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**DRAFT INITIAL ASSESSMENT REPORT
PERFLUOROOCTANE SULFONIC ACID
AND ITS SALTS**

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EXECUTIVE SUMMARY

Introduction

Perfluorooctane sulfonic acid and its salts (PFOS) are fully fluorinated organic molecules produced synthetically by electrochemical fluorination or from the degradation of other fluorochemical products produced by electrochemical fluorination. As a fully fluorinated organic molecule, PFOS is very stable and resists further degradation. Substantial information related to human and environmental exposures to PFOS has been developed providing evidence of widespread distribution in humans and the environment. PFOS at very low levels has been identified in serum and tissue samples from both occupationally and non-occupationally exposed human populations, in various species of wildlife, and in surface waters and other environmental media. An extensive database has been developed and continues to be developed on the possible biological effects of these exposures. The information available as of July, 2000, together with an assessment of human and environmental risks, is contained in this report. The report follows the methods and procedures outlined in the Screening Information Data Set (SIDS) Manual of the Organization for Economic and Cooperative Development Investigation of High Production Volume Chemicals.

Based on the currently available information presented in this report, the observed levels of PFOS from a wide variety of samples have not been associated with identifiable adverse effects on human health, wildlife, or the environment. Additional data currently under development from ongoing studies will be used in the future to refine this initial assessment.

The primary manufacturer of PFOS and its precursor molecules (3M Company) announced on May 16, 2000 that it will voluntarily cease manufacturing perfluorooctanyl-based products. The company is cooperating with the U.S. Environmental Protection Agency and its customers in implementing a phase-out plan calling for most manufacturing to cease by the end of 2000 and all remaining manufacturing to cease by the end of 2002.

Environmental Exposure and Effects

Releases of PFOS and its precursor molecules can occur during their manufacture, during both commercial and end use application, and after product use. Analyses of manufacturing waste streams and those associated with commercial and end use applications indicates that most of the waste generated is in the form of solid waste which is either incinerated or disposed of in landfills. Smaller amounts are released in waste water or to air. Environmental fate and transport and subsequent exposure to PFOS are still the subjects of several on-going studies.

The exposure potential of PFOS in the environment is being determined by a number of monitoring efforts. A multi-city study designed to obtain information about the dispersion of fluorochemicals in the environment, uptake into foods, and their presence in surface water is currently underway. Another study involves a global biosphere monitoring program aimed at understanding the presence of PFOS in mammals, fish and birds. Finally, an additional study involves gathering samples of groundwater, surface water, sediments and fish and bird species from the vicinity of a manufacturing facility. Monitoring data collected through July, 2000, are presented in this report.

Numerous acute and chronic toxicity studies involving freshwater and marine organisms have been conducted, and the data from these studies provide a substantial basis for characterizing potential risks to aquatic animals. Effects on the particular species of terrestrial wildlife and birds in which PFOS has been found have not been studied, but considerable data on surrogate mammalian species have been generated. In situations where specific species effects data are not available, inter-species extrapolation is an accepted practice in ecological risk assessment, with the recognition that there are some uncertainties associated with such extrapolation.

Initial Assessment of Ecological Risk

This initial assessment report applies a well-accepted methodology, wherein levels of environmental and wildlife concentrations are compared with toxicity thresholds that are derived from the various studies. Under this approach to risk assessment, the currently available data indicate that the observed levels of PFOS from a wide variety of environmental samples have not been associated with identifiable adverse effects to wildlife and the environment. Calculated ratios indicate a wide margin of safety, but it should be recognized that uncertainty exists in this analysis. Use of serum and liver data as a measure of internal dose reduces some of the uncertainty in this extrapolation. A number of additional environmental studies are underway or planned to refine this initial assessment.

Human Exposures

In the 1960's and 1970's, organic fluorine was identified in human serum at levels less than 0.10 ppm. In the 1990's, as a result of improved analytical techniques, routine measurements of specific organofluorine chemicals became feasible. This allowed for the measurement of PFOS in serum from humans with occupational and non-occupational exposures. Occupationally exposed fluorochemical production workers have measured serum PFOS levels that average 2.0 ppm with highest levels at approximately 10 ppm. In a limited number of non-occupational samples, serum PFOS levels have ranged between 0.01 - 0.10 ppm. Research is ongoing to better characterize the distribution of serum PFOS in non-occupational populations.

Health Effects

The database includes several sub-chronic studies in rodents and non-human primates, multigeneration reproduction/developmental studies, an extensive array of genotoxicity tests, toxicokinetic work and chronic studies. In addition, several epidemiological investigations in exposed workers are available. The studies are of good quality and acceptable for use in hazard evaluation.

Several investigations involving fluorochemical production workers show no evidence of excess mortality or specific clinical signs attributable to PFOS exposure. 3M has conducted medical surveillance of perfluorochemical production workers for over 20 years. A battery of clinical tests (including lipids, hematological parameters, enzymes and 11 different hormone assays) showed no association with PFOS levels; these findings were based on serum levels in workers up to 6 ppm. A mortality study showed no excess mortality for any cause of death, including cancer. All of this information suggests that workers are not at risk at the serum levels reported.

It is reasonable to assume these workers have the highest level of human exposure to PFOS. Since the production workforce studied is largely male, these studies do not adequately represent women. It is noted that male and female monkeys responded similarly in sub-chronic dosing studies with respect to the nature of adverse effects observed.

Results from several repeat dose toxicological studies consistently demonstrate that the liver is the primary target organ. Manifestations of liver tissue response to high doses of PFOS include enlargement of the liver and apparent alterations in metabolic processes. Liver enlargement and reduction in serum cholesterol are early responses to PFOS. These effects occur in rats as well as monkeys.

Also at high doses PFOS adversely affects survival of rat pups in the neonatal period of life as a result of maternal exposure during fetal development. Reduced weight gain, abortions and resorptions are seen in developmental studies at the higher doses tested. There were no effects on developmental milestones, including post-natal neurological development, or on fertility and estrous cycling in offspring in multi-generation studies.

Multiple genotoxicity assays covering a variety of endpoints demonstrate that PFOS does not present a hazard from interaction with genetic material. The results of a two-year cancer study in rats will be available in the next several months.

PFOS is well-absorbed orally and very slowly eliminated from the body. The mean serum elimination half-life for PFOS is approximately 300 days in humans, based on a continuing study of retired fluorochemical workers. PFOS is not metabolized in any of the multiple species studied, although it can be formed metabolically from other fluorochemicals containing the perfluorooctanesulfonyl moiety. Unlike many chemicals of environmental consequence, PFOS does not preferentially distribute to fatty tissue, preferring instead to associate with proteins in blood and liver. It has been found to cross the placenta, and there is evidence for distribution in milk. Due to the good oral absorption, poor elimination and extensive protein binding, PFOS concentrations in liver and serum are proportional to cumulative exposure. Therefore, serum PFOS concentrations can be used as an integrated measure of exposure over time, regardless of source.

Several investigations have been conducted or are in progress to better understand the mechanism(s) through which PFOS exerts its toxicity. While not completely understood at this time, the mechanisms(s) of toxicity is thought to include changes in metabolic processes associated with fat metabolism.

Initial Assessment of Human Risk

Retrospective cohort mortality assessments of fluorochemical production workers have not revealed excess mortality in any category of disease. PFOS levels up to 6 ppm in production workers have not been associated with abnormalities in clinical testing (chemistries included lipids, hematologic parameters and 11 different hormone values). Considering the study information available to date, and given the distinctly higher PFOS levels in animals necessary to produce toxicity (cholesterol lowering and liver abnormalities), it is unlikely that workers would have these abnormalities at the levels to which they have been exposed. Current knowledge

indicates that general population sera levels are approximately two orders of magnitude lower than production workers at the higher end of the range in these studies.

Although there is some species variation in the levels at which PFOS induces toxicity, the toxic effects are consistent. Furthermore, the fact that the same measure of exposure (serum values for PFOS) is used to extrapolate across species reduces the uncertainty in understanding exposure and ultimately human risk. The information available to date on toxicity associated with PFOS serum levels indicates that PFOS levels in the general population are approximately two to three orders of magnitude lower than serum levels in animal studies that are associated with no adverse health effects.

Conclusion

This report summarizes the information that is available as of July 20, 2000. There is a substantial body of data relating human and environmental exposures to PFOS and the possible biological effects of these exposures. This information suggests that human serum PFOS levels found in occupational and non-occupational populations are not associated with adverse effects. Similarly, levels found in the environment and in wildlife are not associated with adverse effects. Additional research now underway will be used to refine this initial assessment.

INTRODUCTION

Background

The human health and ecological risk assessment process for PFOS contained in this report follows the methods and procedures outlined in the Screening Information Data Set (SIDS) Manual of the Organization for Economic Cooperation and Development (OECD) Programme On the Co-Operative Investigation of High Production Volume Chemicals (OECD, 1997). In particular, this report follows the provisional guidance for the outline of the SIDS Initial Assessment Report, which is discussed in Chapter 4 of the manual. The environmental portion of the SIDS Initial Assessment Report is effectively a Tier I Screening-Level Risk Assessment as envisioned by U.S. EPA. This format was followed recognizing that this initial assessment report is to be part of the OECD Existing Chemicals Program.

Overall, the assessment presents, evaluates, explains, and combines information on PFOS exposure and effects into an initial assessment of potential risk. Based on current information, the observed levels of PFOS from a wide variety of samples are not associated with identifiable adverse effects in humans, wildlife, or the environment. The information in this report is based on a substantial body of data and represents our current knowledge. Additional information is currently under development and will aid in improving this initial assessment of risk of PFOS to human health and the environment.

Section 1 of this report provides the PFOS CAS number, molecular formula, and composition; describes its physicochemical properties; and discusses the behavior implications of these characteristics, such as anticipated sources, sinks, and bioaccumulation. Section 2 presents general information on ecological and human exposures to PFOS, including its uses and function, production volume and expected exposure pathways. Ecological exposure, effects on aquatic and terrestrial ecosystems, and other ecological effects are described in Section 3. Human health exposures, potential hazards, and risks are evaluated in Section 4. Section 5 presents the conclusions and recommendations of this assessment, and the literature and data sources upon which this document is based are listed in Section 6. Summary Reports for the compound's physicochemical properties are included in Appendix I. Appendix II presents Summary Reports for aquatic toxicological studies. Reports summarizing the environmental exposure studies are included in Appendix III. Appendix IV lists planned environmental studies on PFOS. Appendix V contains summary reports of mammalian toxicology studies.

Overview of PFOS

The fluorochemicals discussed in this document are produced by an electrochemical process that exchanges all of the hydrogen atoms of an organic feedstock with fluorine atoms from hydrogen fluoride. The highest volume perfluorochemical produced in this way is perfluorooctane sulfonyl fluoride (POSF). Over eight million pounds of this compound will be produced in 2000. Using this perfluoroorganic molecule as a basic building block, unique chemistries can be created by further reactions with functionalized hydrocarbon molecules. These compounds repel water and oil, reduce surface tension, catalyze oligomerization and polymerization, and maintain their properties under extreme conditions. Depending upon the specific functional derivatization

or the degree of polymerization, such POSF-based compounds may degrade or metabolize to PFOS. PFOS is the stable and persistent end-product that has the potential to bioaccumulate.

In the environment, PFOS is resistant to chemical and biological changes and does not degrade under any observed conditions except for combustion. PFOS or precursors enter the environment through factory discharges, as manufacturing residuals in products or as products themselves. Mechanisms by which PFOS can be transported through the environment include transport in surface water, adsorption onto particles present in air, surface water and sediments, and uptake by aquatic, avian or terrestrial organisms.

The ability to detect and quantify PFOS and its precursor compounds at very low levels in the environment has been limited until recently, when reliable and sensitive methods for extracting, separating, and identifying and quantifying them in tissues and environmental samples became available. For this reason, knowledge of the environmental fate of this class of chemicals has been enhanced. Research is underway to assess PFOS accumulation in the environment, atypical partitioning behavior, and significant surface activity. Uncertainties about the applicability of existing fate-and-transport models and gaps in physicochemical and environmental data also complicate the characterization of the environmental fate of PFOS. More information is being gathered to better understand environmental fate. This includes efforts to help characterize releases from product manufacture and use, including developing fate-and-transport models and designing methods to sample various habitats and species of interest.

PFOS has been found at low levels in samples of human serum from several sources and locations. It is persistent and widespread in human populations. The mechanisms and pathways leading to its presence in human blood are not well characterized, but it is likely there are multiple sources of the compound. Some may arise from environmental exposure to PFOS or precursor molecules, or from residual levels of precursors to PFOS in commercial products. 3M production workers have the highest known blood levels of PFOS. Epidemiological and medical surveillance studies of these workers have not associated adverse health effects with this exposure. An extensive toxicological database on PFOS and specific precursor molecules continues to be developed. While much work in this area is still in progress, the available information indicates that current levels of PFOS are not associated with identifiable adverse effects to human health.

The analytical values represented in this report result from various methodologies and represent the most accurate information available as of July, 2000. For this data, final reports have been issued or are in preparation. To ensure the most accurate analytical results possible, work is presently being conducted to completely characterize samples used in testing and analysis over the last few years. Based on the results of further purity analysis, the values in the reports upon which this document is based will be revised. Therefore, the numbers in this document would also be modified in future revisions.

1.0 IDENTITY

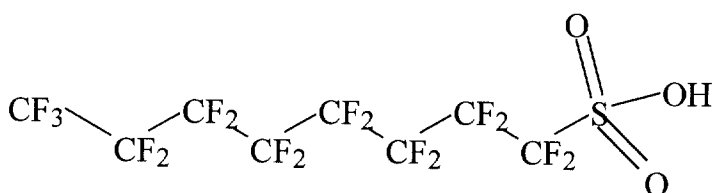
Chemical Name: Perfluorooctane Sulfonic Acid

CAS Number: Various, including: 1763-23-1 (acid)
29081-56-9 (ammonium salt)
70225-14-8 (DEA salt)
2795-39-3 (potassium salt)
29457-72-5 (lithium salt)

The perfluorooctane sulfonate anion (PFOS) has no specific CAS number. The above-listed acid and salts are all considered perfluorooctane sulfonates.

Molecular formula: $C_8HF_{17}O_3S$

Structural formula:



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

Table 1-1. Physical and Chemical Properties (See Appendix I for Robust Summaries)

Parameter	Report Date	Results
Melting Point	2/24/99	> 400 °C
Vapor Pressure	5/05/99	3.31 x 10 ⁻⁴ P @ 20 °C
n-Octanol/Water Partition Coefficient	2/11/00	Not measurable
Air-Water Partition Coefficient	3/19/00	0 (< 2 x 10 ⁻⁶)
Solubility in Pure Water	5/03/99	519 mg/L

1.1 Discussion

PFOS appears to be stable in the environment. This compound is not biodegradable and does not undergo hydrolysis or photolysis. Based on an analysis of bond strength the compound is destroyed by combustion at high temperatures. The very low vapor pressure and immeasurable air/water coefficient indicate that volatility of the compound is insignificant.

PFOS has a solubility of about 519 mg/L in pure water. Solubility decreases significantly in seawater to about 25 mg/L. 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.

PFOS is not anticipated to be present in the atmosphere because of its extremely low volatility. In fact, obtaining an air/water partition coefficient reading for PFOS has not been possible because the partitioning has been too small to measure. Therefore, atmospheric dispersion of PFOS is considered unlikely.

Because of the compound's surface active properties and the test protocol itself, determining the n-octanol/water partitioning coefficient has not been possible. The difficulty in measuring this coefficient is caused by the formation of a third layer between the water and the n-octanol. Because the octanol/water coefficient is used extensively in models to predict bioconcentration and transport mechanisms, a different test method will be used to determine the coefficient. An actual bioconcentration study will also be performed.

