fish per tank, using mean fish weight at the end of the study and volume of water that passed through test chamber in 24-hours).

**Fish age:** Approximately 7 months at test initiation

**Analytical monitoring:** Concentration of PFOS in water and fish.

**Pretreatment:** None

**Number of concentrations:** Two plus a negative control

**Test concentrations (mean measured):** Negative control, 0.086 and 0.87 mg/L

**Uptake period:** 62-days (0.086 mg/L exposure)  
35-days (0.87 mg/L exposure – this exposure ended after 35-days due to fish mortality)

**Depuration period:** 56-days (0.086 mg/L exposure)  
None (0.087 mg/L exposure)

**Test conditions:**

**Dilution water:** Moderately-hard well water

**Dilution water chemistry:**

**Specific conductance:** 313 (310 – 315 µmhos/cm)

**Hardness:** 130 (128 – 132 mg/L)

**Alkalinity:** 178 (176 – 178)

**pH:** 8.1 (8.0 – 8.2)

Measured during the 4 -week period immediately preceding the test.

**Stock and test solution preparation:** Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless.

**Diluter flow rate:** Approx. 6.3 volume additions per 24-hours

**Exposure vessels:** 104 L stainless steel aquaria filled with approximately 80 L solution.

**Number of replicates:** None – one vessel per concentration

**Number of fish per vessel:** 90

**Diet:** Flake food, Ziegler Brothers, Inc., Gardners, PA

**Water chemistry ranges during the study:**

|                         | Neg. Control | 0.086 mg/L  | 0.87 mg/L   |
|-------------------------|--------------|-------------|-------------|
| Dissolved oxygen, mg/L: | 6.8 – 8.6    | 6.8 – 8.6   | 6.4 – 8.2   |
| Temperature, °C:        | 21.8 – 22.0  | 21.7 – 22.0 | 21.7 – 21.9 |
| pH:                     | 7.9 – 8.2    | 7.9 – 8.2   | 7.9 – 8.2   |

**Photoperiod:** 16 hours light and 8 hours dark with a 30-minute transition period.

**Light intensity:** 278 lux at surface of the negative control vessel at test initiation

**Collection of tissue samples:** Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed and measured. Fish then rinsed with dilution water, blotted dry again and dissected into edible and non-edible tissue fractions. The fractions were individually weighed. The head, fins and viscera were considered to be non-edible tissue. The remaining tissue, including skin was considered to be edible tissue.

**Statistical methods:** Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different ( $p > 0.05$ ). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, data was transformed in an attempt to correct the data. Mean tissue concentrations were then compared using ANOVA and Dunnett's test.

The kinetic bioconcentration factor (BCFK), uptake rate ( $k_1$ ) and depuration rate ( $k_2$ ) were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L PFOS using BIOFAC computer software. BIOFAC is a nonlinear parameter estimate routine which estimates rate constants from a set of sequential time-concentration data. These rate constants were then used to calculate a BCFK ( $BCFK = K / K_{12}$ ).

## RESULTS

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**Nominal concentrations:** Negative control, 0.1 and 1.0 mg/L

**Mean measured concentrations:** < 0.05, 0.086 and 0.87 mg/L

**Bioconcentration factors (BCF):**

**0.086 mg/L apparent steady-state BCF**

| Edible | Non-edible | Whole Fish |
|--------|------------|------------|
| 484    | 1124       | 856        |

**0.87 mg/L (study ended prior to achieving steady-state) BCF:**

| Edible | Non-edible | Whole Fish |
|--------|------------|------------|
| 136    | 386        | 278        |

**BIOFAC Estimates (using 0.086 mg/L exposure)**

|                              | Edible   | Non-edible | Whole Fish |
|------------------------------|----------|------------|------------|
| BCFK:                        | 1866     | 4312       | 3614       |
| Time to reach 50% clearance: | 146 days | 133 days   | 152 days   |

**PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L**

Values are from 4 individual fish at each sample period.

| Uptake Day     | Edible Tissue, mg/kg       | Non-edible Tissue, mg/kg   | Whole Fish Conc., mg/kg    |
|----------------|----------------------------|----------------------------|----------------------------|
| 0 (4-hours)    | 0.167, 0.155, 0.144, 0.182 | 0.415, 0.519, 0.417, 0.497 | 0.293, 0.351, 0.286, 0.363 |
| 1              | 0.734, 0.726, 0.631, 0.806 | 1.68, 1.85, 1.72, 2.07     | 1.26, 1.34, 1.29, 1.53     |
| 3              | 1.73, 2.07, 2.03, 2.11     | 4.59, 5.50, 5.47, 5.97     | 3.21, 4.04, 4.18, 4.38     |
| 7              | 3.73, 4.25, 4.73, 6.25     | 10.2, 10.6, 11.9, 15.2     | 7.33, 7.66, 8.73, 11.4     |
| 14             | 11.4, 9.07, 13.7, 12.6     | 27.3, 23.2, 35.3, 32.6     | 20.2, 16.9, 26.0, 24.6     |
| 21             | 11.7, 12.0, 12.9, 10.6     | 33.3, 22.7, 24.6, 24.4     | 23.3, 18.4, 19.8, 18.5     |
| 28             | 18.3, 13.7, 23.9, 23.1     | 49.4, 40.7, 65.3, 57.9     | 35.3, 29.2, 45.4, 44.1     |
| 35             | 22.6, 27.7, 23.8, 20.6     | 67.1, 73.3, 62.0, 59.1     | 46.3, 53.8, 46.6, 40.9     |
| 42             | 27.6, 25.3, 21.2, 27.6     | 64.0, 68.1, 54.4, 79.6     | 50.1, 49.4, 40.9, 56.3     |
| 49             | 33.3, 36.2, 39.0, 30.6     | 85.0, 95.1, 93.1, 77.7     | 62.8, 69.6, 70.8, 57.4     |
| 56             | 48.3, 38.9, 44.1, 38.3     | 122, 94.2, 73.2, 106       | 90.6, 71.6, 63.3, 74.8     |
| 62             | 42.4, 66.2, 42.2, 39.2     | 101, 112, 105, 96.4        | 77.0, 92.7, 79.6, 73.1     |
| Depuration Day |                            |                            |                            |
| 14             | 48.5, 31.8, 31.6, 42.0     | 124, 79.4, 81.8, 113       | 90.3, 60.4, 61.6, 85.3     |
| 28             | 26.0, 33.3, 38.7, 55.8     | 85.7, 95.1, 85.7, 94.8     | 58.2, 70.1, 68.1, 81.1     |
| 42             | 24.1, 31.2, 30.0, 33.0     | 71.7, 80.6, 78.3, 82.1     | 51.4, 61.4, 61.0, 62.2     |
| 56             | 21.1, 37.6, 32.9, 31.2     | 57.7, 80.3, 85.4, 84.4     | 41.6, 66.5, 65.8, 62.1     |

**PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L**

Values are from 4 individual fish at each sample period.

| Uptake Day        | Edible Tissue, mg/kg   | Non-edible Tissue, mg/kg | Whole Fish Conc., mg/kg |
|-------------------|------------------------|--------------------------|-------------------------|
| 0 (4-hours)       | 1.46, 1.48, 1.19, 1.39 | 3.52, 4.37, 4.22, 4.06   | 2.71, 3.08, 2.84, 2.89  |
| 1                 | 4.68, 6.59, 5.56, 5.64 | 11.1, 14.2, 13.3, 12.1   | 8.00, 10.9, 10.2, 9.47  |
| 3                 | 17.3, 15.8, 19.0, 20.8 | 39.3, 42.0, 43.8, 51.8   | 30.5, 30.7, 34.5, 39.1  |
| 7                 | 42.0, 44.0, 57.7, 46.8 | 100, 102, 102, 120       | 74.9, 77.0, 85.3, 89.8  |
| 14                | 87.1, 81.6, 90.7, 73.3 | 177, 207, 245, 214       | 141, 157, 180, 158      |
| 21                | 79.4, 117, 104, 102    | 201, 278, 246, 229       | 146, 210, 185, 172      |
| 28 <sup>(1)</sup> | 102, 131, 107, 133     | 289, 372, 320, 361       | 205, 267, 232, 263      |

<sup>(1)</sup> Sampling of fish stopped after Uptake Day 28 due to mortality.

**Test organism mortality:**

**Negative control:** None during the uptake phase (62 days) or depuration phase (35 days)

**0.086 mg/L exposure:** One fish died after 49 days and one after 59 days of exposure in the uptake phase, none during the depuration phase.

**0.87 mg/L exposure:** Mortality first noted on Day 9 and continued through Day 35 of the uptake phase at which time all of the fish had either died or had been sampled

**Analytical methodology:** Analyses of test solutions and fish tissues were performed at Wildlife



International, Ltd. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

## CONCLUSIONS

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PFOS bioconcentrated in the tissues of bluegill sunfish during this study. Apparent steady-state was attained on Day 49 for the fish exposed to 0.086 mg a.i./L. Although Day 49, 56 and 62 tissue residues were not statistically significantly different, PFOS concentrations appeared to be still increasing during this time. Apparent steady-state BCF values for edible, non-edible and whole fish tissues were calculated to be 484, 1124, and 859, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, non-edible and whole fish tissues were 146, 133 and 152 days, respectively.

## DATA QUALITY

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**Reliability:** Klimisch ranking = 1

## REFERENCES

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This study was conducted at Wildlife International, Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

## OTHER

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**Last changed:** 7/19/01

**Robust Study Report Reference No. 42 - Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) in freshwater**

TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, 1 H-HMR, 19 F-NMR and elemental analyses techniques.

METHOD

**Method:** OPPTS 850.1075 and OECD 203

**Type:** Static acute

**GLP:** Yes

**Date completed:** Study completed 2001, report completed 2002

**Species:** *Oncorhynchus mykiss*

**Supplier:** Thomas Fish Company, Anderson, CA

**Analytical monitoring:** PFOS measured at 0, 48, 96-hours

**Exposure period:** 96-hours

**Statistical methods:** LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

**Test fish age:** juveniles

**Average Total Length and weight:** 3.6 (3.4–4.0) cm, 0.34 (0.25-0.47) g

**Loading:** 0.23 g fish/L

**Pretreatment:** None

**Test conditions:**

**Dilution water:** 0.45 µm filtered moderately hard well water

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**hardness:** 130 (128-132) mg/L as CaCO<sub>3</sub>

**alkalinity:** 177 (176-178) mg/L as CaCO<sub>3</sub>

**pH:** 8.3 (8.2 – 8.4)

**TOC:** Not given

**Conductivity:** 311 (310-315) µmhos/cm

**Stock and test solution preparation:** Primary stock prepared in dilution water at 150 mg/L and mixed for ~23 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the five test concentrations.

**Concentrations dosing rate:** Once

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** 25L polyethylene aquaria containing approximately 15L of test solution; water depth approx 17.5 cm.

**Number of replicates:** two

**Number of fish per replicate:** ten

**Number of concentrations:** five plus a negative control

**Water chemistry during the study:**

**Dissolved oxygen range** (0 – 96 hours) :9.4 – 10.7 mg/L (control exposure)  
9.2 – 10.8 mg/L (50 mg/L exposure)

**pH range** (0 – 96 hours)  
8.1 – 8.4 (control exposure)  
8.2 – 8.4 (50 mg/L exposure)

**Test temperature range** (0 – 96 hours)  
12.1 – 12.6°C (control exposure)  
11.8 – 12.9°C (50 mg/L exposure)

**- Method of calculating mean measured concentrations:** arithmetic mean

**RESULTS**

**Nominal concentrations:** Bk control, 3.1, 6.3, 13, 25, 50 (exposure), 50 (abiotic) mg/L

**Measured concentrations:** <LOQ, 3.0, 6.3, 13, 25, 50, 52 mg/L

**Element value:** 24-hour LC50 = > 50 mg/L (C.I. not calculable)

48-hour LC50 = > 50 mg/L (C.I. not calculable)

72-hour LC50 = > 50 mg/L (C.I. not calculable)

96-hour LC50 = 22 (18 - 27) mg/L

All element values based on mean measured concentrations

**Statistical Evaluation of Mortality:**

Element values and confidence limits for 24, 48, and 72-hours could not be calculated due to lack of mortality. Probit Analysis was used to calculate the 96-hour LC50.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used.

No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.200 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 101. Samples collected at test initiation had measured values from 93.2 to 103% of nominal. Measured values for the biotic samples taken at 48-hours ranged from 93.6 to 103% of nominal, while abiotic samples ranged from 105 to 106% of nominal. Measured values for biotic samples taken at 96-hours ranged from 91.4 to 105% of nominal, while the abiotic samples were 102% of nominal.

**Summary of analytical chemistry data:**

| Nominal Test Concentration, Mg/L | Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|--|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ  | <LOQ                              | -                  |
| 3.1                              | 3.15, 3.02, 2.90, 3.01, 2.83, 2.97                                   | 3.0                               | 97                 |
| 6.3                              | 6.22, 6.21, 6.16, 6.43, 6.15, 6.60                                   | 6.3                               | 100                |
| 13                               | 13.2, 12.1, 12.7, 12.3, 13.1, 12.6                                   | 13                                | 100                |
| 25                               | 25.0, 25.7, 24.3, 25.7, 25.7, 26.2                                   | 25                                | 100                |
| 50                               | 49.7, 49.8, 51.1, 51.5, 49.6, 50.8                                   | 50                                | 100                |
| 50 (abiotic) (1)                 | 53.1, 52.6, 50.9, 51.0   | 52                                | 104                |

(1) Samples taken at 48 and 96-hours only

**Biological observations after 96-hours:**

Fish in the negative control and the 3.0 and 6.3 mg/L exposure concentration appeared normal with no mortalities or overt signs of toxicity. All surviving fish in the 13 and 25 mg/L exposures appeared normal with no overt signs of toxicity after 96-hours.

**Cumulative percent mortality:**

| Mean Measured Test Conc., mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|--------------------------------|----------|----------|----------|----------|
| Neg. Control                   | 0        | 0        | 0        | 0        |
| 3.0                            | 0        | 0        | 0        | 0        |
| 6.3                            | 0        | 0        | 0        | 0        |
| 13                             | 0        | 0        | 0        | 20       |
| 25                             | 0        | 0        | 0        | 50       |
| 50                             | 0        | 5        | 35       | 100      |

**Lowest concentration causing 100% mortality:** 50 mg/L

**Mortality of controls:** None

**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour LC50 for rainbow trout was determined to be 22 mg/L with a 95% confidence interval of 18 –27 mg/L. The 96-hour no mortality and no effects concentration was 6.3 mg/L.

**Submitter:** 3M, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

**Reliability:** Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of 3M.

OTHER

**Last changed:** 1/24/02

**Robust Study Report Reference No. 43 - Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*) in saltwater**

TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, 1 H-HMR, 19 F-NMR and elemental analyses techniques.

METHOD

**Method:** OPPTS 850.1075  
**Type:** Static renewal  
**GLP:** Yes  
**Date completed:** Study completed 2001, report completed 2002  
**Species:** *Cyprinodon variegatus*  
**Supplier:** Aquatic BioSystems, Inc., Fort Collins, CO  
**Analytical monitoring:** PFOS measured at initiation, prior to and after renewal at 24, 48, and 72 hours and at test termination (96-hours)  
**Exposure period:** 96-hours  
**Statistical methods:** The use of a single test concentration (at water solubility) precluded the statistical calculation of LC50 values.  
**Test fish age:** Juveniles  
**Average Total Length and weight:** 3.0 (2.4–3.5) cm, 0.44 (0.21-0.66) g  
**Loading:** 0.29 g fish/L  
**Pretreatment:** None

**Test conditions:**

**Dilution water:** Natural seawater, filtered and diluted to a salinity of approximately 20 parts per thousand with well water

**Dilution water chemistry (during the 4-week period immediately preceding the test):**

**Salinity:** 20 (20 – 20) parts per thousand  
**pH:** 8.2 (8.1 – 8.3)

**Stock and test solution preparation:**

Primary stock prepared in methanol at 40 mg/L, sonicated for approximately 20 minutes and inverted to mix prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the one test concentration. Each solution was stirred with a stainless steel whisk for approximately one minute. All test solutions appeared clear and colorless.

**Solvent:** Methanol

**Solvent concentration (treatment and solvent control groups):** 0.5 mL/L

**Concentrations dosing rate:** Daily static renewal

**Stability of the test chemical solutions:** Extremely stable

**Exposure vessels:** 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.1 cm.

**Number of replicates:** three (biotic), two (abiotic)

**Number of fish per replicate:** ten

**Number of concentrations:** One plus a negative and a solvent control, and an abiotic solution.

**Water chemistry during the study:**

|                               |  |
|-------------------------------|--|
| <b>Dissolved oxygen range</b> | (0 – 96 hours):                            |
|                               | 2.8 – 7.4 mg/L (negative control exposure) |
|                               | 1.7 – 7.6 mg/L (solvent control exposure)  |
|                               | 1.6 – 7.6 mg/L (15 mg/L exposure)          |
| <b>pH range</b>               | (0 – 96 hours)                             |
|                               | 7.9 – 8.3 (negative control exposure)      |
|                               | 7.9 – 8.3 (solvent control exposure)       |
|                               | 7.9 – 8.3 (15 mg/L exposure)               |
| <b>Test temperature range</b> | (0 – 96 hours)                             |
|                               | 21.9 – 22.6°C (negative control exposure)  |
|                               | 22.1 – 22.9°C (solvent control exposure)   |
|                               | 22.2 – 23.1°C (15 mg/L exposure)           |

- **Method of calculating mean measured concentrations:** arithmetic mean

## RESULTS

**Nominal concentrations:** Bk control, solvent control, 20 mg/L (biotic), 20 mg/L (abiotic)

**Measured concentrations:** <LOQ, <LOQ, 15, 13 mg/L

**Element value:** 24-hour LC50 = >15 mg/L (C.I. not calculable)  
 48-hour LC50 = >15 mg/L (C.I. not calculable)  
 72-hour LC50 = >15 mg/L (C.I. not calculable)  
 96-hour LC50 = >15 mg/L (C.I. not calculable)

All element values based on mean measured concentrations

### Statistical Evaluation of Mortality:

Element values and confidence interval could not be calculated due to lack of mortality.

### Analytical Methodology:

Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 5.00 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.2. Samples collected in exposure

vessels at test initiation had measured values from 78.6 to 82.0% of nominal. New samples collected at Hours 24, 48 and 72 had measured concentrations from 74.9 to 81.2 and 86.9 to 92.9, and 82.9 to 87.1% of nominal, respectively. Old samples collected at Hours 24, 48, and 72 had measured concentrations ranging from 66.6 to 76.2, 56.4 to 66.9, and 75.8 to 89.9% of nominal, respectively. Mean measured concentrations of PFOS in samples collected at test termination were 55.6 to 68.4% of nominal. The measured concentrations of PFOS from the abiotic treatment group were slightly lower than those from the exposure treatment group. This may have been due to increased deposition of test substance at the limit of solubility, which could have resulted from the absence of the natural mixing action that was provided by the movement of the fish in the exposure treatment group.

#### Summary of analytical chemistry data:

| Nominal Test Concentration, mg/L | Measured Replicate Values of old and new Test Solutions Respectively, mg/L (1)  | Mean Measured Concentration, mg/L | Percent of Nominal |
|----------------------------------|---|-----------------------------------|--------------------|
| Negative Control                 | All < LOQ   | <LOQ                              | -                  |
| Solvent Control                  | All < LOQ   | <LOQ                              | -                  |
| 20 <sup>(2)</sup>                | 16.4, 15.7, 16.0; 15.2, 13.3, 15.2;<br>16.2, 15.7, 15.0; 13.4, 11.3, 12.7;<br>17.4, 18.2, 18.6; 15.2, 18.0, 16.2;<br>17.0, 16.6, 17.4; 13.7, 11.1, 13.4 | 15                                | 75                 |
| 20 (3)<br>(abiotic)              | 10.9, 9.22; 16.1, 16.6; 9.62, 9.69;<br>16.6, 17.5; 9.01, 9.25; 17.7, 15.9;<br>9.84, 9.22  | 13                                | 65                 |

(1) Replicate samples are listed in this order: Day 0 (new), 24-hours (old), 24-hours (new), 48-hours (old), 48-hours (new), 72-hours (old), 72-hours (new), 96-hours (old)

(2) Triplicate samples

(3) Day 0 not measured, Duplicate samples

#### Biological observations after 96-hours:

Fish in the negative control and solvent control appeared healthy and normal throughout the exposure period. No mortalities appeared in the 15 mg/L treatment group during the study. However, upon transfer to the new test solution at approximately 48 and 72 hours, some fish were observed swimming erratically and turning a dark color. The fish appeared normal within approximately two hours after transfer, although one fish still appeared to be discolored at test termination.

#### Cumulative percent mortality:

| Mean Measured Test Conc., mg/L | 24-hours | 48-hours | 72-hours | 96-hours |
|--------------------------------|----------|----------|----------|----------|
| Negative Control               | 0        | 0        | 0        | 0        |
| Solvent control                | 0        | 0        | 0        | 0        |
| 15                             | 0        | 0        | 0        | 0        |

**Lowest concentration causing 100% mortality:** none – mortality limit apparently greater than solubility limit.



**Mortality of controls:** None

#### CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC50 for sheepshead minnow was determined to be >15 mg/L, the limit of solubility in this study. The 96-hour no mortality concentration was 15 mg/L and no observed effects concentration was <15 mg/L (1 fish out of 30 was discolored at 96-hours).

**Submitter:** 3M, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

#### DATA QUALITY

**Reliability:** Klimisch ranking = 1

#### REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of 3M.

#### OTHER

**Last changed:** 1/24/02

**Robust Study Report Number 44. Bioconcentration test of Salt (Na, K, Li) of perfluoroalkyl (C=4-12) sulfonic acid [This test was performed using Perfluorooctane sulfonic acid, potassium salt (Test substance number K-1520)] in carp**

TEST SUBSTANCE

**Identity:** K-1520. Perfluorooctane sulfonic acid, potassium salt. Lot number A37626B

**Remarks:** Test substance number K-1520. The test substance is a white powder. Purity determined to be 100%

METHOD:

**Method/guideline followed:** Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body” stipulated in the “Test Method for New Chemical Substance” July 13, 1974, Revised October 8, 1998, No. 5, Planning and Coordination Bureau, Environmental Agency; No.615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare: and No. 392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan), and Bioconcentration : Flow-through Fish test ( Guideline 305, June 14, 1996)” in the OECD Guidelines for Testing of Chemicals.

**Type:** Flow-through system

**GLP (Y/N):** Yes

**Year:** October 16- 20, 2000

**Species:** Carp (*Cyprinus carpio*)

**Supplier:** Kishida Chemical Co., Ltd. Fukuokaken yabegawa fisherman’s cooperative association 193-1 Yamauchi, Yame-shi, Fukuoka 834-0012, Japan

**Length and weight at test termination:**

Mean length = 6.4 – 9.6

Mean weight = No weight recorded

**Loading:** 2 and 20 ug/L respectively

**Fish Age:** Yearling fish

**Analytical monitoring:** High-performance liquid chromatography-Mass spectrometry

**Pretreatment:** The fish were checked visually in the receiving and those demonstrating any abnormality were removed. The fish were reared for 8 days in a flow thorough system following an external disinfection. After rearing, the fish were medicated to eliminate parasites and transferred to an acclimatizing aquarium. After the second external disinfection, they were acclimatized. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 15 days in a flow through system at temperatures of  $25 \pm 2^\circ$  C. The fish were transferred to test tanks and reared at the same temperature in the flow through system for another 27 days.

The fish were starved for 24 hours before sampling.

**Number of concentrations:** Two plus a negative control

**Test concentration:** (mean measured): Negative control, 2 and 20 ug/L

**Uptake period:** 58 days

**Depuration period:** 37 days

**Test conditions:**

**Dilution water:** Groundwater from the premises of Kurume Laboratory

**Dilution water chemistry:**

**Specific conductance:** Not recorded

**Hardness:** 111 mg/L

**Alkalinity:** 96.1

**pH:** 7.6 to 7.8

**Dissolved Oxygen:** 7.9 to 8.1

**Temperature:** 25.0 to 25.4°C

**Stock and test solution preparation:** Based on preliminary test results for the 96 hour LC50 value and analytical detection limits, test concentrations of the test substance were decided as follows. The control

was set as a blank test. Level 1 was 20 ug/L and Level 2 was 2 ug/L. The test substance was dissolved with ion-exchanged water to prepare 500 mg/L stock solution.

**Diluter flow rate:** 2 mL/min for stock solution and 1600 mL/min for dilution water; 2307 liters/day for test water were supplied.

**Exposure Vessels:** 100 liter tank

**Number of replicates:** None

**Number of fish per vessel:** 40

**Diet:** Nippon Formula Feed Mfg. Co., Ltd.

#### Water chemistry ranges during the study:

|                   | Neg. Control    | 2 ug/L           | 20 ug/L         |
|-------------------|-----------------|------------------|-----------------|
| Dissolved Oxygen: | 80.0 – 8,1 mg/L | 7.9.0 – 8,1 mg/L | 80.0 – 8,1 mg/L |
| Temperature °C:   | 25.1 – 25.4°C   | 25.5 – 25.8°C    | 25.5 – 25.8°C   |
| pH:               | 7.6 – 7.8       | 7.6 – 7.8        | 7.6 – 7.8       |

**Photoperiod:** Artificial light of white fluorescent lamp (14 hrs./day)

**Light intensity:** Artificial light of white fluorescent lamp

**Collection of tissue samples:** Analysis of test fish was performed six times at each level in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. Analysis of control fish was performed before the experimental starting and after the experimental completion. Six fish were taken out at each sampling time and divided into three groups, and then both were analyzed individually. The fish were separated into parts; tegument, head viscera except liver, liver and remaining matter were weighted separately. The tegument consisted of the skin except head, scales, fin, alimentary canal or gills. The viscera consisted of internal organs except alimentary canal. Two fish were employed to measure the lipid content because of insufficient size.

**Statistical methods:** Whole fish concentrations were calculated based on the sum of tissues parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different.

## RESULTS

**Nominal concentrations:** Negative control,

**Mean measured concentrations:** 2 and 20 ug/L

**Bioconcentration factors (BCF):**

**Level 1 (2 ug/L) apparent steady-state BCF**

|                      | Parts    |       |       |       |         |       |       |      |                 |      |
|----------------------|----------|-------|-------|-------|---------|-------|-------|------|-----------------|------|
|                      | Tegument |       | Head  |       | Viscera |       | Liver |      | Remainder Parts |      |
|                      | T1       | T2    | T1    | T2    | T1      | T2    | T1    | T2   | T1              | T2   |
| Concentration (ng/g) | 2280     | 16700 | 23400 | 17500 | 36000   | 45300 | 37800 | 3200 | 6260            | 5380 |
| BCF                  | 1400     | 1000  | 1400  | 1100  | 2200    | 2700  | 2300  | 1900 | 380             | 320  |

**Level 1 (20 ug/L) apparent steady-state BCF**

|                      | Parts    |      |      |      |         |      |       |      |                 |      |
|----------------------|----------|------|------|------|---------|------|-------|------|-----------------|------|
|                      | Tegument |      | Head |      | Viscera |      | Liver |      | Remainder Parts |      |
|                      | T1       | T2   | T1   | T2   | T1      | T2   | T1    | T2   | T1              | T2   |
| Concentration (ng/g) | 5490     | 4750 | 5600 | 4730 | 9900    | 7410 | 9190  | 7650 | 1810            | 1390 |
| BCF                  | 2800     | 2400 | 2900 | 2400 | 5100    | 3800 | 4700  | 3900 | 930             | 720  |

Time to reach 50% clearance:

**PFOS Concentration in test water:**

| Conc.   | After 1 Day | After 7 Days | After 14 Days | After 21 Days | After 28 Days | After 43 Days | After 58 Days | Average (STD) |
|---------|-------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 2 ug/L  | 15.1        | 14.5         | 15.7          | 16.5          | 17.7          | 15.4          | 16.8          | 16 + 1.12     |
| 20 ug/L | 1.78        | 1.76         | 1.87          | 1.93          | 1.89          | 1.92          | 2.01          | 1.88 + 0.087  |

**PFOS Concentration in Tissue of Carp Exposed to 2 and 20 ug/L:**

| Conc.   | After 7 Days | After 14 Days | After 21 Days | After 28 Days | After 43 Days | After 58 Days | Average (STD) |
|---------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 2 ug/L  | 260          | 440           | 300           | 690           | 750           | 720           | 16 + 1.12     |
| 20 ug/L | 240          | 520           | 410           | 860           | 890           | 1300          | 1.88 + 0.087  |

**Test organism mortality:**

**Negative control:** None documented.

**Level 1 (2 ug/L):** None documented.

**Level 2 (20 ug/L):** None documented.

**Analytical methodology:**

Analysis of PFOS in the test water and carp was performed using high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The test water of each level was analyzed once before first analysis of test fish and at the same time as the analysis of the test fish. The fish were separated into parts; tegument, head, viscera except liver, liver and remaining matter. Steady state was reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within + 20% of each other. When BCFs were less than 100, it was evaluated that a steady-state had been reached after 28 days.

Recovery was good in both water and fish tissues, ranging from 89.3% to 90.6%. Analytical results were corrected for procedural recovery.

**CONCLUSIONS**

In this study, PFOS bioconcentrated in the tissues of carp. Test concentrations of 2 and 20 ug/L were used. The fish were exposed for 58 days to make up for excessive mortality. Bioconcentration factors were calculated to be 720 for 2 ug/L and 200- 1500 for 20 ug/L.

**REFERENCES**

Kurume Laboratory (2001). Chemicals Evaluation and Research Institute, Japan. Test number: 51520

### **Annex 3. Application of equilibrium partitioning models to determining effect concentrations for PFOS salts in soil and sediment**

The review of the complete set of ecotoxicity test reports presented in section 2.2.1, 2.3.1 and 2.3.2 has highlighted the absence of data describing the toxicity of PFOS salts to sediment and soil-dwelling organisms and terrestrial plants. Since tests with aquatic invertebrates and plants have demonstrated toxicity of PFOS salts, and there is generally a correlation between toxicity to aquatic organisms living in the water column and toxicity to sediment- and soil-dwelling organisms. There is as a consequence a need to consider the possibility of deriving effect concentrations for PFOS in soil and sediment to fill the data gaps based on data that already exist for aquatic organisms. One approach to deriving such data is to use equilibrium partitioning models.

Equilibrium partitioning models are used in two ways:

- To calculate soil/sediment pore water concentration from overall soil/sediment concentration
- To calculate  $PNEC_{\text{soil/sediment}}$  from  $PNEC_{\text{water}}$ .

In both cases, the equilibrium constant for distribution between water and soil/sediment,  $K_{p\text{soil/sediment}}$ , is needed. For many substances,  $K_{p\text{soil/sediment}}$  is replaced by  $K_{oc}$ , which is equivalent to assuming that only the organic components of soil or sediment are important in affecting the equilibrium. However, for an anionic surfactant, such as PFOS, it is very likely that interaction with the inorganic substrate will also be important (Salloum et al, 2000), as has been demonstrated in a recent study with PFOS (Ellefson). Therefore  $K_p$  values for an appropriate soil or sediment would have to be used. It should also be noted that salinity affects the solubility of PFOS; the high ionic content of interstitial water could also affect the adsorption behaviour of PFOS. Furthermore, it is uncertain over what time-scale equilibrium would be achieved (Ellefson), and as a consequence the extent of adsorption may be dependent upon the concentration of PFOS in the aqueous phase.

Whilst for all substances extrapolation of PNEC from aquatic data to the terrestrial or sediment compartment is subject to uncertainty, that uncertainty is compounded when the mode of uptake is different for organisms present in different environmental compartments. The mode of toxic action and the mechanism of uptake of surfactants are complex. Therefore the use of equilibrium partition models to obtain  $PNEC_{\text{soil}}$  or  $PNEC_{\text{sediment}}$  is subject to considerable doubt.

Should any laboratory soil or sediment organism test results become available then there is a further complication in extrapolating from these results to PNEC values appropriate to these compartments. The normal method of extrapolation is to apply a correction that takes into account the organic matter content of the matrix. For substances that adsorb preferentially to inorganic matter it would be necessary to correct for both the type of inorganic matrix, the composition of the aqueous phase and the concentration of the substance. This is unlikely to be straightforward.

A search of the open literature was carried out in order to assess the environmental fate and behaviour properties of substances with similar chemistry and properties to PFOS. Published papers are relevant to the consideration of equilibrium partitioning as a relevant model for PFOS environmental fate. Several papers were located and the abstracts are summarised below. It is beyond the scope of this work to investigate the papers in detail but it can be noted that the technical quality of the work, as with all open literature, has already been peer reviewed (although not subject to GLP audit). The findings of these papers do not preclude the possibility of applying equilibrium partitioning to PFOS but do highlight the technical difficulties that might be encountered.

## **Conclusion**

It is concluded that on the basis of the presently available data for PFOS that equilibrium partitioning theory cannot be applied to determine either concentrations in interstitial water of soil and sediments or PNEC values for soil and sediments for the following reasons:

- the nature of the adsorption process cannot be assumed to be linearly dependent upon concentration;
- the adsorption is likely to be highly dependent upon soil composition, particularly the inorganic component; and
- the rate at which equilibrium might be achieved is unknown

## **REFERENCES**

Salloum, M.J., Dudas, M.J., McGill, W.B. and Murphy, S.M. (2000). Surfactant sorption to soil and geologic samples with varying mineralogical and chemical properties. *Environ. Toxicol. Chem.* 19(10), 2436-2442.

Soil Adsorption/Desorption Study of Potassium Perfluorooctanesulfonate (PFOS), Mark E. Ellefson, 3M Laboratory Report No: E00-13 11

**Table III.I. Review of open literature**

| <b>Report title</b>   | <b>Summary of abstract</b>   | <b>Author(s)</b>                         | <b>Journal</b>   |
|---|--|--|--|
| Terrestrial risk assessments for linear alkylbenzenesulfonate (LAS) in sludge-amended soils | Reviews investigations of factors affecting fate of LAS in the terrestrial environment. Refers to precipitating effect of calcium/magnesium ions in addition to primary sorption. Sorbed form is noted as being different to the commercially available form (for which data were available).  | DeWolfe, Watze; Feijtel, Tom             | Chemosphere, vol 36, no. 6, pp 1319-1343, 1998             |
| Effect of sediment organic-carbon on the toxicity of a surfactant to <i>Hyalella azteca</i> | Investigation of the effects of sediment organic carbon levels on the sorption and toxicity to <i>Hyalella azteca</i> of anionic surfactants, specifically ABS (alkylbenzene sulfonate). Studies indicated that higher levels of organic carbon led to higher apparent sorption coefficients. A higher sediment concentration was required to elicit the toxic response at higher organic carbon levels. | Cano, M.L.; Dyer, S.C.; Decarvalho, A.J. | Environ. Toxicol. Chem., vol 15, no. 8, pp 1411-1417, 1996 |
| Anionic surfactant transport characteristics in unsaturated soil                            | Investigations showed that for an alkyl ether sulfate and a linear alkylbenzenesulfonate, soil-surfactant interactions were reversible and that equilibrium conditions were quickly achieved.  | Allred, Barry; Brown, Glenn O.           | Soil Sci., vol 161, no. 7, pp 415-425. 1996                |

#### Annex 4. Summary of the lowest acceptable effect concentrations

| Endpoint  | Species  | Protocol                                     | Result (mg/l)  |      |
|---|--|--|--|------|
| Freshwater fish - acute toxicity  | <i>Pimephales promelas</i> (Fathead minnow)                                    | Not given                                    | 96-hour LC <sub>50</sub>   | 4.7  |
| Freshwater fish - chronic toxicity  | <i>Pimephales promelas</i> (Fathead minnow)                                    | OECD 210 & OPPTS 850.1400                    | 42-day NOEC <sub>surv/growth</sub>   | 0.30 |
| Invertebrate - acute toxicity (Freshwater)                                    | <i>Daphnia magna</i> (Water flea)  | ASTM 1981 & OECD 1981                        | 48-hour EC <sub>50</sub>   | 27   |
| Invertebrate - acute toxicity (Salt water)                                    | <i>Mysidopsis bahia</i> (Mysid shrimp)   | OPPTS 850.1035                               | 96-hour LC <sub>50</sub>   | 3.6  |
| Invertebrate - chronic toxicity (Freshwater)                                  | <i>Daphnia magna</i> (Water flea)  | ASTM 1981 & OECD 1981 (Semi-static)          | 28-day NOEC <sub>repro</sub>   | 7    |
| Invertebrate - chronic toxicity (Salt water)                                  | <i>Mysidopsis bahia</i> (Mysid shrimp)   | OPPTS 850.1350                               | 35-day NOEC <sub>repro/growth</sub>  | 0.25 |
| Aquatic plants – growth inhibition of freshwater algae - Short-term exposure  | <i>Selenastrum capricornutum</i> (now <i>Pseudokirchneriella subcapitata</i> ) | OECD 201, OPPTS 850.5400 & ASTM 1218-90E     | 96-hour EC <sub>50</sub> cell density  | 71   |
|   |  |  | 96-hour E <sub>b</sub> C <sub>50</sub> area under the curve                    | 71   |
|   |  |  | 96-hour E <sub>r</sub> C <sub>50</sub> growth rate                             | 126  |
|   |  |  | 96-hour NOEC <sub>growth rate, cell density, area under the growth curve</sub> | 44   |
|   |  |  | 72-hour EC <sub>50</sub> cell density  | 70   |
|   |  |  | 72-hour E <sub>b</sub> C <sub>50</sub> area under the curve                    | 74   |
|   |  |  | 72-hour E <sub>r</sub> C <sub>50</sub> growth rate                             | 120  |
|   |  |  | 72-hour NOEC <sub>growth rate, cell density, area under the growth curve</sub> | 70   |
| Aquatic plants – growth inhibition of Saltwater algae – Short-term exposure   | <i>Skeletonema costatum</i>  | OPPTS 850.5400                               | 96-hour EC <sub>50</sub> growth rate   | >3.2 |
|   |  |  | 96-hour NOEC <sub>growth rate</sub>  | >3.2 |
| Aquatic plants – growth inhibition of freshwater algae - Longer-term exposure | <i>Selenastrum capricornutum</i> (now <i>Pseudokirchneriella subcapitata</i> ) | OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23 | 14-day EC <sub>50</sub> cell density   | 95   |
|   |  |  | 14-day NOEC <sub>cell density</sub>  | <26  |
|   |  |  | 14-day EC <sub>10</sub> cell density   | 16   |
| Freshwater higher plants – growth inhibition                                  | <i>Lemna gibba</i>   | OPPTS 850.4400                               | 7-day IC <sub>50</sub>   | 108  |
| Amphibians – embryo survival, growth and development                          | <i>Xenopus laevis</i> (African clawed frog)                                    | ASTM E1439-91                                | 96-hour LC <sub>50</sub>   | 13.8 |
|   |  |  | 96-hour EC <sub>50</sub> malformations   | 12.1 |
|   |  |  | Minimum concentration to inhibit growth  | 7.97 |
| Sewage treatment organisms  | Activated sludge   | OECD 209                                     | 3-hour IC <sub>50</sub> respiration inhibition                                 | >905 |



| Endpoint                  | Species   | Protocol                                      | Result (mg/kg of food)   |                  |
|---------------------------|---|---|--|------------------|
| Dietary toxicity to birds | <i>Anas platyrhynchos</i><br>(Mallard duck)             | OECD 205, OPPTS<br>850.2200 & FIFRA E<br>71-2 | LC <sub>50</sub><br>NOEC <sub>mortality</sub><br>NOEC <sub>body weight</sub> | 628<br>146<br>37 |
|                           | <i>Colinus virginianus</i><br>(Northern Bobwhite quail) | OECD 205, OPPTS<br>850.2200 & FIFRA E<br>71-2 | LC <sub>50</sub><br>NOEC <sub>mortality</sub><br>NOEC <sub>body weight</sub> | 220<br>73<br>73  |

| Endpoint                 | Species                           | Protocol   | Result (µg/bee)                          |              |
|--------------------------|-----------------------------------|--|--|--------------|
| Oral toxicity to bees    | <i>Apis mellifera</i> (Honey bee) | OECD 213, EPPO<br>170                              | 72-hour LD <sub>50</sub><br>72-hour NOEL | 0.40<br>0.21 |
| Contact toxicity to bees |                                   | OECD 214, EPPO<br>170, OPPTS<br>850.3020 (Contact) | 96-hour LD <sub>50</sub><br>96-hour NOEL | 4.78<br>1.93 |

## Annex 5. Robust Summaries for Physical Chemical Properties and Environmental Fate Studies

### VAPOR PRESSURE

**Title:** Impinger Studies of Volatility of FC-95 and FC-143

#### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1- Octanesulfonic acid, 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The 3M production lot number was not noted. The test sample is a white powder of uncharacterized purity.