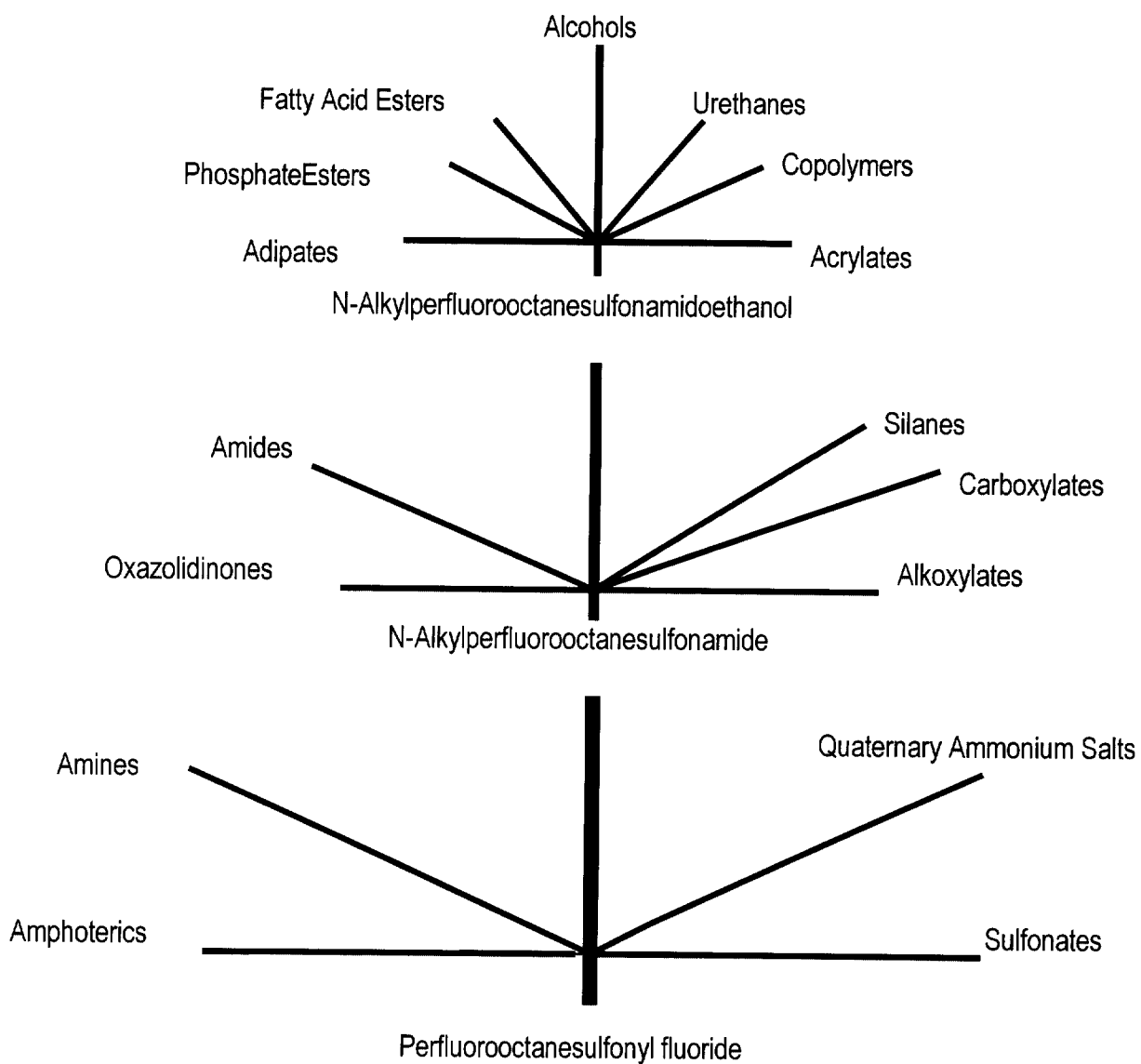
Even with this information, classic models (which are based on log P, or K_{ow} for predicting bioconcentration) may not be appropriate. Studies performed on laboratory rats show PFOS does not bioconcentrate in the lipid fraction but tends to bind to certain proteins. These findings may negate the utility of the conventional models, which are based on measures of affinity for lipids.

2.0 GENERAL INFORMATION ON EXPOSURE

General Information on Exposure

Perfluorooctane sulfonic acid (PFOS) is a fully fluorinated organic acid produced by electrochemical fluorination. The starting feedstock for the electrochemical fluorination reaction is 1-octane sulfonyl fluoride and the primary product produced is perfluorooctane sulfonyl fluoride (POSF). POSF is a commercialized product to some extent, but it is primarily an important intermediate in the synthesis of higher molecular weight fluorochemical products. PFOS is itself a commercialized product produced from the hydrolysis of POSF. 3M sells approximately 50,000 pounds per year of PFOS in various salt forms. It is used for a variety of surfactant applications (mainly fire-fighting foams and coating additives). Unique chemistries are created by further derivatizing POSF through the sulfonyl fluoride moiety using conventional hydrocarbon reactions. POSF is reacted with methyl or ethyl amine to produce either N-methyl or N-ethyl perfluorooctane sulfonamide. At this stage, these intermediates can be used to make amides, oxazolidinones, silanes, carboxylates and alkoxylates as commercial products. These intermediates can be subsequently reacted with ethylene carbonate to form either N-methyl or N-ethylperfluorooctanesulfonamidethanol. These intermediates can be used to make adipates, phosphate esters, fatty acid esters, urethanes copolymers, and acrylates as commercialized products. See Figure 2.1.

Figure 2.1 POSF Fluorochemical Reaction Tree



The secondary reactions producing all of these derivatives are single or sequential batch processes that do not necessarily produce pure products. There may be varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials or intermediates) that are carried forward to the final product. Typically, these residuals are currently present at a concentration of 1% or less in commercialized product. The non-fluorochemical moieties added to the sulfonyl fluoride group of these residuals can be removed through a variety of degradation processes (chemical, environmental, and metabolic). The terminal fluorochemical moiety of such degradation will be PFOS. Higher molecular weight polymeric fluorochemical products tend to be stable and do not degrade to PFOS by these same processes.

Total worldwide POSF production by 3M in 2000 will be 8,000,000 lbs. POSF-derived fluorochemicals (polymers and monomers) are formulated with water or solvent, with the fluorochemical component (or fluorochemical solids) representing a variable percent of the formulation. Total fluorochemical solids include the hydrocarbon reactants combined with the fluorochemical starting material and do not represent the POSF molecule itself. 3M produced fluorochemical solids represent the majority of the total production of sulfonyl based fluorochemicals in the world. The breakdown of 3M fluorochemical production into different product categories include:

Surface Treatments (High Molecular Weight (MW) polymers or formulated products with low percentages of non-polymeric FC solids)

Carpet Protector
Fabric/Upholstery Protector
Apparel and Leather Protector
Protective Products for After Markets and Consumer Application

Paper and Packaging Protectors (Phosphate esters or high MW polymers)

Food Packaging
Paper Products

Performance Chemicals (Low MW chemical substances)

Fire Extinguishing Foam Concentrates
Mining and Oil Surfactants
Electroplating and Etching Bath Surfactants
Household Additives
Chemical Intermediates
Coatings and Coating Additives
Carpet Spot Cleaners
Insecticides Raw Materials

Potential sources of human or environmental exposure to PFOS include the producer's manufacturing operations and waste streams, the manufacturing operations and waste streams of users of POSF-based fluorochemical products, and the use or degradation of some final commercial products containing POSF-based fluorochemicals.

The primary manufacturer of PFOS-based chemicals capable of potentially degrading to PFOS (3M) has announced that its production of these materials will be substantially discontinued by the end of 2000. Manufacture and distribution of a few, well-defined products having essential uses will continue for a limited time period thereafter. The company is working with the U.S. EPA and with its customers to implement an orderly transition away from all such perfluorooctanyl chemical products by the end of 2002.

3.0 ENVIRONMENT

3.1 Environmental Exposure

This section discusses available information on the presence of perfluorooctane sulfonate (PFOS) in the environment. As part of a larger study, monitoring data have been collected for surface water column samples. Summary reports are presented in Appendix III. A global biosphere monitoring program has resulted in analysis of more than 400 archived serum and liver specimens from a wide range of aquatic and terrestrial species. These data are described in Section 3.1 and are summarized in Tables 3-2, 3-3 and 3-4.

3.1.1 General Discussion

Exposure Potential

One potential source of PFOS exposure is the release to the environment of POSF-derived materials in the waste streams generated from the manufacturing process, supply chain operations, and consumer use. Based on extensive engineering calculations and mass balance determinations, estimates of 3M waste stream generation have been derived. 3M has no information on waste streams from other producers and users of these materials.

Generally, the wastes generated from the manufacture and use of POSF derived materials are not in the form of PFOS itself, but rather in the form of high molecular weight polymeric materials, which contain small amounts of residual molecules which may be precursors to PFOS. The degradation of the polymeric substances is very complex and efforts are underway to understand the mechanism and the extent to which they may degrade to PFOS.

From the standpoint of waste type, approximately 90% of the waste generated from all sources is in the form of solid waste, which is either incinerated or disposed of in landfills. The remaining wastes are discharged as wastewater (~9%) or as air emissions (~1%). Several ongoing studies will improve the understanding of the possible mechanisms associated with environmental fate and transport of PFOS. (See Appendix IV.)

The exposure potential from waste generation sources and actual environmental concentrations are being determined through a series of monitoring efforts. For example, a multi-city study was designed to obtain information about the dispersion of fluorochemicals in the environment, uptake into foods, and presence in surface water. The purpose of the study, which has been initiated but not completed, is to improve the understanding of the potential sources of human and ecological exposure. Where possible, samples were taken or will be taken from the surface water column and surface microlayer, sediment, river fish, drinking water, influent and effluent to publicly owned treatment works, sludge, and landfill leachate. Additionally, a "market basket" of several food products will be sampled. The data from this study will provide a more definitive exposure analysis. The surface water column data collected as part of this study were used to create the Initial Assessment for the Environment (Section 3.3.1).

Two other studies are underway to characterize the exposure potential of PFOS. The first study involves a global biosphere monitoring program aimed at understanding the distribution of PFOS in a variety of organisms, such as mammals, fish, and birds. These data provide a direct measure of PFOS exposure to these organisms. In this assessment, this information is combined with the results of ecotoxicological testing and mammalian toxicity testing to understand the potential risk of those exposures, if any. The discussion of the findings from this biosphere program and results of ecological effects studies are presented in Section 3.2.

The second study is being conducted in the vicinity of a manufacturing facility (Decatur, Alabama). Samples of groundwater, surface water, sediments, and fish and bird species have been collected and analyses are in progress. This study will also provide information on PFOS concentrations in progressive levels of the food web and, in conjunction with other information, will be used to develop an analysis of the biomagnification characteristics of this compound.

Exposure and Release Management

3M has already announced that it will voluntarily cease production of POSF chemistry. Even prior to that announcement, several activities to reduce the waste and emissions from 3M manufacturing operations and to improve product quality had been implemented. Product and process understanding have been enhanced, the level of residuals and wastes from the manufacturing operations have been reduced (through improved process controls and installation of treatment equipment), most fluorochemical solid waste materials from 3M manufacturing receive thermal treatment, and the program of product phase-out is underway. All of these steps have had and will continue to have a significant impact on reducing the exposure potential from 3M manufactured fluorochemicals.

3.1.2. Predicted Environmental Concentrations (PEC)

As the SIDS Manual indicates, the predicted environmental concentration or PEC should be derived based on monitoring data and/or calculation using exposure models. At this time, the development of predictive models is in a very early stage. The physical/chemical properties of PFOS and the overall characteristics of the products and waste streams may hinder the development of predictive models. Typically, the models call for the use of log P or K_{ow} factors, which are not available for this compound. These factors are required for predicting bioconcentration and, because of the surface active properties of the compound, the factors cannot be determined. In addition, the nature of exposure routes would add considerable complexity to the development of these models. For example, PFOS degradation in the atmosphere or its binding potential in ecological species or compartments such as sediments is not fully understood. Consequently, the analysis presented in Section 3.2, Effects in the Environment, is based on actual monitoring data which are likely to be more reliable than modeling estimates for this compound.

Data are available from the multi-city study and the global biosphere monitoring program. With these data and the extensive ecotoxicological and animal testing results, the predicted environmental concentrations (PECs) and the predicted no effect concentrations (PNECs) of the compound can be thoroughly compared. This information is presented in the following sections.

3.1.2.1 Surface Waters

PFOS concentrations in surface waters were analyzed in samples from six cities (Nishioka and Strauss, 2000). For each city, a list of key 3M supply chain customers was generated, and the locations of the customers were determined. Examining the various locations resulted in the identification of surface water bodies in both the upstream and downstream vicinity of the key 3M customers. The locations were chosen to integrate the potential PFOS exposure due to all POSF-based manufacturing and fluorochemical use by key supply chain sources. In addition, a “quiet water” site was located at each city except for Cleveland, Tennessee. Details on the sites and locations are contained in the study design. A description of the sites for each city is found in Table 3-1.

At each city sampling site, two water samples were analyzed for PFOS concentration. Table 3-1 presents the results of the laboratory analysis. A site average and a maximum value are presented for each city. The reporting limits and method detection limits are indicated in the table. The average and maximum values are used in the risk assessment analyses presented in Section 3.2. The largest concentration of PFOS was found in the quiet surface water in Port St. Lucie 2,930 parts per trillion (ppt) and the second largest at Decatur (114 ppt). The concentrations of PFOS at Cleveland, Tennessee, were all less than the limit of quantification of 25 ppt. Of those cities with average PFOS concentrations greater than the reporting limit, Columbus, Georgia, had the second largest average PFOS concentration of 80.0 ppt while Pensacola, Florida, had the smallest detectable average PFOS concentration of 25.7 ppt.

The analytical findings of the Port St. Lucie quiet water sampling location are not completely understood but are considered to be an anomaly in the multi-city data set. The PFOS levels at this site are considerably higher than other quiet water sites in the study and are not consistent with other findings at Port St. Lucie. The sampling site is a relatively small and stagnant pond about 200 feet by 200 feet. The pond contained considerable manmade debris including plastic, styrofoam and bottles.

3.1.2.2 Biota

The biosphere monitoring program was designed to assess the global distribution of PFOS. Samples of blood, liver, and other tissues were collected from archived specimens of a variety of species from several locations and analyzed for PFOS. Areas of focus included North America (the Great Lakes and coastal marine locations), the Arctic, and Europe. Analyses of these samples indicated that PFOS is present in the livers and sera of animals, especially in piscivorous (fish-eating) animals. Tables 3-2 and 3-3 summarize the results of these analyses. The highest average concentrations of PFOS in biota was found in the blood of bald eagles and the liver of

minks. Average concentrations in other tissues and other species were relatively lower (Table 3-4).

Concentrations of PFOS measured in blood and liver of wildlife appear to be valid measures of environmental exposure. In laboratory toxicity tests with rats and monkeys, PFOS was found to distribute to blood and liver. In a 26-week capsule toxicity study with *Cynomolgus* monkeys, serum PFOS values increased almost linearly at doses up to about 100 ppm, although serum levels did not increase linearly at higher doses (Table 3-5). In 14-week dietary and two-generation reproductive studies in rats, serum and liver concentrations also increased approximately linearly with dose (Table 3-5). In a radiolabel study the majority of the administered dose was found in blood and liver. Thus, primate and rat studies indicate that serum PFOS concentrations resulting from daily exposure over a broad range of PFOS doses are directly proportional to cumulative dose up to serum concentrations of 100 ppm (see section 4.3.1.2)..

3.2 Effects on the Environment

3.2.1. Effects on Aquatic Animals from Exposure to Aqueous Concentrations

Since 1974 numerous acute and chronic toxicity tests using a variety of freshwater and estuarine aquatic organisms have been conducted (Table 3-6). Over this time, protocols for conducting the toxicity tests have changed. For a given type of test and test endpoint, however, the effect of PFOS to aquatic organisms has been demonstrated to be within a relatively small range. Table 3-6 summarizes the aquatic toxicity test results that are currently available. A variety of effect level test endpoints are available including the EC10, EC50 or EL50 or LL50 or LC50, LOEC and EC90. No effect levels (NOEC and NOEL) are also available. Appendix II contains the robust summaries for each of the tests shown in Table 3-6. Ongoing and planned aquatic toxicity test results are presented in Appendix IV.