#### METHOD

**Method:** Internal

**GLP:** No

**Year study performed:** 1993

**Remarks:** Details outlined in the results section. As described below, there are a number of methodological concerns with this study. In addition, the document submitted is a combination of two reports. The first report contained Parts I and II, and a second report contained Experiment # 3. In addition, the first report contains portions of two versions. As neither of the first report versions is dated, it is unknown which is the final version. **Part I Procedure:** A mixture of 10 ppm FC-95 (PFOS) and 10 ppm FC-143 (PFOA) was prepared in water or water/isopropanol solutions of the polar-organic compounds listed in the table below. Aliquots of each solution were analyzed in triplicate for fluorochemical content by LC-thermospray mass spectrometry both before and after bubbling 280 liters of air through them. The flow rate of the air passing through the stock solutions was 1 L/min. The solutions were kept in an ice bath during bubbling. The concentrations before and after bubbling were compared after adjusting for volume lost during bubbling. **Part II Procedure:** Air was passed through an apparatus containing dry test material and then through glass wool at room temperature to a chain of impingers. A 50:50 propanol:water solution containing 500 ppm ammonium acetate was used in the impingers to catch any volatilized PFOS. All impingers were in ice water. **Experiment #3 Procedure:** The same study as in Part II was conducted in duplicate at 90°C.

#### RESULTS

**Vapor Pressure Value:** Part II-  $8.7 \times 10^{-8}$  torr, Experiment # 3-  $1.2 \times 10^{-7}$  torr

**Temperature °C:** 90°C in experiment # 3

**Decomposition:** not stated

**Part I- Analysis before and after passing 280 liters of air through various stock solutions.**

**Results:**

| <b>Solution</b>                                      | <b>Original PFOS Conc., ppm</b> | <b>% PFOS retained</b> |
|--|---------------------------------|------------------------|
| 500 ppm Tetrabutylammonium hydroxide                 | 10.0                            | 90                     |
| 500 ppm Ammonium acetate                             | 10.0                            | 71                     |
| 503 ppm Laurylpyridinium chloride                    | 10.0                            | 90                     |
| 500 ppm N-Alkyldimethylbenzyl-ammonium chloride      | 10.0                            | 100                    |
| 500 ppm Cetyltrimethylammonium bromide               | 10.0                            | 95                     |
| 505 ppm Tallowtrimethylammonium chloride             | 10.0                            | 93                     |
| 500 ppm Dicoctadimethylammonium chloride             | 10.0                            | 84                     |
| Water/1-Propanol (50:50)                             | 10.0                            | 89                     |
| Water/1-Propanol (50:50)                             | 0                               | 0                      |
| 500 ppm Ammonium acetate in water/1-propanol (50:50) | 0.10                            | 96                     |
| 500 ppm Ammonium acetate in water/1-propanol (50:50) | 0.20                            | 101                    |
| 500 ppm Ammonium acetate in water/1-propanol (50:50) | 0.40                            | 90                     |
| 500 ppm Ammonium acetate in water/1-propanol (50:50) | 0.80                            | 95                     |
| 500 ppm Ammonium acetate in water/1-propanol (50:50) | 2.0                             | 96                     |

**Remarks:** These findings appear to suggest a small loss of PFOS. However, in comments dated 12/7/93, Dr. Edwin Tucker of the Chemistry Department at the University of Oklahoma indicates that it is very unlikely that these fluorochemicals were removed by bubbling air through water due to their vapor pressures, which are very low. Tucker thought more likely mechanisms for loss from the solution phase were concentration of the surfactants in foam and loss from the bubbled solutions as foam or micro-droplets.

**Part II- Analysis of impinger ammonium acetate solutions.**

**Results:** No test material was found to be present in either the first or second impinger. This indicates that any test material transported from the solids to air and then into the ammonium acetate solutions in the impingers is below the detection limit. The calculated maximum pressure was  $8.7 \times 10^{-8}$  torr.

**Remarks:** In the report, the maximum vapor pressure calculations section contains errors. Equation 1 should use the value 0.625:g, not 0.625:g/mL. In addition, the "maximum" vapor pressure calculated was erroneously called "minimum" vapor pressure in the text below both equations 5 and 7.

Dr. Edwin Tucker of the Chemistry Department at the University of Oklahoma states that the experimental conditions do not provide firm evidence that the number is reasonable. There is no evidence that vapor pressure equilibrium was attained between the solid and the flowing gas.

**Experiment # 3- Measuring Vapor Pressure at 90°C**

**Results:** The report concludes that PFOS has a measurable vapor pressure at this temperature. The

minimum vapor pressure for the test material was purported to be  $1.2 \times 10^{-7}$  torr at 90°C. It was considered a minimum because the impinger trains may not have caught all of the fluorochemical that had been volatilized.

**Remarks:** There are reasons to consider this a questionable result. No notation was made about the sensitivity of the analytical measurements in this study, but the quantification limit in the analysis in Part II was 0.625 µg. The concentration range from impinger train 1 in Experiment # 3 was 0.07-0.075 µg. Impinger concentrations ranged from 0-0.18 µg in impinger train 2. These concentrations are near or below the quantification limit reported in Part II.

### CONCLUSIONS

No reliable conclusions can be made based on this study. The general observation is that this compound has very low volatility or a very low vapor pressure under ambient conditions.

**Remarks:** none

### **DATA QUALITY**

**Reliabilities:** Klimisch ranking 3. There is no information on the validity of the test method for determining volatility of the test substance. This study lacks characterization of the purity of the test substance. There is no information on the validity of the analysis method.

### REFERENCE

3M Environmental Laboratory. 1993. 3M Lab Request Number L3306, 3M Company, St. Paul, MN.

## WATER SOLUBILITY STUDY

**Title:** Solubility of PFOS in water

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analysis techniques.

### METHOD

**Method:** Based on OECD 105, OPPTS 830.7840.

**GLP:** Yes

**Year study performed:** 2001

**Remarks:** Water source is ASTM Type I water, Millipore. The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 225 rpm at 30°C for 24, 48, or 72 hours followed by 24-hours of equilibration at 24-25°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

### RESULTS

**Value (mg/L) at temperature °C:** 680 mg/L at 24-25°C

**Description of solubility:** Slightly soluble

**pH value and concentration at temperature °C:** not stated

**pKa value at 25°C:** not stated

**Remarks:** The 24-, 48-, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations.

### CONCLUSIONS

The overall mean solubility concentration of the test substance in pure water was 680 mg/L.

**Remarks:** none

### DATA QUALITY

**Reliabilities:** Klimisch ranking 1

ENV/JM/RD(2002)17

REFERENCE

Ellefson, M. 2001c. Solubility of PFOS in Water. 3M Company, 3M Environmental Laboratory, Project Number E00-1716.

## WATER SOLUBILITY STUDY

**Title:** Solubility of PFOS in Natural Seawater and an Aqueous Solution of 3.5% Sodium Chloride

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analysis techniques.

### METHOD

**Method:** Based on OECD 105, OPPTS 830.7840

**GLP:** Yes

**Year study performed:** 2001

**Remarks:** The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 150 rpm at 30°C for 24, 48, and 72 hours followed by 24 hours of equilibration at 22-24°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

Water Sources: Natural Seawater = Ocean Scientific, lot # LN 58, salinity = 3.5%

Sodium Chloride = EM Science, 99% pure, mixed with ASTM Type 1 water to achieve salinity of 3.5%

### RESULTS

**Value (mg/L) at temperature °C:** Natural Seawater: 12.4 mg/L at 22-23°C; 3.5% NaCl Solution: 20.0 mg/l at 22-24°C

**Description of solubility:** Slightly soluble

**pH value and concentration at temperature °C:** not stated

**pKa value at 25°C:** not stated

**Remarks:** The 24-, 48-, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations for the natural seawater. The 24-hour values were not included in the mean solubility of the sodium chloride calculation because when the coefficient of variation was calculated for all of the replicate analyses for that day, it was >15%.

### CONCLUSIONS

The overall mean solubility concentration of the test substance in natural seawater was 12.4 mg/L. In a 3.5% NaCl solution it was 20.0 mg/L. PFOS solubility decreases with increasing ionic strength of the medium.

ENV/JM/RD(2002)17

**Remarks:** none

#### **DATA QUALITY**

**Reliabilities:** Klimisch ranking 1

**Remarks:** none

#### **REFERENCE**

Ellefson, M. 2001a. Solubility of PFOS in Natural Seawater and an Aqueous Solution of 3.5% Sodium Chloride. 3M Company, 3M Environmental Laboratory, Lab Project Number E00-1716.



## SOLUBILITY STUDY

**Title:** Solubility of PFOS in Octanol

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR and elemental analysis techniques.

### METHOD

**Method:** Based on OECD 105, OPPTS 830.7840

**GLP:** Yes

**Year study performed:** 2001

**Remarks:** Octanol Source: Aldrich

Screen Test: Lot JU0873804, 99+%

Definitive Test: Lot 06238CI, 99.9+% HPLC Grade

The definitive test consisted of placing ~0.010 g test substance with ~10 mL octanol in centrifuge tubes. The tubes were shaken at ~150 rpm at ~30°C for 24, 48, or 72 hours followed by 24 hours of equilibration at 22-23°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

### RESULTS

**Value (mg/L) at temperature °C:**

24 hours: 56.9 mg/L

48 hours: 55.7 mg/L

72 hours: 55.4 mg/L

Mean solubility of PFOS in octanol = 56.0 mg/L

**Description of solubility:** not stated

**pH value and concentration at temperature °C:** not stated

**pKa value at 25°C:** not stated

**Remarks:** The 24, 48, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentration.

### CONCLUSIONS

The overall mean solubility concentration of the test substance in pure octanol was 56.0 mg/L.

**Submitters' Remarks:** Typically, the Column Elution Method is recommended for use with substances with solubility screening results of <10 mg/L. However, the shake flask method was utilized in this study because of the difficulty in obtaining tubing compatible with octanol and the possible explosion hazard posed by possible leaks of a flammable solvent in an incubator.

#### **DATA QUALITY**

**Reliabilities:** Klimisch ranking 1

**Remarks:** none

#### **REFERENCE**

Ellefson, M. 2001b. Solubility of PFOS in Octanol. 3M Company, 3M Environmental Laboratory, Laboratory Project Number E00-1716.

## SOIL ADSORPTION

**Title:** Pilot Study on Soil Adsorption

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The sample was radiolabeled (isotope and labeling position not specified). The 3M production lot number was not noted. The test substance was a white powder of uncharacterized purity.

### METHOD

**Method/guideline followed:** The study explored the use of autoradiography of Thin Layer Chromatography Plates for determining the soil mobility of PFOS. This method was developed by 3M while looking for alternatives for studying the adsorption/desorption properties of the test substance.

**GLP (Y/N):** No

**Year study performed:** 1978

**Statistical methods:** None

**Temperature:** Not indicated

**Remarks:** Little detail was available regarding the study method.

### RESULTS

**Results:** The study used TLC greenhouse soil plates. The radiolabeled spot due to PFOS was too faint to be visualized.

### CONCLUSIONS

No conclusion could be reached in this study.

### DATA QUALITY

**Reliability:** Klimisch ranking 3. These studies lacked sufficient detail regarding methodology. Isotope specific activity and chemical/radiochemical purity of the test substance were not provided. The method for radiosynthesis was also not provided. The analytical methodology lacked validation and a means of identifying and quantifying potential degradation products.

### REFERENCES

Boyd, S.A. 1993. Review of Technical Notebook. Soil Thin Layer Chromatography. Number 48277, p30. Michigan State University.

Mendel, A. 1978. Soil Thin Layer Chromatography—FC-95, FC-143, FM-3422. Excerpt from 3M Technical Notebook. October 13, 1978. Number 48277, p30. Project Number 9970612600.

## BIODEGRADATION STUDY

**Title:** Determination of Methylene Blue Active Substance - FC-95

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS #2795-39-3)

**Remarks:** The 3M production lot was 158. The test substance was a white powder of uncharacterized purity.

### METHOD

**Method/guideline followed:** EPA Method 425.1

**Test Type:** Methylene Blue Active Substance

**GLP (Y/N):** No

**Year study performed:** 1989

**Contact time units:** Not indicated

**Inoculum:** Not indicated

### RESULTS

**Degradation % after time:** Not indicated

#### **Results:**

| 3M Lab Request Number | MBAS Result   |
|-----------------------|---------------|
| G1512-1               | 835,000 mg/kg |
| G1827-2               | 831,000 mg/kg |
| G1828-2               | 730,000 mg/kg |

**Kinetic (for sample, positive and negative controls):** Not indicated

**Breakdown products (yes/no):** Not indicated

**Remarks:** None

### CONCLUSIONS

**Reviewer's remarks:** The test indicated that the test sample exhibited high activity in this MBAS study.

### DATA QUALITY

**Reliability:** Klimisch ranking 3. This study lacked information regarding methodology and testing

parameters. Test substance purity within the test sample was not sufficiently characterized. The sample description from Twin City Testing was “liquid”, but FC-95 was a solid material. This may indicate that the FC-95 was sent in a solution and that the actual MBAS may be higher than the values cited.

#### REFERENCE

Determination of Methylene Blue Active Substance - FC-95. 1989. Pace Analytical and Twin City Testing. Minneapolis, Minnesota—at the request of the 3M Company, 3M Lab Request numbers: G1512, G1827, and G1828.

## SOIL ADSORPTION

**Title:** Soil Adsorption/Desorption Study of Potassium Perfluorooctane Sulfonate (PFOS)

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

**Remarks:** The test substance was a white powder. The sample was recrystallized from a production lot of FC-95. The purity was determined to be 97.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR, and elemental analysis techniques.

### METHOD

**Method/guideline followed:** OECD 106

**GLP (Y/N):** Yes

**Year study performed:** 2001

**Statistical methods:** Statistical analysis and plotting of the data was done according to OECD Method 106 using Microsoft Excel.

**Temperature:** Room temperature (19-30°C)

**Stock and test solution preparation:** The test concentrations and conditions were determined in a preliminary experiment. For the definitive experiment, test solutions were made by diluting a stock solution of unradiolabeled perfluorooctanesulfonate to a final test substance concentration of approximately 0.5 mg/L in aqueous 0.01 M CaCl<sub>2</sub>.

## Soil Characteristics

| Soil Class                     | Clay                               | Loam                               | Clay Loam                          | Sandy Loam                         | River Sediment                     | Domestic Sludge                  |
|--------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|----------------------------------|
| Source                         | Agvise Laboratories, Northwood, ND | Agvise Laboratories, Northwood, ND | Agvise Laboratories, Northwood, ND | Agvise Laboratories, Northwood, ND | Agvise Laboratories, Northwood, ND | NIST, from Denver, CO POTW       |
| Lot Number                     | 00-2407                            | 00-2404                            | 00-2405                            | 99-2564                            | 00-2046                            | 2781                             |
| Physical Description           | 1.00 mm air-dried, 0-6" deep       | 1.00 mm air-dried, 0-6" deep       | 1.00 mm air-dried, 0-6" deep       | 1.00 mm air-dried, 0-6" deep       | 1.00 mm air-dried, 0-6" deep       | 200 mesh, oven-dried, sterilized |
| % Organic Carbon               | 2.6%                               | 4.9%                               | 2.6%                               | 2.8%                               | 1.3%                               | Not analyzed                     |
| % Sand                         | 16%                                | 39%                                | 21%                                | 58%                                | 39%                                | Not analyzed                     |
| % Silt                         | 22%                                | 50%                                | 46%                                | 22%                                | 42%                                | Not analyzed                     |
| % Clay                         | 62%                                | 11%                                | 33%                                | 20%                                | 19%                                | Not analyzed                     |
| CEC (meq/100g)                 | 54.5                               | 23.9                               | 24.7                               | 23.3                               | 17.5                               | Not analyzed                     |
| pH in 0.01 M CaCl <sub>2</sub> | 7.2                                | 7.4                                | 6.0                                | 7.8 <sup>1</sup>                   | 7.7                                | Not analyzed                     |

<sup>1</sup>Value is for pH in water, not pH in 0.1 M CaCl<sub>2</sub>

## Test Conditions:

**Adsorption kinetics:** Replicate study samples containing the soils (or sediments or sludges) were equilibrated by shaking for at least 12 hours at room temperature with 0.01M CaCl<sub>2</sub>. Study samples were dosed with the test substance at approximately 0.5 mg/L and placed on an orbital shaker. Replicate sets of these study samples were removed at designated time points throughout a 48-hour time period. Study samples were then prepared and analyzed for the target analyte. The adsorption kinetics were determined using this data. The last set of study samples (48-hour) were saved and used for the desorption kinetics portion of the method.

**Desorption kinetics (one concentration):** After the adsorption kinetics experiment, the 48-hour study samples were centrifuged and the aqueous phase removed. The volume of solution removed was replaced by an equal volume of 0.01 M CaCl<sub>2</sub> without test substance. The new mixture was agitated until the desorption equilibrium was reached. During a 48-hour period, at defined time intervals, small aliquots of the aqueous phase were removed and analyzed for the target analyte. The desorption kinetics were determined using this data.

## RESULTS

## Adsorption Kinetics of PFOS, 1:5 Soil:Solution Ratio, 48-hour Time Point

| Soil Type | Average Distribution Coefficient, K <sub>d</sub> , L/g | Percentage of Organic Carbon in Soil | Average Organic Carbon normalized Adsorption Coefficient, K <sub>oc</sub> , L/g |
|-----------|--|--------------------------------------|---|
| Clay      | 0.0183   | 2.6                                  | 70.4  |
| Clay Loam | 0.00972  | 2.6                                  | 37.4  |

|                        |         |               |                |
|------------------------|---------|---------------|----------------|
| <b>Sandy Loam</b>      | 0.0353  | 2.8           | 126            |
| <b>River Sediment</b>  | 0.00742 | 1.3           | 57.1           |
| <b>Domestic Sludge</b> | <0.120  | Not available | Not calculable |

All matrices adsorbed the test substance strongly. The sludge demonstrated very strong adsorption (>96%) and PFOS was not detected in the extracts. The data indicated that adsorption occurred within the first few hours of exposure and the test substance concentration did not vary significantly after 16 hours.

**Apparent Desorption Kinetics of PFOS, 1:5 Soil:Solution Ratio,  
48-hour Time Point**

| <b>Soil Type</b>       | <b>Desorption Coefficient, <math>K_{des}</math>, L/g</b> |
|------------------------|--|
| Barnes Loam            | 0.0000471  |
| <b>Clay Loam</b>       | 0.0000158  |
| <b>Clay</b>            | 0.0000349  |
| <b>River Sediment</b>  | 0.0000100  |
| <b>Domestic Sludge</b> | <0.000237  |

The test substance was poorly desorbed from the soil/sediment/sludge matrices during the 48-hour study period. The river sediment displayed the most desorption at 39% after 48 hours. The sludge samples did not desorb a detectable amount of test substance. Desorption that did occur was accomplished rather quickly; after the 8-hour time point the test substance concentration did not vary significantly.

**Adsorption Isotherms**

| <b>Soil Type</b>       | <b>Log <math>K_F^{ads}</math></b> | <b><math>K_F^{ads(1)}</math></b> | <b>Regression constant, 1/n</b> | <b>Regression Constant, n</b> |
|------------------------|-----------------------------------|----------------------------------|---------------------------------|-------------------------------|
| <b>Clay</b>            | -1.2515                           | 0.0560                           | 0.884                           | 1.13                          |
| <b>Clay Loam</b>       | -1.3762                           | 0.0421                           | 0.841                           | 1.19                          |
| <b>Sandy Loam</b>      | -1.0369                           | 0.0919                           | 0.829                           | 1.21                          |
| <b>River Sediment</b>  | -2.0261                           | 0.0094                           | 0.989                           | 1.01                          |
| <b>Domestic Sludge</b> | -1.246                            | 0.0568                           | 1.2581                          | 0.795                         |

(1) Freundlich adsorption coefficient

**Desorption Isotherms**

| <b>Soil Type</b> | <b>Log <math>K_F^{des}</math></b> | <b><math>K_F^{des(1)}</math></b> | <b>Regression Constant, 1/n</b> | <b>Regression Constant, n</b> |
|------------------|-----------------------------------|----------------------------------|---------------------------------|-------------------------------|
| Clay             | <b>-0.653</b>                     | <b>0.222</b>                     | <b>0.935</b>                    | <b>1.07</b>                   |
| Clay Loam        | <b>-1.084</b>                     | <b>0.082</b>                     | <b>0.954</b>                    | <b>1.05</b>                   |
| Sandy Loam       | <b>-0.981</b>                     | <b>0.104</b>                     | <b>1.01</b>                     | <b>0.988</b>                  |
| River Sediment   | <b>-1.41</b>                      | <b>0.039</b>                     | <b>1.02</b>                     | <b>0.984</b>                  |
| Domestic Sludge  | <b>1.47</b>                       | <b>29.5</b>                      | <b>0.327</b>                    | <b>3.06</b>                   |

(1) Freundlich desorption coefficient



Freundlich adsorption isotherms were used to relate the amount of test substance adsorbed on the soil to the amount present in the aqueous solution at equilibrium. The values calculated for the regression constant indicate that the data obtained for the test substance over two orders of magnitude were slightly non-linear.

## CONCLUSIONS

Perfluorooctanesulfonate (PFOS) appeared to adsorb strongly to all of the soil/sediment/sludge matrices tested. PFOS would not be considered to be qualitatively mobile as per OECD Guideline 106 (1/21/00) as the  $K_d$  values are  $>1$  mL/g. The test substance, once adsorbed, does not desorb readily, even when extracted with an organic solvent. In either case, adsorption or desorption, an equilibrium is achieved in less than 24 hours, with substantial adsorption ( $>50\%$ ) occurring in some of the time 0 samples after approximately 1 minute of contact. The test substance exhibited low mobility in all of the adsorbants tested. The shape of the PFOS adsorption isotherm (H-type) indicated a very strong chemical/adsorbent interaction. Because PFOS is a strong acid, it likely forms strong bonds with soils, sludge, and sediment via the mechanism of chemisorption.

## DATA QUALITY

**Reliability:** Klimisch ranking 1.

## REFERENCES

Ellefson, M.E. 2001d. Soil Adsorption/Desorption Study of Potassium Perfluorooctanesulfonate (PFOS). 3M Technical Report. Project Number E00-1311, Completion date June 4, 2001.

## BIOCONCENTRATION (PARTITION COEFFICIENT)

**Title:** Kow (Solubility in Water, Natural Seawater, An Aqueous Solution of 3.5% Sodium Chloride, and n-Octanol with Subsequent Calculation of the n-Octanol Water Partition Coefficient (Kow) of PFOS for each of the Aqueous Matrices)

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS #2795-39-3)

**Remarks:** The test substance was a white powder. The sample was recrystallized from a production lot of FC-95, and assigned the internal reference number of TCR-00017-046. Purity was determined to be 97.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR, and elemental analysis techniques.

### METHOD

**Method/guideline followed:** Calculated from n-octanol solubility and water solubility according to OPPTS 830.7550 and OECD 107.

**Test Type:** n-Octanol/Water partition coefficient (at saturation)

**GLP (Y/N):** Yes

**Year study performed:** 2001

**Test temperature:** n-Octanol solubility value at 23-24°C  
Water solubility value at 24-25°C

**Remarks:** The physical properties of PFOS did not allow a determination of the partition coefficient by the shake flask method per guidance provided in the OPPTS and OECD guidelines. Therefore, this study did not bring the two phases (n-octanol and water) into contact with PFOS at the same time, and this testing reflected the partition coefficient for the subject material at saturation only. The n-Octanol/Water partition coefficient was calculated by dividing the solubility of PFOS in n-octanol by the solubility in water and expressing it as the logarithmic value.

### RESULTS

The calculated log Kow for PFOS was determined to be -1.08 at saturation (log(56 mg/L in n-octanol/680 mg/L in water)).

### CONCLUSIONS

**Remarks:** No conclusions could be derived from this information. It applied only to a saturated system, which would not likely exist in the environment.

### DATA QUALITY

**Reliability:** Klimisch ranking 1

**Remarks:** The solubility studies were conducted properly. However, the applicability of this data point is

limited. Application of this value in a risk assessment has limited or no value as it only applies to saturated systems.

#### REFERENCES

Kow (Solubility in Water, Natural Seawater, An Aqueous Solution of 3.5% Sodium Chloride, and n-Octanol with Subsequent Calculation of the n-Octanol Water Partition Coefficient (Kow) of PFOS for each of the Aqueous Matrices). 2001. 3M Company. St. Paul, Minnesota. Environmental Laboratory Project Number E00-1716.

## BIODEGRADATION STUDY

**Title:** Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) II. Aerobic Soil Biodegradation

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt) CAS #2795-39-3

**Remarks:** White powder, 86.9% purity, also called PFOS or FC-95

### METHOD

**Method/guideline followed (experimental/calculated):** Method was designed by Springborn Laboratories, Inc.

**Test Type (aerobic/anaerobic):** Aerobic

**GLP (Y/N):** No

**Year study performed:** 2000

**Contact time/units:** 20 weeks

**Inoculum:** The inoculum sources were soils collected from a hardwood forest in Hanson, MA, a pine forest in Onset, MA, and a river bank in Bridgewater, MA; and sediments collected from brackish sites below the Wareham, MA wastewater treatment plant outfall and from the Narrows area in Wareham, MA. Biomass was determined on day 83 by both the fumigation/extraction and standard plate count methods and was reported as 17.4 mg C/100 g soil and  $6 \times 10^5$  cells/g, respectively.

**Remarks:** Soil and sediment samples were air-dried, 2.0 mm-sieved, and mixed together in equal dry weight portions. A nutrient mixture was prepared by combining a sterile potting soil extract, a trace mineral solution, a yeast extract, and reagent water. The soil/sediment mixture was adjusted to 75% of the water holding capacity using the above nutrient mixture. Soil moisture was monitored weekly during the study, and adjusted using reagent water as needed. The nominal test concentration was approximately 21.2 mg/kg. The incubation temperature was  $22 \pm 3^\circ\text{C}$ , and the test was conducted in the dark. Information on test solution agitation was not provided. Test vessels were 40-mL I-Chem glass vials with silicone/Teflon-lined septum screw caps containing 10 g (dry weight) soil/sediment mixture. Each test flask received all components at test initiation. Samples were taken at days 7, 14, 21, 28, 35, 42, 49, 56, and 63. Entire samples were extracted with methanol via accelerated solvent extraction. Extracts passed through a 0.2  $\mu\text{m}$  nylon filter prior to analysis. Samples were diluted as necessary in methanol and analyzed via LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, which is approximately twice the water solubility of PFOS. No day 0 samples were taken to determine starting concentrations.

### RESULTS

**Degradation % after time:** Not specified

**Results:** Essentially no PFOS metabolism occurred during the study.

**Kinetic (for sample, positive and negative controls):** Not specified

**Breakdown products (yes/no):** None indicated

**Remarks:** None

#### CONCLUSIONS

**Remarks:** PFOS is recalcitrant in the activated soil/sediment system.

#### **DATA QUALITY**

**Reliability:** Klimisch ranking = 2. Study not conducted according to Good Laboratory Practices. The stock solution used to dose the systems was prepared at twice the water solubility of PFOS. No Day 0 samples were taken to determine starting concentrations.

REFERENCE The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

## BIODEGRADATION STUDY

**Title:** Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) III. Anaerobic Sludge Biodegradation

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt) CAS #2795-39-3

**Remarks:** White powder, 86.9% purity, also called PFOS or FC-95

### METHOD

**Method/guideline followed (experimental/calculated):** Method was designed by Springborn Laboratories, Inc.

**Test Type (aerobic/anaerobic):** Anaerobic

**GLP (Y/N):** No

**Year study performed:** 2000

**Contact time/units:** 56 days

**Inoculum:** The inoculum source was an anaerobic digester at the Rockland, MD wastewater treatment plant.

**Remarks:** A mixture containing dried sludge extract (from rotating biological contactor wastewater treatment, Bridgewater, MA), unspecified OECD mineral media, and resazurin indicator served as the test medium. The media were prepared under nitrogen purge to exclude oxygen. The nominal test concentration was approximately 20.8 mg/L, and loading in test vessels was 300 mL anaerobic sludge per liter of medium. The test temperature was 35°C, and the test was conducted in the dark. Information on test solution agitation was not provided. Twenty 160-mL serum bottles containing 100 mL of test solution (purged with nitrogen after filling) with crimped butyl rubber tops served as test flasks. Each flask received all components at test initiation. Samples were taken from the test bottle on days 7, 14, 21, 28, 35, 42, 49, and 56, and from inoculum control bottles on days 7 and 56. For analysis, an aliquot was removed from each bottle and centrifuged. Both the supernatant and the solid biomass portion were analyzed via LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, approximately twice the water solubility of PFOS. No day 0 samples were taken to determine starting concentrations.

### RESULTS

**Degradation % after time:** Not specified

**Results:** No apparent PFOS biodegradation occurred over the 56-day period.

**Kinetic (for sample, positive and negative controls):** Not specified

**Breakdown products (yes/no):** None indicated

**Remarks:** None

#### CONCLUSIONS

**Remarks:** PFOS is recalcitrant in the anaerobic system.

#### **DATA QUALITY**

**Reliability:** Klimisch ranking = 2. Study not conducted according to Good Laboratory Practices. The stock solution used to dose the systems was prepared at twice the water solubility. No Day 0 samples were taken to determine starting concentrations.

REFERENCE The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

## BIODEGRADATION STUDY

**Title:** Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) IV. Pure Culture Study

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt) CAS #2795-39-3

**Remarks:** White powder, 86.9% purity, also called PFOS or FC-95

### METHOD

**Method/guideline followed (experimental/calculated):** Two study types were used (Pure Culture and Closed Vial Headspace) and Springborn Laboratories, Inc./Betts et al., 1974 is cited.

**Test Type (aerobic/anaerobic):** Aerobic

**GLP (Y/N):** No

**Year study performed:** 2000

### **Pure Culture Studies**

**Contact time/units:** 7 days

**Inoculum:** Four separate pure cultures from the American Type Culture Collection (ATCC) were tested:

*Cunninghamella echinulata* var. *echinulata* (fungi, ATCC #9244)

*Mucor circinelloides* f. *griseocyanus* (fungi, ATCC#1207a)

*Phanerochaete chrysosporium* (fungi, ATCC #24725)

*Streptomyces griseus* (actinomycete, ATCC #13273)

**Remarks:** The test medium was soybean grits-glucose (SGG) and test vessel loading was 6 mL of Stage II cultures into 60 mL media, with 24 hours of agitation on a shaker table at 250 rpm prior to the addition of PFOS. The nominal test concentration was approximately 20.9 mg/L. The test temperature was 26°C. The test vessel type was not noted. Each test flask received all necessary components at test initiation. All work utilized strict aseptic technique until harvest at day 7. Samples were taken on days 0 and 7. Analysis of the broth and cells collected via centrifugation was performed by LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, approximately twice the water solubility of PFOS.

**Degradation % after time:** Not specified

**Results:** The studies with *Cunninghamella*, *Mucor*, and *Streptomyces* did not provide any indication of biotransformation of PFOS. Possible biotransformation of PFOS (90% mass balance) by *Phanaerochaete* was noted, and closed vial studies were performed to confirm this.

**Kinetic (for sample, positive and negative controls):** Not specified



**Breakdown products (yes/no):** None indicated

**Remarks:** None

### **Closed Vial Headspace Study**

**Contact time/units:** 3 days

**Inoculum:** *Phanerochaete chrysosporium* (fungi, ATCC #24725)

**Remarks:** The test medium was soybean grits-glucose (SGG) medium at 1/10 and 1/100 strength plus resazurin. The test concentration was 0.2 mg/L. The incubation temperature was 26°C, and a shaker table in an environmental chamber at 250 rpm was used in incubation. Sterile 22-mL vials served as test vessels. Initial inoculum test vessel loading details were not noted. Each test flask received all necessary components at test initiation. Headspace was purged with oxygen and vials were immediately crimped. Samples were taken once on day 3. Analysis of the broth and cells collected via centrifugation was performed by LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,011 mg/L, approximately twice the water solubility of PFOS. No initial measured concentrations were taken in this closed vial study. Difficulties were encountered in maintaining aerobicity and only 3 days of exposure were maintained.

### RESULTS

**Degradation % after time:** Not specified

**Results:** The results indicated no significant biotransformation of PFOS by *Phanaerochaete* fungi.

**Kinetic (for sample, positive and negative controls):** Not specified

**Breakdown products (yes/no):** None indicated

**Remarks:** None

### CONCLUSIONS

**Remarks:** It did not appear that the four species were capable of metabolizing PFOS.

### **DATA QUALITY**

**Reliability:** Klimisch ranking = 2. The stock solution used to dose the systems was prepared at twice the water solubility. There were no initial measured concentrations taken in the closed vial study. These studies were not conducted in accordance with Good Laboratory Practices.

REFERENCE The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 11/3/00. Lab Project number E01-0434.

## BIODEGRADATION STUDY

**Title:** The 18-day aerobic biodegradation study of perfluorooctanesulfonyl-based chemistries.

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or  $C_8F_{17}SO_3^-K^+$ . (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS# 2795-39-3)

**Remarks:** PFOS is a white powder. The original 3M product lot number was not noted. The PFOS was HPLC purified and assigned 3M standard identification #TCR-00017-046. Upon receipt at the testing laboratory, the test article was given a test, control, and reference (TCR) number CA-TCR02-014. The submitter suggests that an Interim Certificate of Analysis reports the purity to be 97.9%. All results in the study were calculated assuming 100% purity. However, in the study report, the purity is noted as 86.4%. No explanation of this discrepancy was given.

### METHOD

**Method/guideline followed:** Based on EPA guidelines OPPTS 835.3200

**Test Type:** Aerobic

**GLP:** No

**Year study performed:** 2001

**Contact time:** 18 days

**Inoculum:** Activated sludge collected 7/31/00 from the aeration basin at the Metro Wastewater Treatment Plant, St. Paul, MN. The MLSS was determined to be 2,280 mg/L when first collected. The MLSS was stored at 4 °C for approximately 5 weeks prior to being used for this study. The sludge was allowed to settle and the solids used for inoculum. The settled sludge constituted approximately 20% of the volume (~200 mL) of the MLSS used.

**Test Medium:** Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Methanol (1 mL per liter) was added per liter of mineral medium. Fifty mL of settled sludge was added per liter of mineral salts medium. Mineral medium plus sludge was prepared 9/7/00, while fresh medium without sludge (abiotic controls) was prepared 8/10/00.

**Remarks:** Three types of samples were prepared for this study: blank sludge controls (mineral medium, inoculum), abiotic controls (mineral medium, PFOS), and test substance samples (mineral medium, inoculum, PFOS). The test vessels, sterile 125 mL Nalgene polycarbonate culture flasks containing 25 mL of media, were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

The test concentration used was 2.455 mg/L. The samples were agitated at ~200 rpm at a temperature of  $25 \pm 3$  °C. The test vessels were spiked with 6 µL of a 10,230 mg/L solution of PFOS in methanol yielding 2.455 mg/L. Sampling was done on days 0 and 18. The day 0 test vessels were prepared and immediately placed in a freezer that was maintained at -20 °C until analyzed. After 18 days, the test vessels were removed from the incubator and frozen until final sample preparation by solid phase extraction (SPE). Following thawing, test vessel contents were adjusted to 1% acetic acid and then passed through a

conditioned SEP-VAC C18 6cc SPE cartridge. Methanol was then added to the emptied culture flask, shaken vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative-ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to PFOS, the additional compounds quantified are specified below. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

| Compound Name   | Acronym          | Chemical Formula                   |
|---|------------------|------------------------------------|
| 2-(N-ethyl Perfluorooctane sulfonamido) ethyl alcohol | N-EtFOSE Alcohol | $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$ |
| 2-(N-ethyl Perfluorooctane sulfonamido) acetic acid   | N-EtFOSAA        | $C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$ |
| 2-(Perfluorooctane sulfonamido) acetic acid           | M556             | $C_8F_{17}SO_2NH(CH_2COOH)$        |
| N-Ethyl perfluorooctane sulfonamide                   | N-EtFOSA         | $C_8F_{17}SO_2NH(C_2H_5)$          |
| Perfluorooctane sulfinat, potassium salt              | PFOSulfinat      | $C_8F_{17}SO_2^-K^+$               |
| Perfluorooctanoate, ammonium salt                     | PFOA             | $C_8F_{15}COO^-NH_4^+$             |
| Perfluorooctane sulfonamide                           | FOSA             | $C_8F_{17}SO_2NH_2$                |

No reference substance was used. However, when the results from an EtFOSE alcohol study conducted at the same time are compared to the previous EtFOSE alcohol 35-day study, the viability of the microbial inoculum is confirmed.