Examination of Table 3-6 shows that the lowest NOEC found was 0.3 mg/L PFOS, which is calculated from a 47-day chronic test of fathead minnow conducted in 1999. The value represents the PFOS concentration at which no effect was found using a post-hatch survival of eggs as the test endpoint. In keeping with the SIDS approach of using the most sensitive indicator of PFOS effect as the PNEC, this report uses 0.3 mg/L in the risk assessment for aquatic species.

3.2.2 Effects on Terrestrial and Aquatic Birds and Mammals from Bioaccumulation of PFOS

In situations where specific species effects data are not available, inter-species extrapolation of toxicity endpoints is an accepted practice in ecological risk assessment. The magnitude of the uncertainties in such extrapolations is unknown, and could be large (Chapman et al., 1998). If fewer steps are involved in the extrapolation process, the uncertainty will be less (Sample et al., 1996). For PFOS, or any other chemical, these uncertainties should be smaller when extrapolating from mammals to mammals (e.g., rats to mink) and larger when extrapolating from mammals to birds (e.g., rats to eagles). A potentially large source of uncertainty associated with predicting effects of chemicals on birds and mammals comes from the variability associated with

sensitivity among species to toxic chemicals. Toxicity data for laboratory rats represent the most common, relatively consistent test endpoints for human health risk assessment that can be applied to terrestrial vertebrates. These endpoints will also be applied to bird species subject to the increased extrapolation uncertainties mentioned above. Use of a tissue concentration of PFOS rather than an external dose estimate reduces this uncertainty by eliminating some of the pharmacokinetic variables that exist between species. Avian chronic studies are also underway to better characterize the effects of PFOS in birds.

The preliminary results of three recent studies (see Table 3-5) evaluated the subchronic or chronic effects of PFOS on rats and monkeys, which included measurements of PFOS concentrations in serum and liver. As explained above, these results provide the best available information for characterizing the potential effects and risks of PFOS to piscivorous wildlife.

Section C of Table 3-5 presents the results of a growth and reproduction study conducted with rats. Growth and reproduction are typical endpoints used in ecological risk assessments, and have a direct ecological effects correlation at the individual and population level. Examination of the table shows that the NOEL occurred at the 0.4 mg/kg/d dosing level. No toxicologically important effects on pup survival or growth occurred at this dose. At 1.6 mg/kg/d and higher, pre-implantation loss increased and litter size, pup viability, growth, and survival decreased.

For the ecological risk characterization for piscivorous wildlife, the NOEL from the dam pre-mating group was chosen. This value is 47.1-ppm serum PFOS. Several reasons support this choice. First, this value is taken from a study on reproduction which has direct population level relevance. Second, the mammal serum values from 3M's biosphere program are generally derived from adult, nonpregnant animals. Third, end gestation values are affected by the physiologic changes occurring during pregnancy, introducing greater intra- and interspecies variability. In addition, fetal values tend to be more uncertain than adult values, because neonatal sera are difficult to collect, and the risk for contamination or dilution during collection is higher. (Note: There is a NOEL of 62.9 ppm in Section B of Table 3-5 for female rats. This number was not used because it does not represent a reproductive effect). For the ecological risk characterization for piscivorous wildlife, the 0.4 mg/kg/day dam pre-mating value of 47.1-ppm serum PFOS NOEL was used as the PNEC concentration.

Two recent studies were done to assess human health effects related PFOS toxicity to liver concentrations—the 26-week capsule toxicity study with *Cynomolgus* monkeys and the 14-week dietary study with rats. In the 26-week monkey study, the mean NOEL for liver concentrations of PFOS at the 0.15 mg/kg/day dose was 80 ppm, (Table 3-5). For the 14-week rat study, the mean (male and female average) NOEL for liver concentrations for PFOS at the 2.0 ppm dose was 72.5 ppm. (Table 3-5). Either the monkey liver value or the rat liver value could be used as PNECs for this risk assessment. The rat liver value was chosen for this risk assessment because it results in the most conservative risk estimates. Therefore, the 72.5-ppm liver PFOS concentration was used as the PNEC.

3.3 Initial Assessment for the Environment

Effects to the environment are calculated using a ratio of the PEC to PNEC for both aquatic and mammalian assessments. As indicated in the SIDS manual a ratio of greater than one (>1) indicates a specific hazard may be posed. A ratio far below one (<1) indicates a hazard cannot be identified and the chemical can be considered to present a low potential for risk. In this assessment actual environmental data are used to evaluate risk and safety factors are not explicitly incorporated in the PNEC's or risk calculations. The inverse of the risk ratio, or $PNEC/PEC$, is the margin of safety.

In addition to calculating the $PEC / PNEC$ ratios, the effects and exposure data are presented in graphical form (Figures 1, 2 and 3). The graphics provide a method for visually comparing the effects and exposure data. From the graphs, the range of effects concentrations, or range of exposure concentrations, that are contained in the current data set can be assessed. Cumulative frequency distributions of effects and exposure are created for both the aquatic and terrestrial risk assessments, and plots of the data are presented. Unlike the $PEC / PNEC$ ratios which focus on the highest possible risk estimates, the graphical approach provides a comprehensive assessment of the potential risk of PFOS across the entire range of effects and exposure information.

3.3.1 Risks to Aquatic Biota from Exposure to PFOS in Surface Waters

Table 3-7 presents the ratio of PEC to PNEC for each of the cities in which aquatic exposure concentrations are available. The ratios range from 0.000016 at Port St. Lucie (Site 2) to 0.0096, also at Port St. Lucie (quiet surface water). The second largest ratio (0.00037) was determined for the quiet surface water sample in Decatur. All but one of the ratios is less than one by a margin of safety greater than 2000. The quiet water at Port St. Lucie is the exception, with a margin of safety of over 100. A comparison of all toxicological endpoints to the maximum PFOS concentration obtained at each of the six cities is shown in Figure 3-1. The Figure indicates that for the currently available water column data the concentration of PFOS in all samples are well below the entire range of ecotoxicological effects data. Within the bounds of uncertainty (based on this very conservative risk approach), the current information indicates that no adverse effects to aquatic biota would be associated with measured concentrations of PFOS. It should be recognized that aquatic exposure data is limited.

3.3.2. Potential Risks to Terrestrial Wildlife from Bioaccumulation of PFOS

All $PEC/PNEC$ ratios for blood and liver PFOS concentrations in terrestrial organisms were less than one (Tables 3-8 and 3-9, respectively). These tables provide ratios for each species using the lowest tissue concentration, the maximum tissue concentration, and the average of all available tissue samples. The largest maximum ratio for PFOS in blood was 0.0552 for bald eagles. This ratio represents the degree of risk for the individual eagle with the highest measured blood concentrations of PFOS. Note that this ratio represent a margin of safety of 18. The highest mean ratio is also associated with eagles (0.0073), and represents a margin of safety of 137. Mean $PEC/PNEC$ ratios were less for all other species.

Figure 3-2 compares the PNEC for serum concentration with the cumulative distribution of blood PFOS concentrations for all species sampled. Each value in the cumulative distribution is the mean of all samples for a particular species. Labels on the figure indicate the species used in the analysis. Examination of the figure shows that the median PFOS serum concentration for all species is approximately 0.03 ug/ml. The 5th and 95th percentile PFOS serum concentrations are approximately .004ug/ml and 0.28 ug/ml, respectively. In other words, based on current data, only 5% of the species mean PFOS serum concentrations exceed 0.64 ug/ml. Figure 3-2 shows that for the currently available environmental data the serum concentrations of PFOS in wildlife samples are well below the PNEC.

For PFOS in liver, the largest maximum PEC/PNEC ratio was 0.0676 for the mink with the highest liver concentration. This ratio represents a margin of safety of 15. The largest mean PEC/PNEC ratio is also associated with mink liver (0.0170), representing a margin of safety of approximately 59. Figure 3-3 compares the PNEC for liver concentration to the cumulative distribution of liver PFOS concentrations for all species sampled. Again, species mean values are used in the cumulative distribution. Examination of the figure shows that the median PFOS liver concentration for all species is approximately 0.08 ug/g. The 5th and 95th percentile concentrations are approximately 0.02 ug/g and 0.4 ug/g, respectively. Figure 3-3 indicates that for the currently available environmental data the liver concentrations of PFOS in wildlife samples are well below the PNEC.

The remaining species have PEC/PNEC ratios that are much smaller than those of eagles and mink. For example, the second highest maximum serum ratio is associated with herring gull (0.0046), representing a margin of safety of 217. The largest margin of safety for the maximum serum concentrations is 10,000 for the northern fur seal and the stellar sea lion. In liver, the second highest maximum ratio is associated with the river otter (0.0137), representing a margin of safety of 73. The largest margin of safety for the maximum liver concentration is seen in swordfish (5,000).

Sources of uncertainty in these risk characterizations include the following:

1. The relative sensitivities of rats, monkeys, and piscivorous wildlife species to PFOS are unknown; therefore, using rat and monkey laboratory PFOS toxicity data to predict effects to piscivorous wildlife may over- or underestimate risks. Use of a tissue concentration of PFOS rather than an external dose estimate reduces this uncertainty by eliminating some of the pharmacokinetic variables that exist between species.
2. The amount of chronic toxicity data for PFOS available at this time for predicting risks to piscivorous wildlife is limited
3. The degree to which the limited data in the biosphere sampling program for PFOS concentrations in blood and liver in piscivorous wildlife are representative of PFOS concentrations in piscivorous wildlife in general is unknown.

In summary, it can be concluded from currently available data that the observed levels of PFOS from a wide variety of samples cannot be associated with identifiable adverse effects in wildlife or the environment. PEC/PNEC ratios are substantially less than one (<1). It should be

recognized, as indicated above, that uncertainty exists in this analysis. Additional avian chronic studies are underway that will increase the amount of data available for characterizing risk to birds. Finalizing biosphere monitoring studies also will provide more information on the presence of PFOS in the environment.

3.3.3 Other Effects

Standardized whole effluent toxicity tests required under the discharge permit for the manufacturing plant located in Decatur, Alabama were reviewed. Quarterly testing with *Daphnia* and fathead minnows is required. For the past two years, there has been 100% survival in the test organisms exposed to the plant effluent, which does contain PFOS.

Table 3-1. Environmental Concentrations (PECs) for PFOS in Surface Water

Location	Sampling Site	PEC (ng PFOS/L) ppt			Location Maximum
		Sample 1	Sample 2	Site Average	
Columbus, Georgia Sampling locations near the Columbus Water Works Influent	Site 1	63.8	59.9	61.9	83.3
	Site 2	76.6	83.3	80	
	Site 3	55.4	55.4	55.4	
	Quiet surface water	< 25	< 25	< 25	
Pensacola, Florida Sampling locations in Texas Bayou (selected for ease of access)	Site 1	20.8	19	19.9	28.5
	Site 2	28.5	22.8	25.7	
	Site 3	20.9	18.5	19.7	
	Quiet surface water	15.7	17.4	16.6	
Mobile, Alabama Sampling locations near a customer's industrial wastewater treatment plant	Site 1	23.9	22	23	42.8
	Site 2	39.6	42.8	41.2	
	Site 3	34.7	36.3	35.5	
	Quiet surface water	33.3	31.5	32.4	
Decatur, Alabama Sampling locations upstream of the Decatur POTW effluent and downstream of Water Plant	Site 1	23.1	8.3	15.7	114
	Site 2	14.5	22	18.3	
	Site 3	< 25	< 25	< 25	
	Quiet surface water	108	114	111	
Cleveland, Tennessee Sampling locations upstream of the Cleveland Municipal POTW	Site 1	14.7	< 25	19.9	< 25
	Site 2	< 25	< 25	< 25	
	Site 3	< 25	< 25	< 25	
	Quiet surface water	< 25	< 25	< 25	
Port St. Lucie, Florida Sampling locations in the vicinity of the Northport Wastewater Treatment Plant and Port St. Lucie landfill	Site 1	5.2	4.1	4.7	2,930
	Site 2	7.7	9.3	8.5	
	Site 3	7.8	5.4	6.6	
	Quiet surface water	2,930	2,850	2,890	

^aLimit of quantitation is 25 ng/L and method detection limit is 2.5 ng/L.

Table 3-2. Environmental Concentrations (PECs) of PFOS in Blood, Plasma, and Serum Samples from Wildlife Species

Species	Number of Samples	PEC (µg PFOS/mL) ppm			
		Minimum	Maximum	Mean ^(b)	Standard Deviation
Albatross	13	0.0035	0.039	0.011	0.011
Bald Eagle	26	0.0012 ^(a)	2.6	0.34	0.64
Caspian Seal	14	0.012	0.018	0.013	0.0021
Cormorant	11	0.0012 ^(a)	0.43	0.17	0.12
Herring Gull	4	0.066	0.45	0.22	0.18
Northern Fur Seal	44	0.0058 ^(a)	0.0058 ^(a)	0.0058 ^(a)	0.0
Otter	1	0.039	0.039	0.039	--
Polar Bear	14	0.0029 ^(a)	0.052	0.030	0.013
Stellar Sea Lion	12	0.0058 ^(a)	0.0058	0.0058	0.0

^a Level of Quantification for the Specific Analysis

^b The mean concentrations were calculated using all data points, including those that were below the limit of quantification.

Table 3-3. Environmental Concentrations (PECs) of PFOS in Liver Samples from Wildlife Species

Species	Number of Samples	PEC (µg PFOS/g) ppm			
		Minimum	Maximum	Mean ^b	Standard Deviation
Albatross	9	0.035 ^a	0.62	0.099	0.19
Baikal Seal	24	0.035 ^a	0.23	0.094	0.054
Blacktailed Gull	15	0.071	0.50	0.17	0.12
Bottlenose Dolphin	5	0.0070 ^a	0.43	0.22	0.16
Brown Pelican	2	0.046	0.29	0.17	0.18
Brown Trout	10	0.017 ^a	0.026	0.018	0.0026
California Sea Lion	6	0.035 ^a	0.049	0.038	0.0058
Chinook Salmon	6	0.033	0.17	0.11	0.061
Cormorant	12	0.032	0.47	0.096	0.12
Elephant Seal	5	0.035 ^a	0.035 ^a	0.035 ^a	0
Ganges Dolphin	2	0.035	0.081	0.058	0.033
Gozzi	1	0.13	0.13	0.13	--
Green Frog	4	0.035	0.29	0.097	0.13
Harbor Seal	3	0.035 ^a	0.057	0.042	0.013
Lake Whitefish	5	0.033	0.081	0.067	0.02
Loon	8	0.035 ^a	0.69	0.22	0.21
Map Turtle	6	0.039	0.70	0.19	0.26
Mink	30	0.093	4.9	1.2	1.3
Northern Fur Seal	13	0.035 ^a	0.12	0.043	0.024
Polar Bear	17	0.18	0.68	0.35	0.14
River Otter	5	0.15	0.99	0.39	0.35
Sea Otter	8	0.035 ^a	0.035 ^a	0.035 ^a	0
Striped Dolphin	4	0.065	0.16	0.19	0.041
Swordfish	5	0.0070 ^a	0.013	0.0084	0.0028
Terrapin	3	0.035 ^a	0.035 ^a	0.035 ^a	0
Tuna	20	0.035 ^a	0.25	0.08	0.052
Turtle	3	0.099	0.36	0.23	0.13
Weddell Seal	1	0.035 ^a	0.035 ^a	0.035	—

^a Level of Quantification for the Specific Analysis

^b The mean concentrations were calculated using all data points, including those that were below the limit of quantification.

Table 3-4. Environmental Concentrations (PECs) of PFOS in Other Tissue Samples from Piscivorous Wildlife Species

Species	Tissue	Number of Samples	PEC (µg PFOS/g) ppm			
			Minimum	Maximum	Mean ^b	Standard Deviation
Albatross	kidney	7	0.035 ^a	0.035 ^a	0.035 ^a	0
Brown trout	eggs	3	0.049	0.075	0.064	0.013
	muscle	10	0.0070 ^a	0.046	0.011	0.012
Carp	body	4	0.017	0.028	0.022	0.0057
	muscle	10	0.060	0.30	0.12	0.079
Chinook salmon	muscle	6	0.0070	0.19	0.091	0.065
Cormorant	yolk	4	0.035 ^a	0.32	0.19	0.13
Frog	muscle, whole body	8	0.017 ^a	0.024	0.019	0.0027
Green frog	eggs	4	0.017 ^a	0.017 ^a	0.017 ^a	0
Gull	yolk	3	0.035 ^a	0.15	0.078	0.059
Lake whitefish	eggs	2	0.15	0.38	0.26	0.17
	muscle	5	0.097	0.17	0.13	0.035
Sea otter	brain	2	0.035 ^a	0.035 ^a	0.035 ^a	0
	kidney	3	0.035 ^a	0.035 ^a	0.035 ^a	0

^a Level of Quantification for the Specific Analysis

^b The mean concentrations were calculated using all data points, including those that were below the limit of quantification.

Table 3-5. PFOS Toxicity Data for Mammals: Observed Effects, Serum and Liver PFOS Concentrations, and Cumulative Dose

Group ¹	Observed Effect	Serum PFOS Concentration (ppm)	Liver PFOS Concentration (ppm)	Cumulative Dose (mg/kg)
26-week Capsule-Dosing Study in Cynomolgus Monkeys				
0.15 mg/kg/d	NOEL	85	80	27.3
0.75 mg/kg/d	Hepatomegaly; hepatocyte enlargement	> 100, < 300	415 (average)	> 27.3, < 137
0.75 mg/kg/d	Decreased cholesterol in females	> 134 ± 25	415 (average)	46.5
0.75 mg/kg/d	Decreased cholesterol in males	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Decreased T3	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Death or early sacrifice for 2/6 males	> 150, < 300	415 (average)	> 100, < 137
14-Week Dietary Study in Sprague Dawley Rats				
2.0 ppm Males	NOEL	17.9	76.8	~ 17
2.0 ppm Females	NOEL	26.9	68.25	~ 13
5.0 ppm Males	Hepatocellular hypertrophy and vacuolization	45.6	386.53	24.4
5.0 ppm Females	NOEL	62.9	362.45	38.2
20 ppm Males	Hepatocellular hypertrophy and vacuolization; decreased cholesterol; increased AAT	134	599.94	106
20 ppm Females	Hepatocellular hypertrophy and vacuolization	216	617.52	141
Reproduction PK Dosing 6 weeks prior to mating and 21 days of gestation				
0.4 mg/kg/d Dam PM	NOEL	47.1 ± 5.00 (n = 16)	--	16.8
0.4 mg/kg/d Fetus EG	NOEL	39.7 ± 5.90 (n = 5)	--	N/A
0.4 mg/kg/d Dam EG	NOEL	30.3 ± 17.0 (n = 6)	--	28
1.6 mg/kg/d Dam PM	Slight body weight	185 ± 14.0 (n = 16)	--	67.2
1.6 mg/kg/d Fetus EG	Survival, body weight	117 ± 14.5 (n=2)	--	N/A

Table 3-5. PFOS Toxicity Data for Mammals: Observed Effects, Serum and Liver PFOS Concentrations, and Cumulative Dose

Group¹	Observed Effect	Serum PFOS Concentration (ppm)	Liver PFOS Concentration (ppm)	Cumulative Dose (mg/kg)
1.6 mg/kg/d Dam EG	Slight body weight	158 ± 86.6 (n = 4)	--	112
3.2 mg/kg/d Dam PM	Body weight	368 ± 23.6 (n = 16)	--	134
3.2 mg/kg/d Fetus EG	Stillbirth, survival	191 ± 26.4 (n = 6)	--	N/A
3.2 mg/kg/d Dam EG	Body weight	180 ± 41.5 (n = 6)	--	224

¹ PM = Pre-Mating, after 42 days of dosing; and EG = End of Gestation, day 21 of gestation

Table 3-6. Aquatic Species Ecotoxicity Tests with a Survival Endpoint Using PFOS

Ref. No.	Study Date	Species	Chronic or Acute	Toxicity Test Result (mg PFOS/L)
73	1999	<i>Daphnia magna</i>	Acute	48-h EC10 = 53
73	1999	<i>Daphnia magna</i>	Acute	48-h EC50 = 61
73	1999	<i>Daphnia magna</i>	Acute	48-h EC90 = 63
73	1999	<i>Daphnia magna</i>	Acute	24-h EC50 > 91
71	1999	Fathead minnow	Acute	96-h LC50 = 9.5
71	1999	Fathead minnow	Acute	48-h LC50 > 28
75	1999	Freshwater mussel	Acute	96-h LC50 = 59
77	1999	Mysid shrimp	Acute	96-h LC50 = 3.6
77	1999	Mysid shrimp	Acute	72-h LC50 = 4.4
504	1996	<i>Daphnia magna</i>	Acute	48-h NOEL = 6.25
504	1996	<i>Daphnia magna</i>	Acute	48-h EL50 = 11.3
503	1996	Fathead minnow	Acute	96-h NOEL < 490
503	1996	Fathead minnow	Acute	96-h LL50 = 562
87	1994	<i>Daphnia magna</i>	Acute	48-h EC50 = 210
86	1994	Fathead minnow	Acute	96-h LC50 = 19
93	1991	<i>Daphnia magna</i>	Acute	48-h EC50 = 14
90	1979	Bluegill sunfish	Acute	96-h NOEC = 18
90	1979	Bluegill sunfish	Acute	96-h LC50 = 31
184	1974	Fathead minnow	Acute	96-h LC50 = 85
185	1974	Fathead minnow	Acute	96-h LC50 = 100
79	1999	<i>Daphnia magna</i>	Chronic	21-d NOEC = 12
78	1999	Fathead minnow	Chronic	47-d NOEC = 0.3^a
78	1999	Fathead minnow	Chronic	47-d LOEC = 0.6
80	1999	Mysid shrimp	Chronic	35-d NOEC = 0.55
84	1978	Fathead minnow	Chronic	30-d NOEC = 1
84	1978	Fathead minnow	Chronic	30-d LOEC = 1.9

^a Bold indicates the value used in this risk assessment.

Table 3-7. Aquatic Species PEC/PNEC Ratios for Six Locations

Location	PEC ^a /PNEC ^b Ratio			
	Site 1	Site 2	Site 3	Quiet Surface Water
Columbus, GA	2.1 x 10 ⁻⁴	2.7 x 10 ⁻⁴	1.8 x 10 ⁻⁴	8.3 x 10 ⁻⁵
Pensacola, FL	6.6 x 10 ⁻⁵	8.6 x 10 ⁻⁵	6.6 x 10 ⁻⁵	5.5 x 10 ⁻⁵
Mobile, AL	7.7 x 10 ⁻⁵	1.4 x 10 ⁻⁴	1.2 x 10 ⁻⁴	1.1 x 10 ⁻⁴
Decatur, AL	5.2 x 10 ⁻⁵	6.1 x 10 ⁻⁵	8.3 x 10 ⁻⁵	3.7 x 10 ⁻⁴
Cleveland, TN	6.6 x 10 ⁻⁵	8.3 x 10 ⁻⁵	8.3 x 10 ⁻⁵	--
Port St. Lucie, FL	1.6 x 10 ⁻⁵	2.8 x 10 ⁻⁵	2.2 x 10 ⁻⁵	9.6 x 10 ⁻³

^a Site average PEC, in units of ng PFOS/L, presented in Table 3-1.

^b The value used as the PNEC is 0.3 mg PFOS/L, based on study reference no. 78 as presented in Table 3-6. Note that the value 0.3 mg PFOS/L is an actual measured value. Safety factors are not used in the calculation of the PEC/PNEC ratio.

Table 3-8. Piscivorous Wildlife Species PEC/PNEC Ratios for Serum

Species	PEC ^a /PNEC ^b Ratio		
	Minimum	Maximum	Mean
Albatross	0.0001	0.0008	0.0002
Bald Eagle	0.0000	0.0552	0.0072
Caspian Seal	0.0003	0.0004	0.0003
Cormorant	0.0000	0.0091	0.0036
Herring Gull	0.0014	0.0096	0.0047
Northern Fur Seal	0.0001	0.0001	0.0001
Otter	0.0008	0.0008	0.0008
Polar Bear	0.0001	0.0011	0.0006
Stellar Sea Lion	0.0001	0.0001	0.0001

^a Minimum, maximum, and mean PECs (µg PFOS/mL) are presented in Table 3-2.

^b The value used as the PNEC for serum is 47.1 mcg or µg/l.

Table 3-9. Piscivorous Wildlife Species PEC/PNEC Ratios for Liver

Species	PEC ^a /PNEC ^b Ratio		
	Minimum	Maximum	Mean
Albatross	0.0005	0.0086	0.0014
Baikal Seal	0.0005	0.0032	0.0013
Blacktailed Gull	0.0010	0.0069	0.0023
Bottlenose Dolphin	0.0001	0.0059	0.0030
Brown Pelican	0.0006	0.0040	0.0023
Brown Trout	0.0002	0.0004	0.0002
California Sea Lion	0.0005	0.0007	0.0005
Chinook Salmon	0.0005	0.0023	0.0015
Cormorant	0.0004	0.0065	0.0013
Elephant Seal	0.0005	0.0005	0.0005
Ganges Dolphin	0.0005	0.0011	0.0008
Gozzi	0.0018	0.0018	0.0018
Green Frog	0.0005	0.0040	0.0013
Harbor Seal	0.0005	0.0008	0.0006
Lake Whitefish	0.0005	0.0011	0.0009
Loon	0.0005	0.0095	0.0030
Map Turtle	0.0005	0.0097	0.0026
Mink	0.0013	0.0676	0.0166
Northern Fur Seal	0.0005	0.0017	0.0006
Polar Bear	0.0025	0.0094	0.0048
River Otter	0.0021	0.0137	0.0054
Sea Otter	0.0005	0.0005	0.0005
Striped Dolphin	0.0009	0.0022	0.0014
Swordfish	0.0001	0.0002	0.0001
Terrapin	0.0005	0.0005	0.0005
Tuna	0.0005	0.0034	0.0011
Turtle	0.0014	0.0049	0.0032
Weddell Seal	0.0005	0.0005	0.0005

- a) Minimum, maximum and mean PEC's ($\mu\text{g PFOS/g}$) are presented in Table 3-1.
b) The value used as the PNEC for liver is $72.5 \mu\text{g/g}$ (Table 3-5)

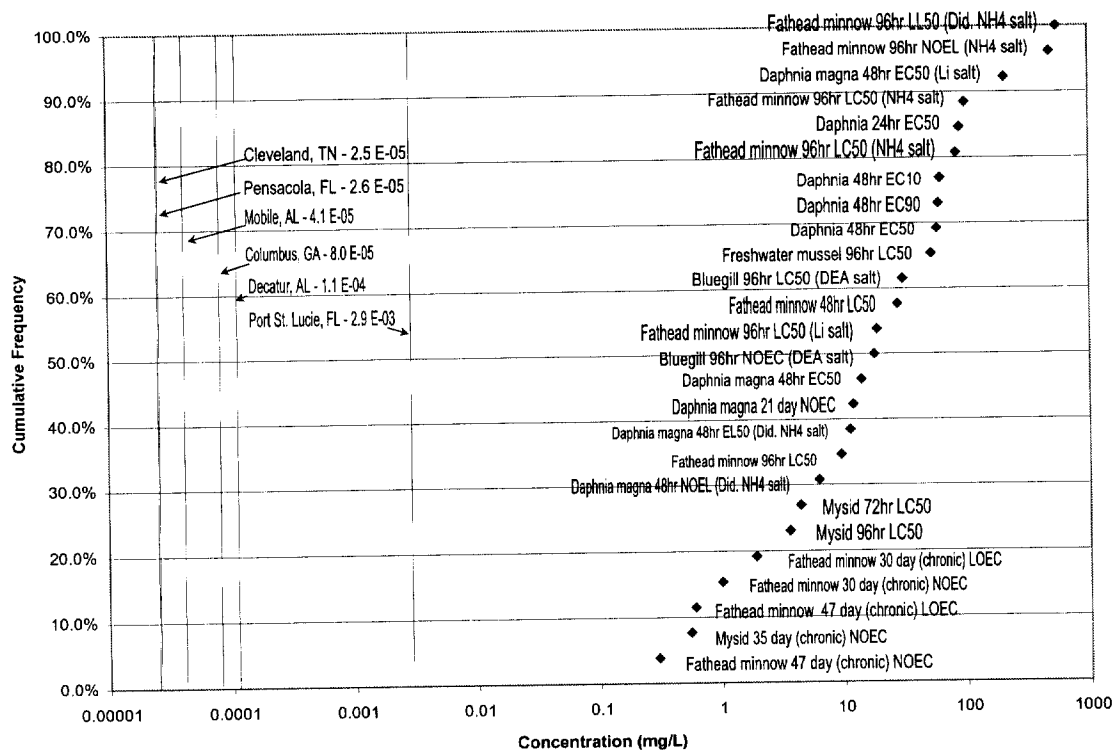


Figure 3-1. Cumulative frequency distribution of aquatic species ecotoxicity test results. [Note: Cumulative frequency of the ecotoxicity test results is calculated by first ordering the data from lowest to highest, while keeping track of the order number. The order number is then divided by the total number of observations and then converted to a percentage. The resulting cumulative frequency is a value that ranges from 0 to 100 percent.]

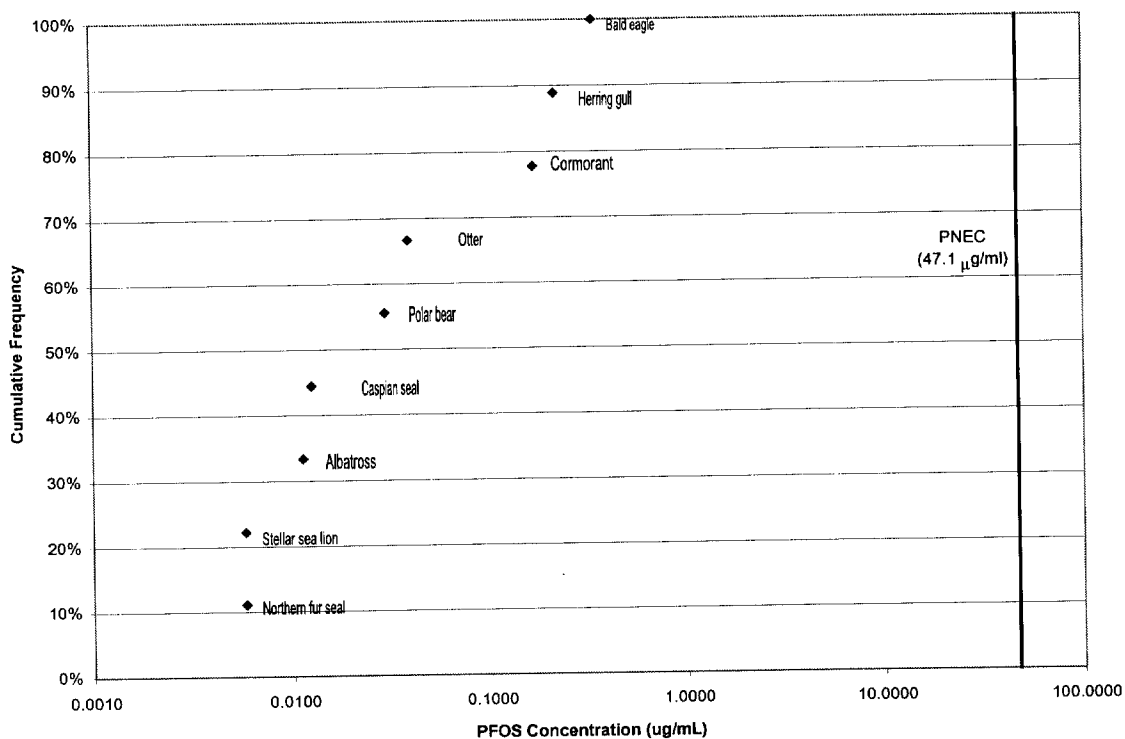


Figure 3-2. Cumulative frequency distribution of mean PFOS concentrations in blood samples of piscivorous wildlife species. [Note: Cumulative frequency of the exposure results is calculated by first ordering the data from lowest to highest, while keeping track of the order number. The order number is then divided by the total number of observations and then converted to a percentage. The resulting cumulative frequency is a value that ranges from 0 to 100 percent.]