## RESULTS

### Degradation % after time: 0

**Results:** After 18 days, the analytical results demonstrate that after exposure to municipal wastewater treatment sludge, 2.455 mg/L PFOS was not measurably degraded biotically or abiotically. Mass balance for PFOS test vessels was excellent and ranged from 104-108%.

**Breakdown products:** No

**Remarks:** None

## CONCLUSIONS

No loss of PFOS was demonstrated. Mass balance was 104-108%. The results from this study confirm the results from other aerobic biodegradation studies of PFOS.

**Submitters' Remarks:** The submitters suggested a Klimisch data quality ranking of 2 because the study was conducted as a non-GLP study, but with the understanding that good data quality objectives be met.

REFERENCE

Lange, C. 2001a. The 18-day aerobic biodegradation study of perfluorooctanesulfonyl-based chemistries. Pace Analytical Services, Inc. Minneapolis, MN. 3M Company. Minneapolis, MN.

## STABILITY IN WATER STUDY

**Title:** Hydrolysis Reactions of Perfluorooctane Sulfonate (PFOS)

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS-potassium salt, 1-perfluorooctanesulfonic acid-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorosulfonate-potassium salt (CAS #2795-39-3).

**Remarks:** 3M production lot number was 171. Test substance is a light-colored powder at 25 °C.

### METHOD

**Method/guideline followed:** Based on OPPTS: 835.2110.

**Test Type:** Hydrolysis as a function of pH

**GLP (Y/N):** No

**Year study performed:** 2001

**Test sample preparation:** Test solutions consisted of 1.0 mL buffered aqueous solutions at 6 pH levels (1.5, 3.0, 5.0, 7.0, 9.0, 11.0). The resulting PFOS concentration in all test samples (sample triplicates and matrix spike samples) was approximately 500 µg/L. Samples were shielded from light during incubation at 50 °C for periods of 0 to 49 days. Control samples and blanks addressed potential non-hydrolytic degradation routes.

**Analytical Procedures:** Samples were analyzed by quantitative HPLC/MS.

**Remarks:** This study was conducted at 50 °C in order to facilitate hydrolysis. Rates derived at 50 °C were extrapolated to 25 °C by dividing by a factor of 10, which is valid for reactions with Arrhenius heats of activation near 18 kcal/mole.

### RESULTS

**Degradation %:** Not applicable; no degradation was reported.

**Half-life ( $t_{1/2}$ ):**  $\geq 41$  years

**Breakdown products (yes/no):** No

**Remarks:** The analytical results indicate no degradation of PFOS or dependence on pH. The mean and standard deviation of all observed PFOS concentrations, pooled over the 6 observed pH levels, indicate that the pseudo-first order hydrolytic half-life of PFOS is greater than 41 years.

### CONCLUSIONS

The analytical results indicate no degradation of PFOS or dependence on pH. The study indicates that the hydrolytic half-life of PFOS in water is greater than 41 years.

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Submitters' Remarks: The authors assigned a Klimisch ranking of 2 for the reliability of this study. The authors also noted that the study was well-conducted, but not under GLP.

**Reviewer's Remarks:** None

REFERENCE

Hatfield, T. 2001a. Hydrolysis Reactions of Perfluorooctane Sulfonate (PFOS). 3M Environmental Laboratory and Pace Analytical Services. Lab request number W1878. Minneapolis, MN.

## BIODEGRADATION STUDY

**Title:** The 35-day aerobic biodegradation study of PFOS

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or  $C_8F_{17}SO_3^-K^+$ . (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS# 2795-39-3)

**Remarks:** PFOS is a white powder. The original 3M production lot number was not noted. The PFOS was HPLC purified. An Interim Certificate of Analysis reports the purity to be 97.9%. All results in this report are based on a purity of 86.4%, however. The lower purity value was associated with a standard the laboratory was using during this study.

### METHOD

**Method/guideline followed:** Based on EPA Guidelines OPPTS 835.3200, OPPTS 835.3210, OPPTS 835.5045.

**Test Type:** Aerobic

**GLP:** No

**Year study performed:** 2001

**Contact time:** 35 days

**Inoculum:** Activated sludge collected 9/18/00 from the aeration basin at the Metro Wastewater Treatment Plant, St. Paul, MN. The suspended solids were allowed to settle for approximately 2 days at 4EC and the settled sludge, approximately 20% of the volume, was used to prepare cultures for the biodegradation study. The mixed liquor suspended solids, MLSS, was not noted.

**Test medium:** Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Fifty mL of settled sludge was added per liter of mineral salts medium.

**Remarks:** Three types of samples were prepared for this test: blank sludge controls (mineral medium, inoculum), abiotic controls (mineral medium, PFOS), and test substance samples (mineral medium, inoculum, PFOS).

Test vessels, sterile 125 mL Nalgene polycarbonate culture flasks containing 25 mL media, were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

The test concentration used was 2.582 mg/L. The samples were agitated at 200 rpm at  $25 \pm 3$  °C. Test vessels were spiked 6  $\mu$ L of 10,760 mg/L solution of PFOS in methanol yielding a 2.582 mg/L PFOS solution. Samples were taken on days 0, 2, 5, 7, 14, and 35.

The day zero test vessels were prepared and immediately placed in a freezer that was maintained at  $-20 \pm 7$  °C. Upon removal from the incubator, test vessels on other days were either immediately frozen, or prepared by solid phase extraction (SPE). Following thawing, if needed, test vessel contents were adjusted to 1% acetic acid and then passed through a conditioned SEP-VAC C18 6cc SPE cartridge containing a plug of quartz wool to deter plugging. Methanol was then added to the emptied culture flask, shaken

vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was then collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative-ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to the parent, PFOS, the compounds below were quantified. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

| Compound Name   | Acronym             | Chemical Formula                   |
|---|---------------------|------------------------------------|
| 2-(N-ethyl Perfluorooctane sulfonamido) ethyl alcohol | N-EtFOSE<br>Alcohol | $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$ |
| 2-(N-ethyl Perfluorooctane sulfonamido) acetic acid   | N-EtFOSAA           | $C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$ |
| 2-(Perfluorooctane sulfonamido) acetic acid           | M556                | $C_8F_{17}SO_2NH(CH_2COOH)$        |
| N-Ethyl perfluorooctane sulfonamide                   | N-EtFOSA            | $C_8F_{17}SO_2NH(C_2H_5)$          |
| Perfluorooctane sulfinatate, potassium salt           | PFOSulfinatate      | $C_8F_{17}SO_2^-K^+$               |
| Perfluorooctanoate, ammonium salt                     | PFOA                | $C_8F_{15}COO^-NH_4^+$             |
| Perfluorooctane sulfonamide                           | FOSA                | $C_8F_{17}SO_2NH_2$                |

## RESULTS

### **Degradation % after time: 0**

**Results:** The analytical results demonstrate that when exposed to municipal wastewater treatment sludge for 35 days, the 2.582 mg/L PFOS samples generated no quantifiable degradation products. PFOS was recovered at  $2.553 \pm 0.102$  mg/L in the pooled study samples and at  $2.653 \pm 0.083$  mg/L in the pooled abiotic samples. The measured concentration of PFOS was always  $100 \pm 7\%$  of the expected concentration.

**Breakdown products:** No

**Remarks:** None

## CONCLUSIONS

The six-sample point screening study established that PFOS is not biodegraded by the microbial populations of the municipal waste treatment inoculum used under the conditions tested.

**Submitters' remarks:** The submitters' assigned a data quality rating of 2 for this study since it was conducted as a non-GLP study, but with the understanding that good data quality objectives be met.

No sample matrix spikes were included in this study. However, PFOS was recovered at expected concentrations, and previous duplicated results obtained during a related project showed excellent PFOS recoveries from sludge using the same extraction method.

A series of positive controls were not run with this study. However, the sludge used in this study was also



used for the preparation of samples in other biodegradation studies, and in some of those studies, preliminary results demonstrated positive biological activity for degradation of test samples.

REFERENCE

Lange, C. 2001b. The 35-day aerobic biodegradation study of PFOS. Pace Analytical Services, Inc. Minneapolis, MN. 3M Company. Minneapolis, MN.

## PHOTODEGRADATION STUDY

**Title:** Screening studies on the aqueous photolytic degradation of potassium perfluorooctane sulfonate (PFOS)

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS-potassium salt, 1-perfluorooctanesulfonic-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoropotassium salt.

**Remarks:** 3M production lot number 171. The test substance is a light-colored powder at 25 °C. The purity was determined to be 86.4% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR, and elemental techniques.

### METHOD

**Method/guideline followed:** Based on OPPTS: 835.5270 and OECD Draft Document "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis," August 2000.

**Test Type:** Direct and indirect photolysis

**GLP (Y/N):** No

**Year study performed:** 2001

**Light Source:** Suntest CPS+ or Suntest XLS+ lamp

**Light Spectrum (nm):** 290-800 nm

**Relative Intensity based on Intensity of Sunlight:** 680 w/m<sup>2</sup>

**Spectrum of substance (max lambda, max epsilon and epsilon 295):** A UV/Vis spectrum of a saturated aqueous solution of PFOS was recorded between 190 and 110 nm.

**Test sample preparation:** Aliquots of PFOS were added to 3 separate sets of VOA screw cap vials (exposed, unexposed, and control vials) containing 5 mL of appropriate matrix. Test vials were placed in the photoreactor. Control vials were wrapped in aluminum foil, sealed in a plastic bag, and placed in the photoreactor.

**Analytical Procedures:** Samples were analyzed by quantitative LC/MS and GC/MS techniques.

**Remarks:** The duration of the study was 67-167 hours. The test media used were water, H<sub>2</sub>O<sub>2</sub>/water (1:1 molar equivalent), Fe<sub>2</sub>O<sub>3</sub>/water (Fe<sup>3+</sup> at 24X molar excess), Fe<sub>2</sub>O<sub>3</sub>/water with H<sub>2</sub>O<sub>2</sub>, and commercial (Aldrich) humic material prepared as in OPPTS 835.5270. All tests included a series of unexposed controls (kept in the dark) for the evaluation of any degradation reactions occurring without the presence of light. Solvent, matrix, and control blanks and spikes were tested under each condition.

### RESULTS

**Concentration of Substance:** Not stated

**Temperature EC:**  $25 \pm 3$

**Direct photolysis:** No decomposition was observed.

**Indirect photolysis:** Data obtained from the  $\text{Fe}_2\text{O}_3$  matrix samples (with and without  $\text{H}_2\text{O}_2$ ) were pooled to provide sufficient data to estimate the minimum half-life. The mean standard deviation of these data indicate that the minimum environmental half-life of PFOS due to indirect photolysis at 25 °C is greater than 3.7 years.

**Breakdown products (yes/no):** No

**Remarks:** No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. Direct photolytic decomposition of PFOS was not observed based on loss of starting material, nor were any of the predicted degradation products detected above their limits of quantitation.

### CONCLUSIONS

No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. The mean and standard deviation of the observed PFOS concentrations in an aqueous  $\text{Fe}_2\text{O}_3/\text{H}_2\text{O}_2$  matrix indicate that the indirect photolytic half-life of PFOS at 25 °C is greater than 3.7 years.

**Submitters' Remarks:** The authors assigned a Klimisch ranking of 2 for the data quality of this study.

**Reviewer's Remarks:** None

### REFERENCE

Hatfield, T. 2001b. Screening studies on the aqueous photolytic degradation of potassium perfluorooctane sulfonate (PFOS). 3M Environmental Laboratory. 3M Company, St. Paul, MN. Report number W2775.

## BIOCONCENTRATION IN FISH

1)

**Title:** Perfluorooctanesulfonate, potassium salt (PFOS): A flow-through bioconcentration test with bluegill (*Lepomis macrochirus*)

### TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS #2795-39-3)

**Remarks:** Sample from 3M production lot number 217. The test substance was a white powder. The purity was determined to be 86.9% by LC/MS, <sup>1</sup>H-NMR, <sup>19</sup>F-NMR, and elemental analysis techniques.

### METHODS

**Method/guideline followed:** USEPA OPPTS 850.1730 and OECD 305

**Test type:** Flow-through exposure with flow-through depuration phase (flow rate: approximately 6 volume additions per 24 hours)

**GLP:** Yes

**Year study performed:** 2001

**Species:** Bluegill sunfish (*Lepomis macrochirus*); mean length = 62 mm (56-66 mm); mean weight: 2.70 g (2.03-3.32 g); age ~ 7 months old

**Supplier:** Osage Catfisheries, Inc. Osage Beach, Missouri

**Concentrations tested:** 0, 0.086, and 0.87 mg/L

**Uptake period:** 62 days (0.086 mg/L exposure); 35 days (0.087 mg/L exposure– this exposure ended after 35 days due to fish mortality)

**Depuration period:** 56 days (0.086 mg/L exposure); None (0.87 mg/L exposure)

**Analytical monitoring:** Analyses of test solutions and fish tissues were performed at Wildlife International, Ltd. Water samples were diluted and analyzed by HPLC with a single quadruple mass spectrometric detection. Tissue samples were homogenized, extracted, diluted, and analyzed by HPLC with triple quadruple mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

**Collection of tissue samples:** Fish were collected from test chambers by random selection at 12 time

points during the 62-day uptake phase. They were euthanized, blotted dry, weighed, and measured. Fish were then rinsed with dilution water, blotted dry again, and dissected into edible and nonedible fractions. The fractions were individually weighed. The head, fins, and viscera were considered to be nonedible tissue. The remaining tissue including skin was considered to be edible tissue.

**Statistical methods:** Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different ( $p > 0.05$ ). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, data were transformed in an attempt to correct the data. Mean tissue concentrations were then compared using ANOVA and Dunnett's test.

The kinetic bioconcentration factor (BCFK), uptake rate ( $k_1$ ), and depuration rate ( $k_2$ ) were calculated for the edible, nonedible, and whole fish exposed to 0.086 mg/L PFOS using BIOFAC computer software. BIOFAC is a nonlinear parameter estimate routine, which estimates rate constants from a set of sequential time-concentration data. These rate constants were then used to calculate a BCFK ( $BCFK = k_1/k_2$ ).

**Test conditions:** Stainless steel aquaria (104 L) filled with approximately 80 L solution were used as exposure vessels. One vessel per concentration was used. The loading rate was 0.48 g fish/L/day (90 fish per vessel). The fish were fed flake food (Ziegler Brothers, Inc., Gardners, PA) and given 16 hours of light and 8 hours of dark with a 30 minute transition period. The light intensity was 278 lux at the surface of the negative control vessel at test initiation.

Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions were stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks were injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless. The dilution water was moderately-hard well water with specific conductance of 313 umhos/cm, hardness of 130 mg/L, alkalinity of 178, and pH of 8.1. During the test, the dissolved oxygen levels ranged from 6.4-8.6 mg/L, the temperature ranged from 21.7 to 22.0 °C, and the pH ranged from 7.9 to 8.2.

**Remarks:** None

## RESULTS

### **Bioconcentration factors (BCF):**

#### **0.086 mg/L apparent steady-state BCF:**

**Edible:** 484

**Nonedible:** 1124

**Whole fish:** 856

#### **0.87 mg/L(study ended prior to achieving steady-state) BCF:**

**Edible:** 136

**Nonedible:** 386

**Whole fish:** 278

**BIOFAC Estimates (using 0.086 mg/L exposure):**

|   | <u>Edible</u> | <u>Nonedible</u> | <u>Whole fish</u> |
|---|---------------|------------------|-------------------|
| <b>BCFK:</b>                            | 1866          | 4312             | 3614              |
| <b>Time to reach<br/>50% clearance:</b> | 146 days      | 133 days         | 152 days          |

**PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L:**  
(Values are from 4 individual fish at each sample period.)

| <b>Uptake day</b>         | <b>Edible tissue, mg/kg</b>   | <b>Nonedible tissue, mg/kg</b> | <b>Whole fish conc., mg/kg</b> |
|---------------------------|-------------------------------|--------------------------------|--------------------------------|
| 0 (4 hours)               | 0.167, 0.155, 0.144,<br>0.182 | 0.415, 0.519, 0.417, 0.497     | 0.293, 0.351, 0.286, 0.363     |
| 1                         | 0.734, 0.726, 0.631,<br>0.806 | 1.68, 1.85, 1.72, 2.07         | 1.26, 1.34, 1.29, 1.53         |
| 3                         | 1.73, 2.07, 2.03, 2.11        | 4.59, 5.50, 5.47, 5.97         | 3.21, 4.04, 4.18, 4.38         |
| 7                         | 3.73, 4.25, 4.73, 6.25        | 10.2, 10.6, 11.9, 15.2         | 7.33, 7.66, 8.73, 11.4         |
| 14                        | 11.4, 9.07, 13.7, 12.6        | 27.3, 23.2, 35.3, 32.6         | 20.2, 16.9, 26.0, 24.6         |
| 21                        | 11.7, 12.0, 12.9, 10.6        | 33.3, 22.7, 24.6, 24.4         | 23.3, 18.4, 19.8, 18.5         |
| 28                        | 18.3, 13.7, 23.9, 23.1        | 49.4, 40.7, 65.3, 57.9         | 35.3, 29.2, 45.4, 44.1         |
| 35                        | 22.6, 27.7, 23.8, 20.6        | 67.1, 73.3, 62.0, 59.1         | 46.3, 53.8, 46.6, 40.9         |
| 42                        | 27.6, 25.3, 21.2, 27.6        | 64.0, 68.1, 54.4, 79.6         | 50.1, 49.4, 40.9, 56.3         |
| 49                        | 33.3, 36.2, 39.0, 30.6        | 85.0, 95.1, 93.1, 77.7         | 62.8, 69.6, 70.8, 57.4         |
| 56                        | 48.3, 38.9, 44.1, 38.3        | 122, 94.2, 73.2, 106           | 90.6, 71.6, 63.3, 74.8         |
| 62                        | 42.4, 66.2, 42.2, 39.2        | 101, 112, 105, 96.4            | 77.0, 92.7, 79.6, 73.1         |
| <b>Depuration<br/>day</b> |                               |                                |                                |
| 14                        | 48.5, 31.8, 31.6, 42.0        | 124, 79.4, 81.8, 113           | 90.3, 60.4, 61.6, 85.3         |
| 28                        | 26.0, 33.3, 38.7, 55.8        | 85.7, 95.1, 85.7, 94.8         | 58.2, 70.1, 68.1, 81.1         |
| 42                        | 24.1, 31.2, 30.0, 33.0        | 71.7, 80.6, 78.3, 82.1         | 51.4, 61.4, 61.0, 62.2         |
| 56                        | 21.1, 37.6, 32.9, 31.2        | 57.7, 80.3, 85.4, 84.4         | 41.6, 66.5, 65.8, 62.1         |

**PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L:**

(Values are from 4 individual fish at each sample period.)

| <b>Uptake day</b> | <b>Edible tissue, mg/kg</b> | <b>Nonedible tissue, mg/kg</b> | <b>Whole fish conc., mg/kg</b> |
|-------------------|-----------------------------|--------------------------------|--------------------------------|
| 0 (4 hours)       | 1.46, 1.48, 1.19, 1.39      | 3.52, 4.37, 4.22, 4.06         | 2.71, 3.08, 2.84, 2.89         |
| 1                 | 4.68, 6.59, 5.56, 5.64      | 11.1, 14.2, 13.3, 12.1         | 8.00, 10.9, 10.2, 9.47         |
| 3                 | 17.3, 15.8, 19.0, 20.8      | 39.3, 42.0, 43.8, 51.8         | 30.5, 30.7, 34.5, 39.1         |
| 7                 | 42.0, 44.0, 57.7, 46.8      | 100, 102, 102, 120             | 74.9, 77.0, 85.3, 89.8         |
| 14                | 87.1, 81.6, 90.7, 73.3      | 177, 207, 245, 214             | 141, 157, 180, 158             |
| 21                | 79.4, 117, 104, 102         | 201, 278, 246, 229             | 146, 210, 185, 172             |
| 28 <sup>1</sup>   | 102, 131, 107, 133          | 289, 372, 320, 361             | 205, 267, 232, 263             |

<sup>1</sup>Sampling of fish stopped after Uptake day 28 due to mortality.

**Remarks:** Test organism mortality was none in the negative control (during both the uptake and depuration phases). At 0.086 mg/L, one fish died after 49 days and one died after 59 days. None died during the depuration phase. At 0.87 mg/L, mortality was first noted on day 9 and continued through day 35 of the uptake phase, at which time all fish either died or had been sampled.

**Was control response satisfactory:** Yes**Statistical results:** NoneCONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. Apparent steady-state was attained on Day 49 for the fish exposed to 0.086 mg a.i./L. Although Day 49, 56, and 62 tissue residues were not statistically significantly different, PFOS concentrations appeared to be still increasing during this time. Apparent steady-state BCF values for edible, nonedible, and whole fish tissues were calculated to be 484, 1124, and 859, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible, and whole fish tissues were 146, 133, and 152 days, respectively.

**Submitters' remarks:** The authors assigned this study a Klimisch data reliability ranking of 1.**Reviewers' remarks:** NoneREFERENCE

Drottar, K., VanHoven, R., and H. Krueger. 2001. Perfluorooctanesulfonate, potassium salt (PFOS): A flow-through bioconcentration test with bluegill (*Lepomis macrochirus*). Wildlife International, Limited. Project number 454A-134. 3M Company. St. Paul, MN.

## Annex 6. Robust Summaries of Toxicology and Human Biomonitoring Studies

### PHARMACOKINETIC STUDIES

**Title:** Absorption of FC-95-14C in Rats after a Single Oral Dose (1979)

#### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS 2795-39-3

**Remarks:** FC-95-14C (carbon-14 label alpha to sulfur atom, Riker Isotope Inventory Number 442). The specific activity is 0.459 +- 0.008 uCi/mg. Thin-layer and column chromatography showed the FC-95-14C to be at least 99% radiochemically pure. The FC-95-14C was found to be suitable for metabolism studies. (Synthesis described in Johnson and Behr, 1979).

#### METHOD

**Method/guideline followed:** NA

**Test type:** in vivo

**Species/strain/cell type or line:** rat, Charles River CD

**Sex:** male

**Age and body weight range of animals used:** 8 weeks, bw mean 285 g (range 243-315)

**Number of animals/sex/dose:** 24

**Route of administration:** oral

**Vehicle:** 0.9% NaCl solution containing 1.2 mg FC-95-14C/2.0 ml

**Doses:** 4.2 mg/kg average, single dose

#### **Excretion routes, body fluids, and tissues monitored and/or sampled during study:**

red blood cells, plasma, urine, feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and carcass

**Statistical methods used:** mean, log mean concentration versus time least squares line

#### **Method remarks:**

Rats were conditioned to individual metal metabolism cages for 24 hours prior to dosing. Rats were allowed free access to Purina Ground Chow and water before and after dosing. Each non-fasted rat was weighed immediately before being given a single oral dose of FC-95-14C. The dosing solution was prepared by adding ~200 mg of FC-95-14C to 0.9% NaCl, shaking for one half hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting. The dose was delivered with a 2.0 cc glass syringe (Trylon) fitted with a stainless steel intubation tube. Recovery of total carbon-14 from series of FC-95-14C spiked, blank biological samples was used to adjust the recovery of the test samples to account for label loss during the experimental manipulations.



Groups of three rats were sacrificed by exsanguination at 1, 2, 6, 12, 24, 48, 96, and 144 hours post dose. Rats were anesthetized with diethyl ether and blood was drawn from the descending aorta of each rat and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. In addition to plasma and red blood cells, total urine, total feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and remainder of carcass were saved from each of the three rats in the 24 and 48 hours post dose groups for carbon-14 analysis.

## RESULTS

### **Detailed results:**

After a single oral dose of FC-95-14C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours. The half-life for elimination of total carbon-14 from plasma is 7.5 days.

The digestive tract and contents contained on the average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the FC-95-14C dose was absorbed from solution after administration to non-fasted rats. The major portion of the radioactivity recovered was found in the carcass. The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ~0.2%. The concentrations of total carbon-14 in red blood cells and plasma were compared. The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-14C, there is no selective retention of carbon-14 in red blood cells.

The half-life of elimination from plasma was determined by analysis of plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after a single oral dose of FC-95-14C. The log of mean concentration versus time for these data was plotted. The least squares line through the individual points from 24 to 144 hours for these data fits the equation:  $C_p = 15.65e^{(-0.00387t)}$  where  $C_p$  is plasma concentration. The half-life of elimination from plasma is 179 hours (7.5 days). Thus, elimination from plasma of total carbon-14 after a single oral dose of FC-95-14C is slow.

**Metabolites measured:** none

## CONCLUSIONS

agree

## REFERENCE

Absorption of FC-95-14C in Rats after a Single Oral Dose. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN. Project No. 890310200. Johnson, JD, Gibson, SJ, and Ober, RF, October 26, 1979.

**Title:** Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-14C (1979)

## TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS 2795-39-3

**Remarks:** FC-95-14C (carbon-14 label alpha to sulfur atom, Riker Isotope Inventory Number 442). The specific activity is 0.459 +- 0.008 uCi/mg. Thin-layer and column chromatography showed the FC-95-14C to be at least 99% radiochemically pure. The FC-95-14C was found to be suitable for metabolism studies. (Synthesis described in Johnson and Behr, 1979).

## METHOD

**Method/guideline followed:** NA

**Test type:** in vivo

**Species/strain/cell type or line:** rat, Charles River CD

**Sex:** male

**Age and body weight range of animals used:** 8 weeks, bw mean 288 g (range 262-303)

**Number of animals/sex/dose:** 6

**Route of administration:** iv, via tail vein

**Vehicle:** 0.9% NaCL solution containing 1.2 mg FC-95-14C/2.0 ml

**Doses:** 4.2 mg/kg average, single dose

### **Excretion routes, body fluids, and tissues monitored and/or sampled during study:**

urine, feces, liver, plasma, kidney, lung, spleen, bone marrow, adrenals, skin, testes, muscle, fat, eye, brain

**Statistical methods used:** mean, standard deviation

### **Method remarks:**

Rats were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The rats were allowed free access to Purina Ground Chow and water before and after dosing. Each rat was weighed, anesthetized with diethyl ether, then given a single iv dose using a 3.0 cc disposable plastic syringe fitted with a 26 gauge 1/2" needle. Urine and feces were collected at intervals for each of the six rats for 89 days. At 89 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta, animals were sacrificed by exsanguination, and tissue samples were collected.

## RESULTS

### **Detailed results:**

By 89 days post dose, mean urinary excretion was 30.2+-1.5% of total C-14 administered. Mean cumulative fecal excretion was 12.6+-1.2%. The authors note that radioactive content in feces was too low to measure after 64 days. At day 89, mean tissue C-14 concentrations above one ug FC-95-14C

equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1. Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 ug/g. There was a difference in C-14 content of subcutaneous fat (0.2 ug/g) and abdominal fat ( $\leq 0.08$  ug/g). Very little C-14 was found in whole eye (0.16 ug/g) and no detectable C-14 was found in brain. Only liver and plasma contained a substantial percentage of dose at 89 days post dose, 25.21% and 2.81%, respectively. The low levels of radioactivity found for kidney, lung, testes, and spleen are due in part to blood still contained in these organs when homogenized.

#### Mean Excretion of Total Carbon-14 in Urine Over Time

| Collection Period<br>(Days) | Percent Dose<br>During Period |
|-----------------------------|-------------------------------|
| 0-0.5                       | 0.91                          |
| 0.5-1                       | 0.77                          |
| 1-2                         | 1.21                          |
| 2-3                         | 1.03                          |
| 3-4                         | 0.93                          |
| 4-5                         | 0.83                          |
| 5-6                         | 0.71                          |
| 6-7                         | 0.76                          |
| 7-8                         | 0.75                          |
| 8-9                         | 0.68                          |
| 9-10                        | 0.68                          |
| 10-11                       | 0.59                          |
| 11-12                       | 0.58                          |
| 12-13                       | 0.59                          |
| 13-14                       | 0.55                          |
| 14-15                       | 0.54                          |
| 15-16                       | 0.51                          |
| 16-17                       | 0.48                          |
| 17-18                       | 0.43                          |
| 18-19                       | 0.39                          |
| 19-21                       | 0.84                          |
| 21-23                       | 0.78                          |
| 23-25                       | 0.66                          |
| 25-27                       | 0.68                          |
| 27-29                       | 0.68                          |
| 29-32                       | 0.86                          |
| 32-36                       | 1.05                          |
| 36-40                       | 0.99                          |
| 40-43                       | 0.75                          |
| 43-47                       | 0.92                          |
| 47-50                       | 0.68                          |
| 50-54                       | 0.78                          |
| 54-57                       | 0.61                          |
| 57-61                       | 0.79                          |
| 61-69                       | 1.50                          |
| 69-78                       | 1.64                          |
| 78-89                       | 2.08                          |
| Total                       | 30.2                          |

#### Mean Excretion of Total Carbon-14 in Feces Over Time

| Collection Period<br>(Days) | Percent Dose<br>During Period |
|-----------------------------|-------------------------------|
| 0-0.5                       | 0.049                         |
| 0.5-1                       | 0.842                         |
| 1-2                         | 0.795                         |
| 2-3                         | 0.649                         |
| 3-4                         | 0.656                         |
| 4-5                         | 0.577                         |
| 5-6                         | 0.510                         |
| 6-7                         | 0.588                         |
| 7-8                         | 0.482                         |
| 8-9                         | 0.421                         |
| 9-10                        | 0.387                         |
| 10-11                       | 0.370                         |
| 11-12                       | 0.296                         |
| 12-13                       | 0.310                         |
| 13-14                       | 0.281                         |
| 14-15                       | 0.276                         |
| 15-16                       | 0.272                         |
| 16-17                       | 0.187                         |
| 17-18                       | 0.163                         |
| 18-19                       | 0.129                         |
| 19-21                       | 0.311                         |
| 21-23                       | 0.302                         |
| 23-25                       | 0.262                         |
| 25-27                       | 0.208                         |
| 27-29                       | 0.202                         |
| 29-32                       | 0.223                         |
| 32-36                       | 0.526                         |
| 36-50                       | 1.530                         |
| 50-64*                      | 0.833                         |
| Total                       | 12.6                          |

\*The radioactive content of the feces was too low to measure after 64 days.

**Metabolites measured:** none.

**CONCLUSIONS**

agree

REFERENCE

Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95- 14 C. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN. Johnson, JD, Gibson, SJ, and Ober, RE , December 28, 1979.

**Title:** Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [<sup>14</sup>C]Perfluorooctanoate or Potassium [<sup>14</sup>C]Perfluorooctanesulfonate (1984)

## TEST SUBSTANCE

### Identity:

Potassium perfluorooctanesulfonate (14C-PFOS)

Ammonium perfluorooctanoate (14C-PFO)

### Remarks:

14C-PFOS: sp act 0.46 uCi/mg, radiochemical purity >99%,

14C label in PFOS is adjacent to sulfur

14C-PFO: sp act 0.51 uCi/mg, radiochemical purity >98%

## METHOD

**Method/guideline followed:** NA

**Test type:** in vivo

**Species/strain/cell type or line:** rat, Charles River CD

**Sex:** male

**Age and body weight range of animals used:** 12 weeks, 300-342 g

**Number of animals/sex/dose:** 5

**Route of administration:** iv

**Vehicle:** 0.9% NaCl, 2 ml/rat

### Doses:

Potassium [<sup>14</sup>C]Perfluorooctanesulfonate (PFOS): 3.4 mg/kg mean, single dose, 0.56 mg/ml

PFOS control animals: 3.5 mg/kg mean

Ammonium [<sup>14</sup>C]Perfluorooctanoate (PFO): 13.3 mg/kg mean, single dose, 2.1 mg/ml

PFO control animals: 13.5 mg/kg mean

### Excretion routes, body fluids, and tissues monitored and/or sampled during study:

Urine, plasma, red blood cells, liver

**Statistical methods used:** mean, standard deviation, Student's t test

### Method remarks:

Rats were housed in individual stainless-steel metabolism cages and fasted with free access to water for 24 hrs prior to receiving the fluorochemicals. The radiolabeled compounds were administered as single intravenous doses (lateral tail vein). Two ml of dosing solution was administered to each rat. Ten rats were dosed with each compound. Five rats from each group were fed cholestyramine (dried and ground resin Z-620), 4% in feed (Purina Lab Chow), for 14 days after administration of PFO and for 21 days after administration of PFOS. Control rats were administered radiolabeled fluorochemical but were not treated with cholestyramine. In order to allow comparison of the radiometric results on an absolute basis, the

radiolabel doses were not adjusted for individual body weights. Urine and feces samples were collected at intervals for individual rats in each group until 14 days after 14C-PFO administration and 21 days after 14C-PFOS administration. At these times, rats were anesthetized with diethyl ether and exsanguinated by drawing blood from the descending aorta. Plasma and red blood cells were prepared promptly by centrifugation. Liver was collected as the whole organ and stored frozen until analysis.

## RESULTS

### **Detailed results:**

After 21 days of cholestyramine treatment, the mean percentage of 14C-PFOS dose eliminated via feces (75.8 ± 5.0) was 9.5-fold the mean percentage of dose eliminated via feces by control rats (8.0 ± 0.8). After adjustment for the amount of carbon-14 excreted in urine (18% for controls and 5% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 19% for cholestyramine-treated rats and 74% for control rats. After 14C-PFOS administration, the mean liver carbon-14 content at 21 days represents 11% and 40% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 21 days of cholestyramine treatment.

After 14 days of cholestyramine treatment, the mean percentage of 14C-PFO dose eliminated via feces (43.2 ± 5.5) was 9.8-fold the mean percentage of dose eliminated via feces by control rats (4.4 ± 1.0). After adjustment for the amount of carbon-14 excreted in urine (67% for controls and 41% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 16% for cholestyramine-treated rats and 28% for control rats. After 14C-PFO administration, the mean liver carbon-14 content at 14 days represents 4% and 8% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 14 days of cholestyramine treatment.

Carbon-14 Concentration (expressed as ug eq/g tissue or ml fluid)

| Treatment Group | Liver     | Plasma   | Red Blood Cells |
|-----------------|-----------|----------|-----------------|
| <b>14C-PFOS</b> |           |          |                 |
| Cholestyramine  | 9.4±1.6*  | 0.9±0.1* | 0.3±0.1*        |
| Control         | 35.6±5.6  | 6.9±0.6  | 1.8±0.4         |
| <b>14C-PFO</b>  |           |          |                 |
| Cholestyramine  | 12.1±2.1* | 5.1±1.7* | 1.8±0.7*        |
| Control         | 22.3±6.2  | 14.7±6.8 | 4.2±2.4         |

\*Significantly different from control values (p<0.05)

The authors conclude that the high concentration of 14C-PFOS or 14C-PFO in liver at 2 to 3 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 by nearly 10-fold suggest that there is a considerable enterohepatic circulation of 14C-PFOS and 14C-PFO.

**Metabolites measured:** none

## CONCLUSIONS

agree

## REFERENCE

Johnson, J. D., Gibson, SJ, and Ober, RE (1984). Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium

[14C]Perfluorooctanesulfonate. *Fundamental and Applied Toxicology* 4, pages 972-976.

See also Johnson, J. D., Gibson, SJ, and Ober RE (1984). Enhanced elimination of FC-95-14C and FC-143-14C in rats with cholestyramine treatment. Project No. 8900310200, Riker Laboratories, Inc. St. Paul, MN.

**Title:**

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats, Analytical Laboratory Report, Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine, and Feces Samples

**TEST SUBSTANCE**

**Identity:** Perfluorooctylsulfonate, potassium salt, CAS 2795-39-3

**Remarks:** Purity 86.9%, Lot # 217

**METHOD**

**Method/guideline followed:**

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions noted on page 3 of report.

**Test type:** in vivo

**Species/strain/cell type or line:** rat/Sprague-Dawley/pregnant CrI:CD(R)BR VAF/Plus(R)

**Sex:** F0: female, F1: both

**Age and body weight range of animals used:** 60 days, 200-225 g

**Number of animals/sex/dose:** F0: 16, F1: 5 male and 5 female pups/litter

**Route of administration:** oral

**Vehicle:** 0.5% Tween(R) 80 in R.O. deionized water, dosage volume 5 ml/kg

**Doses:** 0 (vehicle), 0.1, 0.4, 1.6, and 3.2 mg/kg/day in volume of 5 ml/kg, once daily beginning 42 days prior to cohabitation, and continued through day 14 or day 20 of presumed gestation. Only the F0 females were dosed.

**Excretion routes, body fluids, and tissues monitored and/or sampled during study:**

F0 urine, feces, serum, liver. F1 liver and serum.

**Statistical methods used:** mean and standard deviation.

**Method remarks:** Serum, urine, and feces specimens were collected from adult female rats (F0 dams) before mating and at gestation day (GD) 7, GD 15 and GD 21. Liver specimens were collected from F0 dams at termination of the study (GD 21). A total of 54 pooled serum and liver specimens were collected from fetuses on GD 21. Specimens were sent to the 3M Environmental Laboratory and the contract labs to be analyzed for PFOS.



**RESULTS****Detailed results:****Average Results for the Analysis of Serum Samples (ug/ml)**

|              | 0 mg/kg | 0.1 mg/kg | 0.4 mg/kg | 1.6 mg/kg | 3.2 mg/kg |
|--------------|---------|-----------|-----------|-----------|-----------|
| Day 0 dam    | 0.0723  | 8.89      | 40.7      | 160       | 318       |
| Day 7 dam    | 0.126   | 7.82      | 40.9      | 154       | 105       |
| Day 15 dam   | 0.0926  | 8.80      | 41.4      | 156       | 275       |
| Day 21 dam   | 0.0714  | 4.24      | 26.2      | 136       | 155       |
| Day 21 Fetal | 0.125   | 9.07      | 34.3      | 101       | 165       |

**Average Results for the Analysis of Liver Samples (GD 21)**

| Dose Group<br>(mg/kg/day) | PFOS Conc. (ug/g) |                  |
|---------------------------|-------------------|------------------|
|                           | Female Adult (F0) | Fetal Liver (F1) |
| 0                         | 0.288             | 0.169            |
| 0.1                       | 29.2              | 7.93             |
| 0.4                       | 107               | 30.6             |
| 1.6                       | 347               | 86.7             |
| 3.2                       | 610               | 230              |

**Average Results for the Analysis of Urine Samples from F0 Dams**

|        | 0 mg/kg | 0.1 mg/kg | 0.4 mg/kg | 1.6 mg/kg | 3.2 mg/kg |
|--------|---------|-----------|-----------|-----------|-----------|
| Day 0  | <LOQ    | 0.0497    | 0.302     | 0.959     | 1.53      |
| Day 7  | <LOQ    | 0.0620    | 0.308     | 1.10      | 1.60      |
| Day 15 | 0.00905 | 0.0685    | 0.526     | 0.622     | 0.563     |
| Day 21 | 0.0194  | 0.0574    | 0.555     | 2.71      | 1.61      |

**Average Results for the Analysis of Feces Samples from F0 Dams**

|        | 0 mg/kg | 0.1 mg/kg | 0.4 mg/kg | 1.6 mg/kg | 3.2 mg/kg |
|--------|---------|-----------|-----------|-----------|-----------|
| Day 0  | 0.0380  | 0.499     | 2.42      | 10.3      | 23.9      |
| Day 7  | 0.0155  | 0.490     | 2.16      | 9.19      | 33.0      |
| Day 15 | 0.0322  | 0.662     | 2.93      | 11.1      | 29.5      |
| Day 21 | 0.0342  | 0.416     | 2.39      | 9.94      | 20.1      |

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the fetuses. PFOS was also observed in the control dams, as well as the control fetuses. On sacrifice on GD21, the levels of PFOS were much higher in the liver than in the serum for the dams. The levels of PFOS remained fairly steady in the serum of the dams from GD0 – GD 15, but the levels dropped at GD21. In the GD21 fetuses, the level of PFOS in the serum was generally comparable to the level observed in the dams, whereas the level of PFOS in the fetal livers was well below that seen in the dams.

**Results Remarks:**

As stated in the report. "It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to 50% or greater."

**Metabolites measured:** none

## CONCLUSIONS

Conclusions: Under the conditions of the present studies, PFOS was observed in the livers, urine, feces and sera of all female rats dosed with PFOS during the in-life phase of the study. Additionally, PFOS was observed in fetal liver and serum taken during gestation from the same group of female rats.

Agree

## REFERENCE

Study Title: Oral (Gavage) Pharmacokinetic Study of PFOS in Rats  
Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine, and Feces Samples  
3M Medical Department Study: T-6295.12, Argus In-Life Study: #418-013, FACT TOX-110, 3M Laboratory Request No. U2849, 3M Environmental Laboratory, May 4, 2001.

**Title:**

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, Analytical Laboratory Report, Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of Crl:CDBR VAF/Plus ® Rats Exposed to PFOS via Gavage

**TEST SUBSTANCE**

**Identity:** Perfluorooctylsulfonate, potassium salt (FC-95), CAS 2795-39-3

**Remarks:** Purity 86.9%, Lot # 217

**METHOD****Method/guideline followed:**

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions noted on page 3 of report. It does not appear these exceptions would significantly impact the results or conclusions. The analytical phase completed at the 3M Environmental Laboratory was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

**Test type:** in vivo

**Species/strain/cell type or line:** rat/Sprague-Dawley/pregnant Crl:CD(R)BR VAF/Plus ®

**Sex:** F0: female, F1: both

**Age and body weight range of animals used:** 65 days, 192-231 g

**Number of animals/sex/dose:** F0: 8, F1: 5 male and 5 female pups/litter

**Route of administration:** oral

**Vehicle:** 0.5% Tween(R) 80 in R.O. deionized water, dosage volume 5 ml/kg

**Doses:** 0 (vehicle), 0.1 and 1.6 mg/kg/day in volume of 5 ml/kg (0.00, 0.02, and 0.32 mg/mL), once daily beginning 43 days prior to cohabitation until confirmed evidence of mating. Only the F0 females were dosed.

**Excretion routes, body fluids, and tissues monitored and/or sampled during study:**

F0 urine, feces, serum, liver. F1 liver and serum.

**Statistical methods used:** means, standard deviations, and percentages

**Method remarks:**

Rat dams were exposed to PFOS via gavage prior to and during mating. Exposure to PFOS was halted on the first day of presumed gestation. Pups were not directly exposed to PFOS, but may have been exposed in utero and during lactation. Male rats of the same source and strain were used only as breeders and were not administered the test article or considered part of the test system.

Urine and fecal samples were collected from F0 female rats for the following intervals: one day prior to initiation of cohabitation to the following morning, days 6 to 7, 14 to 15, and 20 to 21 of presumed

gestation (DG 6 to 7, 14 to 15, and 20 to 21), and days of lactation (DL) 21 to 22. Blood samples were collected from each of the maternal rats on the day cohabitation was initiated (prior to cohabitation), DG 7, 15 and 21, and DL 14 and 22. Day 1 of lactation was defined as the day of birth. On DL 4, litters were culled to five male pups and five female pups per litter, where possible. Sera specimens were collected from pooled litter samples on DL 21. On DL 22, all surviving Generation F0 and Generation F1 animals assigned to the study were sacrificed, and a liver specimen was collected from each animal. The liver from each pup was collected and pooled per litter. Blood samples were collected and pooled per liter. Urine, fecal, serum and liver samples were shipped to the Sponsor for analysis. On days 1 to 4 of the 43-day pre-mating period, F0 female rats received 25% greater dose due to an incorrect calculated amount of test substance in vehicle.

## RESULTS

### Detailed results:

#### Average Results for the Analysis of Sera Samples (PFOS Conc. ug/ml)

|              | DG0   | DG7    | DG15   | DG21 | DL14   | DL21   | DL22   |
|--------------|-------|--------|--------|------|--------|--------|--------|
| 0.0 mg/kg F0 | 0.100 | 0.0796 | 0.0742 | <LLQ | 0.0542 | NS     | 0.0492 |
| 0.0 mg/kg F1 | NS    | NS     | NS     | NS   | NS     | 0.0531 | NS     |
| 0.1 mg/kg F0 | 9.21  | 7.24   | 5.68   | 2.58 | 1.63   | NS     | 0.979  |
| 0.1 mg/kg F1 | NS    | NS     | NS     | NS   | NS     | 1.80   | NS     |
| 1.6 mg/kg F0 | 161   | 129    | 90.6   | 39.5 | 20.6   | NS     | 14.1   |
| 1.6 mg/kg F1 | NS    | NS     | NS     | NS   | NS     | 27.1   | NS     |

#### Average Results for the Analysis of Liver Samples (PFOS Conc. ug/g)

|              | DL22  |
|--------------|-------|
| 0.0 mg/kg F0 | 0.243 |
| 0.0 mg/kg F1 | 0.174 |
| 0.1 mg/kg F0 | 6.15  |
| 0.1 mg/kg F1 | 5.00  |
| 1.6 mg/kg F0 | 59.7  |
| 1.6 mg/kg F1 | 56.2  |

#### Average Results for the Analysis of Urine Samples (PFOS Conc. ug/ml)

|              | DG0     | DG6/7  | DG14/15 | DG20/21 | DL21/22 |
|--------------|---------|--------|---------|---------|---------|
| 0.0 mg/kg F0 | 0.00819 | 0.0100 | 0.00685 | 0.00614 | <LOQ    |
| 0.1 mg/kg F0 | 0.0905  | 0.0307 | 0.0327  | 0.0231  | 0.00555 |
| 1.6 mg/kg F0 | 2.11    | 0.888  | 0.613   | 0.340   | 0.0334  |

#### Average Results for the Analysis of Feces Samples (PFOS Conc. ug/g)

|              | DG0   | DG6/7 | DG14/15 | DG20/21 | DL21/22 |
|--------------|-------|-------|---------|---------|---------|
| 0.0 mg/kg F0 | ND    | ND    | ND      | ND      | ND      |
| 0.1 mg/kg F0 | 0.601 | 0.399 | 0.294   | 0.119   | 0.0522  |
| 1.6 mg/kg F0 | 10.9  | 8.39  | 4.83    | 2.06    | 0.387   |

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the levels in the serum decreased with time. The levels of PFOS were much higher in the liver than in the serum of the dams and the pups. The levels of PFOS were similar in the liver of the dams and pups, while the levels in the serum were slightly higher in the pups than in the dams.

### Results Remarks:

As stated in the report. "It is not possible to verify true recovery of endogenous analyte from tissues

without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to 50% or greater.”

**Metabolites measured:** none

## CONCLUSIONS

### Statement of Conclusion

Under the conditions of the present study, perfluorooctanesulfonate was observed in all sample types of all Generation 0 test system animals dosed with the test substance during the in-life phase of the study, and in all sample types of their offspring (Generation F1).

Agree

## REFERENCE

Study Title: Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats Analytical Laboratory  
Report Title: Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of CrI:CDBR VAF/Plus ® Rats Exposed to PFOS via Gavage, 3M Environmental Laboratory Report No. FACT TOX-111, Laboratory Request No. U2994, 3M Ref. No. T-6295.14, Argus In-Life Study 418-015, May 4, 2001.

ENV/JM/RD(2002)17

**Title:** Half-Life Study of PFOS in Serum, 2000

**TEST SUBSTANCE**

**Identity:** PFOS

**METHOD**

**Study design:** PFOS half-life study on retired workers from the Decatur, Alabama plant

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the half-life of PFOS in retired perfluorochemical production workers.

**Study period:** Nov. 1998 to 2003

**Setting:** N/A

**Total population:** 27 retirees from the Decatur, Alabama and Cottage Grove, Minnesota plants

**Subject selection criteria:** The participants volunteered for this study

**Total # of subjects in study:** 18 males

**Comparison population:** N/A

**Participation rate:** Unknown

**Subject description:** Retirees ranged in age from 55-74 years, worked in the plant for an average of 28 years, average time from retirement to start of study was 30 months (range: 5-130 months).

**Health effects studied:** N/A

**Data collection methods:** Blood sera samples collected every 6 months

**Details on data collection:** No information was provided as to how the blood was drawn, stored, etc.

**Exposure period:** Unknown.

**Description/delineation of exposure groups/categories:** N/A

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** Medians and ranges calculated.

**Other methodological information:** Half-lives were calculated assuming a one-compartment model. A

log-linear relationship was used to estimate the serum fluorochemical elimination half-life in participating retirees. In this log linear relationship, the slope of the line is related to the elimination constant via the equation slope =  $-k_{el}(2.303)$ . Once the elimination constant is calculated, the half-life is determined using the relationship  $t_{1/2} = 0.693/k_{el}$ .

If 3 data points were not available for any of the subjects and if there was a lack of fit to the model, that retiree was not included in the analyses. Eighteen participants met these requirements.

## RESULTS

**Describe results:** The median serum half-life of PFOS was 270 days, with a range of 139 to 640 days. It should be noted that the difference in serum PFOS levels between retirees was quite large (0.2 - 2.0 ppm).

**Study strengths and weaknesses:** For most of the participants not included in the analysis, the second measurement was higher than the first. Therefore, the data did not fit the model and they were excluded. Although this may justify not including those participants in the analysis, it is an indication of the many limitations of the data. It is stated in the report that neither age nor number of months retired was associated with the serum PFOS half-life calculations; however, this statement is not supported with any data in the report. In addition, no individual data were provided in the report and the relationship between number of years exposed in the workplace and PFOS levels and half-life were excluded. Retirees were excluded from these calculations if their second measurement was higher than the first. It is unknown why this occurred, but the exclusion of those retirees introduces bias to the results. Also, elimination of PFOS occurs via urine and feces; however, these measurements were not taken. Therefore, it cannot be determined that the half-life suggested by the preliminary results reported here represents a true elimination half-life from the body. Finally, the effect of continued non-occupational, low-level exposure on the half-life is unknown.

**Research sponsors:** 3M Environmental Lab

**Consistency of results:** These results are not consistent with the first study in which 3 retirees were followed over a 5-year period. In that study, the estimated half-life was 3 to 4 years. There is no explanation in this current report as to why there is such a large difference in the results or why none of the retirees in the current study didn't fall within the range of the first study.

## CONCLUSIONS

N/A

## REFERENCE

Determination of Serum Half-Lives of Several Fluorochemicals, June 8, 2000, 3M Company. FYI-0700-1378, 8(e) Supplemental Submission, 8EHQ-0373/0374,.

## ACUTE TOXICITY STUDIES

**Title:** An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. 1979

### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

**Remarks:** Dust, PFOS (T-2306 CoC). Purity not specified.

### METHOD

**Method/guideline followed:** Similar to OECD 403

**GLP:** N, no QA/QC indicated

**Year study performed:** 1979

**Species/Strain:** Rat/Sprague-Dawley

**Sex (Males/females/both):** Both

**No. of animals/sex/dose:** 5/sex/group

**Route of Administration:** Inhalation

**Remarks:** Concentrations of 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09, 45.97 mg/l PFOS were administered to eight test groups. A Wright dust-feed mechanism with dry air at a flow rate of 12 to 16 liters per minute was used to administer the PFOS dust. Rats were exposed for 1 hour. The test group rats weighed 201-299 g at study initiation. The control group rats weighed 203-263 g at study initiation. The control rats were exposed to dry air at a flow rate of 12 liters per minute. All other protocols were the same as the test group rats. The rats were observed for abnormal signs prior to exposure, at 15-minute intervals during the 1-hour exposure, at removal from the exposure chamber, hourly for four hours after exposure, and daily thereafter for 14 days. Individual bodyweights were recorded on Day 0 (prior to exposure), Day 1, Day 2, Day 4, Day 7, and Day 14. It is reported that all animals dying spontaneously were necropsied as soon as possible after death. Blood samples were collected on Day 14 from all surviving animals, but analyses were not provided.

### RESULTS

LC<sub>50</sub> = 5.2 (4.4 – 6.4) mg/l, (95% confidence limits); referenced method of Litchfield and Wilcoxon



**Number of deaths at each dose level (by sex):**

0.0 mg/l: 0/10; 1.89 mg/l: 0/10; 2.06 mg/l: 1/10; 4.88 mg/l: 2/10; 6.49 mg/l: 8/10;  
7.05 mg/l: 8/10; 24.09 mg/l: 10/10 (authors did not provide summary by sex)

**Remarks:** The highest dose group, 45.97 mg/l, was not used in the LC<sub>50</sub> calculations and terminated on Day 2. At that point, only 5 animals survived and blood samples were taken at termination. The 13.9 mg/l group was also terminated early (Day 1) because of a mechanical problem during exposure. These animals were also not used in the LC<sub>50</sub> determination.

In the 24.09 mg/l exposure group, all animals died by Day 6. At 7.05 and 6.49 mg/l there was 80% mortality with last deaths at Day 10. At 4.88, 2.86, and 1.89 mg/l there was 20%, 10%, and 0% mortality, respectively. At 2.86 mg/l, deaths occurred on Day 7 and 10. At 1.89 mg/l, one death occurred on Day 12. The rats in all these groups showed signs of toxicity including emaciation, red material around the nose or other nasal discharge, yellow material around the anogenital region, dry rales or other breathing disturbances, and general poor condition. Abnormal in-life observations were reported to be less frequent in the lower exposure groups.

The most common abnormality was discoloration of the liver and lung. Discoloration of the lung was also observed in control rats and therefore may not be treatment related. Therefore, the most significant treatment-related abnormality was varying degrees of discoloration of the liver. Among animals that died prematurely, decreased body weight, discoloration of the lung, and discoloration and distention of the small intestine were also observed.

**CONCLUSIONS**

LC50 = 5.2 (4.4 – 6.4) mg/l, (95% confidence limits). Only conclusion provided; seems reasonable with available data

**REFERENCE**

Rusch, G.M., W.E. Rinehart and C.A. Bozak. 1979. An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. Project No. 78-7185, Bio/dynamics Inc.

ENV/JM/RD(2002)17

**Title:** Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD<sub>50</sub>) Study in Rats. 1978.

## **TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

**Remarks:** FC-95. Purity not specified.

## **METHOD**

**Method/guideline followed:** Similar to OECD 401

**GLP (Y/N):** N, no QA/QC indicated

**Year study performed:** 1978

**Species/Strain:** Rat/Charles River CD

**Sex (Males/females/both):** both

**Number of animals/sex/dose:** 5/sex/dose

**Vehicle:** 20% acetone/80% corn oil

**Route of Administration:** gavage

**Remarks:** Levels of 100, 215, 464, and 1000 mg/kg PFOS were tested. All dose levels were administered as volumes of 10ml/kg body weight. The rats weighed 172-212 g at the beginning of the study immediately prior to dosing and weights were recorded at Day 7 and Day 14. The rats were observed for abnormal signs during the four hours after exposure, and daily thereafter for 14 days. It is reported that all animals dying spontaneously were grossly necropsied, as well as all rats that survived to the end of the 14-day study.

## **RESULTS**

LD50: 251 (199-318) mg/kg, (95% confidence limits); 3 references for statistical tables are given.

**Number of deaths at each dose level (by sex):** 100 mg/kg: 0/5 males, 0/5 females;  
215 mg/kg: 2/5 males, 1/5 females; 464 mg/kg: 5/5 males, 5/5 females;  
1000 mg/kg: 5/5 males, 5/5 females

**Remarks:** All rats in the 464 and 1000 mg/kg dose groups died before the end of the study. Three animals in the 215 mg/kg group died prematurely. It appears signs of toxicity most frequently observed included: hypoactivity, decreased limb tone, and ataxia. At necropsy observations included: yellow-stained urogenital region, stomach distention and signs of irritation of the glandular mucosa, and lung congestion. No differences between sexes were noted.

LD50 male rats: 233 (160-339) mg/kg (95% confidence limits)

LD50 female rats: 271 (200-369) mg/kg (95% confidence limits)

### CONCLUSIONS

None specified beyond LD50

### **REFERENCE**

Dean, W.P., D.C. Jessup, G. Thompson, G. Romig, and D. Powell. 1978. Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD<sub>50</sub>) Study in Rats. Study No. 137-083, International Research and Development Corporation. (Includes Acute Oral Toxicity Study in Rats with T-2297 CoC. Project No. 78-1433A, Biosearch, Inc.) .

## SKIN IRRITATION

**Title:** Eye and Skin Irritation Report on Sample T-1117. 1974

### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

**Remarks:** FC-95, Sample T-1117. Purity not specified.

### METHOD

**Note pH of test material:** Not specified

**Method/Guideline followed:** Not specified

**Test Type:** in vivo

**Species/strain/cell type:** Rabbits/ albino

**Sex (males/females/both):** Not specified

**Number of animals/sex/dose:** 6 total

**Total dose:** appears to be 1.0 gram, 0.5g placed on each of 2 prepared test sites (intact-wet, abraded-wet); total dose not specified

**Vehicle:** None? Not specified

**Length of time test material is in contact with animal/cell:** 72 hr

**Grading scale:** Separate scores for erythema formation and edema formation are summed. Reference source not provided.

**Remarks:** Six albino rabbits had their hair clipped from their backs and flanks, and five tenths of one gram (0.5 g) of test material was placed on abraded-wet or intact-wet prepared test sites, then covered with gauze patches. After 24 hours and 72 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale.

## RESULTS

**Results:** In all cases it is reported the primary skin irritation scores were 0; which indicates no reddening or swelling detected.

**Primary irritation score:** zero

**Remarks:** No indication of reliability. No QA/QC. No effects reported.

## CONCLUSIONS

No irritation. Inadequate information is presented in report to evaluate quality of study and validity of conclusion.

## REFERENCE

J. A. Bieseimer and D.L. Harris. 1974. Eye and Skin Irritation Report on Sample T-1117. Project No.4102871, WARF Institute Inc.

## EYE IRRITATION

**Title:** Eye and Skin Irritation Report on Sample T-1117. 1974

### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

**Remarks:** T-1117. Purity not specified.

### METHOD

**Note pH of test material:** Not specified

**Method/Guideline followed:** Not specified

**Test Type:** in vivo

**Species/strain/cell type or line:** Rabbit/New Zealand White

**Sex (males/females/both):** Not specified

**Number of animals/sex/dose:** 6/single dose

**Total dose:** it appears 0.1 gram, (protocol states either 0.1 ml or 0.1g and T-1117 is reported to be a solid {FC-95} by 3M), total dose not specified

**Length of time test material is in contact with animal/cell:** could be 72 hours,  
But at 1 hr observation and at each scoring after “any accumulated discharge or residue of test material was flushed from the eye.”

**Observation period:** 1hr, 24 hr, 48 hr, 72 hr

**Scoring method used:** Not specified or referenced - “The reaction to the test material was read according to the scale of scoring for damage to the cornea, iris, and the bulbar and palpebral conjunctivae...”

**Remarks:** Rabbits were placed in collars so they could not rub their eyes. One tenth of a gram (0.1 g) of the test substance was instilled in one eye, the other eye was left untreated as a control. It is reported that the reaction to the test material was read against a scale of damage to the cornea, iris, and the bulbar and palpebral conjunctivae at 1, 24, 48, and 72 hours after treatment. The scale criteria are not presented or referenced. Each time the eyes were scored, any accumulated discharge or residue of test material was flushed from the eye. It appears that scores were maximal at 1 hour and 24 hours after treatment then decreased over the rest of the study.

## RESULTS

**Corrosive:** no

**Irritation score:** Only total scores provided.

1 hr: 8.00; 24 hr: 9.33; 48 hr: 3.33; 72 hr: zero

**Tool used to assess score:** Not specified

**Description of lesions:** none

**Remarks:** Inadequate description and discussion in report. Scores appear reduced in all rabbits over time. Decreases were noted at 48hrs and at 72hrs values were zero.

#### CONCLUSIONS

Only conclusion provided in study is that test substance is irritating to eyes. Inadequate information is presented in report to evaluate quality of study and validity of conclusion.

#### REFERENCE

J. A. Bieseimer and D.L. Harris. 1974. Eye and Skin Irritation Report on Sample T-1117. Project No. 4102871, WARF Institute Inc.

## GENETIC TOXICITY STUDIES

**Title:** MUTAGENICITY EVALUATION OF T-2014 CoC IN THE AMES SALMONELLA/MICROSOME PLATE TEST, 1978

### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS #2795-39-3

**Remarks:** T-2014 CoC, FC-95, Purity not specified.

### METHOD

**Method/Guideline followed:** Ames, 1975

**Test type:** Reverse mutation

**Test system:** Bacteria; Yeast

**GLP:** N

**Year study performed:** 1977

**Species/Strain/cell-type/cell line:** Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA09, Saccharomyces cerevisiae D4

**Metabolic activation:** 0.1 ± .05 ml S9 homogenate of Aroclor 1254 induced Sprague Dawley rat liver

**Concentrations tested:** 0.01 µg/plate, 1.0 µg/plate, 10.0 µg/plate, 100 µg/plate, 500 µg/plate nonactivated; 0.1 µg/plate, 1.0 µg/plate, 10.0 µg/plate, 100 µg/plate, 500 µg/plate activated

**Statistical methods used:** None

**Remarks:** There were no significant protocol variations. (1) For the time when the test was done (1977) a single plate per concentration was routine; (2) the negative control was the solvent DMSO; the positive controls were chosen according to strain being tested and activation condition and included ethyl methanesulfonate, quinoline hydroxide, nitrofluorene, 2-anthramine; and 2-dimethylnitrosamine (3) a limited repeat study was done with strain TA100 both with and without activation because the testing laboratory believed that there was some evidence of mutagenicity with this strain. The doses tested without activation were 100 µg/plate, 500 µg/plate, and 1000 µg/plate and 500 µg/plate, 1000 µg/plate, and 2000 µg/plate with activation. However, a review of the data shows that was originally thought to be mutagenicity was within the normal variation of the assay. The repeat was inadequate because the doses tested were too high and too toxic to shed any light on possible mutagenic activity; there were no signs of mutagenicity in any of the other strains tested. The test with Saccharomyces was also negative. (4) criteria to evaluate results were as follows: dose-response over 3 concentrations with lowest increase equal to 3X the solvent control for TA1535, TA1537 and TA1538. Dose-response over 3 concentrations with lowest increase equal to 3X background for TA100 and 2x-3X background for TA98 and D4.

### RESULTS

**Overall results: positive, negative, ambiguous:** Negative



**Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):** Negative with and without activation

**Cytotoxic concentration:** 1000 µg/ml both with and without activation.

**Statistical results:** No statistics performed.

**Remarks:** None

#### CONCLUSIONS

Author's conclusions are accurate but for the wrong reasons. The test chemical is negative not because it was negative on repeat testing but because what was taken as mutagenicity in the first test was within normal variation of the assay.

#### REFERENCE

Litton Bionetics, Inc. Kensington, Maryland 20795 1978. Mutagenicity Evaluation of T-2014 CoC in the Ames Salmonella/Microsome Plate Test. Final Report. Submitted to: 3M Company, Saint Paul, Minnesota 55101

**Title:** *SALMONELLA – ESCHERICHIA COLI/MAMMALIAN-MICROSOME REVERSE MUTATION ASSAY WITH PFOS*, 1999

### TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS #2795-39-3

**Remarks:** FC-95, T-6295, purity not specified.

### METHOD

**Method/Guideline followed:** Ames et al., 1975; Green and Muriel, 1976; Maron and Ames, 1983

**Test type:** Reverse mutation

**Test system:** Bacterial

**GLP:** Y

**Year study performed:** 1999

**Species/Strain/cell-type/cell line:** Salmonella typhimurium TA1535, TA100, TA98, TA1537  
Escherichia coli WP2<sub>uvrA</sub>

**Metabolic activation:** 0.1 ml S9 liver homogenate from Aroclor 1254 induced Sprague-Dawley rats

**Concentrations tested:** S. typhimurium: 33.3 µg/plate, 100 µg/plate, 333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000µg/plate µg/plate with activation and 0.333 µg/plate, 1.00 µg/plate, 3.33 µg/plate, 10.0 µg/plate, 33.3 µg/plate plate, 100 µg/plate, 3333 µg/plate, 1,000 µg/plate and 5,000 µg/plate without activation.

E. coli: 33.3 µg/plate, 100 µg/plate, 3333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000 µg/plate both with and without activation.

**Statistical methods used:** None

**Remarks:** There were no significant protocol deviations. (1) There were 3 plates per test concentration and control; the positive controls were strain and activation condition specific and included benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, ICR-191 and 4-nitroquinoline-N-oxide. The vehicle control was DMSO; (2) the solvent was DMSO; (3) the assay was not repeated. (4) For the test article to be considered positive in strains TA98, TA100 and WP2<sub>uvrA</sub>, there had to be at least a 2-fold increase in the mean revertants per plate over that of the appropriate vehicle control. The increase had to be accompanied by a dose response to increasing concentrations of the test article. For strains TA1535 and TA1537 there had to be at least a 3-fold increase in the mean revertants per plate over that of the appropriate vehicle control. The increase had to be accompanied by a dose response to increasing concentrations of the test article.

### RESULTS

**Overall results: positive, negative, ambiguous:** Negative

**Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):** PFOS was not

genotoxic when tested either with or without metabolic activation.

**Cytotoxic concentration:** Cytotoxicity was noted at 5000 µg/plate without metabolic activation. This cytotoxicity was evidenced by a slight reduction in the bacterial lawn.

**Statistical results:** Results were not evaluated statistically.

**Remarks:** There were no test-specific confounding factors. Mutation frequencies were within the range of the vehicle controls.

## CONCLUSIONS

Author's conclusions are that PFOS is negative in this assay. This is accurate.

## REFERENCE

Mecchi, M.S. 1999. *Salmonella – Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay with PFOS*. Covance Laboratories Inc. (Covance) Vienna, Virginia 22182 Final Report Covance Study No.: 20784-0-409. Submitted to: 3M Corporate Toxicology St. Paul, Minnesota 55144-1000

**Title:** CHROMOSOMAL ABERRATIONS IN HUMAN WHOLE BLOOD LYMPHOCYTES WITH PFOS, 1999

**TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS #2795-39-3

**Remarks:** FC-95, purity not specified.

**METHOD**

**Method/Guideline followed:** Galloway, 1994

**Test type:** In vitro cytogenetics

**Test system:** Human cells in culture

**GLP:** Y

**Year study performed:** 1999

**Species/Strain/cell-type/cell line:** Human lymphocytes

**Metabolic activation:** Aroclor 1254 induced rat liver S9 homogenate, 15.0 µL/ml, plus NADP and isocitric acid.

**Concentrations tested:** 12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 199 µg/ml, 249 µg/ml, 299 µg/ml, 349 µg/ml, 449 µg/ml, 599 µg/ml without activation.

12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 199 µg/ml, 249 µg/ml, 349 µg/ml, 449 µg/ml with activation

**Statistical methods used:** Cochran-Armitage test for linear trend; Fisher's Exact Test

**Remarks:** There were no significant protocol deviations. (1) Each concentration was tested in replicate; each replicate was considered an independent unit. The negative control for the nonactivation assay was DMSO at 10 µl/ml, which was the highest concentration used in the test cultures; in the activation assay it was DMSO plus the S9 mix; the positive control was mitomycin C for the nonactivation assay and cyclophosphamide for the activation assay. Three concentrations of each positive control were tested. Cultures were exposed to chemical for 3 hours and harvested 22 hours later. One hundred metaphases from each replicate of the useable treatment cultures and the solvent and one dose of the positive control were used; mitotic index was evaluated by analysing the number of mitotic cells in at least 1000 cells per culture; (2) the solvent for the chemical was DMSO; (3) there was no follow up study done although in a study such as this where there are negative results after 3 hours incubation with a 22 hour harvest time a second study with a continuous exposure of 22 hours for the nonactivated portion of the assay is recommended. (4) The test article would have been considered positive if there had been a significant increase ( $p \leq 0.01$ ) in the number of cells with chromosomal aberrations at one or more concentrations. The test article was considered negative because there was no significant increase observed in the number of cells with chromosomal aberrations at any concentration tested.

## RESULTS

**Overall results: positive, negative, ambiguous:** Negative

**Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):** Negative both with and without activation.

**Cytotoxic concentration:** 299 µg/ml without metabolic activation and 199 µg/ml with activation were the first cytotoxic concentrations tested as evidenced by a reduction in mitotic index.

**Statistical results:** Negative

**Remarks:** Mitotic index was reduced 38%, 8%, 15%, 15%, 12%, 19%, 24%, 69% and 92% in cultures treated with 12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 249 µg/ml, 299 µg/ml, 149 µg/ml and 449 µg/ml without activation. Aberrations were analysed from cultures treated 199 µg/ml, 249 µg/ml, 299 µg/ml, and 349 µg/ml.

With metabolic activation, mitotic index was reduced by 12%, 41%, 71%, and 53% in cultures treated with 49.7 µg/ml, 199 µg/ml, 249 µg/ml, and 299 µg/ml. Aberrations were analysed from cultures treated with 99.3 µg/ml, 149 µg/ml, 199 µg/ml, and 299 µg/ml. Only 27 and 4 metaphases were available for analysis from cultures treated with 299 µg/ml.

## CONCLUSIONS

Author's conclusions are that PFOS does not cause mutation in human lymphocytes; this is correct as stated..

## REFERENCE

Murli, H. 1999. Chromosomal Aberrations in Human Whole Blood Lymphocytes with PFOS. Covance Laboratories Inc. (Covance) Final Report. Covance Study No.: 20784-0-449. Submitted to: 3M Corporate Toxicology, St. Paul Minnesota 55144-1000.

**Title:** Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS, 1999

**TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS #2795-39-3

**Remarks:** T-6295, purity not specified.

**METHOD**

**Method/Guideline followed:** Williams, 1977; Williams, 1980; Butterworth et al., 1987

**Test type:** Unscheduled DNA Synthesis in Mammalian Cells in Culture

**Test system:** Primary cells in culture

**GLP:** Y

**Year study performed:** 1999

**Species/Strain/cell-type/cell line:** Primary hepatocytes from a Fischer 344 rat male rat.

**Metabolic activation:** None

**Concentrations tested:** 15 concentrations between 0.025 µg/ml and 4000 µg/ml. Six, 0.5 µg/ml, 1.0 µg/ml, 2.5 µg/ml, 5 µg/ml, 10.0 µg/ml and 25.0 µg/ml, chosen for evaluation based upon cytotoxicity.

**Statistical methods used:** None

**Remarks:** There were no significant protocol deviations. (1) Triplicate cultures on coverslips were incubated for 19.6-20.0 hours, then the assay was terminated and <sup>3</sup>H-thymidine added to the cultures for 30 minutes after which the cells were fixed, dried over night, coverslips were mounted on slides, dipped in emulsion and stored for 6 days at 2-8° C after which the emulsions were developed, fixed and stained. 150 cells per dose were read (50 from each coverslip) and the mean net nuclear grain count determined. (2) The solvent for the assay was DMSO; (3) there was no follow-up repeat study; (4) the positive control was 2-AAF; (5) for a treatment to be considered positive, there must be an increase in the mean net nuclear grain count to at least 5 grains per nucleus above the concurrent vehicle control value, and/or an increase in the number of nuclei with five or more net grains such that the percentage of these nuclei in test cultures is 10% above the percentage seen in the vehicle control cultures. The positive control satisfied both of these criteria.

**RESULTS**

**Overall results: positive, negative, ambiguous:** Negative

**Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):** Negative

**Cytotoxic concentration:** Excessive cytotoxicity at and at 50.0 µg/ml; weak cytotoxicity at 25.0 µg/ml. Cell morphology was suitable for analysis at and below 25.0 µg/ml.

**Statistical results:** The results were not evaluated statistically.

**Remarks:** There were no test-specific confounding factors.

#### CONCLUSIONS

The author concludes that PFOS is negative in this assay. This is accurate.

#### REFERENCE

Cifone M.A. 1999. Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS. Covance Laboratories Inc. Vienna, VA 22182 Final Report. Covance Study No.: 20780-0-447. Submitted to 3M Corporate Toxicology St. Paul, MN 55144-1000

**Title:** MUTAGENICITY TEST ON T-6295 IN AN IN VIVO MOUSE MICRONUCLEUS ASSAY, 1996

**TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS #2795-39-3

**Remarks:** T-6295, FC-95, purity not specified.

**METHOD**

**Method/Guideline followed:** Heddle, 1983

**Test type:** Micronucleus

**GLP:** Y

**Year study performed:** 1996

**Species/Strain:** Mouse; CrI:CD-1<sup>®</sup>(ICR)BR

**Sex:** Males & Females

**No. animals/sex/dose:** 5/sex/dose

**Vehicle (if used):** Deionized water

**Route of administration:** Oral

**Doses:** 237.5 mg/kg, 450 mg/kg, 950 mg/kg

**Frequency of treatment:** Single dose

**Statistical methods used:** Analysis of variance; Dunnet's t-test

**Remarks:** There were no significant protocol deviations. (1) Animals were 9 weeks and 1 day old at start of dosing males; weight range for the males was 29.9 –37.0 g; for females it was 23.1-29.2 g; (2) the vehicle was deionized water; (3) the test lasted 72 hours; (4) the test material was administered as a single oral dose; (5) all treatment groups were sampled at 24, 48 and 72 hours; (6) the vehicle control was H<sub>2</sub>O; the positive control was 80 mg/kg cyclophosphamide dissolved in water and administered by gavage. Controls were sampled at 24 hours only. Control groups consisted of 5 males and 5 females each. (7) No clinical examinations were made. (8) No necropsies or other gross examinations were made on these animals. (9) Micronuclei were evaluated in the bone marrow of treated animals. Frequency of PCEs vs. NCEs was determined by scoring the number of PCEs and NCEs in the optic fields while scoring the first 1000 erythrocytes. A positive was judged by an increase in micronucleated polychromatic erythrocytes over levels observed in the vehicle controls in either sex or at any harvest time. Bone marrow toxicity was judged by a significant reduction in PCE/NCE ratios in either sex at any harvest time. (10) The M.T.D. was chosen on the basis of 2 preliminary dose selection assays both of which showed significant toxicity at the highest dose tested.



## RESULTS

*Effect on mitotic index or PCE/NCE ratio by dose level and sex:*

PCE:NCE Ratio

237.5 mg/kg

24 hours: males  $0.57 \pm 0.11$ ; females  $0.52 \pm 0.10$

48 hours: males  $0.48 \pm 0.04$ ; females  $0.80 \pm 0.10$

72 hours: males  $0.39 \pm 0.11$ ; females  $0.42 \pm 0.14$

450 mg/kg

24 hours: males  $0.75 \pm 0.11$ ; females  $0.59 \pm 0.08$

48 hours: males  $0.71 \pm 0.05$ ; females  $0.37 \pm 0.07$

72 hours: males  $0.29 \pm 0.06$ ; females  $0.40 \pm 0.12$

950 mg/kg

24 hours: males  $0.56 \pm 0.13$ ; females  $0.59 \pm 0.08$

48 hours: males  $0.54 \pm 0.08$ ; females  $0.44 \pm 0.11$

72 hours: males  $0.17 \pm 0.05$ ; females  $0.17 \pm 0.05$

**Genotoxic effects (unconfirmed, dose-response, equivocal):** Negative

### Statistical results:

The PCE:NCE ratio was reduced in 237.5 mg/kg males at 48 and 72 hours; in 450 mg/kg males at 72 hours and in 450 mg/kg females at 48 hours and in 950 mg/kg males at 48 and 72 hours and in 950 mg/kg females at 72 hours. There was no statistically significant increase in the number of micronucleated PCEs over the controls in any treatment group. The positive control induced a significant increase in the number of mPCE in both males and females and reduced the PCE:NCE ratio in females only at 24 hours.