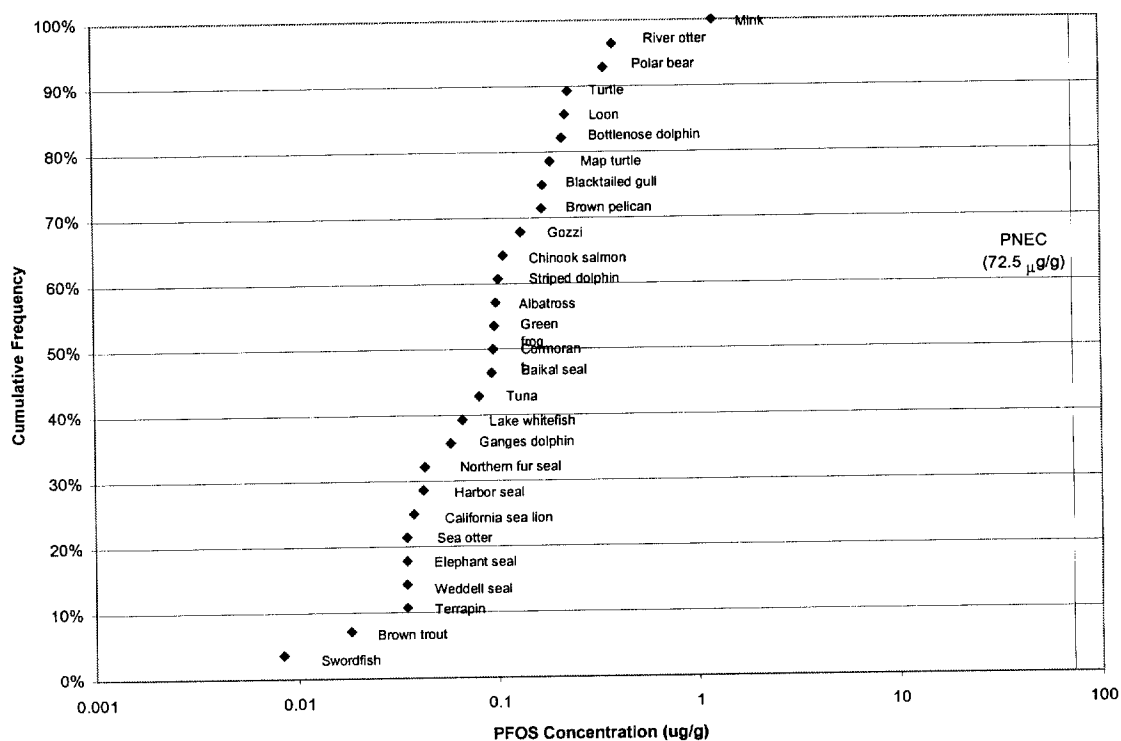


Figure 3-3. Cumulative frequency distribution of mean PFOS concentrations in liver samples of piscivorous wildlife species. [Note: Cumulative frequency of the exposure results is calculated by first ordering the data from lowest to highest, while keeping track of the order number. The order number is then divided by the total number of observations and then converted to a percentage. The resulting cumulative frequency is a value that ranges from 0 to 100 percent.]

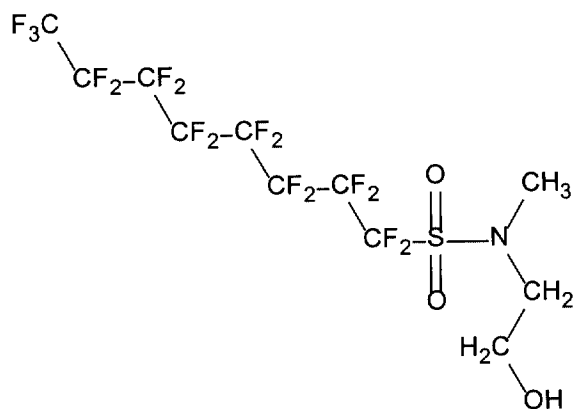
4.0 HUMAN HEALTH

4.1 Introduction

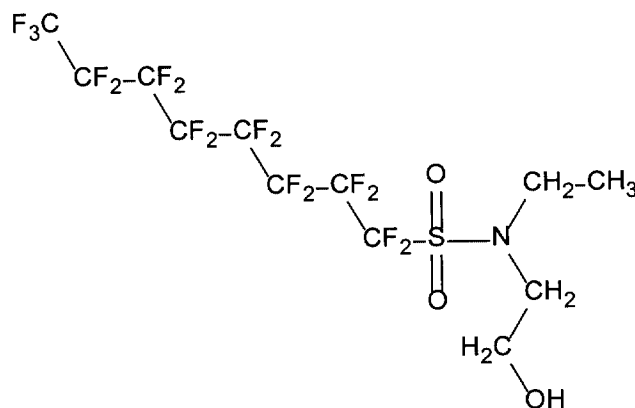
This section contains a summary of the available data relating to the potential for PFOS to induce adverse effects in humans. The available data consist of a large body of epidemiological, animal, and other types of experimental data, reported over the past 25 years. Substantial dose-response data now exist relating serum levels to adverse outcomes in experimental animals, including primates. Included are repeat-dose studies, two-generation reproduction-development studies, teratology studies, and genotoxicity studies. Still in progress, and not reported here, are chronic animal studies; results from these long-term bioassays will be available by the end of 2000. The majority of citations in this Section are from studies and reports from 3M sponsored work, the details of which are found in Robust Summaries. These Robust Summaries are contained in Appendix V of this SIAR. The authors of this section wish to acknowledge the effort of U.S. EPA scientists in preparing many initial Robust Summary drafts that were further developed and edited by the SIAR authors.

The principle focus of all of this work is PFOS. The toxicity testing program includes two related compounds, N-ethylperfluorooctane sulfonamidoethanol (N-EtFOSE) and N-methylperfluorooctane sulfonamidoethanol (N-MeFOSE). The structures of these chemicals are shown below. Both are components of commercial products, and both are known to be absorbed and undergo metabolism to yield PFOS.

N-Methylperfluorooctane sulfonamidoethanol



N-Ethylperfluorooctane sulfonamidoethanol



Both of these compounds have been subjected to repeat dose toxicity and genotoxicity testing, and the ethyl derivative has also been evaluated in teratology and two-generation reproduction studies. As will be seen, both compounds produce effects very similar to those produced by PFOS. This suggests that the toxicities of PFOS precursor molecules may be due to PFOS accumulation as opposed to the precursor molecules themselves, or to any of the intermediary metabolites leading to PFOS. This conclusion can not be verified until a careful quantitative evaluation of PFOS serum levels in relation to toxicity is completed. Thus, for the present, the data on the N-ethyl and N-MeFOSE alcohol derivatives is presented for completeness, and because they confirm, at least qualitatively, the PFOS results.

4.2 Human Exposure

4.2.1 Background

The data on PFOS levels in human serum samples are presented in this section. The presence of organic fluorine in human serum was observed 30 years ago. The advancement of analytical chemistry has had a significant influence on knowledge of fluorocarbons in human sera. The techniques developed and used by researchers in the 1960's and 1970's were time-intensive, requiring hours for a single analysis. The methods were generally nonspecific, measuring "organic fluorine compounds" (any compound having fluorine covalently bonded to carbon) rather than specific molecules. The development of a rapid analytic technique in the late 1970's decreased analytic time for total organic fluorine to under an hour, allowing large-scale medical surveillance of production employees at higher detection limits (about 0.5 parts per million organic fluorine) that were adequate for the levels found in occupationally exposed individuals. The advancement of chromatographic/mass spectroscopy technology enabled, by the early 1990's, rapid analysis of specific fluorochemicals from small volumes of sera. This technology was first used in medical surveillance in 1993. Detection limits for PFOS in serum were lowered to 50 parts per billion by 1997.

This section presents: 1) a brief summary of the historical information regarding organic fluorine in human sera; 2) data from 3M employees involved in fluorochemical production; and 3) data from various sources of human sera that may represent non-occupational exposures.

Historical Finding of the Organic Form of Fluorine in Blood

Taves (1968a) described two forms of fluorine in serum, one that was exchangeable with radioactive fluorine-18 and one that was not. Pothapragada et al. (1971) also described two forms, ionic and nonionic. Taves (1968b) showed that the non-exchangeable fluorine was bound to albumin. This finding, along with results of extraction and precipitation and the need for ashing to release this form of fluorine, led to the conclusion that the non-exchangeable or nonionic fluorine was "organic", i.e. covalently bound to carbon (Taves et al. 1976). Using NMR spectroscopy, these authors tentatively identified a component of the organic fluorine as perfluorooctanoic acid (PFOA). There was some variation in the observed spectra from an authentic sample of PFOA, however, leading the authors to suggest that branching, or the presence of a sulfonate, was possible. Subsequent efforts by 3M researchers identified both PFOS and PFOA in blood.

A number of studies over the past 25 years reported levels of organic fluorine in human blood serum from presumably non-occupationally exposed persons. Table 4-1 presents the study author, level measured, populations studied and methods of analysis. The variety of methods used for determination of fluorine suggests that some caution be used in interpreting results. All reported means were in the tens-of-parts per billion levels. The average of reported values from United States sources is 0.038 ppm.

Table 4-1. Historical Findings of Serum Organic Fluorine Levels in the General Population

Year	Author	OF* (ppm)	N	Method**	Source
1972	Guy	0.030	65	Ash	US
1975	Pothapragada	0.036	2	O bomb	US
1976	Guy, Taves	0.025	106	Ash	US
1978	Belisle	0.020	9	O bomb	US
1979	Singer	0.045	264	Ash	US
1980	Paez	0.085	Pooled	Ash	Argentina
1980	Ubel	0.045	4	Mod O bomb	US
1981	Belisle	0.011	8	O bomb	China
1989	Yamamota	0.032	11	LOPA	Japan
* Organic fluorine, specific identities not provided.					
** Varied methods were used to measure organic fluorine. See papers for details.					

4.2.2 Occupational Exposures

3M manufactures POSF, a starting material for other fluorochemicals that may then degrade or metabolize, to an undetermined degree, to PFOS. Employees may be exposed by one or more routes (i.e., inhalation, skin contact/absorption, or ingestion) to fluorochemicals in the manufacturing environment. The primary route of exposure varies among employees and depends on several factors, including process conditions, job tasks, work location, personal hygiene, personal habits and general work practices. Exposure estimation has been exceedingly difficult when multiple sources of exposure are probable. Biological monitoring data (e.g., serum levels) may address this problem as it allows for an internal measurement assessment from all exposure sources. Biological monitoring data are especially relevant where they reflect dose to the target organ (e.g., liver). This is likely to be the situation for PFOS as it has been observed in cynomolgus primates that the liver to serum ratio is approximately 1:1 up to serum levels approaching 100 ppm (see section 4.3.1).

Table 4-2 presents PFOS serum values obtained from plant employees in Decatur (Alabama, USA), Antwerp, (Belgium), and Sagamihara Japan [RS6, RS8, RS9]. The Decatur and Antwerp plants are involved in fluorochemical production, whereas the Sagamihara facility handles, but does not produce, sulfonated perfluorochemicals. Although voluntary biennial medical surveillance of production employees began in the late 1970's, routine specific measurement of serum PFOS levels did not commence until the mid-1990's. [Note: Prior to this time period, total organic fluorine was measured. Fluorine is 65% of the molecular weight of PFOS. The contribution of PFOS to organic fluorine, in ppm, will therefore be 0.65 x (PFOS value in ppm). PFOS was measured in 5 Decatur employee sera samples in 1979 but analytical techniques were too time consuming and required too large a volume of serum for routine medical surveillance.]

A voluntary medical surveillance program may not lend itself to an adequate characterization of the distribution of employee fluorochemical serum levels if participation rates are low as a consequence of respondent (i.e., selection) bias. Because the extent of this potential bias was unknown for the fluorochemical medical surveillance programs at the Decatur and Antwerp

manufacturing plants, a cross-sectional study was designed in 1998 to randomly sample employees at the Decatur (Alabama) manufacturing site (RS6). The purpose of the study was to determine whether the distribution of employee serum fluorochemical levels observed in the voluntary medical surveillance programs was a reasonable reflection of the plant population. A total of 232 Decatur employees were randomly sampled; 186 (80%) participated. Respondents and non-respondents ($n = 46$) were comparable with respect to age, gender and employment duration. Of the randomly sampled participating employees, 126 were from the chemical plant and 60 from the film plant (where fluorochemicals were not manufactured, although in one production run a fluorochemical is occasionally used). Blood levels of the fluorochemicals were analyzed according to the employees' demographics, current or longest-held job, and the locations of jobs within the chemical plant. Serum PFOS levels were weakly associated in a linear fashion with years worked in the chemical plant ($r^2 = 0.11$). No positive association was observed between frequency of self-reported hand-to-mouth usage or hand cleanliness and serum PFOS levels.

Table 4-2. PFOS Serum Concentrations: Occupational Populations

<i>Location & Year</i>	<i>Mean (ppm)</i>	<i>Range (ppm)</i>
Decatur (Alabama): Plant		
1994 (n = 100) ^a	2.44	0.25 – 12.83
1997 (n = 84) ^a	1.96	0.10 – 9.93
1998 Chemical (n = 126) ^b	1.51	0.09 – 10.60
1998 Film (n = 60) ^b	0.17	0.02 – 0.95
Antwerp (Belgium): Plant		
1995 (n = 88) ^a	1.93	0.00 – 9.90
1997 (n = 65) ^a	1.48	0.10 – 4.83
Sagamihara (Japan): Plant		
1999 Production (n = 32) ^c	0.14	≤0.03 – 0.63
1999 Management (n = 32) ^c	0.04	≤0.03 – 0.06
^a Voluntary study. Estimated participation rates < 50% of employees who routinely or periodically worked in the chemical plant. ^b Random sample cross-sectional study. ^c Voluntary cross-sectional study.		

The distribution of serum PFOS levels measured in this random sample was similar to that previously reported from the voluntary medical surveillance examinations. Thus, it was unlikely that employee serum PFOS levels higher than that observed in the medical surveillance programs existed in these fluorochemical manufacturing populations. Results from this random sample assessment are currently being used in the construction of an exposure matrix for the updated retrospective cohort mortality study of the Decatur employee population (see Section 4.4.3). These biological monitoring data were also used in the ongoing analysis of health claims comparison analysis between chemical and film plant employees from 1993-1998 (see Section 4.4.3).

There are many industrial uses of fluorochemical-containing compounds, and it is likely that PFOS could be found in serum samples from workers in these various user industries. 3M production employees are exposed to concentrated PFOS precursors whereas downstream workers are generally exposed to PFOS precursors in concentrations of less than 1% as residual starting materials in polymer products. This suggests downstream workers should have lower serum levels of PFOS than those observed in 3M production employees. The Sagamihara sampling data is consistent with this viewpoint.

4.2.3 Non-occupational Exposures

In 1998, three questions were addressed to delineate the extent to which PFOS might be present in the blood of members of the general population not exposed occupationally to precursor molecules:

- 1) Is PFOS detectable in human blood samples from a corporate-based 3M employee population known not to have worked in 3M fluorochemical manufacturing or use plants?
- 2) Is PFOS detectable in pooled blood samples from the general population and from

- different geographical locations across the United States and outside the United States?
- 3) Is PFOS detectable in historical human blood samples collected prior to the introduction of POSF-based chemicals into the marketplace?

To address the first two of questions several studies were conducted and the data from them are summarized in Table 4-3. PFOS could be detected in the serum of adults in the United States (RS27, RS29, RS30) and could be detected in children using a very small amount of serum (0.1 ml) (RS31). PFOS could also be detected in the serum of adults in Belgium, the Netherlands, Germany, Sweden and Japan (RS27, RS29, RS28). No inferences could be developed regarding associations between PFOS and demographics (e.g., age, and gender), time trends and source(s).

Table 4-3. PFOS Serum Concentrations: Non-Occupational Populations (1998-1999)

<i>Population</i>	<i>Mean (ppm)</i>	<i>Range (ppm)</i>
3M Corporate Center (n = 31)	0.047	0.028 - 0.096
Tokyo (Japan): Head Office (n = 30)	0.052	≤0.03 – 0.097
<i>Commercial Laboratories</i> ^a		
Intergen (pooled: approx 500 donors)	0.044	0.043 - 0.044
Sigma (pooled: approx 200 donors)	0.033	0.026 – 0.045
35 Lots Commercial Labs	0.035	0.005-0.085
US Blood Banks (n = 18 pooled samples) ^b	0.030	0.009 - 0.056
European Blood Banks (pooled samples) ^c		
Belgium (n = 5)	0.017	0.005-0.022
Netherlands (n = 6)	0.053	0.039-0.061
Germany (n = 6)	0.037	0.032-0.046
Sweden (n = 39 individuals) ^d		
28 individuals	< 0.032 (LLOQ)	-
11 individuals	0.048	0.032-0.085
Children (U.S., n = 10 individuals) ^e	0.054	0.031 - 0.115
^a Donor pool information, such as age, sex, or geographical location, not available. ^b 3 to 6 samples per blood bank, 5 to 10 donors per sample. Geographically distributed across the continental US and Alaska. Not a statistically valid sample of the US population (RS29). ^c pooled samples, 10 donors per sample in Belgium and the Netherlands; 30 donors per sample in Germany (RS28). ^d RS7 ^e Pilot analysis. Limited serum (0.1 ml) from children aged 6 or 12 who were enrolled in a group A streptococcal study. Study is in progress. RS31		

To address the third exploratory question, historical samples were obtained to learn whether PFOS could be detected in samples obtained prior to the manufacture of precursor compounds (the late 1940's). PFOS was not detected in ten pooled samples (10 donors per sample) from U.S. military recruits of the Korean War era (1948-1951). Analysis of 10 Swedish samples collected in 1958 resulted in a range of values from non-detect to 2 ppb. A limited number of samples taken during the conduct of several post-1969 epidemiological studies conducted in the United States were analyzed and serum PFOS levels ranging from non-detect to 59 ppb could be identified. Analysis of serum samples from two Chinese rural provinces (Linxian, Shandong) that were collected in 1984 and 1994 showed no detectable PFOS.

It appears from this limited sampling that PFOS presence in human serum coincides with the introduction of PFOS-precursor molecules; the conclusion is highly uncertain because of the sparseness of the database. Definitive statements regarding time trends cannot be made at this time.

4.2.4 Forthcoming Studies

Several additional studies to address human exposure are being sponsored. These include the following:

- 1) An analysis of 500 individual, contemporary adult blood samples from the American Red Cross in order to obtain a cross sectional analysis of serum PFOS levels in adults by age, gender and in up to 5 U.S. geographical locations;
- 2) An analysis of approximately 250 individual elderly adult samples (ages 65 - 99) from an ongoing study of cognitive function among the elderly in the Seattle, Washington area;
- 3) An analysis of 600 children's samples (ages 2 - 12) from 23 states who were enrolled in a group A streptococcal clinical trial in the mid-1990's;
- 4) A comparison of liver to serum PFOS ratios from 30 human organ donors; and
- 5) A time trend analysis of fluorochemicals, including PFOS, in sera that were collected in Maryland in 1974 and 1989 from the same 59 individuals as well as 120 different individuals in the two time periods (ages 20-60+).

All of these research efforts are scheduled for completion by the first quarter of 2001.

4.2.5 Indirect Exposure via the Environment

Human exposure via the environment is discussed as part of section 3.1.1.

4.3 Effects on Human Health

4.3.1 Mode of Action, Toxicokinetics, Metabolism

4.3.1.1 Mode of Action

The mechanisms governing the biological responses to PFOS exposure observed in toxicological studies are currently under investigation. Several studies provide clues to the potential modes of toxicity. Competition with fatty acids for carrier protein binding sites (Nabbefeld et al 1998, Nabbefeld 1988), cholesterol synthesis (Haugham and Øystein, 1992) and bioenergetics (Wallace and Starkov, 1998) have been studied. In addition PFOS has been reported to induce peroxisome proliferation (Sohlenius et al., 1993, Ikeda et al., 1987). No conclusions have been reached on the importance of any of these possible mechanisms toxicity at this time.

4.3.1.2 Toxicokinetics and Metabolism

The absorption, tissue distribution, potential metabolism and excretion of PFOS have been studied most extensively in rats by both radiolabel and direct quantitation. Data relating oral dose to serum and liver concentrations of PFOS in the cynomolgus monkey during dosing and recovery is available from direct quantitation. In addition, serum PFOS concentrations in retired 3M chemical workers have been followed in an attempt to estimate an elimination rate constant for the human.

Absorption

PFOS is well absorbed from the digestive system. A radiolabel study in which adult male rats were given a single oral dose of 4.2 mg/kg [¹⁴C]PFOS demonstrated that > 95 % of the dose was absorbed in the first 24 hours (RS60).

Dermal absorption of PFOS appears to be possible but is limited. In one study, PFOS was applied under occlusion to approximately 40 % of the body surface area of male and female New Zealand White rabbits at 5,000 mg/kg and left in place for 24 hours (RS54). Blood samples were obtained on days 1, 7, 14, and 28. Analysis for total blood fluoride was performed on day one and day 28 samples from a single male and single female. Total serum fluoride values for the male were 10.3 ppm for day one and 130 ppm for day 28. The respective values for the female were 0.9 ppm and 128 ppm. Although this study indicated some dermal absorption at a high dose, it is limited in that the values from only two animals were measured, and only from the day one and day 28 samples (O'Malley and Ebbens, 1981).

No quantitative information is available on the absorption of PFOS from inhalation exposure. Due to the exceptionally low vapor pressure of PFOS, inhalation exposure would be unlikely. If it does occur it would be associated with aerosols or particulates containing PFOS.

Distribution

PFOS distributes predominantly to the blood and liver, with liver concentrations being potentially several times higher than serum concentrations, depending on species and dose. A radiolabel study in which adult male rats were given a single intravenous dose of 4.2 mg/kg [¹⁴C]PFOS demonstrated that the carbon-14 in liver and plasma represents 25 and 3% of the dose, respectively, after 89 days. During the 89-day post-dose period, the rats excreted a mean of 30.2% of the total carbon-14 via urine and the mean cumulative fecal excretion was 12.6%. At 89 days, mean tissue concentration of total carbon-14 expressed as µg [¹⁴C]PFOS equivalents/g were: liver, 20.6; plasma, 2.2; kidney, 1.1; lung, 1.1; spleen, 0.5; and bone marrow, 0.5. Lower concentrations (<0.5) were measured in adrenals, skin, testes, muscle, fat and eye. No radioactivity (<0.05) was detected in brain. (RS57).

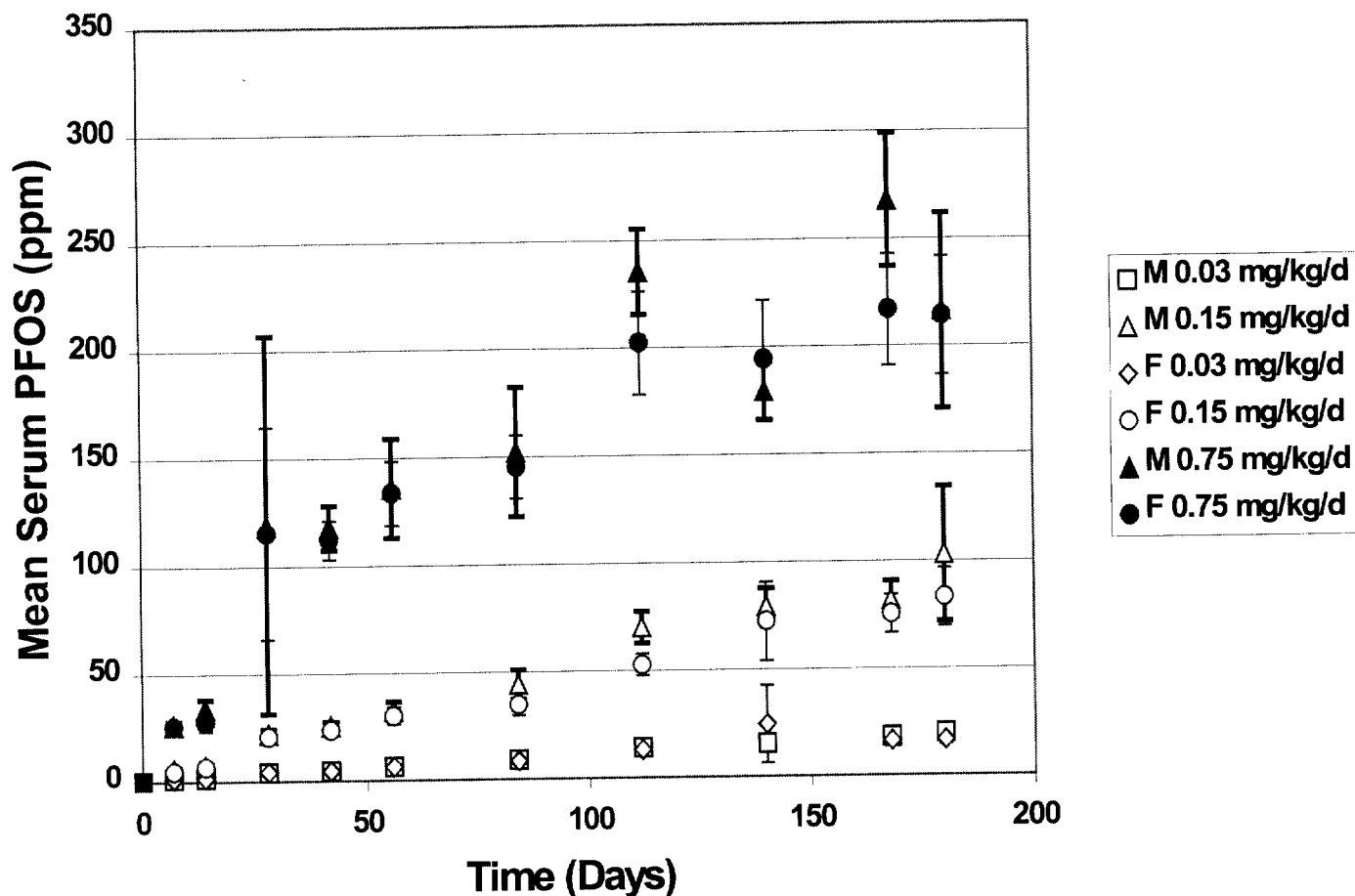
Significant enterohepatic circulation of PFOS has been reported as evidenced by the fact that cholestyramine (four percent by weight in diet) treatment of rats given single intravenous doses of PFOS increased fecal elimination 9.5 times over control (RS58).

The volume of distribution in male and female cynomolgus monkeys on daily dosing has been

estimated as 0.2 L/kg (RS34). Monkeys dosed by oral capsule with PFOS (0.02 and 2.0 mg/kg/day) demonstrated a linear (r-squared > 0.99) increase in serum concentration throughout the exposure period (28 days). There was no apparent sex difference and the individual slopes of the cumulative PFOS dose versus serum PFOS concentration curves appeared to be virtually identical for the monkeys in the two dose groups. The average slope of the curve in the 0.02 mg/kg/day group (n = 6) was 5.22 ± 0.74 ppm PFOS in serum per mg/kg cumulative dose. The two monkeys in the 2.0 mg/kg/day dose group had an average slope of 5.40 ± 0.61 ppm PFOS in serum per mg/kg PFOS cumulative dose. At the end of the 28-day dosing period, serum concentration in the 0.02 and 2.0 mg/kg/day groups were approximately 3 ppm and 300 ppm. These data suggest a volume of distribution of 0.2 L/kg for continuous dosing over a two order of magnitude range.

In a 26-week capsule-dosing study in cynomolgus monkeys, a similar pattern of increasing serum concentration with cumulative dose was observed (RS32). At the lower doses, 0.03 and 0.15 mg/kg/day, serum levels increased in fairly linear fashion and reached means of 18 ppm and 85 ppm at the end of the dosing period, respectively. At the 0.75 mg/kg/ dose, the increase in serum PFOS appeared to saturate, reaching a mean of 215 ppm by the end of the dosing period. The accumulation of PFOS in the serum of male and female monkeys appeared similar in all dose groups. Figure 4.1 shows the trend in group mean serum concentrations during the dosing period.

Figure 4-1. Mean serum PFOS concentrations for males and females during 182 days of oral dosing at either 0.03, 0.15 or 0.75 mg/kg/day.



Male and female CR:CD rats continuously exposed to PFOS in the diet for 14 weeks (RS39) also showed a linear relationship between dose and serum concentration as is shown by the data in Table 4-4.

Table 4-4. Serum concentrations in male and female rats after 14-weeks of exposure to PFOS in the diet.

Males	Dose Group ^a			
	0.5 ppm (0.036 mg/kg) ^b	2.0 ppm (0.1 mg/kg) ^b	5.0 ppm (0.35 mg/kg) ^b	20 ppm (1.37 mg/kg) ^b
Estimated Cumulative Dose (mg/kg)	3.2	12.6	31.1	123.3
Serum PFOS concentration	4.22 ppm (8.5 µM)	17.9 ppm (35.9 µM)	45.6 ppm (91.4 µM)	134 ppm (304.6 µM)

Females	Dose Group ^a			
	0.5 ppm (0.04 mg/kg) ^b	2.0 ppm (0.16 mg/kg) ^b	5.0 ppm (0.41 mg/kg) ^b	20 ppm (1.6 mg/kg) ^b
Estimated Cumulative Dose (mg/kg)	3.6	14.6	37.0	144.0
Serum PFOS concentration	6.7 ppm (13.3 µM)	26.9 ppm (53.9 µM)	62.9 ppm (126.0 µM)	216 ppm (432.9 µM)

^a Dietary concentration of PFOS in ppm (µg PFOS/g diet)

^b Estimated dose based on feed analysis, feed consumption and body weight during study

Distribution across the placenta and exposure of the fetus *in utero* has been demonstrated. Table 4-5 presents serum concentrations from a pharmacokinetic study conducted during pregnancy (RS52). Dams were treated with vehicle only (control), 0.1, 0.4, 1.6 or 3.2 mg/kg/day PFOS by oral gavage for 42 days prior to mating, during mating and during gestation. Serum was obtained from dams prior to mating and on gestation days 7, 14 and 21. Serum was obtained from pooled fetal litters on gestation day 21. The pre-mating and gestation day 21 values are represented in the Table 4-5.

Table 4-5. Maternal and fetal PFOS serum and liver PFOS concentrations associated with gestation (rats).

Dose Group Mg/kg/d ^a	Media	Mean Serum and Liver [PFOS] in ppm		
		Pre-mating ^b	Gestation Day 21	
		Dams (n)	Dams (n)	Fetal Litter (n)
0.1	Serum	10.3 ± 1.26 (16)	4.91 ± 1.23 (7)	10.5 ± 1.01 (7)
	Liver	---	23.4 ± 3.76 (8)	9.17 ± 1.08 (7)
0.4	Serum	47.1 ± 5.00 (16)	30.3 ± 17.0 (6)	39.7 ± 5.90 (5)
	Liver	---	107 ± 25.2 (7)	42.5 ± 19.4 (6)
1.6	Serum	185 ± 14.0 (16)	158 ± 86.6 (4)	117 ± 14.5 (2)
	Liver	---	440 ± 316 (5)	100 ± 30.6 (2)
3.2	Serum	367 ± 23.6 (16)	180 ± 41.5 (6)	191 ± 26.4 (6)
	Liver	---	598 ± 83.9 (6)	265 ± 70 (6)

- a) Dams were treated daily by oral gavage for 42 days prior to mating, during mating and through gestation.
b) After 42 days dosing

The apparent lack of correspondence in the relationship of dose to serum level when comparing the 14-week dietary study and the reproduction study may be explained by the fact that the reproduction study was a gavage study, as opposed to the 14-week dietary study. In the latter study, mg/kg dose levels were estimated based on feed analysis, feed consumption and body weight during the course of the study. Feed consumption is an error-prone measurement, due to the possibility of spilled (unconsumed) feed. Dietary doses also represent a continuous source of exposure as opposed to pulse dosing by gavage.