### Remarks:

(1) Animals were examined approximately 1-2 hours before sampling for signs of toxicity and mortality. Animals in the 237.5 mg/kg group remained healthy throughout the treatment period. (2) Both males and females in the 950 mg/kg dose group began dying about 22 hours after treatment. Also at 22 hours 2 males in the 950 mg/kg dose group went into convulsions when their cage was opened but recovered in a few minutes. At about 46 hours after treatment 1 female from the 450 mg/kg dose group and more males and females from the 950 mg/kg dose group were found dead and at about 71 hours after treatment, one male from the 950 mg/kg dose group was found dead. All surviving animals appeared normal at that point. (3) No other clinical signs were noted or reported. (4) Body weight changes were not reported. (5) Food and water consumption were not reported. (6) There was no increase in the percent of micronucleated PCEs at any dose level tested or at any time period sampled.

## CONCLUSIONS

The author concludes that PFOS is negative in the mouse bone marrow micronucleus assay. This is an accurate assessment.

## REFERENCE

Murli, H. 1996. Mutagenicity Test on T-6295 in an *In Vivo* Mouse Micronucleus Assay. Corning Hazelton

ENV/JM/RD(2002)17

Inc. (CHV), Vienna, Virginia 22182. Final Report. CHV Study No.: 17403-0-455. Submitted to 3M St. Paul, Minnesota 55144-1000

**Title:** IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2247 CoC AND T-2248 CoC, 1978

## TEST SUBSTANCE

**Identity:** T-2247 CoC; L-4299, a 50% by weight solution of the diethanolammonium salt of perfluorooctanesulfonate in water T-2248 CoC; 22.5% of a reaction product of ethyl and methyl methacrylates and 22.5% of the pyridinium chloride salt of an: N-methylperfluorooctanesulfonamidoethanol-based glutaryl amide.

**Remarks:** T-2247 CoC, T-2248 CoC, purity not specified

## METHOD

**Method/Guideline followed:** Ames et al., 1975; Zimmermann and Schwaier, 1967; Brusick and Mayer, 1973

**Test type:** Reverse Mutation; Recombination

**Test system:** Salmonella typhimurium; Saccharomyces cerevisiae

**GLP:** N

**Year study performed:** 1978

**Species/Strain/cell-type/cell line:** Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100; Saccharomyces cerevisiae D3

**Metabolic activation:** 0.5 ml of 10% S9 liver homogenate from Aroclor 1254 induced rats.

**Concentrations tested:** Plate incorporation assay: 10 µg/plate, 50 µg/plate, 100 µg/plate, 500 µg/plate, 1000 µg/plate, 5000 µg/plate

*Dessicator method: 0.1 ml/dessicator, 0.5 ml/dessicator, 1.0 ml/dessicator, 5.0 ml/dessicator*

Yeast recombination: 0.1%, 0.5%, 1.0%, 5.0%

Yeast repeat assay at 1.0%, 2.0%, 4.0%, 5.0%

**Statistical methods used:** None

**Remarks:** There were no significant protocol deviations. (1) The plate incorporation assay and the S. cerevisiae assay were performed with both chemicals and with one plate per test concentration; the dessicator assay was performed with T-2247 CoC using two plates per concentration but used only strains TA 98 and TA100 for the test. However, given the complexity of the dessicator assay and the limitations involved in setting it up, this is acceptable; (2) the positive controls were chosen according to the strain and activation conditions and included sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-anthramine for the plate incorporation assay; 1,1-dichloroethylene for the desiccator assay with T-2247 CoC and 1,2,3,4-diepoxybutane for the S. cerevisiae assay. The negative control group for all assays was water. (3) The plate incorporation assay with both agents and the yeast assay with T-2248 were repeated; the desiccator assay was run only once. (4) For the desiccator assay, plates were prepared as for the standard assay but no test chemical was added to the agar. The strains tested were S. typhimurium TA98 and TA 100. The test was performed both with and without metabolic activation. Plates without lids were placed side by side in

a perforated shelf in a 9-liter desiccator. A known volume of T-2247 was added to a glass Petri dish that was placed in the center of and attached to the bottom of the shelf. In decreasing order, 5.0 ml, 1.0 ml, 0.5 ml and 0.1 ml of test chemical were added to the desiccator. The negative control chemical was water; the positive control chemical was 1,1-dichloroethylene. Both were treated in the same manner as T-2247. The desiccator was sealed and placed on a magnetic stirrer plate in a room maintained at 37° C. A magnetic stirrer with vanes was placed in the base of each desiccator to ensure adequate dispersion of the chemical. Plates were incubated for 8 hours, removed from the desiccators, their lids replaced and they were incubated at 37° C for an additional 42 hours before revertants were counted.

## RESULTS

**Overall results: positive, negative, ambiguous:** All tests were negative.

**Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):** Negative both with and without activation.

**Cytotoxic concentration:** T-2247 was not cytotoxic.

In the plate incorporation assay, T-2248 was toxic to strain TA1538 at 1000 µg/plate and to all other strains at 5000 µg/plate when tested without activation. It was toxic at 1000 µg/plate to strain TA1537 and at 5000 µg/plate for all other strains when tested with metabolic activation.

T-2248 was slightly toxic to *S. cerevisiae* D3 at 5% concentration without metabolic activation.

**Statistical results:** No statistical results were determined.

**Remarks:** In the first assay with T-2248 and *S. cerevisiae* D3 without metabolic activation there seemed to be some slight indication of mutagenicity at the highest concentration tested, 5%. The assay was repeated at 1%, 2%, 4%, and 5% concentrations with and without activation. There was no indication of a mutagenic dose response and the testing laboratory concluded that T-2248 did not cause recombination in *S. cerevisiae* D3. There were no test-specific confounding factors in any aspect of the test.

## CONCLUSIONS

The testing laboratory concluded that T-2247 and T-2248 were nonmutagenic for *S. typhimurium* TA1535, TA100, TA1537, TA1538, and TA98 when tested in a plate incorporation assay with and without metabolic activation; that T2247 did not induce mutation in *S. typhimurium* TA98 and TA100 when tested in a dessicator assay for volatile chemical and that neither chemical induced recombination in *S. cerevisiae* D3.

These conclusions are accurate.

## REFERENCE

Simmon, V.F. 1978. IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2247 CoC AND T-2248 CoC. SRI International, Final Report. Prepared for 3M Company, St. Paul, Minnesota 55101.

**REPEAT DOSE STUDIES**

**Title:** First ninety-day rhesus monkey toxicity study, 1979

**TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS # 2795-39-3

**Remarks:** FC-95, purity not specified.

**METHOD**

**Method/guideline followed:** None

**Study duration:** 90 days

**GLP (Y/N):** No

**Year study performed:** 1978

**Species/strain:** Rhesus monkey

**Sex:** Males and females

**Number of animals per dose group:** 2/sex/group

**Route of administration:** Gavage

**Doses tested and frequency:** 0, 10, 30, 100, 300 mg/kg/day

**Post-observation period:** None

**Statistical methods used:** None

**Remarks:** Distilled water was used for the vehicle control. The males weighed 3.05-3.80 kg at study initiation and the females weighed 2.75-4.10 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study. The study was terminated after 20 days due to the death of the monkeys. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

## RESULTS

**NOAEL (dose and effect):** None

**LOAEL (dose and effect):** None

**Toxic response/effects by dose level:** All of the monkeys in the treated groups died.

**Statistical results:** None

**Remarks:** The monkeys in the 300 mg/kg/day group died between days 2-4, the monkeys in the 100 mg/kg/day group died between days 3-5, the monkeys in the 30 mg/kg/day group died between days 7-10, and the monkeys in the 10 mg/kg/day group died between days 11-20 of treatment. The monkeys from all the groups showed similar signs of toxicity including decreased activity, emesis with some diarrhea, body stiffening, general body trembling, twitching, weakness, convulsions and prostration. At necropsy, several of the monkeys in the 100 and 300 mg/kg/day groups had a yellowish-brown discoloration of the liver; histologic examination showed no microscopic lesions. Congestion, hemorrhage and lipid depletion of the adrenal cortex was noted in all treated groups. No other lesions were noted.

## CONCLUSIONS

**Remarks:** Authors conclusions stated above in results. Reviewer agrees.

## REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1979. Ninety-day subacute rhesus monkey toxicity study. Study No. 137-087, International Research and Development Corporation, Mattawan, MI.

Title: **Second ninety-day rhesus monkey toxicity study, 1978**

## TEST SUBSTANCE

**Identity:** Potassium perfluorooctylsulfonate, CAS # 2795-39-3

**Remarks:** FC-95, purity not specified.

## METHOD

**Method/guideline followed:** None

**Study duration:** 90 days

**GLP (Y/N):** No

**Year study performed:** 1978

**Species/strain:** Rhesus monkey

**Sex:** Males and females

**Number of animals per dose group:** 2/sex/group

**Route of administration:** Gavage

**Doses tested and frequency:** 0, 0.5, 1.5, 4.5 mg/kg/day

**Post-observation period:** None

**Statistical methods used:** Body wts, hematological, biochemical and urinalysis and organ wts were compared by analysis of variance (one-way classification), Bartlett's test and the appropriate t-test using Dunnett's multiple comparison tables to judge significance of differences.

**Remarks:** Distilled water was used for the vehicle control. The males weighed 2.55-3.55 kg at study initiation and the females weighed 2.7-3.75 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

## RESULTS

**NOAEL (dose and effect):** None

**LOAEL (dose and effect):** 0.5 mg/kg/day: Soft stools, diarrhea, anorexia, emesis, occasional decreases in activity; slight reduction in serum alkaline phosphatase.

**Toxic response/effects by dose level:** 4.5 mg/kg/day - 4/4 monkeys died between weeks 5-7, clinical signs (anorexia, emesis, black stool, dehydration), significant reduction in serum cholesterol, marked diffuse lipid depletion in the adrenals, moderate diffuse atrophy of pancreatic acinar cells, moderate diffuse atrophy of serous alveolar cells.

1.5 mg/kg/day – clinical signs (soft stools, diarrhea), reduced body weight, reduced serum alkaline phosphatase activity and serum potassium (females), reduced serum cholesterol (1/2 females), reduced inorganic phosphate (1/2 females).

0.5 mg/kg/day – clinical signs, (soft stools, diarrhea), soft stools, diarrhea, anorexia, emesis, occasional decreases in activity; slight reduction in serum alkaline phosphatase.

**Statistical results:** The statistical results are presented, but should be viewed with caution due to the small number of animals.

4.5 mg/kg/day - significant reduction in serum cholesterol

1.5 mg/kg/day – significant reduction in serum alkaline phosphatase activity and serum potassium (females)

**Remarks:** All monkeys in the 4.5 mg/kg/day group died or were sacrificed in extremis between week 5 and 7 of the study. Beginning on the first or second day of the study, these monkeys exhibited signs of gastrointestinal tract toxicity including anorexia, emesis, black stool and dehydration. All of the monkeys had decreased activity and just prior to death showed marked to severe rigidity, convulsions, generalized body trembling and prostration. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.7 kg at week 5. After 30 days of treatment, there was a significant reduction in serum cholesterol and a 50% reduction in serum alkaline phosphatase activity. At necropsy, mean organ weights were comparable among the control and treated monkeys. Histologic examination showed several treatment related lesions. All the male and females had marked diffuse lipid depletion in the adrenals. One male and two females had moderate diffuse atrophy of the pancreatic exocrine cells with decreased cell size and loss of zymogen granules. Two males and one female had moderate diffuse atrophy of the serous alveolar cells characterized by decreased cell size and loss of cytoplasmic granules.

All monkeys in the 1.5 mg/kg/day group survived until the end of the study. During the first week of the study, the monkeys had decreased activity. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included black stool, diarrhea, mucous in the stool and bloody stool; at the end of the study, anorexia, dehydration or general body trembling were noted. Although statistical significance was not achieved, the mean body weight of the males dropped from 3.15 kg at the beginning of the study to 2.93 kg at the end of the study, and the mean body weight of the females dropped from 3.22 kg to 2.75 kg. One of the females had very low serum cholesterol and another had a reduction in inorganic phosphate. Necropsy revealed no treatment related lesions.

All monkeys in the 0.5 mg/kg/day group survived until the end of the study. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included diarrhea, soft stools, anorexia and emesis. Occasionally, decreased activity was noted in three of the monkeys. Necropsy revealed no treatment related lesions.

## CONCLUSIONS

**Remarks:** Authors conclusions stated above in results. Reviewer agrees.



REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1978. Ninety-day subacute rhesus monkey toxicity study. Study No. 137-092, International Research and Development Corporation, Mattawan, MI.

ENV/JM/RD(2002)17

**Title:** Ninety day study in rats, 1978

#### **TEST SUBSTANCE**

**Identity:** Potassium perfluorooctylsulfonate, CAS # 2795-39-3

**Remarks:** FC-95, purity not specified.

#### **METHOD**

**Method/guideline followed:** None

**Study duration:** 90 days

**GLP (Y/N):** No

**Year study performed:** 1978

**Species/strain:** CD rat

**Sex:** Males and females

**Number of animals per dose group:** 5/sex/group

**Route of administration:** Diet

**Doses tested and frequency:** 0, 30, 100, 300, 1000, 3000 ppm  
Equivalent to 0, 2, 6, 18, 60, 200 mg/kg/day

**Post-observation period:** None

**Statistical methods used:** Body wts, hematological, biochemical and urinalysis and organ wts were compared by analysis of variance (one-way classification), Bartlett's test and the appropriate t-test using Dunnett's multiple comparison tables to judge significance of differences.

**Remarks:** The males weighed 196-232 g and the females weighed 165-206 g at study initiation. The animals were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

## RESULTS

**NOAEL (dose and effect):** None

**LOAEL (dose and effect):** 30 ppm (2 mg/kg/day) based on a significant increase in relative and absolute liver weights.

**Toxic response/effects by dose level:** 3000 ppm – 10/10 rats died between days 7-8. 1000 ppm – 10/10 rats died between days 8-14. 300 ppm – 5/5 male rats died between days 13-25; 5/5 female rats died between days 18-28. At 300, 1000 and 3000 ppm – histologic lesions in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. 100 ppm – 2/5 males and 2/5 females died during week 5 and a third male died during week 11, mean body weights were reduced by 16.7% (males) and 16.3% (females) at study termination, food consumption significantly reduced, significant reduction in hematocrit (males), erythrocyte (males), hemoglobin (males & females), leukocyte (males), and reticulocyte (females) counts, significant increase in absolute (females) and relative (males & females) liver weight and relative kidney weight. At 100, 300, 1000 and 3000 ppm – slight to marked focal necrosis of hepatocytes.

30 ppm – Significant reduction in food consumption (males), significant increase in absolute and relative liver weight (females).

At all dose levels – very slight to slight cytoplasmic hypertrophy of hepatocytes in the centrilobular to midzonal regions, especially in males.

**Statistical results:** 100 ppm – significant reduction in food consumption

**Remarks:** All of the rats in the 300, 1000 and 3000 ppm groups died. Death occurred between days 13-25 and days 18-28 for the males and females, respectively, in the 300 ppm group. At 1000 ppm, death occurred between days 8-14, and at 3000 ppm, the rats died between days 7-8 of treatment. The rats in all groups showed signs of toxicity including emaciation, convulsions following handling, hunched back, red material around the eyes, yellow material around the anogenital region, increased sensitivity to external stimuli, reduced activity and moist red material around the mouth or nose.

Three males and two females in the 100 ppm group died prior to scheduled sacrifice. Two of the males and the two females died during week 5 and the third male died during week 11 of the study. At study termination, mean body weights were reduced by 16.7% and 16.3% in the male and female groups, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for males and females at 100 ppm. After 30 days of treatment, hematologic values were comparable among the control and 100 ppm groups. Clinical chemistry analyses at one month showed a significant increase in mean glucose in males, blood urea nitrogen values in males and females, and creatinine phosphokinase and alkaline phosphatase values for females. After 90 days of treatment at 100 ppm, the two surviving males had significantly reduced erythrocyte, hemoglobin, hematocrit and leukocyte counts; the three surviving females had significantly reduced hemoglobin and reticulocyte counts, as well as slightly lower erythrocyte, hematocrit and leukocyte counts. Two of the surviving females showed slight to moderate increases in plasma glutamic oxalacetic and pyruvic transaminase activities. Urinalysis results were comparable among treated and control groups at 30 and 90 days. Relative liver weight was significantly increased in the males and absolute and relative liver weights were significantly increased in the females.. Relative kidney weights were significantly increased in both sexes.

All rats in the 30 ppm group survived until the end of the study. At study termination, mean body weights were reduced by 8.7 and 8% in the males and females, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for the males at 30 ppm. Hematologic values

were comparable among the control and 30 ppm group at 30 and 90 days. One female showed a slightly elevated glucose level and one male showed a slightly increased alkaline phosphatase level at 30 days. At 90 days, one male showed moderate increases in glucose, blood urea nitrogen and  $\gamma$ -glutamyl transpeptidase activity. The females had significant increases in absolute and relative liver weights. The males had significant decreases in absolute and relative adrenal weights, absolute thyroid/parathyroid weight and absolute pituitary weight. The biological significance of the changes in male organ weights is unclear since similar changes were not noted in higher dose groups.

At necropsy, treatment related gross lesions were present in all treated groups and included varying degrees of discoloration and/or enlargement of the liver and discoloration of the glandular mucosa of the stomach. Histologic examination also showed lesions in all treated groups. Centrilobular to midzonal cytoplasmic hypertrophy of hepatocytes and focal necrosis was observed in the liver; the incidence and relative severity were greater in the males. In addition, especially among rats in the 300, 1000 and 3000 ppm groups, treatment related histologic lesions were noted in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. In the thymus, this consisted of depletion in the number and size of the lymphoid follicles and in the bone marrow hypocellularity was noted. The spleen was slightly atrophied with a corresponding decrease in the size and number of lymphoid follicles and cells and a similar depletion was noted in the mesenteric lymph nodes. Mucosal hyperkeratosis and/or acanthosis was observed in the forestomach and mucosal hemorrhages were noted in the glandular portion of the stomach. Decrease atrophy in the height and thickness of the villi were noted in the small intestine. Atrophy of the skeletal muscle was noted, as well as epidermal hyperkeratosis and/or acanthosis was noted in the skin.

## CONCLUSIONS

**Remarks:** Authors conclusions stated above in results. Reviewer agrees.

## REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1978. Ninety-day subacute rat toxicity study. Study No. 137-085, International Research and Development Corporation, Mattawan, MI.

Title: 4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys. Unaudited Draft. 1998.

### TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: T-6295, purity not specified.

### METHOD

Method/guideline followed: Range finding – Unaudited Draft report, sections missing

Study duration: 28 days

GLP (Y/N): Y, report contains GLP statement, but as submitted, unaudited draft report with sections missing

Year study performed: 1998

Species/Strain: Monkey/Cynomolgus

Sex: both

Number of animals per dose: 2 per sex, 0 mg/kg/day; 3 per sex, 0.02 mg/kg/day; 1 per sex, 2.0 mg/kg/day

Route of administration: Capsule in stomach

Doses tested and frequency: 0 mg/kg/day; 0.02 mg/kg/day; 2.0 mg/kg/day

Post-observation period: none

Statistical methods used: none

Remarks: Monkeys were observed at least daily for general clinical signs and body weights were recorded twice weekly. Hematological and clinical chemistry analyses were conducted on samples collected before the beginning of the study at day -7 (baseline values) and day 29. Additional blood samples for clinical chemistry were collected on study days 2, 7, and 14. Blood samples for serum PFOS concentrations were taken on days -7, 2, 3, 7, 14, and 29. In addition, samples from day -7 and day 29 were analyzed for levels of estradiol, estrone, estriol, thyroid stimulating hormone, triiodothyronine, and thyroxin. The study animals were terminated as scheduled at 30 days. At necropsy a sample of liver was collected from each animal for palmitoyl CoA oxidase activity analyses. Samples of liver, testes, and pancreas were collected for proliferation cell nuclear antigen evaluation. A sample of liver was also collected from each animal for PFOS concentration analysis. The following organs from control and all treated groups were examined microscopically: adrenals, eye, kidney, liver, lung, spleen, pancreas, femoral bone marrow, testes, and thymus.

**RESULTS**

NOAEL (dose and effect): None determined.

LOAEL (dose and effect): None determined

Toxic response/effects by dose level: None determined

Statistical results: None, high-dose too few animals (1 male, 1 female)

Summary Hormone Analyses Data in Males on Females on Days -7 and 29

| Dose Level | Sex     | Day | Estradiol<br>pg/mL | Estrone<br>pg/mL | Estriol<br>pg/mL | TSH<br>ØU/mL | Triiodothyronine<br>ng/dL | Thyroxin<br>Øg/dL |
|------------|---------|-----|--------------------|------------------|------------------|--------------|---------------------------|-------------------|
| 0          | Males   | -7  | 32.03±4.002        | 28.23±14.701     | 0.00±0.000       | 2.82±0.686   | 139.74±29.232             | 5.43±2.001        |
| 0          | Males   | 29  | 32.09±0.205        | 21.93±4.30       | 0.00±0.000       | 2.15±2.008   | 186.35±6.935              | 4.32±1.407        |
| 0.02       | Males   | -7  | 28.61±4.874        | 17.32±1.373      | 0.00±0.000       | 2.11±0.799   | 122.49±48.610             | 4.28±0.335        |
| 0.02       | Males   | 29  | 34.25±7.998        | 14.72±4.767      | 0.00±0.000       | 3.10±0.584   | 188.34±26.229             | 4.19±0.612        |
| 2.0        | Males   | -7  | 28.71              | 13.98            | 0.00             | 1.71         | 97.61                     | 3.86              |
| 2.0        | Males   | 29  | 18.16              | 20.12            | 0.00             | 0.95         | 90.73                     | 3.18              |
| 0          | Females | -7  | 45.28±0.212        | 28.77±5.162      | 0.00±0.000       | 4.37±4.398   | 132.65±56.434             | 4.88±0.948        |
| 0          | Females | 29  | 53.47±20.082       | 25.97±2.128      | 0.00±0.000       | 2.95±0.530   | 162.70±29.366             | 4.26±0.438        |
| 0.02       | Females | -7  | 48.12±21.124       | 30.40±10.852     | 0.00±0.000       | 2.51±1.506   | 131.09±56.434             | 4.76±1.057        |
| 0.02       | Females | 29  | 58.72±27.628       | 31.61±0.921      | 0.00±0.000       | 2.76±1.764   | 180.58±15.203             | 4.05±1.108        |
| 2.0        | Females | -7  | 37.37              | 29.50            | 0.00             | 3.92         | 122.38                    | 3.60              |
| 2.0        | Females | 29  | 19.82              | 25.85            | 0.00             |              | 90.17                     | 3.38              |

**CONCLUSIONS**

Estradiol, estrone, thyroid stimulating hormone (TSH), thyroxin, and triiodothyronine levels were lower in the high dose animals at the end of the study. Since the numbers of tested animals are small (one male and one female in the high-dose group) and baseline levels are variable, it is not clear if these hormone level changes are treatment-related.

Remarks: None

**REFERENCE**

Thomford, P.J. 1998. 4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys. Unaudited Draft. Study No.T-6295.6, for 3M , St. Paul, MN, by Covance Laboratories Inc., Madison, WI.

Title: 26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys, 2002

## TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95, T-6295, purity not specified.

## METHOD

Method/guideline followed: No guideline followed.

Study duration: 78 weeks, 26-week treatment period, followed by 52 weeks recovery.

GLP (Y/N): Y

Year study performed: 2000

Species/strain: Cynomolgus monkeys

Sex: Males and females

Number of animals per dose group: 6 animals/sex/dose group for groups 1, 3, and 4; 4 animals/sex for group 2.

Route of administration: Oral capsule

Doses tested and frequency: 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day

Post-observation period: 52 weeks

Statistical methods used: Levene's test for variance homogeneity: ANOVA, Dunnett's t-test ANCOVA, covariate-adjusted means, 5% two-tailed probability level.

Remarks: Animals were observed twice daily for mortality and moribundity and were examined at least once daily for abnormalities and signs of toxicity; food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during weeks 26 and 52. Body weight data were recorded weekly before the start of treatment, on Days -1 and 1 and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and at specified intervals during treatment and recovery. Blood samples were also taken for hormone determinations. Samples of serum were collected at various time points during the study and sent to 3M for analysis of PFOS levels. The following organs were weighed at scheduled and unscheduled sacrifices; paired organs were weighed separately: adrenal (2), brain, epididymis (2), kidney (2), liver, ovary (2), pancreas, testis (2), and thyroid (2) with parathyroid. The following tissues were collected for histopathology: adrenals (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eyes (2), femur with bone marrow, gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mammary gland, mesenteric lymph node, ovary (2), pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, trachea, urinary bladder, uterus, and vagina. Liver specimens from the 0.15 and 0.75

mg/kg/day recovery animals were collected via biopsy and analyzed for PFOS levels.

Serum and liver specimens collected from test animals were sent to the 3M Laboratory and analyzed for the presence of PFOS. Serum was harvested from blood that was centrifuged within one hour of collection. Liver specimens were flash frozen in liquid nitrogen. Both liver and serum samples were stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Samples were shipped frozen and on dry ice from Covance Laboratories to 3M periodically from August 1998 through March 2000 which covered the in-life phase of the study. Once received at 3M specimens were stored in freezer at either -55°C ± 10-20°C or -20°C ± 10°C.

During the first 26 weeks of the study a total of 550 serum specimens and 30 liver specimens were collected. Of the serum specimens, 151 were from Group 1, 99 from Group 2, 152 from Group 3 and 148 from Group 4. Eight liver samples were collected from Group 1, 8 from Group 2, 12 from Group 3 and 14 from Group 4. In the recovery Groups, 72 serum and 4 liver samples were collected from Group 1; 72 serum and 4 liver samples from Group 3 and 80 serum and 4 liver samples from Group 4.

Liver and serum samples were extracted using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to extraction. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external standard calibration.

Liver samples were homogenized in water. An aliquot of each liver homogenate and all serum samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the samples and the ion pairs were partitioned into MtBE. The extracts were evaporated until dry on a nitrogen evaporator and then were reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter.

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of the fluorochemical of interest using HPLC/ES/MS/MS. Molecular ion 499, the primary ion for PFOS (C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup>) analysis, was fragmented to produce ion 99 (FSO<sub>3</sub><sup>-</sup>). Ion 99 was monitored for quantitative analysis.

## RESULTS

NOAEL (dose and effect): 0.15 mg/kg/day.

LOAEL (dose and effect): 0.75 mg/kg/day. Death, liver effects, effect on cholesterol

Toxic response/effects by dose level: Death at 0.75 mg/kg/day; increased absolute liver weight, liver to body weight percentages, liver to brain weight ratios in females at 0.75 mg/kg/day; absolute and relative liver weight; Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and lower high density lipoprotein cholesterol, liver organ weights.

Statistical results: The difference in weight at the end of treatment between the control and the 0.75 mg/kg/day female treatment groups was statistically significant; the effect on total cholesterol and high density lipoprotein cholesterol in the 0.75 mg/kg/dose group was statistically significant; in males in the 0.75 mg/kg/day dose group the liver organ weights and the organ-to body weight percentages were statistically significant and in females the liver weights, the organ-to-body weight percentage and the organ-brain weight ratio were all significant.



## Remarks:

Males weighed 3.3-3.4 kg and females weighed 2.8-2.9 kg at the beginning of the study. At the end of 26 weeks of treatment, males weighed 3.7, 3.8, 3.5, and 3.3 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. Females weighed 3.1, 3.1, 3.1 and 2.8 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. The difference between the control and the 0.75 mg/kg/day female treatment groups was statistically significant. At the end of the recovery period, differences in weight between the control and treated animals were no longer obvious.

Two males from the 0.75 mg/kg/day group did not survive to the scheduled sacrifice. One animal died after dosing on Day 155 (Week 23). Clinical signs noted in this animal included: constricted pupils, pale gums, few, mucoid, liquid and black-colored feces, low food consumption, hypoactivity, labored respiration, dehydration, and recumbent position. In addition, the animal was cold to the touch. An enlarged liver was detected by palpation. Cause of death was determined to be pulmonary necrosis with severe acute inflammation. On day 179, the second male was sacrificed in a moribund condition. Clinical signs noted included low food consumption, excessive salivation, labored respiration, hypoactivity and ataxia. Cause of death was not determined.

Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and males and females in the 0.15 and 0.75 mg/kg/day groups had lower high density lipoprotein cholesterol during treatment. However, only the effect in the 0.75 mg/kg group was statistically significant. The effect on total cholesterol worsened with time. By day 182, mean total cholesterol for males and females in the high dose group were 68% and 49% lower, respectively, than levels in the control animals. The effect on high density lipoprotein was greater than that seen with cholesterol. On day 182, the mean high density lipoprotein levels were 79% and 62% lower in males and females, respectively, from the high dose group than they were in male and female control animals. Males in the high dose group also had lower total bilirubin concentrations and higher serum bile acid concentrations than males in either the control or other treatment groups. The effect on total cholesterol was reversed within 5 weeks of recovery and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of recovery.

Estradiol values were lower in males given 0.75 mg/kg/day on days 62, 91, and 182 by because of variation only the day 182 value was significant. Estrone values were generally higher in the treated females on days 37, 62 and 91 by again because of variation in the data none of these values were significantly different. Triiodothyronine values were notably lower on days 91 and 182 in males and females given 0.15 and 0.75 mg/kg/day. There were other instances in which hormone values in treated groups were different from those of controls but these differences were not consistent over time or between sexes, were not clearly dose-related and did not appear to be related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in the study complicates the interpretation of the hormone data.

At terminal sacrifice, females in the 0.75 mg/kg/day dose-group had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to body weight percentages were increased in the high-dose group compared to the controls. "Mottled" livers were observed in two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had a "mottled" and large liver. Three of 4 high-dose males (including those that did not survive to scheduled sacrifice) had centrilobular or diffuse hepatocellular hypertrophy that was also observed in all high-dose females. Centrilobular or diffuse hepatocellular vacuolation occurred in 2 of 4 females and 2 of 4 males in the high-dose group.

No PFOS related lesions were observed at recovery sacrifice indicating that the effects seen at terminal sacrifice may be reversible.

Although low levels of PFOS were often detected in the sera and liver of the control animals, these levels were significantly lower than those found in the low dose test animals. PFOS levels in the sera of test animals increased with dose during treatment from  $21.0 \pm 1.57$  and  $20.4 \pm 2.71$   $\mu\text{g/ml}$  in the Group 4 males and females respectively at the end of Week 1 to  $194 \pm 8.94$  and  $160 \pm 23.1$   $\mu\text{g/ml}$  in males and females respectively in Group 4 at the end of Week 27. During recovery, PFOS levels in serum samples decreased over time until they reached  $41.1 \pm 25.9$   $\mu\text{g/ml}$  in males and  $41.4 \pm 1.15$   $\mu\text{g/ml}$  in females from Group 4 at 79 weeks post-treatment. Control values were  $< \text{LOQ}$  (the limit of quantitation) at Week 4 in both males and females and  $0.0215 \pm 0.00296$  and  $0.0243 \pm 0.00355$   $\mu\text{g/ml}$  in males and females respectively at the end of Week 79. The serum values for selected weeks of treatment and recovery are shown in the table below. There were no significant differences between PFOS levels in the sera of treated males and females.

**Average PFOS Concentrations ( $\mu\text{g/ml}$ ) in Serum of Monkeys for  
Selected Weeks During Treatment and Recovery**

|         | Group 1<br>0.0 mg/kg/day   |                         | Group 2<br>0.03 mg/kg/day |                      | Group 3<br>0.15 mg/kg/day |                    | Group 4<br>0.75 mg/kg/day |                   |
|---------|----------------------------|-------------------------|---------------------------|----------------------|---------------------------|--------------------|---------------------------|-------------------|
|         | Males                      | Females                 | Males                     | Females              | Males                     | Females            | Males                     | Females           |
| Week 1  | <LOQ                       | <LOQ                    | 0.869<br>$\pm$ 0.147      | 0.947 $\pm$<br>0.110 | 4.60 $\pm$ 0.782          | 3.71 $\pm$ 0.455   | 21.0 $\pm$ 1.57           | 20.4 $\pm$ 2.71   |
| Week 4  | <LOQ                       | <LOQ                    | 3.20 $\pm$ 0.577          | 3.40 $\pm$ 0.291     | 17.8 $\pm$ 1.68           | 16.5 $\pm$ 1.87    | 95.3 $\pm$ 70.4           | 92.7 $\pm$ 39.6   |
| Week 16 | 0.0407<br>$\pm$<br>0.0110  | 0.0432 $\pm$<br>0.0081  | 11.2 $\pm$<br>2.44        | 10.5 $\pm$ 1.90      | 56.2 $\pm$ 5.84           | 42.1 $\pm$ 4.04    | 189 $\pm$ 15.9            | 162 $\pm$ 19.3    |
| Week 27 | 0.0529<br>$\pm$<br>0.0145  | 0.0416 $\pm$<br>0.0148  | 15.9 $\pm$ 5.54           | 11.1 $\pm$ 1.52      | 68.1 $\pm$<br>5.75        | 58.5 $\pm$<br>4.67 | 194 $\pm$<br>8.93         | 160 $\pm$<br>23.9 |
| Week 35 | 0.0459 $\pm$<br>0.00303    | 0.0723 $\pm$<br>0.00352 | Not<br>Determined         | Not<br>Determined    | 84.5 $\pm$ 12.0           | 74.7 $\pm$ 9.53    | 181 $\pm$ 19.5            | 171 $\pm$ 10.1    |
| Week 47 | 0.0355<br>$\pm$<br>0.00221 | 0.0459 $\pm$<br>0.00323 | Not<br>Determined         | Not<br>Determined    | 48.3 $\pm$ 3.69           | 42.6 $\pm$ 6.70    | 124 $\pm$ 25.9            | 98.3 $\pm$ 8.32   |
| Week 57 | 0.0327<br>$\pm$<br>0.00526 | 0.0445 $\pm$<br>0.00385 | Not<br>Determined         | Not<br>Determined    | 30.2 $\pm$ 2.36           | 32.3 $\pm$ 1.34    | 78.0 $\pm$ 16.3           | 106 $\pm$ 3.84    |
| Week 69 | 0.0406<br>$\pm$<br>0.00313 | 0.0400 $\pm$<br>0.00301 | Not<br>Determined         | Not<br>Determined    | 26.4 $\pm$ 2.59           | 34.5 $\pm$ 3.46    | 84.0 $\pm$ 52.4           | 75.0 $\pm$ 5.25   |
| Week 79 | 0.0215<br>$\pm$<br>0.00296 | 0.0243 $\pm$<br>0.00355 | Not<br>Determined         | Not<br>Determined    | 19.1 $\pm$ 0.805          | 21.4 $\pm$ 2.01    | 41.1 $\pm$ 25.9           | 41.4 $\pm$ 1.15   |

LOQ = Lowest Observable Concentration

Liver values behaved in a manner similar to serum values and increased over time. At Week 27 mean PFOS values on an RSD basis were  $22.2 \pm 0.0269$  in Group 1 males and  $16.8 \pm 0.0178$  in females in Group 1;  $27.0 \pm 4.66$  and  $9.73 \pm 2.15$  in males and females in Group 2;  $33.1 \pm 19.5$  in males and  $21.4 \pm 14.9$  in females in Group 3, and  $6.03 \pm 23.9$  in males and  $5.00 \pm 13.6$  in females in Group 4. At Week 79 values in the liver were  $71.0 \pm 33.4$  in males and  $21.4 \pm 10.8$  in females in Group 4. At Week 80, values were  $14.9 \pm 1.38$  in Group 3 males and  $23.5 \pm 4.98$  in Group 3 females.

## CONCLUSIONS

PFOS is toxic to cynomolgous monkeys at 0.75 mg/kg/day causing death, alterations in total cholesterol, and effecting liver weight and causing hepatocellular hypertrophy and vacuolation in both treated males and females. However, the effects on cholesterol and the liver appear to be reversible after a 52 week recovery period.

REFERENCE

Thomford, PJ. (2000). 26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys. Unaudited Draft Final Report Prepared for 3M, St Paul, Minnesota by Covance Laboratories, Inc., Madison Wisconsin 53704-2595. April 12, 2000. 502 pp.

Seacat, AM. Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples. 3M Medical Department Study: T-6295.7; Covance In-Life Study:#6329-223. Analytical Study; FACT TOX-030; 3M Laboratory Request No. U2279.

**DEVELOPMENTAL TOXICITY STUDIES**

**Title:** Oral Teratology Study of FC-95 in Rats - Experiment No. 0680TR0008

**TEST SUBSTANCE**

**Identity:** Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

**Remarks:** FC-95, Lot 640.

**METHOD**

**Method/Guideline followed (i.e., OECD 414, etc.):** Actual guideline followed was not specified but appears to be similar in design to OECD 414.

**GLP (Y/N):** The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study was conducted according to the 1978 Good Laboratory Practice regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

**Year study performed:** 1980

**Species/Strain:** Sprague-Dawley rats

**Number of animals per dose:** 22

**Route of administration:** Gavage

**Dosing regimen (list all with units):** Four groups of 22 time-mated Sprague-Dawley rats were administered Potassium Perfluorooctylsulfonate in corn oil by gavage on gestation days 6-15. Doses were adjusted according to the most recent recorded body weight.

**Doses:** 0, 1, 5, and 10 mg/kg/day

**Statistical methods used:** The animals will be assigned cages according to a computer-generated random numbers table. The statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities.

**Remarks – Detail and discuss any significant protocol parameters and deviations:**

Potassium Perfluorooctylsulfonate was administered in corn oil by gavage to four groups of 22 time-mated Sprague-Dawley rats weighing 175-261g, at doses of 0, 1, 5, and 10 mg/kg/day PFOS on days 6-15 of gestation (Gortner, 1980). Purina Laboratory Chow and water were available ad libitum. The animals were dosed according to a constant dose volume of 5 ml/kg of body weight and observed daily from day 3 through day 20 of gestation for abnormal clinical signs.

Body weights were recorded on days 3, 6, 9, 12, 15, and 20 of gestation and the rats. All animals were sacrificed on day 20 by cervical dislocation and the ovaries, uteri and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning by the Wilson technique. The remaining fetuses were subjected to a

skeletal examination using alizarin red.

## RESULTS

**NOAEL (dose and effect) – maternal and developmental:** A NOAEL of 5 mg/kg/day for maternal toxicity was indicated. No signs of maternal toxicity were reported at doses of 5 mg/kg/day and below. A NOAEL for developmental toxicity could not be established; signs of developmental toxicity were evident at all doses.