Serum levels at the end of lactation after exposure in the PFOS reproduction study are represented in Table 4-6.

Table 4-6. Comparison of serum PFOS levels at the end of lactation after dosing rats with PFOS for 42 days pre-mating, during mating, gestation and through lactation (approximately 13 weeks).

PFOS Dose	[PFOS] in Serum
0.0 mg/kg	0.037 ± 0.020
0.1 mg/kg	5.28 ± 0.358
0.4 mg/kg	18.9 ± 1.30
1.6 mg/kg	82 ± 17.5

There is conclusive evidence from the cross-fostering study that PFOS distributes to milk and also crosses the placenta. These data are discussed in Section 4.3.4.

Metabolism

PFOS is not known to undergo further metabolism or to form conjugates. Preliminary data from analysis of urine, feces and tissues of rats as well as the inherent stability of perfluorinated anions suggest that PFOS is not metabolized (Johnson et al., 1984). Analysis by LC/MS of serum and liver samples from recent studies has not revealed any evidence of metabolism.

Certain chemicals made from perfluorooctanesulfonyl fluoride (POSF) may undergo a degree of metabolism to PFOS. For example, N-EtFOSE can be metabolized to PFOS. In one study, in which rats were administered ^{14}C -N-EtFOSE in feed, at least 28% of the radioactivity found in the liver at 48 hours was PFOS (RS56). This represented 4.4% of the administered dose.

Excretion

Single intravenous doses (mean 4.2 mg/kg) of [^{14}C]PFOS in 0.9% NaCl were administered to male rats (RS57). By 89 days after dosing, 30.2% of the administered ^{14}C had been excreted in the urine and 12.6% had been excreted in the feces. Whole body elimination in the male rat appeared to be biphasic. Initial redistribution from the plasma yielded a plasma elimination half-life of ^{14}C of 7.5 days following single oral administration of [^{14}C]PFOS (mean dose 4.2 mg/kg) to male rats (RS60). In the aforementioned intravenous study, elimination of only 42.8 % of the dose through urine and feces after 89 days indicates that the half-life of elimination from the body is > 89 days in the male rat.

Cynomolgous monkeys have been followed for one year in recovery after six months of daily oral dosing by capsule at 0.15 or 0.75 mg/kg/day (RS32). While the numbers of animals are limited (two per sex per dose group), the values suggest mean serum elimination half-lives of 189 days for the mid-dose recovery group and 175 days for the high-dose recovery group. There are likely no true differences in serum PFOS elimination rates between the mid-dose and high-dose groups. A low-dose recovery group was not part of the study design.

Fecal and total excretion of ^{14}C were markedly increased in male rats administered cholestyramine (~ 2.7 g/kg/d) in their diet following single intravenous doses of [^{14}C]PFOS (Johnson and Gibson, 1984 and RS58). The results suggest that there was significant enterohepatic circulation of PFOS. Cholestyramine administered at 4% by weight in feed to male rats decreased the retention of carbon-14 in liver, plasma, and red blood cells and increased the elimination of carbon-14 via feces after iv dosing with PFOS- ^{14}C . Groups of five rats were dosed intravenously with PFOS- ^{14}C (mean dose, 3.4 mg/kg). Rats were sacrificed at 21 days post dose. The mean liver, plasma, and red blood cell concentration as well as fecal and urinary excretion of ^{14}C for cholestyramine-treated rats were compared to mean control rat values. Mean cholestyramine-treated rat ^{14}C concentrations in liver (9.4 $\mu\text{g/g}$), plasma (0.9 $\mu\text{g/ml}$), and red blood cells (0.3 $\mu\text{g/g}$) represent a decrease from mean control rat concentrations of 3.8, 7.7, and 6.0 fold, respectively. Fecal elimination (75.9% with cholestyramine treatment) was increased 9.5 fold. The extent of urinary ^{14}C elimination, as a result of the relatively high rate of fecal elimination of ^{14}C was lower in cholestyramine-treated rats. The extent of total elimination of ^{14}C (urine plus feces) was higher in the cholestyramine-treated rats.

Human Data

Serum PFOS levels in three retired male 3M chemical workers followed for five and one-half years suggested a mean serum elimination half-life of 1,428 days. A recent initial analysis of 27 3M fluorochemical production workers retirees' sera, collected three times over a 12-month period, has suggested that the serum elimination half-life of PFOS in the human may be much lower, approximately 300 days [RS53]. The range of the initial serum PFOS levels in these retirees was from 0.2 to 4.6 ppm. These retirees have their serum collected and analyzed every six months. More definitive serum PFOS half-life estimates are expected within the next year upon collection of two more samples.

Summary

The data provide no evidence for PFOS metabolism in any species. PFOS is readily absorbed after oral exposure but absorption by the dermal route is low. There is evidence that absorbed PFOS is bound to protein and distributed primarily in blood and liver. PFOS undergoes entero-hepatic recirculation. There is slow whole body elimination in both sexes. At lower and moderate doses, body-burden is proportional to cumulative dose. In rat studies it is clear that PFOS can traverse the placenta and expose the fetus *in utero*. PFOS is also distributed in the milk of lactating females.

4.3.2 Acute Toxicity Studies

Numerous reports of acute studies of PFOS have been performed. Data from an inhalation study in rats, two oral studies of rats, one dermal study of rabbits and one dermal and eye irritation study of rabbits are noted below.

Inhalation Exposure

In a study to determine the median lethal concentration (LC_{50}), Rusch et al. (1979) administered the potassium salt of PFOS as dust in air to Sprague-Dawley rats at levels of 1.89 to 45.97 mg/l PFOS. An LC_{50} of 5.2 mg/l was estimated from this study (RS2).

The rats in all 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. The most significant treatment related abnormality was 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.

Oral

The study of Dean et al 1978 determined an acute oral LD_{50} and 95% confidence limits of

251 (199-318) mg/kg. The study report by Gabriel 1978, tested only two doses and determined the acute oral LD₅₀ was greater than 50 mg/kg and less than 1500 mg/kg (RS3).

Dermal Exposure

No significant toxicity was observed in a 1979 percutaneous absorption study in which male and female albino rabbits were dosed dermally under occlusion with 5000 mg/kg PFOS for 24 hours and observed for 28-days post-dose (RS54).

4.3.3 Repeated Dose Toxicity

4.3.3.1 PFOS

PFOS has been studied in 90-day subchronic dietary studies in rats (RS37), in a 90 day gavage study in rhesus monkeys (RS35, RS36), and in a 26-week oral (capsule) study in cynomolgus monkeys (RS32). A two-year chronic feeding study in rats is currently in final stages of completion (RS39).

The studies in rhesus monkeys and the 90-day rat study were reported in 1978. The more recent chronic study in rats and the 26-week study in cynomolgus monkeys were also designed to provide serum data. The earlier studies are summarized in Table 4-7, and it can be seen that the pattern of toxic effects observed in those studies is similar to that seen in the more recent studies. The older studies included much higher doses on both rats and primates. Areas of overlap on dose showed consistency of toxic effects. In the following, the recent studies are described in detail.

In the recent 26-week cynomolgous study, groups of six monkeys of each sex (4/sex at low dose) received PFOS by capsule at doses of 0, 0.03, 0.15, and 0.75 mg/kg/day (RS32). Two animals per sex from the control, 0.15 and 0.75 mg/kg/day groups were assigned to a recovery group and were followed for at least 52 weeks following the last administration of PFOS. At the end of the dosing period, high-dose females showed reduced body weights compared to controls, but the differences were no longer obvious by the end of the recovery period.

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 appeared to have lower high density lipoprotein cholesterol during treatment. HDL cholesterol values, however, were only determined on study days 153 and 182, and no prestudy values are available for comparison. The effect on total cholesterol was reversed within 5 weeks of recovery and high density lipoprotein cholesterol

increased within 9 weeks of recovery. Triiodothyronine values were lower in males in the 0.75 mg/kg/day dose group on day 182. However, total thyroxine and thyroid stimulating hormone levels were normal and thyroid gland showed normal histology.

Perhaps related to the observed decrease in cholesterol at the 0.75 mg/kg dose was a progressive decrease in estradiol in males.

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 which 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 were reversible.

The LOAEL for this study is 0.75 mg/kg/day based upon death, liver effects, and effects on cholesterol. The NOAEL is 0.15 mg/kg/day. All effects appeared to be reversible.

Interim results at weeks 14 and 53 are available from the ongoing chronic rat study (RS39). That study involves groups of 60 or 70 CrI:CD[®] (SD) IGS BR rats of each sex fed diets containing PFOS at 0, 0.5, 2.0, 5.0, and 20.0 ppm. Through week 53, high dose females showed reduced body weight gain and reduced food consumption. Reduced serum cholesterol and increased serum alanine aminotransferase (males only) was seen in high-dose animals. Mildly increased urea nitrogen was seen in animals fed 5 or 20 ppm, and serum glucose was reduced in high dose males and females, and in males at 2 and 5 ppm at week 53.

At the 53-week sacrifice, high-dose rats showed increased absolute (males only) and relative liver weight. Centrilobular hepatocyte hypertrophy and midzonal to centrilobular vacuolation was increased in incidence in males at 20 ppm, and high-dose females showed an increased incidence of centrilobular hepatocyte hypertrophy and pigment.

Data on serum PFOS levels are available from the 26-week cynomolgus monkey study and for week 14 of the chronic rat study. The relationships between PFOS serum levels and toxic responses for these two studies are presented in Table 4-8. The serum levels presented in Table 4-8 represent preliminary analyses available as of July, 2000. These values will change in the final analytical reports based on adjustments for purity of the samples and other possible corrections.

Table 4.7. Summary of Repeat-Dose Studies for PFOS

Study	Species (Strain)	Dose (units)	n	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology (M = Male; F = Female)
90-Day Dietary (Goldenthal et al. 1978a)	Rat (CD)	0 (ppm)	5 M / 5 F	■ No effect ■ ↓ body weight, ↑ plasma glutamate-pyruvate transaminase, ↑ plasma glutamate-oxalacetate transaminase, liver discoloration ■ 3 deaths; ↑ sensitivity to external stimuli, red material around the eyes or mouth, ↓ food consumption, ↑ plasma creatinine phosphokinase, ↑ alkaline phosphatase, ↑ blood glucose, ↑ blood urea nitrogen, ↓ hemoglobin, ↓ hematocrit, ↓ erythrocyte count, ↓ reticulocyte count (F), ↓ leukocyte count, liver enlargement, necrosis & hepatocellular hypertrophy, stomach discoloration & hemorrhage ■ Death; emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, splenic lymphoid follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy & dermal acanthosis, hyperkeratosis ■ Death; hunched posture ■ Death; hypoactivity
		30	5 M / 5 F	
		100	5 M / 5 F	
		300	5 M / 5 F	
		1000 3000	5 M / 5 F 5 M / 5 F	
14-Week Dietary (Part of ongoing 2-yr study, Covance 1999a)	Rat (Sprague Dawley)	0 (ppm)	10 M / 10 F	■ No effect ■ No effect ■ No effect ■ Hepatocellular hypertrophy and vacuolization in M at 5 ppm ■ ↓ body weight, ↓ cholesterol (M), ↑ liver weight, enlarged & vacuolated liver cells, ↑ palmitoyl CoA oxidase activity
		0.5	10 M / 10 F	
		2	10 M / 10 F	
		5	10 M / 10 F	
		20	10 M / 10 F	

Table 4.7. Summary of Repeat-Dose Studies for PFOS

Study	Species (Strain)	Dose (units)	n	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology (M = Male; F = Female)
90-Day Gavage (Goldenthal et al. 1979)	Rhesus Monkey	0 (mg/kg/day)	2 M / 2 F	■ No effect ■ Death within 11 – 20 days; ↓ body weight, marked weakness, anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage & lipid depletion of adrenal cortex ■ Death within 7 – 10 days; ↓ body weight, marked weakness, anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage & lipid depletion of adrenal cortex ■ Death within 3 – 5 days; ↓ body weight, marked weakness, anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage & lipid depletion of adrenal cortex ■ Death within 2 – 4 days; ↓ body weight, marked weakness, anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage & lipid depletion of adrenal cortex
		10	2 M / 2 F	
		30	2 M / 2 F	
		100	2 M / 2 F	
90-Day Gavage (Goldenthal et al. 1978b)	Rhesus Monkey	0 (mg/kg/day)	2 M / 2 F	■ No effect ■ No Adverse Effect; Slight & intermittent ↓ activity in 3 of 4 animals ■ Blood & mucus in stools, diarrhea, dehydration, tremors, ↓ body weight, ↓ cholesterol, marked ↓ activity ■ Death within 7 weeks; marked ↓ cholesterol, black or bloody stool, dehydration, rigidity, convulsions, prostration, ↓ serum cholesterol, diffuse lipid depletion of adrenals, atrophy of pancreatic exocrine cells & atrophy of submandibular salivary gland serous alveolar cells, ↓ serum alkaline phosphatase, ↑ SGOT
		0.5	2 M / 2 F	
		1.5	2 M / 2 F	
		4.5	2 M / 2 F	

Table 4.7. Summary of Repeat-Dose Studies for PFOS

Study	Species (Strain)	Dose (units)	n	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology (M = Male; F = Female)
26-Week Capsule (Thomford 2000)	Cynomolgus Monkey	0 (mg/kg/day)	6 M / 6 F	■ No effect
		0.03	4 M / 4 F	■ No effect
		0.15	6 M / 6 F	■ No adverse effect
		0.75	6 M / 6 F	■ 2 deaths; ↓ body weight, ↓ food consumption, black & mucoid stools, ↓ activity, dehydration, labored respiration, ↓ cholesterol, ↓ HDL cholesterol, ↓ triiodothyronine (normal T4 & TSH), ↓ estradiol, liver discoloration, ↑ liver weight, vacuolated liver cells

Table 4.8 PFOS Toxicity Data for Mammals:
Key Observed Effects, Serum and Liver PFOS Concentrations, and Cumulative Dose

Group¹	Observed Effect	Serum PFOS Concentration (ppm)	Liver PFOS Concentration (ppm)	Cumulative Dose (mg/kg)
26-week Capsule-Dosing Study in Cynomolgus Monkeys (Thomford 2000)				
0.03 mg/kg/d	NOEL	18	25	5.46
0.15 mg/kg/d	NOEL	85	80	27.3
0.75 mg/kg/d	Hepatomegaly; hepatocyte enlargement	> 100, < 300	415 (average)	> 27.3, < 137
0.75 mg/kg/d	Decreased cholesterol in females	> 134 ± 25	415 (average)	46.5
0.75 mg/kg/d	Decreased cholesterol in males	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Decreased T3 (normal T4 & TSH)	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Death or early sacrifice for 2/6 males	> 150, < 300	415 (average)	> 100, < 137
14-Week Interim of Chronic Dietary Study in Sprague Dawley Rats (Covance 1999a)				
0.5 ppm Males	NOEL	4.22	26.38	~ 4
0.5 ppm Females	NOEL	6.65	18.59	~ 3
2.0 ppm Males	NOEL	17.9	76.8	~ 17
2.0 ppm Females	NOEL	26.9	68.25	~ 13
5.0 ppm Males	Hepatocellular hypertrophy and vacuolization	45.6	386.53	24.4
5.0 ppm Females	NOEL	62.9	362.45	38.2
20 ppm Males	Hepatocellular hypertrophy and vacuolization; decreased cholesterol; increased AAT	134	599.94	106
20 ppm Females	Hepatocellular hypertrophy and vacuolization	216	617.52	141

4.3.3.2 N-EtFOSE

N-EtFOSE has been studied in ninety-day studies in rats (RS49) and rhesus monkeys (RS50). A two-year chronic toxicity study was completed in 1987. That study used a "wide range" material that included many short-chain fluorochemicals and was not representative of the typical material currently used in commercial products. A robust summary of this study is included (RS33). Subsequently, a two-year chronic study in rats using a more representative "narrow-range" product was initiated and is currently in progress (RS51). The pattern of toxic effects seen in these studies is similar to those seen with PFOS. The primary target is the liver, with dose-related increases in liver weight and macroscopic and microscopic liver lesions in rats.