**LOAEL (dose and effect) – maternal and developmental:** A LOAEL of 10 mg/kg/day for maternal toxicity was indicated based on significant reductions in mean body weights during gestation day 12-20 at the high-dose group of 10 mg/kg/day. A LOAEL of 1 mg/kg/day for developmental toxicity was indicated based on abnormalities of the lens of the eye.

**Toxic response/effects by dose level - maternal:** Significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day.

**Toxic response/effects by dose level - developmental:** Unusually high incidences of developmental variations and abnormalities of the lens of the eye were observed in all dose groups.

**Statistical results:** At 10 mg/kg/day, mean maternal body weights were statistically significantly lower than controls (Dunnett's test  $p < 0.05$ ). Mean litter data and pup weights were not significantly different from controls (Dunnett's t test  $p < 0.05$ ). Number of fetuses with gross findings were not significantly different from controls (Chi-square  $p < 0.05$ ).

Number and percent of fetuses with skeleton findings were not significantly different from controls (Chi-square  $p < 0.05$ ).

Number and percent of fetuses with internal findings - - developmental lens abnormalities with secondary lens aberrations were significantly higher than controls (Chi-square  $p < 0.05$ ).

**Remarks – Additional information to adequately assess the data:** Signs of maternal toxicity consisted of significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day. No other signs of maternal toxicity were reported. Developmental toxicity evident at doses of 10 mg/kg/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and the mean numbers of viable male, female, and total fetuses, but the differences were not statistically significant. In addition, unusually high incidences of unossified, assymetrical, bipartite, and missing sternebrae were observed in all dose groups; however, these skeletal variations were also observed in control fetuses at the same rate and therefore were not considered to be treatment-related. The most notable sign of developmental toxicity observed in all dose groups consisted of abnormalities of the lens of the eye, which was not seen in controls. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group. All eye abnormalities appeared to be localized to the area of the embryonal lens nucleus, although a variety of morphological appearances were present within that location. According to the authors, this abnormality appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. An amendment to the results and discussion section concludes that the gross finding of a lens cleft was an artifact created by freehand sectioning and the range of gross lens observations and the differences among the dose group incidences were due to the maner and frequency in which the lens cleft artifact was created by free-hand sectioning and the limitations inherent in visualizing the embryonal nucleus. Additionally, a subsequent study (Wetzel, 1893) of similar design was not able to repeat this finding.

## CONCLUSIONS

Given the explanation that the eye abnormalities reported at doses as low as 1 mg/kg/day in this study may have been due to an artifact of sectioning and that these findings could not be repeated in a second study of similar design, it is entirely plausible that the lens defect observed in this study is not treatment-related.

## REFERENCE

Gortner, E.G. 1980. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0680TR0008, December, 1980. "Oral Teratology Study of FC-95 in Rats".

Title: **Rat Teratology Study T-3351 Final Report – Project No. 154-160**

## **TEST SUBSTANCE**

**Identity:** Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

**Remarks:** T-3351, Lot No. 80275

## **METHOD**

**Method/Guideline followed (i.e., OECD 414, etc.):** Actual guideline followed was not specified but appears to be similar in design to OECD 414.

**GLP (Y/N):** Quality Assurance inspections of the study and review of the final report were conducted according to the standard operating procedures of the Office of Quality Assurance and according to the general requirements of the Good Laboratory Practice regulations that were issued on December 22, 1978, by the Food and Drug Administration for compliance on and after June 20, 1979.

**Year study performed:** 1983

**Species/Strain:** Sprague-Dawley rats

**Number of animals per dose:** 25

**Route of administration:** Gavage

**Dosing regimen (list all with units):** Four groups of 25 pregnant Sprague-Dawley rats were administered Potassium Perfluorooctylsulfonate in corn oil by gavage on gestation days 6-15. Doses were adjusted according to the most recent recorded body weight.

**Doses:** 0, 1, 5, and 10 mg/kg/day

**Statistical methods used:** Statistical methods used for analysis of the data : Dunnett's t test for control vs. compound-treated group mean comparisons. If the variances were proved to be homogeneous, the data were analysed by one-way classification analysis of variance (ANOVA). Mean fetal body weights per litter were statistically analysed as follows: Bartlett's test for homogeneity of variances was performed by one-way classification of covariance (ANCOVA).

If ANCOVA was significant, control vs. treatment group comparisons were analysed using the Games and Howell modification of the Tukey-Kramer honestly significant difference test. Tests for homogeneity of variances, ANOVA, and ANCOVA were evaluated at the 5% one-tailed probability level. Control vs. compound-treated group mean comparisons of the above data were evaluated at the 5% two-tailed probability level. Percent fetal viability, percent fetal loss (dead and resorbing fetuses), percent early, late, and total resorptions, and the number of dead fetuses were analysed by nonparametric one-way ANOVA and the Terpstra-Jonckheere test for trend. The litter was used as the experimental unit. Teratology data were analysed using the Cochran-Armitage test for linear trend in proportions. If a significant trend was noted, the results of Fisher's "exact" test were evaluated at the one-tailed, 5% level. If a significant trend was not observed, or if there was a significant trend with severe departure from it, the results of Fisher's "exact" test were evaluated at the two-tailed, 5% level.



**Remarks – Detail and discuss any significant protocol parameters and deviations:**

Potassium perfluorooctylsulfonate was administered in corn oil by gavage to four groups of 25 pregnant Sprague-Dawley rats at doses of 0, 1, 5, and 10 mg/kg/day PFOS on gestation days (GD) 6-15 (Wetzel, 1983). Sexually mature Sprague-Dawley rats, one per sex per cage, were paired until confirmation of mating or until two weeks had elapsed. Mating was confirmed by daily vaginal examinations for the presence and viability of sperm or the presence of a copulatory plug. The day of confirmation of mating was designated as day 0 of gestation. Purina Rodent Laboratory Chow 5001 and tap water were available ad libitum. A dose volume of 3 ml/kg of body weight was administered and doses were adjusted according to the most recently recorded body weight measurements. Dams were observed twice daily for signs of mortality and moribundity and once daily for clinical signs of toxicity. Individual body weights and food consumption were recorded on GD 6, 8, 12, 16, and 20. Animals were sacrificed on GD 20 by CO<sub>2</sub> asphyxiation and the fetuses were delivered by cesarean section on GD 20. A gross necropsy was performed on all dams. The uterus from each female was excised, weighed and examined for the number and placement of implantation sites, number and of live and dead fetuses, number of early and late resorptions, and any abnormalities and then weighed again after the contents were removed. The ovaries were examined for the number of corpora lutea. Each female was examined by gross necropsy. Each fetus was sexed, weighed, and examined externally. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by the Wilson technique, with particular attention to the eyes, palate, and brain. The remaining fetuses were subjected to a skeletal examination that included evaluation of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles using alizarin red; bone alignment and degree of ossification were also evaluated.

**RESULTS**

**NOAEL (dose and effect) – maternal and developmental:** The NOAEL for maternal toxicity is 1 mg/kg/day. The NOAEL for developmental toxicity is 1 mg/kg/day.

**LOAEL (dose and effect) – maternal and developmental:** The LOAEL for maternal toxicity is 5 mg/kg/day, based on clinical signs of toxicity, decreases in body weight and food consumption, decreases in uterine weights, and an increased incidence in gastrointestinal lesions.

The LOAEL for developmental toxicity is 5 mg/kg/day, based on decreased fetal body weight and increases in external and visceral anomalies and variations.

**Toxic response/effects by dose level - maternal:** Clinical signs of toxicity, decreases in body weights and food consumption at 5 and 10 mg/kg/day; decreases in uterine weights, increased incidence in gastrointestinal lesions, and two deaths at 10 mg/kg/day.

**Toxic response/effects by dose level - developmental:** Decreased fetal weight at 5 and 10 mg/kg/day; external and visceral anomalies and skeletal variations at 10 mg/kg/day.

**Statistical results:** Statistically significant differences between controls and treated were noted for the following maternal endpoints: mean body weight gain, mean total food consumption, and mean gravid uterine weight. Nonparametric analysis of the mean incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss did not indicate statistical significance; however, there was a significant linear trend towards an increased incidence in these data with respect to control. The primary trend component was contributed by the high-dose group. Statistically significant treatment-related increases in the incidences of visceral anomalies and skeletal variants were also observed.

**Remarks – Additional information to adequately assess the data:** Evidence of maternal toxicity, that was observed at the 5 and 10 mg/kg/day dose groups both during and following treatment and considered to be treatment-related, consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough haircoat, and bloody crust. Significant decreases in mean body weight gains during GD 6-8, 6-16, and 0-20 were also observed at the 5 and 10 mg/kg/day dose groups. These reductions were considered to be treatment-related since mean body weight gains were greater than controls during the post-exposure period (GD 16-20). Significant decreases in mean total food consumption were observed on GD 17-20 in the 10 mg/kg/day dose group, and on GD 7-16 and 0-20 in both the 5 and 10 mg/kg/day dose groups. The mean gravid uterine weight in the 10 mg/kg/day dose group was significantly lower when compared with controls. The mean terminal body weights minus the gravid uterine weights were lower in all treated groups, with significant decreases at 5 and 10 mg/kg/day. High-dose animals also exhibited an increased incidence in gastrointestinal lesions. No significant differences were observed in pregnancy rates, number of corpora lutea, and number and placement of implantation sites among treated and control groups. Two dams in the 10 mg/kg/day dose group were found dead on GD 17.

Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although, none of these effects were statistically significantly different from controls. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg/day dose groups. The percent of male fetuses was 52%, 54%, and 60% for 1, 5, and 10 mg/kg/day, respectively, compared to 44% in controls. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups but not in the mid-dose group. Statistically significant increases in the incidences in the number of litters containing fetuses with visceral anomalies, delayed ossification, and skeletal variations were observed in the high dose group of 10 mg/kg/day. These included external and visceral anomalies of the cleft palate, subcutaneous edema, and cryptorchism as well as delays in skeletal ossification of the skull, pectoral girdle, rib cage, vertebral column, pelvic girdle and limbs. Skeletal variations in the ribs and sternbrae were also observed at this dose level.

## CONCLUSIONS

The developmental eye abnormalities that were seen in Gortner (1980) were not observed in the present developmental toxicity study even though the study design and doses were the same. Findings of abnormalities in eye development were initially thought to be treatment-related but then later suggested as being artifacts of sectioning.

## REFERENCE

Wetzel, L.T. 1983. Hazelton Laboratories America, Inc. Project Number: 154-160, December 19, 1983. "Rat Teratology Study, T-3351, Final Report".

**Title:** ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS – 3M T-6295.10, ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.10, 1999.

## TEST SUBSTANCE

**Identity:** Potassium Perfluorooctylsulfonate (PFOS), CAS No. 2795-39-3

**Remarks:** PFOS – Lot 217, 98.4% pure (SMD Analytical Request 53030) Analytical Documentation filed along with final report. Note: Same lot as used in two-year rat PFOS carcinogenicity study (T-6295, Covance 6329-183).

## METHOD

**Method/Guideline followed (i.e., OECD 414, etc.):** The requirements of the International Conference on Harmonization (ICH) Harmonized Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal products, stages C and D of the reproductive process in a non-rodent species were used as the basis for study design (U.S. Food and Drug Administration, 1994. Federal Register, September 22, 1194, Vol. 59, No. 183).

**GLP (Y/N):** The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (MHW) and the European Economic Community (EEC). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study.

**Year study performed:** 1999

**Species/Strain:** New Zealand White rabbits

**Number of animals per dose:** 22 (additional total of 19 rabbits, 3-5 per dose, were included in a satellite study to determine the concentrations of PFOS in maternal liver and serum.)

**Route of administration:** Gavage

**Dosing regimen (list all with units):** Four groups of 22 pregnant New Zealand White rabbits were administered Potassium Perfluorooctylsulfonate (PFOS) in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 ml/kg was administered, adjusted daily on the basis of individual body weights.

**Doses:** 0, 0.1, 1.0, 2.5, and 3.75 mg/kg/day (In the satellite study the number of does was 3, 5, 3, 3 and 5 in the control, 0.1, 1.0, 2.5 and 3.75 mg/kg/day groups, respectively).

## Statistical methods used:

The animals will be assigned to individual housing on the basis of computer-generated random units. The litter was the unit of measurement. Clinical observation and other proportion data were analysed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (e.g., maternal body weights, body weight changes, feed consumption values and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data and fetal ossification site data) were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance. If the Analysis of Variance was significant, Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test was used. In cases, in which Kruskal-Wallis Test was statistically significant ( $p \leq 0.05$ ), Dunn's Method of Multiple

Comparisons was used to identify the statistical significance of the individual groups. Count data obtained at Caesarean-sectioning were evaluated using the procedures described for the Kruskal-Wallis Test.

**Remarks – Detail and discuss any significant protocol parameters and deviations:** Timed-pregnant New Zealand White rabbits (obtained from Covance Research Products, Inc.), 22 per group, were given doses of 0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day PFOS in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weights. The does were observed twice daily for viability, and clinical observations were recorded 1 hour prior to and after dosing during the treatment period and once daily during the post-treatment period (i.e. gestation days 20-29). Maternal body weights were recorded on gestation days 0 and 6-29; food consumption was recorded daily throughout the study. On gestation day 29, the does were euthanized; a gross necropsy of the thoracic, abdominal and pelvic viscera was conducted and the number of corpora lutea in each ovary was recorded. The uteri were examined for number and distribution of implantations, live and dead fetuses, and early and late esorptions. The fetuses were weighed, sexed and examined for external abnormalities. All fetuses were examined for visceral and skeletal abnormalities and the brain of one-half of the fetuses were free-hand cross-sectioned and examined in situ.

In the satellite study, the does were euthanized on gestation day 21, blood samples were collected, and the liver was weighed and sectioned. The fetuses were removed and examined for external abnormalities. Fetuses and placentae were pooled per litter. All samples were sent to the Sponsor (3M) for analysis. At this time, only the liver and serum analyses have been Reported (3M Environmental Laboratory, 2001).

## RESULTS

**NOAEL (dose and effect) – maternal and developmental:** The NOAEL for maternal toxicity is 0.1 mg/kg/day. The NOAEL for developmental toxicity is 1.0 mg/kg/day.

**LOAEL (dose and effect) – maternal and developmental:** The LOAEL for maternal toxicity is 1.0 mg/kg/day, based on abortions, incidences of scant feces, and decreases in body weight gains and food consumption. The LOAEL for developmental toxicity is 2.5 mg/kg/day, based on reductions in body weight and increased incidences in fetal alterations.

**Toxic response/effects by dose level - maternal:** Maternal toxicity was evident at dose levels of 1.0 mg/kg/day and above and consisted of the following: abortions at 2.5 mg/kg/day and above occurring on GD 22-28; increased incidence of scant feces at 1.0 mg/kg/day and above; reductions in mean body weight and body weight gain at doses of 1.0 mg/kg/day and above; reductions in absolute and relative food consumption at 2.5 mg/kg/day and above.

**Toxic response/effects by dose level – developmental:** Developmental toxicity was evident at doses of 2.5 mg/kg/day and above and consisted of the following: reductions in mean fetal body weight at 2.5 mg/kg/day and above; delayed ossification at 2.5 mg/kg/day and above.

### Statistical results:

Maternal data: Statistically significant increases in abortions were observed at 3.75 mg/kg/day. Incidences of scant feces at 3.75 mg/kg/day reached statistical significance ( $p \leq 0.01$ ). Maternal body weight gains were reduced or body weight losses occurred in the 1.0, 2.5, and 3.75 mg/kg/day dosage groups at most measured intervals during dosing; these reductions were significant ( $p \leq 0.05$  or 0.01) in the 3.75 mg/kg/day dose group on GDs 10-13, 13-16, and 16-19. Dosage-dependent, significant body weight reductions or body weight losses ( $p \leq 0.05$  or 0.01) occurred in the 1.0, 2.5, and 3.75 mg/kg/day dosage

groups for the entire dosage period (calculated as GD 7-21). Dosage-dependent reductions in body weight gains occurred in the 2.5 and 3.75 mg/kg/day dosage groups for the entire period of gestation (GD 0-29) and for the gestation period after the initiation of dosing (GD 7-29; significant at  $p \leq 0.01$  in the 2.5 mg/kg/day dosage group). Average body weights were significantly reduced ( $p \leq 0.05$  or  $0.01$ ) on GD 17-24 in the 3.75 mg/kg/day dosage group. Absolute and relative food consumption values were significantly reduced ( $p \leq 0.05$  or  $0.01$ ) in the 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (GD 7-21), and the entire period after the initiation of dosage (GD 7-29).

Fetal data: Fetal body weights (total, male and female) were significantly reduced ( $p \leq 0.05$  and  $p \leq 0.01$ , respectively) in the 2.75 and 3.75 dosage groups. Significant delays ( $p \leq 0.05$  and  $0.01$ ) in litter and fetal averages for ossification were seen at both 2.5 and 3.75 mg/kg/day dosage groups.

#### Remarks:

Maternal toxicity was evident at doses of 1.0 mg/kg/day and above. One doe in the 2.5 mg/kg/day group and nine does in the 3.75 mg/kg/day aborted. All abortions occurred on gestation days 22-28 and were considered treatment-related by the study authors. There was a significant increase in the incidence of scant feces in the 3.75 mg/kg/day group. Scant feces were also noted in one and three does in the 1.0 and 2.5 mg/kg/day groups, respectively. Mean maternal body weight gains were significantly reduced in the 3.75 mg/kg/day group on gestation days 10-13, 13-16, 16-19 and 21-24. Mean body weight gains were also calculated for the treatment period (days 7-21), post-treatment period (days 21-29) and duration of the study (days 7-29). There was a significant reduction in mean maternal body weight gain during the treatment period in the 1.0, 2.5 and 3.75 mg/kg/day groups. Mean body weight gain for the entire study period was also significantly reduced in the 2.5 mg/kg/day group. Mean food consumption (g/kg/day) was significantly reduced in the 2.5 mg/kg/day group on gestation days 16-19, 19-21 and 21-24, as well as for the entire study period (days 7-29). Mean food consumption was significantly reduced in the 3.75 mg/kg/day group on gestation days 13-16, 16-19, 19-21 and 21-24, as well as the entire treatment period (GD 7-21) and the entire period after the initiation of dosage (GD 7-29). Pregnancy occurred in 20 (90%), 19 (86.4%), 19 (86.4%), 17 (77.3%), and 21 (95.4%) rabbits in each dosage group. Cesarean-sectioning observations on GD 29 were based on 20, 18, 19, 16, and 12 pregnant rabbits in each of the five respective dosage groups.

In the satellite study of does euthanized on gestation day 21, the liver and serum analyses were reported by 3M Environmental Laboratory (2001). All serum and liver samples (including those from untreated controls) had detectable levels of PFOS; the values are presented below. Data from analysis of fetal and placenta tissues were not presented in the report.

#### Average Concentration of PFOS in Rabbit Liver and Serum by Dose Group:

| Dose group<br>(mg/kg/day) | PFOS conc.<br>liver (ug/g) | PFOS conc.<br>serum (ug/ml) |
|---------------------------|----------------------------|-----------------------------|
| 0.0                       | 0.239                      | 0.0690                      |
| 0.1                       | 13.1                       | 2.73                        |
| 1.0                       | 133                        | 23.8                        |
| 2.5                       | 317                        | 45.8                        |
| 3.75                      | 416                        | 88.9                        |

Qualitatively, increasing concentrations of PFOS were found in samples of liver and serum as doses of PFOS increased. The levels of PFOS are much higher in the liver than in the serum.

These values should be viewed with caution. It was stated that because radio-labeled reference material

was not available, "it is not possible to verify true recovery of endogenous analyte from tissues." Matrix spike recovery indicates the accuracy of quantitation to be +/- 30%. It is also noted that liver concentrations may be biased high. The only conclusion presented in the laboratory report is that "PFOS was observed in the liver and serum of all rabbits dosed with the test article."

Developmental toxicity was evident at doses of 2.5 mg/kg/day and above. The number of corpora lutea, resorptions, live/dead fetuses, litter size and sex ratio were comparable among treated and control groups. Mean fetal body weight (male, female and sexes combined) was significantly reduced in the 2.5 and 3.75 mg/kg/day groups. There was also a significant reduction in the ossification of the sternum (litter averages) in the 2.5 and 3.75 mg/kg/day groups, and a significant reduction in the ossification of the hyoid (litter averages), metacarpals (litter averages) and pubis (litter and fetal averages) in the 3.75 mg/kg/day group. Other fetal gross external, soft tissue and skeletal alterations (malformations and variations) were considered unrelated to treatment because the incidences were not dosage-dependent and/or were within historical control range.

## CONCLUSIONS

Conclusions are summarized above and this reviewer agrees.

## REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G. 1999. Argus Research Laboratories, Inc. Protocol Number: 418-012, January 1999. "Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits".

3M Environmental Laboratory. 2001. Analytical Laboratory Report, FACT TOX-099, February 9, 2001. "Determination of the Concentration of Potassium Perfluorooctanesulfonate (PFOS) in Rabbit Liver and Serum Samples."

## REPRODUCTIVE TOXICITY STUDIES

**Title:** COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.9, 1999.

### TEST SUBSTANCE

**Identity:** Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3.

**Remarks:** The test article, FC-95 (lot 217), was received on May 20, 1998, and stored at room temperature. Prepared suspensions were stored at room temperature overnight. Information regarding the purity, identity, strength and composition of the test article is on file with the Sponsor.

### METHOD

**Method/Guideline followed (i.e., OECD 414, etc.):** This study was designed to evaluate ICH Harmonized Tripartite Guideline stages A-F. A modification of the requirements of the U.S. Food and Drug Administration (FDA) were used as a basis for the study design.

**Type of study (one-generation, two-generation, etc.):** Two-generation reproductive toxicity

**GLP (Y/N):** The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (MHW) and the European Economic Community (EEC). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study have been documented.

**Year study performed:** 1999

**Species/Strain:** Sprague Dawley rats

**Sex (males/females/both):** Both

**Number of animals per dose:** 35

**Route of administration:** Gavage

**Dosing regimen (list all with units):** Five groups of 35 rats per sex per dose group were administered PFOS by gavage for six weeks prior to and during mating. Treatment in male rats continued until one day before sacrifice (approximately 22 days total); female rats were treated throughout gestation, parturition, and lactation.

**Doses:** 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day

**Premating exposure period for males/females (P and F1, if appropriate):** Six weeks for P; Nine and a half weeks for F1.

**Statistical methods used:** Proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (body weights, body weight changes, and feed consumption) were analyzed using Bartlett's Test of Homogeneity of Variance and Analysis of Variance (ANOVA). If the ANOVA was significant ( $p \leq 0.05$ ), Dunnett's Test was used to identify the statistical significance of the individual groups. If the ANOVA was not appropriate, the Kruskal-Wallis Test was used. In cases where the Kruskal-Wallis Test was statistically significant ( $p \leq 0.05$ ), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups.

If there were greater than 75% ties, Fisher's Exact Test was used. Fisher's Exact Test was also used to evaluate necropsy data for the pups which were stillborn or found dead. Data obtained at Cesarean-sectioning, natural delivery, preweaning reflex/physical developmental data and postweaning behavioral data involving discrete data (number of corpora lutea, number of pups per litter, trials to a criterion) were evaluated by the Kruskal-Wallis Test.

**Remarks – Detail and discuss any significant protocol parameters and deviations:**

F0 Generation:

Parental animals (F0) were observed twice daily for clinical signs. Body weights and food consumption values were recorded weekly during the treatment period in male rats; and weekly during mating and then daily during gestation, and on lactation days 1, 4, 7, 10, 14, and at sacrifice in female rats. Each dosage group consisted of two sets of female rats. One set consisted of the first ten female rats with confirmation of mating; this group was dosed until gestation day (GD) 10 and delivered via Cesarean-sectioning. The remaining females comprised the second set which delivered naturally. During the 21-day lactation period, the dams were evaluated for clinical signs during parturition and length of gestation, and then each litter was evaluated at least twice daily for size and pup viability at birth. Pup observations during the 21-day lactation period included physical signs, body weights, nursing behavior, surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air righting reflex. Pupil constriction was evaluated only on lactation day 21. On lactation day 4, litters were randomly culled to four male and four female pups. The remaining pups were sacrificed and necropsied. The F0 male rats were sacrificed and necropsied after the end of dosing at the time of parturition (lactation day 1). The testes, epididymides, prostate, and seminal vesicles were weighed. Evaluations of sperm number, motility, and morphology were not included in the protocol. The F0 generation females that delivered by Cesarean-sectioning were sacrificed on GD 10 and necropsied. Pregnancy status was confirmed, the ovaries were examined for the number and distribution of corpora lutea, implantation sites were determined, and embryos were examined for viability. The F0 generation females that delivered naturally were sacrificed on lactation day (LD) 21 and necropsied. Ovaries were examined as above and the number and distribution of implantation sites was recorded. The liver from each parental rat was removed, weighed and analyzed. Blood samples were collected from 5 male rats that had mated and from 5 female rats on LD 21 for pharmacokinetic analysis; livers from the pups from the litters of these five dams were also collected for analysis.

F1 Generation:

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosages groups, only the 0.1 and 0.4 mg/kg/day dosage groups were carried into the second generation. Twenty-five F1 generation rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, and 0.4 mg/kg/day beginning on LD 22 and continuing through the day before sacrifice. At 24 days of age, one rat per sex per litter in each dosage group was tested in a passive avoidance paradigm. On LD 28, females were evaluated for the age of vaginal patency and on LD 34, male rats were evaluated for the age of preputial separation. One rat per sex per litter were evaluated in a water-filled M-maze on LD 70. Assignment to cohabitation within each dosage group began on LD 90. Females with evidence of mating



were considered to be at GD 0 and assigned to individual housing for the remainder of the dosing period. The F1 generation male rats were sacrificed after mating, necropsied and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver naturally. Dams that delivered litters were sacrificed and necropsied on LD 21. All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups.

## RESULTS

**NOAEL (dose and effect) – for F0, F1, and F2 (as appropriate):** The NOAEL for the F0 generation male and female parental animals = 0.1 mg/kg/day, the lowest dose tested. The NOAEL for the F1 generation male parental animals could not be determined since treatment related signs of toxicity were observed at 0.1 and 0.4 mg/kg/day; the NOAEL for the F1 generation female parental animals = 0.1 mg/kg/day. The NOAEL for the F1 generation offspring = 0.4 mg/kg/day. The NOAEL for the F2 generation offspring = 0.1 mg/kg/day.

**LOAEL (dose and effect) – for F0, F1, and F2 (as appropriate):** The LOAEL for the F0 generation male and female parental animals = 0.4 mg/kg/day, based on reductions in body weight gain and food consumption. The LOAEL for the F1 generation male animals = 0.1 mg/kg/day based on significant reductions in absolute food consumption; the NOAEL for the F1 generation female animals = 0.4 mg/kg/day based on reductions in body weight and food consumption. The LOAEL for the F1 generation offspring = 1.6 mg/kg/day, based on significant reductions in the number of implantation sites, litter size, pup viability, growth and survival. The LOAEL for the F2 generation offspring = 0.4 mg/kg/day, based on significant reductions in pup growth.

### **Toxic response/effects by dose level – parental/F1:**

Toxic effects in F0 generation animals: reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dosage groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4 and 1.6 mg/kg/day dosage groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Signs of reproductive toxicity in the F0 generation males were seen at the highest dose group of 3.2 mg/kg/day and included significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dosage groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Toxic effects in F1 generation animals: F1 males; the only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels. F1 females; observations at the 0.4 mg/kg/day dosage group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation.

### **Toxic response/effects by dose level – offspring (F1/F2):**

Toxic effects in the F1 generation pups consisted of reduced pup viability at the two highest dose groups (1.6 and 3.2 mg/kg/day). The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. Statistically significant increases were observed in the number of dams with stillborn pups at the 3.2 mg/kg/day dose group.

Viability and lactation indices were significantly reduced in these same dosage groups (viability index = 0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day; lactation index = 94.6% at 1.6 mg/kg/day). Statistically significant reductions in pup body weights were also observed at the two highest dosage groups. Gestation length was significantly reduced at 3.2 mg/kg/day. Significant reduction in the number of implantation sites followed by a concomitant reduction in litter size was observed at 3.2 mg/kg/day. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead.

Toxic effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7-14.

#### **Statistical results:**

F0 generation male animals: Significant reductions ( $p \leq 0.05$  or  $p \leq 0.01$ ) in body weight gains at 0.4 mg/kg/day and higher. Absolute and relative food consumption values were significantly reduced ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the 1.6 and 3.2 mg/kg/day dosage groups. A significant increase ( $p \leq 0.01$ ) in the number of male rats in the 3.2 mg/kg/day dosage group with brown liver. The gross lesions of the liver were considered to be treatment related because the incidences were dosage-dependent. Significant reductions ( $p \leq 0.05$  and  $p \leq 0.01$ ) in terminal body weights were observed in the 1.6 and 3.2 mg/kg/day dosage groups. Significant reductions ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the absolute weights of the seminal vesicles with fluid and the prostate were observed in the 3.2 mg/kg/day dosage group.

F0 generation female animals: Significant increases ( $p \leq 0.05$  or  $p \leq 0.01$ ) in localized alopecia were observed in the 0.4, 1.6, and 3.2 mg/kg/day dosages groups. Significant reductions in body weight and body weight gains and food consumption ( $p \leq 0.05$  or  $p \leq 0.01$ ) were observed in the 1.6 and 3.2 mg/kg/day dosage during pre-mating and gestation and then in 0.4 mg/kg/day dosage group and above during lactation. Significant reductions ( $p \leq 0.01$ ) in gestation length, implantation sites, and litter size were observed at 3.2 mg/kg/day.

F1 generation offspring: Pup viability was significantly reduced ( $p \leq 0.05$  or  $p \leq 0.01$ ) in the 1.6 and 3.2 mg/kg/day dosage groups. Significant increases ( $p \leq 0.05$  or  $p \leq 0.01$ ) were observed in the number of dams with stillborn pups, while significant reductions ( $p \leq 0.05$  or  $p \leq 0.01$ ) were observed in the viability index, lactation index, and averages for surviving pups at 3.2 mg/kg/day. A dosage-dependent pattern of reduced pup body weight was evident in each dosage group, with statistical significance ( $p \leq 0.01$ ) in the 1.6 and 3.2 mg/kg/day dosage groups.

F1 generation adult animals: Males - Significant reductions ( $p \leq 0.05$  or  $p \leq 0.01$ ) in absolute food consumption at 0.1 and 0.4 mg/kg/day; females - significant ( $p \leq 0.05$ ) body weight loss on lactation days 1-4 at 0.4 mg/kg/day; and significant reductions ( $p \leq 0.05$ ) in food consumption at 0.4 mg/kg/day on days 1-8 postweaning.

F2 generation offspring: Pup body weights tended to be reduced in the 0.1 mg/kg/day dosage group on lactation day 4 and 7, but were comparable to controls by lactation day 14. Pup body weights in the 0.4 mg/kg/day dosage group tended to be reduced, though not significantly, on lactation days 4-21, with significant reductions ( $p \leq 0.05$  and  $p \leq 0.01$ , respectively) on lactation days 7 and 14, as compared to controls.

**Remarks:** In the F0 generation male rats, there were no treatment-related clinical signs of toxicity, no mortality, and no effects on mating or on any of the fertility parameters evaluated in any dose group tested. Reported effects included reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dosage groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4 and 1.6 mg/kg/day dosage groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Body weights, body

weight gains, absolute and relative food consumption were unaffected by treatment at the 0.1 mg/kg/day dosage group. Signs of reproductive toxicity in the F0 generation males were seen at the highest dose group of 3.2 mg/kg/day and included significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported.

In the F0 generation female rats, no deaths were reported at any dose level. In dams sacrificed on GD 10 for Caesarean-sectioning, there did not appear to be any effects on estrous cycling, mating and fertility parameters, the numbers of corpora lutea and implantations, or in the number of viable or non-viable embryos. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dosage groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and body weight gain and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Reversible delays in reflex and physical development were observed in the F1 generation offspring. The ability to surface right was significantly delayed in the 1.6 and 3.2 mg/kg/day dosage groups on LDs 3-10 (delays in the 3.2 mg/kg/day dose group were observed on LD 1, after which there were no surviving pups remaining for further observation). By the end of the observation period, however, all surviving pups in the 1.6 mg/kg/day dosage group had the ability to surface right. There were no delays observed in the ability to surface right in dose groups  $\leq 0.4$  mg/kg/day. Similar responses were seen for pinna unfolding and eye opening. Although there were transient delays seen with these signs of physical development across all dose groups, by the end of the observation period responses in pups were similar to controls. The time of development of the acoustic startle reflex and the ability to air right were both significantly reduced in the 1.6 mg/kg/day dosage group. No effects on these reflexes were observed in the low dose group of 0.1 mg/kg/day and only a transient delay (on LD 16 only) in the ability to air right was seen in the 0.4 mg/kg/day group. At the end of lactation (LD 21), all live pups in all dose groups (0, 0.1, 0.4, and 1.6 mg/kg/day) had pupil constriction response.

The most significant finding reported in the offspring was that of reduced pup viability at the two highest dose groups. The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. As a result, the viability index was greatly reduced in these dosage groups (0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day). The lactation index was also significantly reduced (94.6%) in the 1.6 dosage group. In addition, gestation length was significantly reduced in the high-dose group and there also was a significant reduction in the number of implantation sites followed by a concomitant reduction in litter size. Statistically significant reductions in pup body weights were also observed at the two highest dosage groups. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead. The percentage of male pups was comparable across all dosage groups.

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosages groups, only the 0.1 and 0.4 mg/kg/day dosage groups were carried into the second generation.

Clinical observations in the F1 generation male rats appeared unremarkable. No treatment-related deaths were reported and no statistically significant differences were reported for any of the following parameters: body weights/body weight gains, average day of preputial separation; values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating or fertility parameters; necroscopic examinations; absolute or relative weights for the right or left testis, seminal vesicles, right

epididymis, or prostate; and terminal body weights. The only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels.

Clinical observations for the F1 generation females were likewise unremarkable. Observations at the 0.4 mg/kg/day dosage group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation. There were no statistically significant differences reported for any of the following parameters: values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating and fertility parameters; gestation index; pregnancy rates; and necroscopic examinations.

Evidence of treatment-related effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. Body weights were comparable to control levels by LD 14. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7-14. Mean body weights on LD21 continued to remain lower than controls, although the difference was not statistically significant (46.5 g in 0.4 mg/kg/day dose group vs. 50 g in controls). Clinical and necroscopic observations of the F2 generation pups were unremarkable. No other toxicologically significant effects were reported.

Liver and sera samples collected from the initial population of dosed animals (F0) and their offspring (F1) were analyzed for the presence of PFOS. The F0 results for the F0 animals were:

| Dose group<br>(mg/kg/day) | Average PFOS conc.<br>in serum (ug/ml) | Average PFOS conc.<br>in liver (ug/g) |
|---------------------------|--|---------------------------------------|
| 0.0:                      | female 0.0307<br>male 0.0244           | female 0.171<br>male 0.665            |
| 0.1:                      | female 5.28<br>male 10.5               | female 14.8<br>male 84.9              |
| 0.4:                      | female 18.9<br>male 45.4               | female 58.0<br>male 176               |
| 1.6:                      | female 82<br>male 152                  | female 184<br>male 323                |
| 3.2:                      | female NR*<br>male 273                 | female NR*<br>male 1360               |

\*samples not received

Qualitatively, the F0 results indicate all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group. The average PFOS concentrations in pooled liver samples from F1 animals shortly after birth were 0.0511, 6.19, 57.6, and 70.4 ug/g in the 0.0, 0.1, 0.4, and 1.6 mg/kg/day dose groups, respectively. These quantitative values for the PFOS concentration in the liver and serum should be viewed with caution. The accuracy of quantitation is  $\pm 30\%$ , the purity of the analytical reference substance is unknown, and there were several uncorrected dilution errors.

## CONCLUSIONS

Conclusions stated above and this reviewer agrees.

## REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G. 1999b. Argus Research Laboratories, Inc. Protocol Number: 418-008, Sponsor Study Number: 6295.9, June 10, 1999. Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats.

3M Environmental Laboratory. 1999b. Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2795-39-3) in the Serum and Liver of Sprague-Dawley Rats Exposed to PFOS via Gavage. As amended April 19, 2000.

## CROSS-FOSTERING/DEVELOPMENTAL TOXICITY STUDY

**Title:** ORAL (GAVAGE) CROSS-FOSTERING STUDY OF PFOS IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER 418-014, 3M T-6295.13, JULY 1999.

### TEST SUBSTANCE

**Identity:** Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3.

**Remarks:** The test article, FC-95 (Lot 217) was received on October 21, 1998, and stored at room temperature. Prepared suspensions were stored at room temperature. Information regarding the identity, strength, composition, and purity of the test article is on file with the sponsor.

### METHOD

**Method/Guideline followed (i.e., OECD 414, etc.):** For the cross-fostering study: The requirements of the U.S. Food and Drug Administration (FDA) were used as the basis of the study design, i.e., the International Conference on Harmonization: Guideline on detection of toxicity to reproduction for medicinal products. Federal Register, September, 22, 1994, Vol. 59. No. 183. For the PFOS bioanalysis: US FDA GLP Final Rule 21 CFR 58, with exceptions: Two separate study directors were assigned to the in-life phase and the analytical phase of this study. The ABS final report does not have a Statement of Compliance. The QAU statement in the ABS final report indicates compliance with EPA 40 CFR Part 792, rather than FDA 21 CFR Part 58. Dose confirmation analyses were not conducted according to the GLP regulations; analytical method was not fully validated. Not all raw data were verified by the group leader or designee.

**GLP (Y/N):** The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. FDA, the Japanese Ministry of Health and Welfare, and the European Economic Community. There were no deviations from the GLP regulations that affected the quality or integrity of the study. Quality assurance Unit findings derived from the inspections during the conduct of this study are documented.

**Year study performed:** 1999

**Species/Strain:** Sprague-Dawley rats

**Number of animals per dose:** 25

**Route of administration:** Gavage

**Dosing regimen (list all with units):** Two groups of 25 female rats were administered PFOS by gavage beginning 42 days prior to mating to untreated (breeder) males, and continuing throughout gestation and into day 21 of lactation.

**Doses:** 0, 1.6 mg/kg/day

**Statistical methods used:** Averages and percentages were calculated. Litter values were used where appropriate.

**Remarks – Detail and discuss any significant protocol parameters and deviations:** Two groups of 25

female Sprague-Dawley rats were administered 0 and 1.6 mg/kg/day PFOS in 0.5% Tween-80 by gavage, beginning 42 days prior to mating to untreated (breeder) males, and continuing throughout gestation and into day 21 of lactation. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weight. Parental females were observed twice daily for viability and clinical observations were recorded 1 hour prior to and after dosing during the treatment period. Maternal body weights were recorded once during the acclimation period and then daily during the treatment period and at sacrifice; food consumption was also recorded once during the acclimation period and then daily during gestation and on days 1, 4, 7, 10, and 14 of lactation. During parturition, females were continually evaluated for clinical signs and also for duration of gestation, length of parturition, litter sizes, and pup viability at birth. Maternal behavior was recorded daily throughout lactation. All maternal rats were sacrificed by carbon dioxide asphyxiation on day 22 of lactation and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed; any gross lesions were preserved for future analysis. In addition, the number and distribution of implantation sites were recorded. Rats that did not deliver a litter were not included in the cross-fostering procedure and were sacrificed on lactation day 25, examined for gross lesions, and the uteri examined to confirm the presence/absence of implantation sites. Dams with no surviving pups were sacrificed after the last pup was found dead, missing, or presumed cannibalized.

Following completion of parturition, litters were immediately removed from their respective dams and placed with either a control- or PFOS-treated dam for rearing. This cross-fostering procedure resulted in four groups of 12-13 dams or pups as follows: A) control dams with litters from PFOS-treated dams, i.e., in utero exposure only; B) control dams with litters from control dams, i.e., negative control; C) PFOS-treated dams with litters from PFOS-treated dams, i.e., both in utero and post-natal exposure; and D) PFOS-treated dams with litters from control dams, i.e., post-natal exposure only.

On day 1 of lactation (birth), each pup was individually weighed and each litter was evaluated twice daily during lactation for viability. Pups were observed once daily for clinical signs and gross external physical anomalies. Pup body weights were recorded on days 1, 4, 7, 14, and 21 of lactation, and then at sacrifice. On day 4 of lactation, each cross-fostered litter was culled to 5 males and 5 females. On day 21 of lactation, all pups were sacrificed via decapitation and examined for gross lesions. Pups found dead or sacrificed because of moribundity were examined for gross lesions and for the cause of death or the moribund condition. The lungs, liver, and any gross lesions were collected from selected pups at various timepoints (for the F0 generation, blood, milk, and liver samples were collected on LD 14; milk was collected 2-6 hours post-dosing; for the F1 generation offspring, blood was collected on LD 14) and preserved for possible future analysis. Liver samples were evaluated via electron microscopy. In addition, samples of blood, milk (including the milk-secreting glands), and liver were collected from selected maternal rats and pups (blood and liver samples were pooled per litter) at various timepoints for analysis of PFOS concentration. Only the analysis of the sera samples are available at this time.

## RESULTS

**Toxic response/effects by dose level - maternal:** Mean maternal body weight and body weight gains at 1.6 mg/kg/day were reduced compared to controls during pre-mating and continuing throughout gestation. Mean maternal body weight changes in the treated group were comparable to those seen in control animals during lactation. During the pre-mating period, and on into gestation and lactation, food consumption was reduced in treated animals as compared to controls. Reductions in gestation length, the average number of implantation sites, delivered sizes, and live litter size were observed in treated animals.

**Toxic response/effects by dose level – developmental:** Pup mortality was observed in two of the cross-fostered groups. On lactation days 2-4, approximately 19% of the pups in group C were either found dead or presumed cannibalized. Pup mortality was also observed in group A at a rate of 9%. In addition, the

number of live pups on day 4 of lactation, numbers of surviving pups per litter, and live litter sizes were also reduced in these two groups. Pup mortality in groups B and D during lactation days 2-4 were at 1.6% and 1.1%, respectively. Reductions in pup body weights were observed in groups A and C on day 1 of lactation. Pup body weights in group D were comparable to controls during that same period. From lactation day 4-21, pup body weights in groups A, C, and D were reduced when compared to group B (negative control), with the reductions greatest in group C. Two litters in group A and one litter on group C did not nurse. Milk analysis of the stomachs of pups found no milk in the stomachs of 57%, 100%, and 87% of the pups found dead and necropsied in groups A, C, and D, respectively.

Electron microscopic examination of the liver revealed an increase in the number of peroxisomes in pups from dams treated with 1.6 mg/kg/day PFOS.

**Statistical results:** The method of statistical analysis consisted of calculation of averages (mean  $\pm$  S.D.) and percentages. Statistical significance was not assessed. The data were presented according to day of gestation/lactation and compared by dose and by cross-fostered group. Litter values were used where appropriate. The data are too cumbersome to cite here.

**Remarks – Additional information to adequately assess the data:** All maternal rats survived to schedule sacrifice. Signs of clinical toxicity observed in the dams during the study period (e.g., chromorhinorrhea, scaly tail, abrasion on the head, neck, tail and/or forelimb, missing, broken and/or misaligned incisors, and localized alopecia, among others) were not considered to be treatment-related since they also occurred in the control animals. All pregnant animals delivered live offspring.

Following cross-fostering on LD 1, live litter sizes were comparable between treated and control. Sex ratios and the lactation index were comparable among all groups. Signs of clinical toxicity were observed in pups, but were not considered to be treatment-related since they also occurred in group B (negative control) at the same rate. No significant differences were observed between group B and the other groups following examination of pup lungs.

PFOS concentrations in the serum of untreated dams ranged from below the limit of detection (0.05 ug/ml) to 5.34 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with untreated dams, were below the limit of detection. Serum PFOS concentrations in the pups from treated dams, fostered with untreated dams, ranged from 47.6 ug/ml to 59.2 ug/ml. PFOS concentrations in the serum of treated dams ranged from 59.2 ug/ml to 157 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with treated dams, ranged from below the limit of detection to 35.7 ug/ml. Serum PFOS concentrations in the pups from treated dams, fostered with treated dams, ranged from 79.5 ug/ml to 96.9 ug/ml. These data indicate that exposure to PFOS can occur both *in utero* and via milk from treated dams.

## CONCLUSIONS

Pups from control dams that were cross-fostered with PFOS-treated dams (post-natal exposure only) had the same low mortality rate (1.1%) as pups from control dams cross-fostered with control dams (1.6%; negative control). Mortality rates in the remaining two groups, however (i.e. control dams with litters from PFOS-treated dams, i.e., in utero exposure only; and PFOS-treated dams with litters from PFOS-treated dams, i.e., both in utero and post-natal exposure), had higher mortality rates at 9.6 % and 19.2%, respectively. Under the conditions of the study, this limited data appear to indicate that reduced pup survival is mainly a result of in utero exposure to PFOS and that post-natal exposure via milk in conjunction with in utero exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse affect on pup viability. Additionally, analysis of PFOS concentration showed that PFOS was observed in the sera of F0



female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera samples taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed via lactation, but not exposed *in utero*. These are the conclusions of the study authors and this reviewer agrees.

#### REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G.1999c. Testing Facility: Argus Research Laboratories, Inc., Protocol Number 418-014, Sponsor: 3M Corporate Toxicology, Study Number T-6295.13, July 23, 1999. Oral (Gavage) Cross-Fostering Study of PFOS in Rats.

3M Environmental Laboratory. 1999. Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) (CAS Number: 2759-39-3) in the Serum of Sprague-Dawley Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage, Laboratory Report No. U2779, Requestor Project No. 3M Tox 6295.13. Study initiation date: June 10, 1999. Completion at signing (2/11/00). Sample analysis completion June 28, 1999. Study Identification Number: FACT Tox-108

## REPEAT DOSE DATA

**Title:** 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats

### TEST SUBSTANCE

**Identity:** Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295).

**Remarks:** The test substance, Lot No. 217, is a light colored, free flowing powder, and is 86.9% pure. The impurities of the test substance were not indicated in the main body of the study report.

### METHOD

**Method/guideline followed:** Guideline number not stated

**Study duration:** Two years

**GLP (Y/N):** Yes

**Year study performed:** 1998-2000

**Species/strain:** Sprague-Dawley rat [CrI:COBS<sup>R</sup> CD(SD)BR]

**Sex:** Male and female

**Number of animals per dose group:** The control and high-dose groups contained 70 rats/sex and the low-, mid-, and mid-high dose groups contained 60 rats/sex. A high-dose recovery group contained 40 rats/sex.

**Route of administration:** Diet

**Doses tested and frequency:** Control (Group 1): 0 ppm ; low-dose (Group 2): 0.5 ppm ; mid dose (Group 3): 2 ppm; mid-high dose (Group 4): 5 ppm; high-dose (Group 5) : 20 ppm.

**Post-treatment observation period:** A high-dose recovery group (Group 6) was observed for 52 weeks after 52 weeks of treatment.

**Statistical methods used:** Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at  $P < 0.05$ , transformations were used to stabilize the variance. One-way analysis of variance [ANOVA] was used to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, palmitoyl CoA oxidase activities, and organ weight data. ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparisons between treated and control groups. Group comparisons were evaluated at the 5.0%, two-tailed probability level.

**Remarks:** The animals were observed twice daily (a. m. and p.m.) for mortality and moribundity; findings were recorded as they were observed. At least once prior to treatment and weekly thereafter, each animal was removed from its cage and examined; abnormal findings or an indication of normal was recorded. Body weight data were collected weekly through Week 17, once every 4 weeks thereafter, and at Week

105. Food consumption data were collected weekly for the first 16 weeks and once every 4 weeks thereafter. During Weeks 4, 14, 27, and 53, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry tests from 10 animals/sex in Groups 1 through 5. Blood was collected for cholesterol and triglyceride determinations from all animals prior to the terminal sacrifice during Week 105 (Week 103 for females in Group 3) and the recovery sacrifice (Group 6) during Week 106. Blood films were also prepared for animals at the terminal and recovery sacrifices. Five animals/sex in Groups 1 through 5 were sacrificed during Week 4; livers were collected and weighed. [The liver samples were collected for PFOS analysis, mitochondrial activity, hepatocellular proliferation rate measurements by proliferation cell nuclear antigen (PCNA), and determination of palmitoyl-CoA oxidase activity. In addition, liver samples were collected for PFOS analysis (Weeks 14, 53, 103, 105, and 106), hepatocellular proliferation rate measurement [by PCNA at Week 14 and by bromodeoxyuridine (BrdU) immunohistochemistry at Week 53] and palmitoyl-CoA oxidase determination (Week 4 and 14).]

## RESULTS

### **Survival rates:**

There was a significant increased trend in survival that occurred in the males that was due to significant increases in survival in mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control group. None of the other treated groups in the males revealed any significant differences in survival. No significant trend was noted in survival in females. There was a significant decrease in survival in the mid-dose (2.0 ppm) group and not in the mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control.

### **Neoplastic effects:**

The results of the study show that PFOS is carcinogenic, inducing tumors of the liver, and of the thyroid and mammary glands.

| Tumors                    | Tumor incidence (%) |                   |                   |                  |                  |                  |
|---------------------------|---------------------|-------------------|-------------------|------------------|------------------|------------------|
|                           | Male                |                   |                   |                  |                  |                  |
|                           | 0                   | 0.5 ppm           | 2 ppm             | 5 ppm            | 20 ppm           | 20 ppm recovery# |
| <b>Liver</b>              |                     |                   |                   |                  |                  |                  |
| Hepatocellular adenoma*   | 0<br>(0/60)         | 6.0<br>(3/50)     | 6.0<br>(3/50)     | 2.0<br>(1/50)    | 11.7**<br>(7/60) |                  |
| <b>Thyroid</b>            |                     |                   |                   |                  |                  |                  |
| Follicular cell adenoma   | 5.0<br>(3/60)       | 10.2<br>(5/49)    | 8.0<br>(4/50)     | 8.2<br>(4/49)    | 6.8<br>(4/59)    | 23.1**<br>(9/39) |
| Follicular cell carcinoma | 5.0<br>(3/60)       | 2.0<br>(1/49)     | 2.0<br>(1/50)     | 4.1<br>(2/49)    | 1.7<br>(1/59)    | 2.6<br>(1/39)    |
| Combined                  | 10.0<br>(6/60)      | 12.2<br>(26/49)   | 10.0<br>(5/50)    | 10.2<br>(5/49)   | 8.5<br>(5/59)    | 25.6<br>(10/39)  |
|                           | Female              |                   |                   |                  |                  |                  |
| <b>Liver</b>              |                     |                   |                   |                  |                  |                  |
| Hepatocellular adenoma*   | 0<br>(0/60)         | 2.0<br>(1/50)     | 2.0<br>(1/49)     | 2.0<br>(1/50)    | 8.3**<br>(5/60)  |                  |
| Hepatocellular carcinoma  | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 0<br>(1/50)      | 1.7<br>(1/60)    |                  |
| Combined*                 | 0<br>(0/60)         | 2.0<br>(1/50)     | 2.0<br>(1/49)     | 2.0<br>(1/50)    | 10.0**<br>(6/60) |                  |
| <b>Thyroid</b>            |                     |                   |                   |                  |                  |                  |
| Follicular cell adenoma   | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 4.0<br>(2/50)    | 1.7<br>(1/60)    |                  |
| Follicular cell carcinoma | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 2.0<br>(1/50)    | 0<br>(0/60)      |                  |
| Combined                  | 0<br>(0/60)         | 0<br>(0/50)       | 0<br>(0/49)       | 6.0**<br>(3/50)  | 1.7<br>(1/60)    |                  |
| <b>Mammary</b>            |                     |                   |                   |                  |                  |                  |
| Fibroadenoma/adenoma      | 38.3<br>(23/60)     | 60.0**<br>(30/50) | 45.8<br>(22/48)   | 52.04<br>(26/50) | 25<br>(15/60)    |                  |
| Carcinoma                 | 18.3<br>(11/60)     | 24.0<br>(12/50)   | 31.2<br>(15/48)   | 22.0<br>(11/50)  | 23.3<br>(14/60)  |                  |
| Combined                  | 48.3<br>(29/60)     | 72.0**<br>(36/50) | 64.6**<br>(31/48) | 58.0<br>(29/50)  | 40.0<br>(24/60)  |                  |

\*Significant positive trend ( $P < 0.03$ ).

\*\* Significantly increased over the control ( $P < 0.05$ ).

# Recovery group; after 52 weeks of treatment.

#### Remarks:

At terminal of the studies, mean body weights of the surviving males and females were not significantly different from the controls. Food consumption for males and females was similar in all treated groups compared to animals given the control material except for the high-dose females which had statistically significantly lower food consumption during weeks 2 through 44. Except for the mid-dose (2.0 ppm) female group, there were no significant decreases in survival in any other male and female treatment groups as compared to that of the control. Therefore, it appears that higher dose levels could have been tolerated by the high-dose groups.

#### Nonneoplastic effects:

**NOAEL (dose and effect):** 0.5 ppm (Male)  
2 ppm (Female)  
**LOAEL (dose and effect):** 2 ppm (Male)  
5 ppm (Female)

Hepatotoxicity, characterized by significant increases in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm. A significant increase in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration was found in all the male treated groups (0.5, 2, 5, or 20 ppm); however, this liver lesion is believed to be due to old age of the animals and is not considered to be treatment-related. Therefore, based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is considered to be 2 ppm in male rats and 5 ppm in female rats.

#### Results of Statistical Analyses of Nonneoplastic Lesions in Male Rats.

| Group  | 1<br>Control | 2<br>Low | 3<br>Mid | 4<br>Mid-<br>High | 5<br>High | 6<br>High<br>Recovery | Groups 5<br>vs. 6 |
|--|--------------|----------|----------|-------------------|-----------|-----------------------|-------------------|
| Liver – Vacuolation, Hepatocellular Midzonal/Centrilobular |              |          |          |                   |           |                       |                   |
|  | 0/65         | 0/55     | 6/55     | 10/55             | 19/55     | 9/40                  | 0.0060+**         |
| p-values   | .0000+**     | NA       | .1690    | .0024+**          | .0001+**  | .4152+                |                   |
| Liver – Hypertrophy, Hepatocellular Centrilobular          |              |          |          |                   |           |                       |                   |
|  | 0/65         | 2/55     | 4/55     | 22/55             | 42/565    | 3/40                  | .0000-**          |
| p-values   | .0000+**     | .2080+   | .0415+*  | .0000+**          | .0000+**  | .0527+                |                   |
| Liver – Granular Cytoplasm, Eosinophilic, Centrilobular    |              |          |          |                   |           |                       |                   |
|  | 0/65         | 0/55     | 0/55     | 0/55              | 14/65     | 0/40                  | .0007-**          |
| p-values   | .0000+**     | NA       | NA       | BA                | .0139+*   | NA                    |                   |
| Liver – Pigment Hepatocellular, Centrilobular              |              |          |          |                   |           |                       |                   |
|  | 0/65         | 0/55     | 0/55     | 0/55              | 6/65      | 0/40                  | .0513             |
| -values  | .006+**      | NA       | NA       | NA                | .0139+*   | NA                    |                   |
| Liver – Necrosis, Individual Hepatocyte                    |              |          |          |                   |           |                       |                   |
|  | 5/65         | 4/55     | 6/55     | 5/55              | 14/65     | 4/40                  | .1024             |
| p-values   | .0106+*      | NA       | NA       | NA                | .0224+*   | .0699+                |                   |
| Liver – Degeneration, Cystic                               |              |          |          |                   |           |                       |                   |
|  | 5/65         | 15/55    | 19/55    | 17/55             | 22/65     | 15/40                 | NA                |
| p-values   | .0007+**     | .0041+** | .0003+** | .0011+**          | .002+**   | .0002+**              | .4306+            |

#### Results of Statistical Analyses of Nonneoplastic Lesions in the Female Rats.

| Group  | 1<br>Control | 2<br>Low | 3<br>Mid | 4<br>Mid-High | 5<br>High | 6<br>High Recovery | Groups 5<br>vs. 6 |
|--|--------------|----------|----------|---------------|-----------|--------------------|-------------------|
| Liver - Infiltrate, Lymphohistiocytic              |              |          |          |               |           |                    |                   |
|  | 42/65        | 42/55    | 38/55    | 41/55         | 56/65     | 32/40              | .2852             |
| p-values   | .0080 +**    | NA       | .3738+   | NA            | .0038 +** | .0709+             |                   |
| Liver – Hypertrophy, Hepatocellular, Centrilobular |              |          |          |               |           |                    |                   |
|  | 2/65         | 1/55     | 4/55     | 16/55         | 52/65     | 2/40               | .0000 -**         |
| p-values   | .0000 +**    | NA       | .2641+   | .0001 +**     | .0000 +** | NA                 |                   |

|   |                    |                 |                |                   |           |                  |           |
|---|--------------------|-----------------|----------------|-------------------|-----------|------------------|-----------|
| Liver - Granular Cytoplasm, Eosinophilic, Centrilobular |                    |                 |                |                   | 36/65     |                  |           |
| p-values  | 0/65<br>.0000 +**  | 0/55<br>NA      | 0/55<br>NA     | 7/55<br>.0034 +** | .0000 +** | 1/40<br>.3810+   | .0000 -** |
| Liver - Pigment, Hepatocellular, Centrilobular          |                    |                 |                |                   | 36/65     |                  |           |
| p-values  | 0/65<br>.0000 +**  | 0/55<br>NA      | 0/55<br>NA     | 1/55<br>NA        | .0000 +** | 0/40<br>NA       | .0000 -** |
| Liver - Necrosis, Individual Hepatocyte                 |                    |                 |                |                   | 15/65     |                  |           |
| p-values  | 7/65<br>.0359 +*   | 6/55<br>NA      | 6/55<br>NA     | 6/55<br>NA        | .0500 +*  | 3/40<br>.4254-   | .0329 -*  |
| Liver - Degeneration, Cystic                            |                    |                 |                |                   | 4/65      |                  |           |
| p-values  | 0/65<br>.0187 +*   | 1/55<br>NA      | 1/55<br>NA     | 2/55<br>.2080+    | .0596+    | 1/40<br>.3810+   | .3660     |
| Liver - Hypertrophy, Hepatocellular, Periportal         |                    |                 |                |                   | 3/65      |                  |           |
| p-values  | 12/65<br>.0026 -** | 10/55<br>.5796- | 9/55<br>.4778- | 4/55<br>.0614-    | .0127 -*  | 7/40<br>NA       | .0344 +*  |
| Liver - Infiltrate, Macrophage, Pigmented               |                    |                 |                |                   | 23/65     |                  |           |
| p-values  | 2/65<br>.0000 +**  | 3/55<br>NA      | 5/55<br>.1567+ | 6/55<br>.0889     | .0000 +** | 7/40<br>.0147 +* | .0383 -*  |

**List of statistically different non-neoplastic effects (increased compared with controls, p < 0.05):**

Males (0.5ppm):

Liver - Degeneration, Cystic

Males (2 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Males (5 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Vacuolation, Hepatocellular Midzonal / Centrilobular

Males (20 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Vacuolation, Hepatocellular Midzonal / Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Pigment Hepatocellular, Centrilobular

Liver - Necrosis, Individual Hepatocyte

Females (0.5 ppm):

None

Females (2 ppm):

None

Females (5 ppm):

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Infiltrate, Macrophage, Pigmented

Females (20 ppm):

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Infiltrate, Macrophage, Pigmented

Liver - Pigment Hepatocellular, Centrilobular

Liver - Necrosis, Individual Hepatocyte

Liver - Infiltrate, Lymphohistiocytic

Liver - Hypertrophy, Hepatocellular, Periportal

### Remarks:

The cystic degeneration of the liver is believed to be due to old age of the animals and is not considered to be treatment-related. However, A significant increase in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Therefore, the NOAEL for the male rat is considered to be 0.5 ppm. The author concluded that the NOAEL for both male and female rats are 2 ppm (which we do not agree).

There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) at weeks 4 and 14, or by bromodeoxyuridine (BrdU) at week 53.

### Serum and Liver level of PFOS

Under the conditions of the studies, PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals.

Summary of PFOS Concentration-Serum (:g/mL)

| Timepoint            | Sex    | 0 ppm                        | 0.5 ppm                 | 2 ppm                  | 5 ppm                 | 20 ppm                 | Group 6              |
|----------------------|--------|------------------------------|-------------------------|------------------------|-----------------------|------------------------|----------------------|
|                      |        |                              |                         |                        |                       |                        | High Recovery        |
|                      |        | Average ±SD                  | Average ±SD             | Average ±SD            | Average ±SD           | Average ±SD            | Average ±SD          |
| Week 0               | Male   | <LOQ <sup>b</sup><br>(n=5)   | 0.907 ±0.0619<br>(n=5)  | 4.33 ±1.16<br>(n=5)    | 7.57 ±2.17<br>(n=5)   | 41.8 ±7.92<br>(n=5)    |                      |
|                      | Female | 0.0259 ±0.00663<br>(n = 5)   | 1.61 ±0.207<br>(n = 5)  | 6.62 ±0.499<br>(n = 5) | 12.6 ±1.73<br>(n = 5) | 54.0 ±7.34<br>(n = 5)  |                      |
| Week 14 <sup>a</sup> | Male   | <LOQ <sup>c</sup><br>(n = 5) | 4.04 ±0.801<br>(n = 5)  | 17.1 ±1.22<br>(n = 5)  | 43.9±4.90<br>(n = 5)  | 148 ±13.8<br>(n = 5)   |                      |
|                      | Female | 2.67 ±4.58<br>(n = 5)        | 6.96 ±0.993<br>(n = 4d) | 27.3 ±2.34<br>(n = 5)  | 64.4±5.48<br>(n = 5)  | 223±22.4<br>n = 5)     |                      |
| Week 53              | Male   | 0.0249 ±0.0182<br>(n = 5)    |                         |                        |                       | 146 ±33.5<br>(n = 4)   |                      |
|                      | Female | 0.395 ±0.777<br>(n = 5)      |                         |                        |                       | 220±44.0<br>(n = 5)    |                      |
| Day 719              | Male   |                              |                         |                        |                       |                        |                      |
|                      | Female |                              |                         | 20.2 ±13.3<br>(n = 9)  |                       |                        |                      |
| Week 105             | Male   | 0.0118 ±0.0104<br>(n = 11)   | 1.31 ±1.30<br>(n = 10)  | 7.60 ±8.60<br>(n=17)   | 22.5±23.5<br>(n = 25) | 69.3 ±57.9<br>(n = 22) |                      |
|                      | Female | 0.0836 ±0.134<br>(n = 24)    | 4.35 ±2.78<br>(n = 15)  |                        | 75.0±45.7<br>(n=15)   | 233 ±124<br>(n = 25)   |                      |
| Week 106             | Male   |                              |                         |                        |                       |                        | 2.42 ±5.09<br>(n=10) |
|                      | Female |                              |                         |                        |                       |                        | 9.51 ±8.70           |

<sup>a</sup> Not corrected for purity of the standard material.

<sup>b</sup> LOQ-Limit of Quantitation = 0.00910 pg/mL

LOQ-Limit of Quantitation = 0.0457 pg/mL

C92987F sample spilled during extraction, no sample remaining for analysis. It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated (that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Summary of PFOS Concentration-Liver (:g/g)

| Timepoint | Sex    | Group 1<br>Control<br>Average ±SD | Group 2<br>Low<br>Average ±SD | Group 3<br>Mid<br>Average ±SD | Group 4<br>Mid-High<br>Average ±SD | Group 5<br>High<br>Average ±SD    | Group 6<br>High Recovery<br>Average ±SD |
|-----------|--------|-----------------------------------|-------------------------------|-------------------------------|------------------------------------|-----------------------------------|---|
| Week 0    | Male   | 0.104 ±0.0673<br>(n = 5)          | 11.0±2.31<br>(n = 5)          | 31.3 ±5.84<br>(n = 5)         | 47.6 ±12.5<br>(n = 5)              | 282 ±45.3 <sup>o</sup><br>(n = 5) |   |
|           | Female | 0.107 ±0.0486<br>(n = 5)          | 8.71 ±0.552<br>(n = 5)        | 25.0±6.11<br>(n = 5)          | 83.0±14.1<br>(n = 5)               | 373±44.1<br>(n = 5)               |   |



|          |        |                          |                        |                       |                       |                      |                        |
|----------|--------|--------------------------|------------------------|-----------------------|-----------------------|----------------------|------------------------|
| Week 10  | Male   | 0.459 ±0.0573<br>(n = 5) | 23.8 ±3.45<br>(n = 5)  | 74.0 ±6.16<br>(n = 5) | 358 ±28.8<br>(n = 5)  | 568 ±107<br>(n = 5)  |                        |
|          | Female | 12.0 ±22.4<br>(n = 5)    | 19.2 ±3.77<br>(n = 5)  | 69.2 ±3.46<br>(n = 5) | 370 ±22.3<br>(n = 5)  | 635±49.0<br>(n = 5)  |                        |
| Week 53  | Male   | 0635 ±1.04<br>(n = 10)   |                        |                       |                       | 435±96.9<br>(n = 9)  |                        |
|          | Female | 0923 ±1.77<br>(n=10)     |                        |                       |                       | 560 ±180<br>(n=10)   |                        |
| Day 719  | Male   |                          |                        |                       |                       |                      |                        |
|          | Female |                          |                        | 55.1 ±31.5<br>(n = 9) |                       |                      |                        |
| Week 105 | Male   | 0.114 ±0.148<br>(n = 11) | 7.83 ±7.34<br>(n = 10) | 26.4±20.4<br>(n=17)   | 70.5±63.1<br>(n = 25) | 189 ±141<br>(n = 22) |                        |
|          | Female | 0.185 ±0.184<br>(n = 24) | 12.9 ±6.81<br>(n = 15) |                       | 131 ±61.4<br>(n=15)   | 381 ±176<br>(n = 25) |                        |
| Week 106 | Male   |                          |                        |                       |                       |                      | 3.12 ±5.97<br>(n = 10) |
|          | Female |                          |                        |                       |                       |                      | 12.9 ±10.4<br>(n = 17) |

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## CONCLUSIONS

The study results are summarized as follows:

1. Treatment-related changes were found more commonly in males than in females of each of the treatment groups, which were supported by earlier pharmacokinetic studies demonstrating a higher retention of the compound by males than females.
2. The test material was considered to be carcinogenic in the rat, inducing tumors of the liver and the thyroid gland in the males and tumors of the liver and of the thyroid and mammary gland in females.
3. Based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is considered to be 2 ppm in male rats and 5 ppm in female rats.
4. There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) or by bromodeoxyuridine (BrdU).
5. PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals.

**REFERENCE**

3M, (2002). 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats. Final Report, 3M T-6295 (Covance study no.: 6329-183), Volumes I-IX , 4068 pages, January 2, 2002. 3M, St. Paul, Minnesota.

Seacar, A.M., Thomford, P.J., and Butenhoff, J.L. Terminal observations in Sprague-Dawley rats after lifetime dietary exposure to potassium perfluorooctanesulfonate. Toxicol. Sci./Toxicologist, 66 (1-S):185, 2002.

**EPIDEMIOLOGIC DATA**

**Title:** Identification of Fluorochemicals in Sera of American Red Cross Adult Blood Donors

**TEST SUBSTANCE**

**Identity:** PFOS and 6 other fluorochemicals

**Remarks:** The results reported are preliminary. The final report is expected November 2001.

**METHOD**

**Study design:** Cross-sectional

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the levels of PFOS in the serum of American Red Cross blood banks in 6 regions of the U.S.

**Study period:** 2000

**Setting:** N/A

**Total population:** Serum pooled from 6 ARC blood banks in various geographic regions in the US: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD.

**Subject selection criteria:** Unknown

**Total # of subjects in study:** 652 donors, age 20-69 years

**Comparison population:** N/A

**Participation rate:** N/A

**Subject description:** No information was provided on the individuals from whom the sera samples were taken.

**Health effects studied:** PFOS levels in blood

**Data collection methods:** Blood sera samples were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS).

**Details on data collection:** No information was provided as to how the blood was drawn, stored, etc.

**Exposure period:** Unknown--PFOS serum levels used as surrogate for exposure.

**Description/delineation of exposure groups/categories:** N/A

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

**Other methodological information:** N/A

## RESULTS

**Describe results:** The mean serum PFOS level was 43.7 ppb. The range was 4.27 to 1656 ppb. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

**Study strengths and weaknesses:** These data are cross-sectional data used to determine PFOS levels in the general population. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small. Blood donors cannot be considered representative of the general population of the US.

**Research sponsors:** 3M Medical Department, Corporate Occupational Medicine

**Consistency of results:** Mean PFOS levels reported in this study are similar to those reported earlier in pooled blood samples, although they are on the higher end of the range.

## CONCLUSIONS

N/A

## REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of American Red Cross adult blood donors. Interim report. June 25, 2001.

## EPIDEMIOLOGIC DATA

**Title:** An Epidemiologic Analysis of Episodes of Care of 3M Decatur Chemical and Film Plant Employees, 1993-1998

### TEST SUBSTANCE

**Identity:** POSF-based chemicals used at the Decatur plant

**Remarks:** Episodes of care analyses are not often used in occupational epidemiologic studies.

### METHOD

**Study design:** Episode of care comparison

**Manufacturing/Processing/Use:** The 3M Decatur, Alabama plant began production in 1961. It is made up of the film plant and the chemical plant. The 3 major product groups in the chemical plant are protective chemicals, performance chemicals, and fluoroelastomers. Perfluorooctanesulfonyl fluoride (POSF) is the major sulfonate fluorochemical manufactured at Decatur and is used as the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers.

**Hypothesis tested:** To use episodes of care methodology as a screen for morbidity outcomes associated with long-term, high exposure to POSF-based production at the 3M facility in Decatur, Alabama.

**Study period:** Episodes of care experience of 652 chemical employees and 659 film plant employees were analyzed for workers at the plant who were employed for at least 1 year between January 1, 1993 and December 31, 1998.

**Setting:** 3M plant in Decatur, Alabama.

**Total population:** 1311 workers were eligible for the cohort (at least 1 year of employment at the plant). The total worker population was not reported.

**Subject selection criteria:** All workers employed at the Decatur plant for at least 1 year between Jan. 1, 1993 and Dec. 31, 1998. Episodes of care were limited to their Decatur time of employment for employees hired, terminated, or died during the study period. However, records of employees on Medicare, long-term disability or who chose HMO coverage were not in the database and would not be included in the episodes of care for that employee.

**Comparison population:** Chemical and film plant employees were analyzed separately and then compared to each other. Employee comparison groups were defined according to their potential workplace exposure to POSF fluorochemical production. Group A: all chemical plant employees and all film plant employees eligible for the cohort. Group B: all chemical plant employees who worked solely in the chemical plant and all film plant employees who worked exclusively in the film plant. Group C: all chemical plant employees with high fluorochemical exposures compared to their job counterparts in the film plant. Group D: all plant workers with high fluorochemical exposure for at least 10 years prior to the study onset compared to their job counterparts in the film plant.

**Participation rate:** 97% of Decatur employees were eligible for participation in the study.

**Subject description:** 82% of the employees in the cohort were male (530 in the chemical plant and 558 in

the film plant). The mean age was 45.1 in the chemical plant and 48.6 in the film plant. Sixty percent of the chemical plant employees had worked only in the chemical plant and a similar percentage of film plant workers had worked exclusively in the film plant. Seventy-six percent of the chemical plant workers had high exposure jobs.

**Health effects studied:** Morbidity. Based on animal data and epidemiologic studies on PFOA and PFOS, certain episodes of care were considered *a priori*. They included: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, gastrointestinal disorders of the liver and biliary tract, and reproductive disorders.

**Data collection methods:** The Clinical Care Groups episode of care software developed by Ingenix, Inc. was used to provide a comprehensive grouping of all visits (inpatient and outpatient), procedures, ancillary services, and prescription drugs used in the diagnosis, treatment and management of more than 400 diseases or conditions. The software code constructs an episode of care around the index-eligible record by searching backward and forward in time for the health claims records that are related to the disease or condition on the index record. The index record consists of either procedure codes indicative of a face-to-face encounter or a pharmacy record for a delineating drug.

**Exposure period:** The episodes of care that were included in the study were those experienced between Jan. 1, 1993 to Dec. 31, 1998.

**Description/delineation of exposure groups/categories:** Workers were placed into groups according to potential workplace exposures: workers who were employed solely in the chemical or film plants, those who had high exposure jobs, and those who worked at least 10 years in jobs with high potential for fluorochemical exposure.

**Measured or estimated exposure:** estimated based on job history information.

**Exposure levels:** Not measured. Employees were placed into exposure categories based on job description.

**Statistical methods:** A risk ratio episode of care (RREpC) provided the estimate of risk between the observed to expected episodes of care for chemical plant employees compared to the observed to expected episodes of care among film plant employees. The expected number of episodes of care for both the film and chemical plant employees was calculated from health claims data of the 3M manufacturing population in the U.S. Because the chemical and film plant cohorts had slightly different age and gender structures, an adjusted ratio was calculated and compared to the unadjusted risk ratio. In most cases, the risk ratios were comparable. Therefore, 95% confidence intervals were only calculated for the unadjusted risk ratios.

**Other methodological information:** It should be noted that from an epidemiologic perspective, an episode of care could represent any and all incident cases, prevalent cases, and/or misclassified cases (both false positive and false negative). In addition, types and counts of episodes of care may differ by the software used, and it is possible that 2 different diagnoses may be assigned to the same episode. Certain services, such as lab procedures and prescriptions may not be reported for the episode. Also, the endpoint of an episode may vary among software programs. The clinical flexibility of the algorithm may differ depending on the software program.

## RESULTS

**Describe results:** The only increased risk of episodes for the conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths

(which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding is important because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study. However, the update of the study did not confirm this finding.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

**Study strengths and weaknesses:** See “other methodological information” section for limitations of episodes of care software. The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. Episodes of care are reported, not disease incidence; therefore, this parameter cannot be interpreted in any other manner. The data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease. In addition, many of the risk ratios for episodes of care had very wide confidence intervals. The analysis was limited to 6 years. Also, the utilization of health care services may reflect local medical practice patterns. Individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications. Episodes of care may include the same individual several times. Not all employees were included in the database, such as those on long-term disability.

**Research sponsors:** 3M Company

**Consistency of results:** No other morbidity studies have been conducted on fluorochemicals.

CONCLUSIONS

This study should only be used for hypothesis generation regarding workers employed at the Decatur plant who are employed in jobs with high exposure to POSF-based fluorochemicals.

REFERENCE

Olsen, GW, Burlew, MM, Hocking, BB, Skratt, JC, Burris, JM, Mandel, JH. An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. Final Report. May 18, 2001.



**EPIDEMIOLOGIC DATA**

**Title:** Identification of Fluorochemicals in Sera of Children in the United States

**TEST SUBSTANCE**

**Identity:** PFOS and 6 other fluorochemicals

**Remarks:** The results reported are preliminary. The final report is expected November 2001.

**METHOD**

**Study design:** Cross-sectional.

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the serum concentrations of selected fluorochemicals in a sample of children to provide a more specific understanding of the distribution of these compounds in children.

**Study period:** Child sera samples were collected from January 1994 to March 1995. The sera samples were analyzed in Spring 1999.

**Setting:** N/A

**Total population:** Not reported

**Subject selection criteria:** The sera samples were provided to 3M by the University of Minnesota Department of Pediatrics. They were obtained from a large clinical trial on Group A streptococcal infections in children. The children were residents of 23 states in the US. These children presented with signs and symptoms of acute-onset pharyngitis. All of the children had positive throat cultures at the initial visit.

**Total # of subjects in study:** n = 599 children, age 2-12 years

**Comparison population:** N/A

**Participation rate:** N/A

**Subject description:** No information was provided on the children from whom the sera samples were taken.

**Health effects studied:** PFOS serum levels in blood, as well as 6 other fluorochemicals.

**Data collection methods:** Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). The samples were collected from equal numbers of male and female children residing in 23 states.

**Details on data collection:** No information was provided as to how the blood was drawn, stored, etc.

**Exposure period:** N/A

**Description/delineation of exposure groups/categories:** Blood sera samples were collected from children 2 – 12 years old.

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

**Other methodological information:** N/A

## RESULTS

**Describe results:** The mean PFOS serum level was 43.5 ppb. The range was 6.7 - 515 ppb. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

**Study strengths and weaknesses:** These data are cross-sectional data used to determine PFOS levels in U.S. children. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small.

**Research sponsors:** 3M Medical Department, Corporate Occupational Medicine

**Consistency of results:** To date, no other data have been collected on PFOS serum levels in children.

## CONCLUSIONS

N/A

## REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of children in the United States. Interim Report. June 25, 2001.

## EPIDEMIOLOGIC DATA

**Title:** Mortality Study of Workers Employed at the 3M Decatur Facility

### TEST SUBSTANCE

**Identity:** Several perfluorooctanesulfonyl fluoride-based fluorochemicals, including PFOS

**Remarks:** This study is an update of the study published by Mandel and Johnson, 1995.

### METHOD

**Study design:** Retrospective cohort mortality study.

**Manufacturing/Processing/Use:** The 3M Decatur, Alabama plant began production in 1961. It is made up of the film plant and the chemical plant. The 3 major product groups in the chemical plant are protective chemicals, performance chemicals, and fluoroelastomers. Perfluorooctanesulfonyl fluoride (POSF) is the major sulfonate fluorochemical manufactured at Decatur and is used as the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers.

**Hypothesis tested:** To determine whether occupational exposure to fluorochemicals is related to mortality of employees of the 3M facility in Decatur, Alabama

**Study period:** The study population worked at the plant for at least 1 year since it began production in 1961. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

**Setting:** 3M plant in Decatur, Alabama.

**Total population:** 3512 workers were identified. Of these workers, 2083 worked at the plant for at least one year. Eighty-four percent of the cohort was male.

**Subject selection criteria:** All workers employed at the Decatur plant for at least 1 year. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

**Comparison population:** In SMR analyses, Minnesota population death rates for whites were used. Mortality reference rates from 7 regional counties were also used to rule out large variations based on regional mortality reporting differences.

**Participation rate:** Death certificates were obtained for 96% (n = 139) of the cohort who were deceased.

**Subject description:** 84% of the employees in the cohort were male. The mean age at follow-up was 51.1 years, and the mean number of years worked at the plant was 14.9. The number of person-years at follow-up was 50970. There were 145 deaths identified in the cohort. The high exposure group was slightly younger than the other 2 groups but worked 3 years longer at the plant than the other 2 exposure groups.

**Health effects studied:** Mortality

**Data collection methods:** A review of employee work history records of any employee with at least 1 year employment were abstracted to record the workers' name, SSN, 3M identification number, date of

birth, and dates of work history. This cohort was linked to records from the original cohort to update the employment information and verify other data. The National Death Index was searched for all of the workers. Discrepancies with the original cohort were resolved and deaths before 1979 were verified in the Social Security Death Index. A licensed nosologist coded the death certificates to ICD 8.

**Exposure period:** The potential exposure period was from the plant opening in 1961 to Dec. 31, 1997.

**Description/delineation of exposure groups/categories:** Workers were placed into 3 exposure groups based on job history information: high exposure, low exposure, and no exposure. There were 145 deaths in the cohort: 65 in the high exposure group, 27 in the low exposure group and 53 in the non-exposed group.

**Measured or estimated exposure:** estimated based on job history information.

**Exposure levels:** Not measured. Employees were placed into 3 exposure categories based on job description: low exposure (n = 289), high exposure (n = 782), non-exposed (n = 812).

**Statistical methods:** Standardized Mortality Ratios (SMRs) and 95% confidence intervals were derived using the PC Life Table Analysis System software developed by NIOSH. This program computes age, gender, and race-specific SMRs using standard life table methods. The expected number of deaths is estimated by multiplying the age, gender, race, and calendar period tabulated person-years of follow up to the corresponding cause-specific mortality reference rates. Mortality rates for white Minnesotans were used as reference data.

**Other methodological information:**

## RESULTS

**Describe results:** 145 deaths were identified in the cohort: 65 of these deaths were in the high exposure group. When the entire cohort was analyzed, SMRs were not elevated for most of the cancer types and for non-malignant causes. SMRs that were above 1 (cancer of the esophagus, liver, breast, urinary organs, bladder, and skin) were also elevated when the cohort was limited to any employee ever employed in a high exposure job (except breast cancer). Only 2 or 3 deaths were reported for each of these cause-specific categories and were not statistically significant, except for bladder cancer.

Workers who were employed in high exposure jobs were greater than 12 times more likely to die of bladder cancer than the general population of Alabama (SMR = 12.77, 95% CI = 2.63 - 37.35). This effect remained when the data were analyzed using county death rates. Three male employees in the cohort died of bladder cancer, and all of the deaths occurred in employees who had worked in high exposure jobs for at least 5 years (SMR = 24.49, 3 observed deaths, 0.12 expected). All of them had worked at the Decatur plant for more than 20 years. In the previous cohort mortality study, 1 bladder cancer death was reported.

Two deaths were reported for liver cancer. One was in the low exposure group and one in the high exposure group. The SMR for workers who were employed in either high or low exposure jobs was 3.08 (95% CI = 0.37 – 11.10). Five cases of cirrhosis of the liver were reported in this cohort, 2 in the high exposure group, 1 in the low exposure group, and 2 in the non-exposed. The observed did not exceed the expected mortality experience in any of these groups.

**Study strengths and weaknesses:** A larger cohort was followed in this update of the original study, thus reporting twice as many deaths; however, the cohort is fairly young and the number of deaths is still small. There were small numbers of deaths in many of the categories for males and especially for females in all categories, thus limiting the power of the study. Death certificates were located for 96% of the cohort, but

the 6 not obtained could greatly impact the results of the analyses since the number of deaths in most of the sub-cohorts was very small. Biological measurements of fluorochemicals were not available; therefore, exposure categories based on job descriptions were used as a surrogate for exposure. The categories are more specific in this update of the study; however, there is still a potential for misclassification of exposure. Based on the results of the biomonitoring conducted on employees in a random sample of Decatur employees in 1998, the geometric mean of PFOS in film plant employees was 0.136 ppm. However, in this study, film plant employees are considered non-exposed. In addition to fluorochemicals, workers were exposed to other chemicals in the workplace.

**Research sponsors:** University of Minnesota

**Consistency of results:** In the first study, there was an excess of bladder cancer; however, this was based on only 1 death. Three additional deaths have now been reported.

### CONCLUSIONS

Workers employed at the Decatur plant who are employed in jobs with high exposure to POSF-based fluorochemicals are at increased risk of death from bladder cancer.

### REFERENCE

Alexander, B.H. April 26, 2001. Mortality study of workers employed at the 3M Decatur facility. Final Report. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota.

### **OTHER**

This study is the second update of the mortality study.

## EPIDEMIOLOGIC DATA

**Title:** Identification of Fluorochemicals in Human Sera. I. American Red Cross Adult Blood Donors

### TEST SUBSTANCE

**Identity:** PFOS and 6 other fluorochemicals

**Remarks:** This is a final report. The preliminary report was dated June 25, 2001.

### METHOD

**Study design:** Cross-sectional

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the levels of PFOS in the serum of American Red Cross blood banks in 6 regions of the U.S.

**Study period:** 2000

**Setting:** N/A

**Total population:** 6 ARC blood banks in various geographic regions in the US provided 645 serum samples from adult donors. The 6 regions included: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD.

**Subject selection criteria:** Unknown

**Total # of subjects in study:** 645 donors, age 20-69 years

**Comparison population:** N/A

**Participation rate:** N/A

**Subject description:** The only demographic factors known were age, gender, and location.

**Health effects studied:** Levels of 7 fluorochemicals in human blood serum, including PFOS. The other chemicals were: PFOA, PFOSAA, M570, M556, PFOSA, PFHS.

**Data collection methods:** Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex.

**Details on data collection:** Blood sera samples were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). 24 samples were split and analyzed to provide an estimate of the reliability of the analyses conducted. The analytical lab was blind to the identity of these split samples. These analyses were performed concurrently with all other analyses of the study to minimize experimental error.

**Exposure period:** Unknown--PFOS serum levels used as surrogate for exposure.

**Description/delineation of exposure groups/categories:** 332 male donors, 313 female donors. There were 10 or more subjects for both males and females in each age category (20-29, 30-39, etc.) except the 60-69 age group where there were fewer.

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** A reliability assessment was done. Geometric means, 95% confidence intervals of the geometric means, range, interquartile range, raw cumulative 90<sup>th</sup> percentile, and frequency distributions, were calculated. Central tendency and distribution of the data by age, gender, and location were presented. Bootstrap analysis was done to calculate mean serum PFOS values for each 6 locations adjusted for 10-year age intervals, gender, and their interaction terms. Multivariable regression analyses. A reliability assessment was also done for the chemical analyses.

**Other methodological information:** N/A

## RESULTS

**Describe results:** The results of the reliability analysis indicate that there was a strong correlation between the split samples ( $r = .9$ ) for PFOS (as well as PFOA and PFHS).

PFOS is the only chemical that met the criteria for a log-normal distribution based on the Shapiro-Wilk test.

The geometric mean serum PFOS level for all locations and gender was 34.9 ppb (95% CI, 33.3-36.5 ppb). The range was < LLOQ (4.3 ppb) to 1656 ppb. Males had significantly higher ( $p < .05$ ) geometric mean PFOS levels than females. The geometric mean for all males was 37.8 ppb (95% CI, 35.5-40.3) and was 31.3 ppb for all females (95% CI, 30.0 – 34.3). Age was not an important predictor of adult serum fluorochemical concentrations.

When stratified by geographic location, the highest geometric mean for PFOS was in the samples from Charlotte, NC (51.5 ppb, range: 19.3 – 166.0) and the lowest from Boston (28.0 ppb, range: 4.3 – 87.2). The other PFOS geometric means and ranges by location were: Los Angeles, 40.4 ppb (6.6 – 205.0); Minneapolis/St. Paul, 33.1 ppb (7.7 – 207.0); Portland, 27.0 ppb (6.0 – 1656); Hagerstown, 35.3 ppb (7.6 – 226.0). The cumulative 90% were highest for Charlotte (105.3), Minneapolis/St. Paul (71.7), Los Angeles (70.1), and Hagerstown (69.8). Portland and Boston were much lower (49.4 and 48.7, respectively).

The results from a bootstrap analysis, done to calculate mean serum PFOS values for each 6 locations adjusted for 10-year age intervals, gender, and their interaction terms, resulted in similar means for Boston (29.0 ppb), Los Angeles (35.0 ppb), Minneapolis/St. Paul (34.8 ppb), and Hagerstown (34.9 ppb). However the mean for Charlotte was much lower (39.0 ppb) and the mean for Portland was slightly higher (32.8 ppb).

Bootstrap analyses for PFOS calculated a mean of the 95% tolerance limit of 88.5 ppb with an upper 95% confidence limit of 100.0 ppb. The mean of the 99% tolerance limit was 157.3 ppb with an upper 95% confidence limit of 207.0 ppb. At the lowest tolerance limit analyzed (90%), the mean for PFOS was 70.7 ppb with an upper 95% confidence limit of 74.3 ppb.

The highest serum PFOS measurement in this sample was 1656 ppb from a male blood donor, 67 years old from Portland. The next highest donor level was 329 ppb from a male donor, 62 years old also from

Portland. The next 8 highest serum PFOS values (range 139 – 226 ppb) were measured in 4 females and 4 males representing Charlotte (n=4), Hagerstown (n=2), Los Angeles (n=1) and Minneapolis/St. Paul (n=1).

PFOS and PFOA were strongly correlated ( $r = .63$ ) PFOS had a lower correlation with PFOSAA ( $r = .42$ ) and M570 ( $r = .20$ ). Both PFOSAA and M570, adjusted for age, gender, and their interaction, were significant predictors of PFOS in a multivariable model. PFOSAA was the stronger of the 2 independent variables. Age and gender were not significant predictors in models that examined the significant association between PFOS and PFOA.

**Study strengths and weaknesses:** Blood donors cannot be considered representative of the general population of the US.

**Research sponsors:** 3M Medical Department, Corporate Occupational Medicine

**Consistency of results:** Mean PFOS levels reported in this study are similar to those reported in other pooled blood samples.

## CONCLUSIONS

N/A

## REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in human sera. I. American Red Cross adult blood donors. Final report. February 25, 2002.



## EPIDEMIOLOGIC DATA

**Title:** Identification of Fluorochemicals in Human Sera. II. Elderly Participants of the Adult Changes in Thought Study, Seattle, Washington

### TEST SUBSTANCE

**Identity:** PFOS and 6 other fluorochemicals

**Remarks:** The results reported are final.

### METHOD

**Study design:** Cross-sectional.

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the serum concentrations of selected fluorochemicals in a sample of elderly persons to provide a more specific understanding of the distribution of these compounds in this age group.

**Study period:** 9/29/2000

**Setting:** N/A

**Total population:** 238 serum samples from elderly adult donors from the Adult Changes in Thought study.

**Subject selection criteria:** Donors were 65-96 years old. Subjects were identified during an enrollment phase of this community-based prospective cohort study of dementia and normal aging conducted collaboratively between the U. of Washington and Group Health Cooperative (HMO). Eligible individuals were those with no known history of neuropsychiatric disease or dementia.

**Total # of subjects in study:** 238

**Comparison population:** N/A

**Participation rate:** N/A

**Subject description:** 238 adults--118 males, 120 females. The mean age was 76 years. Female subjects had resided in the Seattle area for 53.3 years, males 50.2 years.

**Health effects studied:** PFOS serum levels in blood, as well as 6 other fluorochemicals.

**Data collection methods:** Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS).

**Exposure period:** N/A

**Description/delineation of exposure groups/categories:** 65-75 years (n= 121), 75+ to 85 years (n = 93), 85+ to 96 years (n = 24).

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms. A reliability assessment was also done.

**Other methodological information:** N/A

## RESULTS

### **Describe results:**

The geometric mean of PFOS for all samples was 31.0 ppb (95% CI, 28.8-33.4). The range was 3.4 – 175 ppb. There was no significant ( $p < .05$ ) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ( $p < .05$ ) associated with PFOS in men but not in women. The mean of the 95% tolerance limit for PFOS was 84.1 ppb with an upper 95% confidence limit of 104.0 ppb.

**Study strengths and weaknesses:** These data are cross-sectional data used to determine PFOS levels in elderly. Very little descriptive information about the subjects is available. The subjects only characterize PFOS levels in the Seattle region. To date, they are the only data available characterizing serum PFOS levels in the elderly.

**Research sponsors:** 3M Medical Department, Corporate Occupational Medicine

**Consistency of results:** To date, no other data have been collected on PFOS serum levels in elderly.

## CONCLUSIONS

**PFOS levels in these Seattle residents are similar to those found in adults and children in the U.S.**

### REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. February 25, 2002. Identification of fluorochemicals in human sera. II. Elderly participants of the Adult Changes in Thought study, Seattle, Washington. Final Report. 3M Company, Medical Department.

## EPIDEMIOLOGIC DATA

**Title:** Identification of Fluorochemicals in Human Sera. III. Pediatric Participants in a Group A Streptococci Clinical Trial Investigation

### TEST SUBSTANCE

**Identity:** PFOS and 6 other fluorochemicals

**Remarks:** The results reported are final.

### METHOD

**Study design:** Cross-sectional.

**Manufacturing/Processing/Use:** N/A

**Hypothesis tested:** To determine the serum concentrations of selected fluorochemicals in a sample of children to provide a more specific understanding of the distribution of these compounds in children.

**Study period:** Child sera samples were collected from January 1994 to March 1995. The sera samples were analyzed in Spring 1999.

**Setting:** N/A

**Total population:** Not reported

**Subject selection criteria:** The sera samples were provided to 3M by the University of Minnesota Department of Pediatrics. They were obtained from a large clinical trial on Group A streptococcal infections in children. The children were residents of 23 states in the US. These children presented with signs and symptoms of acute-onset pharyngitis. All of the children had positive throat cultures at the initial visit.

**Total # of subjects in study:** n = 599 children, age 2-12 years

**Comparison population:** N/A

**Participation rate:** N/A

**Subject description:** 299 male children, 300 female children from 23 states and the District of Columbia.

**Health effects studied:** PFOS serum levels in blood, as well as 6 other fluorochemicals.

**Data collection methods:** Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). Sera were frozen at -20 degrees C prior to the request for analysis.

**Exposure period:** N/A

**Description/delineation of exposure groups/categories:** Blood sera samples were collected from children 2 – 12 years old.

**Measured or estimated exposure:** N/A

**Exposure levels:** N/A

**Statistical methods:** Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms. A reliability assessment was also done.

**Other methodological information:** N/A

## RESULTS

### **Describe results:**

The geometric mean of PFOS for all of the participants was 37.5 ppb (95% CI, 33.3-36.5). The range was 6.7 to 515.0 ppb. Male children had significantly ( $p < .01$ ) higher geometric mean PFOS levels than females: 40.1 ppb and 35.2 ppb, respectively. In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. When stratified by age, the geometric mean tended to rise for each age group from age 2 (28.6 ppb) through age 9 (42.8 ppb) where it was highest, and then started to decrease gradually to 32.8 ppb at 12 years. In simple linear regression analyses, age was not significantly ( $p < .05$ ) associated with PFOS. Although the data were not provided, a graphical presentation of log PFOS levels for each state by gender were similar across the states, however, it is difficult to interpret these data given the limited sample size for each gender/location subgroup.

**Study strengths and weaknesses:** These data are cross-sectional data used to determine PFOS levels in U.S. children. Very little descriptive information about the subjects is available. To date, they are the only data available characterizing serum PFOS levels in children.

**Research sponsors:** 3M Medical Department, Corporate Occupational Medicine

**Consistency of results:** To date, no other data have been collected on PFOS serum levels in children.

## CONCLUSIONS

N/A

## REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of children in the United States. Interim Report. June 25, 2001.

## EPIDEMIOLOGIC DATA

**Title:** A Cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology, and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program

### TEST SUBSTANCE

**Identity:** PFOS, PFOA

**Remarks:**

### METHOD

**Study design:** cross-sectional

**Manufacturing/Processing/Use:** Facilities in Decatur, Alabama and Antwerp, Belgium which manufacture perfluorooctanesulfonyl fluoride products. These fluorochemicals can metabolize in the body to PFOS.

**Hypothesis tested:** To provide an aggregate analysis of the hematology, clinical chemistries, and hormonal parameters of volunteer employees in relation to serum PFOS and PFOA levels as measured in the medical surveillance examinations of Antwerp and Decatur employees in 2000.

**Study period:** March 1, 2000. End date was not reported.

**Setting:** Occupational. 3M plants located in Antwerp, Belgium and Decatur, Alabama.

**Total population:** 340 Antwerp employees and 500 Decatur employees working in the chemical plant area were eligible for inclusion in the surveillance.

**Subject selection criteria:** Voluntary participation in medical surveillance program in Y2000.

**Total # of subjects in study:** 255 Antwerp employees (206 male and 49 female) and 263 Decatur employees (215 male and 48 female).

**Comparison population:** N/A

**Participation rate:** 75% of employees at the Antwerp plant and 50% of the employees at the Decatur plant who were eligible participated. 73% of the participating Antwerp male employees and 75% of the Decatur employees were engaged in production activities. Only 12% of the participating Antwerp female employees were engaged in production activities compared to 63% of the Decatur female employees.

**Subject description:** Male Antwerp employees had lower PFOS and PFOA levels, were significantly younger than Decatur male employees, had lower BMIs, worked fewer years, had higher self-reported daily consumption of alcohol, had lower mean alkaline phosphatase, GGT, AST, ALT and triglyceride values and higher total bilirubin and HDL values. Comparable results were observed for Antwerp female employees vs. Decatur females.

**Health effects studied:** To determine if there were differences in the following parameters based on

PFOS/PFOA levels: hematology (hematocrit, hemoglobin, RBCs, WBCs, platelet count), clinical chemistries (alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, blood urea nitrogen, creatinine, glucose, cholesterol, low density lipoproteins, high density lipoproteins, and triglycerides), and thyroid hormones (thyroid stimulating hormone, serum thyroxine, free thyroxine, serum triiodothyronine, thyroid hormone binding ratio, and free thyroxine).

**Data collection methods:** Medical questionnaire, work history questionnaire, blood sera samples, measurements of height, weight, and blood pressure, urinalysis (Decatur only), and standard clinical chemistry and hematology tests, thyroid hormone measurement, and pulmonary function tests. Values used for reference ranges were not provided.

**Details on data collection:** The site-specific work history questionnaire was administered to all participants. The data were self-reported. Questionnaire content, design, administration, etc. were not provided in this report. Data on blood collection (amount, etc.) not provided. Urinalysis was only assessed for Decatur employees via standard urine microstick analysis which tested for urine glucose, albumin, and RBCs.

TSH, free T4 and T3 were determined by immunochemiluminometric assay. T4 and THBR were determined by a cloned enzyme donor immunoassay. FTI was calculated by multiplying T4 and THBR.

Sera samples were extracted using an ion-pairing extraction procedure. In addition to PFOA and PFOS, the extracts were also analyzed for PFHS, PFOSAA, PFOSA, and M556 (perfluorooctanesulfonamidoacetate) using high-pressure liquid chromatography electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human serum matrix. All serum values for PFOS and PFOA were above the LLOQ.

**Exposure period:** Unknown. PFOS/PFOA serum levels indicate exposure.

**Description/delineation of exposure groups/categories:** Workers were stratified by plant location as well as by serum PFOS distribution, production status (production vs. non-production workers), and gender.

**Mean serum PFOS levels for all employees participating in this study at Antwerp (n = 206) and Decatur (n = 215) were 0.96 and 1.40 ppm, respectively. Levels among production employees were higher. At Antwerp, the mean PFOS level of male production employees was 1.16 ppm and 1.63 ppm at Decatur.**

**Mean PFOA levels for all employees were 1.03 and 1.90 ppm at Antwerp and Decatur, respectively. Levels among production employees were higher. At Antwerp, the mean PFOA level of male production employees was 1.28 ppm and 2.34 ppm at Decatur.**

**Measured or estimated exposure:** Serum PFOS and PFOA levels were used to estimate exposure.

**Statistical methods:** Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance, and multivariable regression were used to evaluate associations between PFOS and PFOA and each hematological and clinical chemistry test and thyroid hormone assay. For stratified analyses, employees were divided into quartiles of their serum PFOS distribution. Potential confounding factors considered in the analyses included: age, BMI, alcohol consumption, cigarette use, years worked at either plant, and type of job.

Multivariable regression models were fitted with PFOS/PFOA analyzed as continuous variables. Natural log transformations of the dependent variables were performed, when necessary, to normalize variables and to enhance model fit. SAS was used to analyze the data.

### **Other methodological information:**

## RESULTS

### **Describe results:**

Antwerp and Decatur employees were different in several ways (see "Subject description" above). Therefore, univariate analyses were initially stratified by location and then those analyses were stratified by gender and production status. They were placed into quartiles depending on production status. Therefore, the PFOS values in the quartiles are different for male production, non-production, and female employees.

#### Antwerp

The mean PFOS level for all employees at this plant was 0.96 ppm (range 0.04 - 6.24 ppm). When stratified by production status, the mean was 1.16 ppm for production employees and 0.42 ppm for non-production employees. The mean for female employees was 0.13 ppm.

Male production employees were placed into the following quartiles based on PFOS levels: Q1 (mean, 0.29 ppm; range, 0.04 – 0.41 ppm), Q2 (mean, 0.58 ppm; range 0.41 – 0.78 ppm), Q3 (mean 1.18 ppm; range 0.79 – 1.66 ppm), Q4 (mean, 2.61 ppm; range, 1.67 – 6.24 ppm).

In male production employees (n = 150), the highest quartile mean serum PFOS level was 2.61 ppm (range 1.76 – 6.24 ppm) and the lowest was 0.29 ppm (range 0.04 – 0.41 ppm). Production employees in the highest quartile were significantly ( $p < .05$ ) older and worked more years at Antwerp than employees in the lowest quartile. The only difference in clinical chemistries for production workers was in BUN. When compared by quartile of serum PFOS distribution, no significant ( $p < .05$ ) differences among male production employees were observed for thyroid (TSH, T4, free T4, T3, THBR, FTI) or for hematology (HCT, HGB, RBC, WBC, platelets). The same held true for non-production employees (n = 56).

For all female employees, BUN was significantly different (higher) between 1<sup>st</sup> quartile and 3<sup>rd</sup> and 4<sup>th</sup> quartile. Thyroid and hematology results were not significantly different between any of the quartiles for females.

#### Decatur

The mean PFOS level for all employees at this plant was 1.40 ppm (range 0.11 – 10.06 ppm). When stratified by production status, the mean was 1.63 ppm for production employees and 0.73 ppm for non-production employees. The mean for female employees was 0.93 ppm. 75% of male employees worked in production jobs (n = 161) and 63% of female employees worked in production jobs.

Male production employees were placed into the following quartiles based on PFOS levels: Q1 (mean, 0.55 ppm; range, 0.11 – 0.75 ppm), Q2 (mean, 1.01 ppm; range 0.76 – 1.30 ppm), Q3 (mean 1.74 ppm; range 1.32 – 2.29 ppm), Q4 (mean, 3.22 ppm; range, 2.31 – 10.06 ppm).

When male production workers were placed into quartiles, the only significant ( $p < .05$ ) difference between the quartiles was in ALT (highest quartile different from all 3 others).

No significant differences between quartiles were observed for thyroid (TSH, T4, free T4, T3, THBR, FTI), hematology (HCT, HGB, RBC, WBC, platelets), or urinalysis (albumin, blood, sugar).

For female employees (both production and non-production employees), there were no significant differences among quartiles for demographics, thyroid, hematology, or urinalysis except for mean platelet count where the third quartile was significantly lower than the 1<sup>st</sup> quartile, but the 4<sup>th</sup> was not.

When results were analyzed by number of employees who had values above the reference range for hepatic clinical chemistry tests and liver enzyme and bilirubin tests, there was a higher percentage of male Decatur production workers in the highest PFOS quartile for ALT, GGT, and total liver panel than the other quartiles. Most notable were the results for ALT where 8% of employees in the lowest exposure group (Q1) and 28% in the highest exposure group (Q4) had values above the reference range, while the percentages for total liver panel (which includes alkaline phosphatase, AST, ALT, GGT, and total and direct bilirubin) were 18% and 35%, respectively. This trend was not evident in Decatur non-production employees (although the n was only 54), in Decatur females, or in any of the Antwerp employees. However, each sub-population had a different serum PFOS quartile distribution. Therefore, they cannot be directly compared.

### **Analyses combining employees from both plants**

When clinical chemistry results of all male employees from both plants were combined (both production and non-production) (n = 421) and placed into quartiles (n = 105 per quartile), mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly (p < .05) higher in the 4<sup>th</sup> quartile (mean PFOS level 2.69, range 1.69 – 10.06 ppm) than in the first (mean PFOS level 0.27 ppm, range 0.04 – 0.42 ppm). It should be noted that the number of Antwerp production employees were evenly distributed among the quartiles while this was not the case for Decatur employees. The highest number of Decatur employees was in the 4<sup>th</sup> quartile. In addition, overall the employees in the 1<sup>st</sup> quartile were slightly younger, had a lower BMI, and worked fewer years than employees in the other quartiles.

Thyroid results for this same group indicated that T3 was significantly higher (p < .05) and THBR was significantly lower (p < .05) in Q4 than Q1. In female employees combined for both plants (n = 97), alkaline phosphatase and GGT were significantly higher (p < .05) and total bilirubin significantly lower in Q4 than in Q1. Most of the Decatur female employees worked in production jobs while most of the Antwerp females worked in non-production jobs. Therefore, Q4 was 92% female production workers and all of them worked at the Decatur plant.

The combined plant data were analyzed for employees who had values above the reference range for alkaline phosphatase, AST, ALT, GGT, and total liver panel. For male employees for all of these measures, the levels increased from Q1 to Q4. In Q1, 4% of the employees had values above the reference range for ALT and 6% for GGT, while 12% was reported for Q4 for both of these tests. For total liver panel, 14% of the employees had values above the reference range in Q1 as compared to 23% in Q4. The numbers of female employees with values above the reference range was very small (n = 8).

The above data were not adjusted for potential confounders; therefore, multivariable regression analyses were conducted. There was a positive significant (p = .04) association between PFOS and cholesterol and also a positive significant (p = .05) association between PFOA and cholesterol. When both PFOS and PFOA were included in the model, neither were statistically significant at p=.05. PFOS was not significant with HDL although PFOA was negatively associated with HDL (p = .04). Triglycerides were positively associated with PFOS (p = .01) and PFOA (p = .002). When both were left in the model, PFOA remained significant (p = .02). Total organic fluorine (TOF) was highly significant for triglycerides (p = .0009). No significant associations were observed with PFOS, PFOA, or TOF in relation to alkaline phosphatase, GGT, AST or total bilirubin. A significant (p = .02) positive association was observed for TOF and ALT. A positive significant (p = .04) association between T3 and PFOS was observed. Plant location was highly significant (p < .0001) in the model. BMI, cigarettes/day, alcohol/day were also significant. [In the



univariate analyses, Antwerp employees had higher mean T3 levels than Decatur employees overall. However, for each plant (individually) T3 values increased by quartile as PFOS serum levels increased, although the differences were not statistically significant.] THBR, as well as the other thyroid hormones, were not significant in the regression analyses. Most of the thyroid hormones, including TSH, T4, THBR, and FTI, were not significantly associated with PFOS, PFOA, or TOF. However, PFOS, PFOA and TOF were positively associated ( $p = .04$ ,  $.01$ , and  $.004$ , respectively) with T3.

**Study strengths and weaknesses:** Cross-sectional design, voluntary participation, the Decatur and Antwerp populations were significantly different in certain demographic and clinical chemistry results as well as in PFOS serum levels, PFOS serum levels are declining thereby making it harder to detect an effect if one is present, plant populations cannot be compared because quartiles are different for each subgroup, only one measurement at a certain point in time was collected for each test, other perfluorinated chemicals, such as PFOA, are present in the plants.

**Research sponsors:** 3M

**Consistency of results:** These results are somewhat consistent with those of the 1995 and 1997 cross-sectional medical surveillance data, in that all of them conclude that there were no significant abnormalities in hematological and clinical chemistry parameters of the Antwerp or Decatur workers. No decline in cholesterol levels was observed as PFOS serum levels increased. The hormone data collected in 1995 was different from that collected in 2000 and therefore cannot be compared. A longitudinal analysis of these data did not reveal any significant changes in hepatic or lipid clinical chemistry values; however, there were many limitations to the study.

## CONCLUSIONS

The authors concluded that these data continue to suggest that Antwerp and Decatur fluorochemical production and non-production employees do not have significant changes in serum cholesterol, lipoproteins, or hepatic enzymes that are consistent with toxicological findings in laboratory animals.

## REFERENCE

Olsen GW, Burlew MM, Burris JM, Mandel JH. October 11, 2001. A cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program. Final report. 3M Medical Department.

## EPIDEMIOLOGIC DATA

**Title:** A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997, and 2000 Fluorochemical Medical Surveillance Program

### TEST SUBSTANCE

**Identity:** PFOS, PFOA

**Remarks:**

### METHOD

**Study design:** longitudinal

**Manufacturing/Processing/Use:** 3M Decatur, Alabama plant and Antwerp, Belgium plant.

**Hypothesis tested:** To determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of 2 3M facilities.

**Study period:** There were 3 time periods during which medical surveillance took place at the plants—1994/95, 1997, and 2000.

**Setting:** 3M plants in Decatur, Alabama and Antwerp, Belgium.

**Total population:** 175 male employees participated in 2000 and at least one of the other sampling periods.

**Subject selection criteria:** Employees participated voluntarily.

**Comparison population:** n/a

**Participation rate:** 106/175 (61%) participated in 1994/95, 110/175 (63%) participated in 1997, and 175 participated in 2000. 24% participated in all 3 sampling periods (n = 41, Antwerp 20, Decatur 20), 37% in 1994/95 and 2000 (n = 65, Antwerp 45, Decatur 20), and 39% (n = 69, Antwerp 34, Decatur 35) in 1997 and 2000.

**Subject description:** Male employees volunteered to participate in biomonitoring offered at the plants. In general, Antwerp male employees were significantly younger, had lower BMIs and a higher daily consumption of alcohol reported than Decatur male employees. Antwerp male employees also had lower mean alkaline phosphatase and triglyceride values and higher total bilirubin and HDL values than the Decatur male employees.

**Health effects studied:** To determine whether workers' lipid and hepatic clinical chemistry results are affected by PFOS and PFOA levels.

**Data collection methods:** Clinical chemistries and hematology collected—cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), triglycerides (mg/dl), alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dl). Demographic data collected via questionnaire.

**Details on data collection:** Details on data collection methods including questionnaire content, design, administration, etc. and blood collection methods were not provided.

PFOS and PFOA methods of analysis differed slightly each year. In 1994/95, the method used tetrabutylammonium to ion-pair with PFOS and PFOA in the serum. The ion-pairs were then extracted with ethyl acetate and the abstraction product was then analyzed using high-performance liquid chromatograph-thermospray mass spectrometry. In 1997, the serum samples were analyzed by liquid chromatography/mass spectrometry, using selected ion monitoring in the negative-ion mode. In 2000, sera samples were extracted using an ion-pairing extraction procedure. High-performance liquid chromatography/electrospray tandem mass spectrometry was used. The samples were evaluated versus an extracted curve from a human serum matrix.

**Exposure period:** Unknown. PFOS, PFOA levels measured in blood serum.

**Description/delineation of exposure groups/categories:** The groups of employees were broken into subpopulations A, B, and C. A was comprised of the employees who participated in all 3 years of surveillance, B contained those employees who participated in 1994/95 and 2000, and C contained those who participated in 1997 and 2000.

**Measured or estimated exposure:** PFOS and PFOA levels were measured in workers' blood serum. No ambient exposure data are available.

**Exposure levels:**

|         | Mean PFOS levels |          |
|---------|------------------|----------|
|         | Antwerp          | Decatur  |
| 1994/95 | 1.87 ppm         | 2.62 ppm |
| 1997    | 1.42 ppm         | 1.85 ppm |
| 2000    | 1.16 ppm         | 1.67 ppm |

|         | Mean PFOA levels |          |
|---------|------------------|----------|
|         | Antwerp          | Decatur  |
| 1994/95 | 1.08 ppm         | 1.90 ppm |
| 1997    | 1.54 ppm         | 1.41 ppm |
| 2000    | 1.43 ppm         | 1.83 ppm |

**Statistical methods:** repeated measures incorporating the random subject effect fitted to a mixed model using SAS. Restricted maximum likelihood estimates of variance parameters were computed. Adjusted regression models were built by introducing all covariates and testing the covariance structure. Covariates included in the model were age, BMI, number of alcoholic drinks per day, and cigarettes smoked per day.

**Other methodological information:** A total of 175 male employees (100 Antwerp and 75 Decatur) who participated in the 2000 surveillance year also participated in at least one previous fluorochemical medical surveillance exam since 1994/95. Therefore, this provided an opportunity to undertake a longitudinal assessment.

## RESULTS

### **Describe results:**

#### **PFOS results**

When mean serum PFOS levels were compared by surveillance year, PFOS levels have been decreasing in the participants in medical surveillance in both plants. When the data were analyzed by the 3 subcohorts (those who participated in 2 or more medical exams between 1995 and 2000), Antwerp and Decatur employees in each of the 3 subcohorts had lower mean serum PFOS levels in 2000 than at their year of entry.

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was no association between PFOS and serum cholesterol or triglycerides in male participants over time. There were also no significant associations between PFOS and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

#### **PFOA results**

When mean serum PFOA levels were compared by surveillance year, PFOA levels in the employees participating in medical surveillance at the Antwerp plant increased between 1994/95 and 1997 and then decreased slightly between 1997 and 2000. At the Decatur plant, PFOA serum levels decreased between 1994/95 and 1997 and then increased between 1997 and 2000. When the data were analyzed by plant and the 3 subcohorts (those who participated in 2 or more medical exams between 1995 and 2000), there were no consistent changes across subcohorts at the Antwerp plant. However, among the 3 Decatur subcohorts, mean PFOA levels tended to increase.

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between PFOA and serum cholesterol ( $p = .0008$ ) and triglycerides ( $p = .0002$ ) over time. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees ( $p = .005$ ) and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years ( $p = .001$ ). However, the association between PFOA and triglycerides was also statistically significant ( $p = .02$ ) for subgroup B (employees who participated in biomonitoring in 1994/95 and 2000). There was not a significant association between PFOA and triglycerides among Decatur workers.

There were no significant associations between PFOA and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

#### *Total Organic Fluorine (TOF) results*

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between TOF and serum cholesterol ( $p = .007$ ) and triglycerides ( $p = .008$ ) over time. However, the interaction term with time (years) was not significant. This association was more consistent for Antwerp employees than Decatur employees.

#### **Study strengths and weaknesses:** Study limitations include the following:

1. A very small number of employees participated in all 3 study periods (only 24%,  $n = 41$ )
2. different labs were used each year for analysis and different analytical techniques for PFOS
3. could not analyze female employees due to small numbers
4. PFOS levels in employees are decreasing over time and are below those levels causing effects in laboratory animals
5. serum PFOS levels were approximately 0.5 ppm lower in Antwerp employees than Decatur
6. more Antwerp employees than Decatur participating in this study (57% vs.43%)

7. PFOA levels fluctuating (direction depending on the plant)
8. there are several consistent differences between the Antwerp and Decatur male populations (eg., statistically significant differences in BMI, age, consumption of alcohol, and differences in chemistry profiles)
9. low levels of PFOS and PFOA measured in each program year among these employees as compared with those that cause effects in laboratory animals
10. blood sampling was conducted only once per sampling period at a certain point in time.

**Research sponsors:** 3M

**Consistency of results:** This is the first longitudinal analysis of the surveillance data and will probably be the last since PFOS is being phased out.

The positive association between PFOA and serum cholesterol and triglycerides is not consistent with the hypolipidemia effect observed in rodents (and not observed in primates). In addition, this effect has not been observed at 3M's Cottage Grove facility where PFOA serum levels in workers are much higher than at the Decatur or Antwerp plant.

#### CONCLUSIONS

A longitudinal analysis over a six-year period of 175 Antwerp and Decatur male employees did not show significant changes, consistent with toxicological data, of lipid or hepatic clinical chemistry values associated with PFOS. A positive statistically significant association was observed between PFOA and cholesterol and triglycerides. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years.

#### REFERENCE

Olsen, G.W., Burlew, M.M, Burris, J.M., Mandel, J.H. A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997, and 2000 Fluorochemical Medical Surveillance Program. 3M Final Report. October 11, 2001.