In the 90-day rat study, N-EtFOSE was fed in the diet at levels of 0, 30, 100, 300, 1,000, 3,000 and 10,000 ppm to groups of five Charles River CD rats of each sex (RS49). All rats at the 1,000-, 3,000- and 10,000-ppm dosage levels died between days 9 and 29 of the study.

Findings in high dose animals were consistent with those found in animals exposed to high doses of PFOS. These included compound-related gross and microscopic liver lesions with consistent changes in biochemical parameters.

N-EtFOSE was administered to groups of two male and two female rhesus monkeys by gavage at dosage levels of 1, 3, 10 or 30 mg/kg/day (RS50). A control group received the vehicle, propylene glycol. On and after the second day of study, all the monkeys, including the control group showed anorexia, slight to marked decrease in activity and ataxia. The ataxia disappeared from all the monkeys directly after the amount of propylene glycol used was reduced from 5 to 2 ml/kg on study day 3.

Most of the monkeys, including those in the control group lost body weight early in the study. At 12 weeks of study all the monkeys were at or near the original body weight except for the groups receiving 10 and 30 mg/kg/day. At the end of the study the differences in the mean body weights of the treated and control groups were not statistically significant.

Microscopically, the adrenals from one male and two female rhesus monkeys at the 30 mg/kg/day dosage level had compound-related slight to severe lipid depletion, and the pancreas from the two female monkeys at the 30 mg/kg/day dosage level had compound-related moderate atrophy of exocrine cells. No liver lesions were seen, however.

In the ongoing two-year bioassay using a test material that is more representative of most material currently used in commercial products, groups of 60 or 70 CrI:CD®(SD)IGS BR rats of each sex were fed diets containing 0, 1, 30, 100, and 300 ppm N-EtFOSE (RS51). Due to excessive toxicity (reduced body weight, reduced food consumption) the 300 ppm group was terminated at about week 8 and the 1 ppm and additional concurrent control group were added. At sacrifice, the 300 ppm animals showed hematologic changes (reduced red blood cell count, hematocrit, and hemoglobin), clinical chemistry changes (lower glucose, globulin, and cholesterol, and higher BUN, albumin, total bilirubin (males only) aspartate aminotransferase (males only) and alanine aminotransferase), liver enlargement, and hepatocellular hypertrophy and necrosis, and hemorrhage in the liver.

A similar (though less severe) pattern of effects was seen at the 30 and 100 ppm groups at interim sacrifices at later measurement times. This pattern included: reduced body weight gain over the first 53 weeks; mildly decreased red blood cell count, hemoglobin, and hematocrit for females in the 100 ppm dose group; mildly lower glucose and mildly higher urea nitrogen for

animals given 100 ppm (predominantly at Weeks 14 and 27; moderately higher albumin and moderately lower globulin (predominantly at Weeks 27 and 53) in 100 ppm males. Hepatocellular hypertrophy and vacuolation were observed in 100 ppm animals at week 53, and hepatocellular hypertrophy was also seen at week 14 in males given 30.

Decreases in body weight and total serum cholesterol were prominent effects in this study. Male body weights for the 30 ppm and 100 ppm dose groups were significantly lower than control values through week 53 of the study. Females also experienced lower body weights. In the 3 ppm dose group females, transient but significantly lower body weights occurred on weeks 14, 15 and 16. Females in the 30 ppm dose group also had significantly lower body weights through week 29 and again on week 37 and weeks 43-53. The 100 ppm dose group females had significantly reduced body weight through week 53.

Cholesterol was significantly lower in males in the 3 ppm, 30 ppm and 100 ppm dose groups at week 14. This effect was present at weeks 27 and 53 in males in the 30 and 100 ppm dose groups. In contrast, females in the 30 and 100 ppm dose groups had significantly lower cholesterol only on week 14.

4.3.3.3 N-MeFOSE

N-MeFOSE has been studied in a 13-Week dietary study in Crl:CD® (SD) IGS BR rats at doses of 3, 30, and 100 ppm (20/sex/dose) (RS38). The only effect seen at 3 ppm was a slightly reduced body weight gain.

Effects seen at 30 ppm were: lower serum globulin, cholesterol and triglycerides in males; lower terminal body weight in males; increased liver-to-body weight ratio in males and females; increased absolute liver weight in females; increased incidence of centrilobular hepatocellular hypertrophy in males and females, and a slight increase in minimal hepatocellular vacuolation in males.

At 100 ppm, the same effects as at 30 ppm were seen (but at higher incidence and/or greater severity), plus lower serum cholesterol and triglycerides in females; lower hematocrit and higher BUN in both sexes; higher serum albumin in males; higher serum AAT in males increases in all liver weight parameters in both sexes (relative and absolute); liver coagulative necrosis, hepatocellular pigment, and erosion of small portions of stomach mucosa in both sexes (RS38).

4.3.3.4 Summary

PFOS, N-EtFOSE, and N-MeFOSE produce similar toxic effects in subchronic studies in rats and monkeys. The effects occurring at the lowest doses are liver cell hypertrophy, in some cases with vacuolation, a decline in weight gain, and a reduction in serum cholesterol levels. The most recent data, summarized in Table 4-9, provides dose-response information on these effects. The fact that the three compounds produce similar effects suggests that these effects may be due to the presence of PFOS and not the precursor compounds. This suggestive evidence can not be confirmed until all of the serum data have been collected and evaluated. Results from the chronic studies of PFOS and N-EtFOSE will become available in the future.

Table 4-9 Comparative Effects Among Three Perfluorooctanesulfonyl Fluoride-Based Chemicals

Effect	Dose at which effect occurs after 14 weeks of dietary compound administration in rats.		
	N-EtFOSE^a	N-MeFOSE^b	PFOS^c
Hepatocellular enlargement	30 ppm males 100 ppm females	30 ppm males 100 ppm females	5 ppm males 20 ppm females
Cholesterol lowering	3 ppm males 30 ppm females	30 ppm males 100 ppm females	20 ppm males -- ND females*
Body weight lowering	30 ppm males 3 ppm females ^d	30 ppm males 100 ppm females	20 ppm males 20 ppm females
Liver to Body weight increase	100 ppm males 100 ppm females	30 ppm males 30 ppm females	20 ppm males 20 ppm females

* ND, not detected.

^a N-Ethyl-perfluorooctanesulfonamido ethanol

^b N-Methyl-perfluorooctanesulfonamido ethanol

^c Perfluorooctane sulfonate

^d Slight but statistically significant effect occurring only at the terminal weighing

4.3.4 Reproductive and Developmental Toxicity

Developmental toxicity studies in rats and rabbits and 2-generation studies in rats for PFOS and N-EtFOSE were reviewed. The N-EtFOSE studies are included for comparative purposes since, as noted above (Section 4.3.1) it is believed to be readily metabolized to PFOS. In addition, the results of a PFOS cross-fostering study are presented and discussed. All studies employed the oral (gavage) route for administering the chemicals.

Developmental Studies

Separate studies with PFOS and N-EtFOSE assessed the effects of pregnant rat exposure on prenatal development of their embryos and fetuses. Each chemical caused maternal and fetal toxicity and in the case of N-EtFOSE, a low litter incidence of anatomical malformations were seen at very high doses. Initial studies with both chemicals reported a lesion in the lens of the eye in all treated groups (RS41, RS45). The causal association between this effect and chemical exposure was subsequently retracted by the study director when it was established that the "lesion" was an artifact associated with the method of free hand sectioning used in the fetal examination. These lesions were not observed in repeat studies in this laboratory. Additional prenatal developmental toxicity studies with PFOS and N-EtFOSE have been performed in the rat and rabbit. Key aspects of all studies are summarized in Tables 4-10 and 4-11.

Table 4-10.

Oral (gavage) PFOS Developmental Toxicity Studies				
Design	NOAEL*	LOAEL*	Effects	Reference
Rat SD Group size: 22 Dose:* 0,1, 5,10	<u>Mat.</u> 5 <u>Dev.</u> 10	<u>Mat.</u> 10, <u>Dev.</u> None	<u>Mat.</u> Body weight.	RS45
Rat SD Group size: 25 Dose:* 0,1, 5,10	<u>Mat.</u> 1 <u>Dev.</u> 1	Mat. 5 Dev. 5	<u>Mat.</u> Body wt. Clinical signs, g.i. lesions. <u>Dev.</u> Body wt, visc. anom., skel. var.	RS46
Rabbit NZW Group size: 22 Dose:* 0, 0.1, 1, 2.5, 3.75	<u>Mat.</u> 0.1 <u>Dev.</u> 1	<u>Mat.</u> 1 <u>Dev.</u> 2.5	<u>Mat.</u> Body wt. Abortions Dev. Body wt. Delayed ossification	RS44

* (mg/kg). Rats dosed on GD 6-15. Rabbits dosed on GD 7-20.

Results from the PFOS studies were similar. Maternal toxicity and developmental toxicity was consistently seen and expressed as reductions in maternal weight gain or fetal body weight. Reductions in food consumption commonly paralleled the effect on maternal weight. Fetal effects were primarily associated with maturational delays, e.g., skeletal variations and delayed ossification. Abortions were observed in rabbits at a dose of 2.5 mg/kg and higher. The lowest developmental toxicity NOAEL for rat and rabbit are the same, 1 mg/kg body weight. The maternal toxicity NOAEL was 0.1 and 1.0 mg/kg for rabbit and rat, respectively.

Table 4-11.

Oral (gavage) N-EtFOSE Developmental Toxicity Studies				
Design	NOAEL*	LOAEL*	Effects	Reference
Rat SD Group size: 22 Dose:* 0,25,37.5,75	<u>Maternal</u> 25 <u>Develop.</u> 25	<u>Maternal</u> 37.5 <u>Develop.</u> 37.5	<u>Mat.</u> Body wt., deaths at 75 <u>Dev.</u> Body wt, cleft palate, sternebrae malform.	RS41
Rat SD Group size: 25 Dose:* 0, 1, 5, 10, 20	<u>Maternal</u> 5 <u>Develop.</u> 5	Maternal 10 Develop. 10	<u>Mat.</u> Body wt. <u>Dev.</u> Body wt, delayed ossification	RS42
Rabbit NZW Group size: 18 Dose:*0,1,5,15	<u>Maternal</u> 5 <u>Develop.</u> 5	Maternal 15 Develop. 15	<u>Mat.</u> Body wt. <u>Dev. Fetal &</u> <u>Neonatal</u> Viability, resorptions	RS55
Rabbit NZW Group size: 22 Dose:* 0, 0.1, 1, 2.5, 3.75	<u>Maternal</u> 0.1 <u>Develop.</u> 1	<u>Maternal</u> 1 <u>Develop.</u> 2.5	<u>Mat.</u> Body wt. abortions <u>Dev.</u> Late resorptions	RS40

* (mg/kg). Rats dosed on GD 6-15. Rabbits dosed on GD 6-18 or 7-20.

The more recent rat study with N-EtFOSE (RS42) showed maternal and developmental toxicity expressed as effects on body weight, this is similar to that of the earlier study (RS41). Concordance is not good between the 2 rat studies as to the NOAEL or LOAEL doses. Morphological defects in fetuses were seen only at doses of 37.5 mg/kg and higher. The results of the rabbit studies (RS40, RS55) are similar as to the nature of the effects but there are differences as to doses that caused maternal toxicity. Fetal survival during the 24 hour incubation period was significantly lower at the 15 mg/kg dose (RS55). The rabbit and the rat study conducted at similar and slightly higher dose levels indicate that the primary early maternal effect is associated with depressed body weight. The lowest NOAELs occurred in the rabbit study; 0.1 and 1.0 mg/kg for maternal and developmental endpoints, respectively.

When results of the PFOS and N-EtFOSE developmental studies are compared, the type of effects, and doses that cause those effects, are similar. Fetal toxicity, as contrasted to anatomical malformations, characterizes the principal effect of both PFOS and N-EtFOSE. Maternal toxicity also occurs at doses associated with developmental toxicity. Rabbits exposed to N-EtFOSE have a tendency to abort litters or resorb fetuses. These effects are possibly causally linked to the maternal effect. This type of response was not prominent in rats exposed to either PFOS or N-EtFOSE.

Two-Generation Studies

Reproductive parameters generally were not affected by PFOS exposure in either generation of either sex in a 2-generation study in the rat (RS47). The exception was a decrease in implantations and litter size in F₀ females at 3.2 mg/kg, the highest dose tested. The early adverse response in adults and in pups is reduced body weight gain in both sexes. Most significant was the death of all F₁ pups in the perinatal period at the maternal dose of 3.2 mg/kg bw/day. Mortality was also seen in F₁ pups from dams that received 1.6 mg/kg. The dose-response for this effect is steep as demonstrated by viability indices (survival from birth to LD 4) of 98.7, 98.3, 98.3, 66.1 and 0.0% for the 0, 0.1, 0.4, 1.6 and 3.2 mg/kg dose, respectively. Severity of effect on F₁ pups in the lactation phase of the study resulted in post weaning dose groups being reduced to 0, 0.1 and 0.4 mg/kg. These 3 dose groups proceeded through a mating, pregnancy and postnatal evaluation phase until F₂ pups were 21 days of age. The F₁ rats in all these groups developed normally as measured by an array of developmental milestones, including neurobehavioral performance. Effects on reproduction, lactation and on postnatal viability of the F₂ pups were modest and transient. The NOAELs from the study and the effect(s) seen at the next higher dose are:

- F₀ generation - 0.1 mg/kg; at the 0.4 mg/kg dose effects on body weight gain
- 1.6 mg/kg; for reproductive effects at 3.2 mg/kg reduced implantions and litter size
- F₁ generation – 0.4 mg/kg; at the 1.6 mg/kg dose pup mortality and decreased body weight
- 0.4 mg/kg for reproductive effects, the highest dose tested
- F₂ generation – 0.4 mg/kg, the highest dose tested.

The N-EtFOSE 2-generation study (RS43) was of a design similar to the PFOS study and conducted in the same laboratory. A design difference was that dosing of the parental (F₀) generation in the N-EtFOSE study commenced 28 days prior to cohabitation of the sexes as contrasted to 42 days in the PFOS study. The doses were 0, 1, 5, 10, 15 mg/kg/day. There was a decrease in F₁ litter size and pup viability in the 10 and 15 mg/kg dose groups during the perinatal period. The dose-response for this effect was steep as seen in viability indices (survival from birth to LD 4) of 92.8, 99.1, 92.0, 30.2 and 1.20% for the 0, 1, 5, 10 and 15 mg/kg dose, respectively. The last 2 pups in the high dose group died on LD 5 and an additional 6 of 60 pups alive on LD 4 in the 10 mg/kg group died between LD 5 and 14. Due to the severe effects seen in F₁ pups at the two higher doses, post weaning portions of the study were reduced to 0, 1, and 5 mg/kg groups. These dose groups proceeded through a mating, pregnancy and postnatal evaluation phase until F₂ pups were 21 days of age. F₁ rats in the 0, 1, and 5 mg/kg groups developed normally in the post weaning period as measured by an array of developmental milestones, including neurobehavioral performance. Effects on F₁ reproduction and lactation and on post natal viability of the F₂ pups were seen at the 5 mg/kg dose. The NOAELs from the study and the effect(s) seen at the next highest dose are:

- F₀ generation - 1 mg/kg; at the 5 mg/kg dose body weight effects
- 5 mg/kg for reproductive; at the 10 mg/kg dose higher incidence of stillborn pups
- F₁ generation – Less than 1 mg/kg, the lowest dose tested, based on body weight effects
- 5 mg/kg for reproductive effects, the highest dose tested

F₂ generation – 1 mg/kg; at 5 mg/kg reduced litter size, pup viability and growth

The results of the 2-generation studies with PFOS and N-EtFOSE are very similar with respect to the types of effect seen, dose response, and lowest doses that cause effects. Maternal body weight changes are observed in the pre-natal teratology studies and the two generation studies with both chemicals. However, the mortality seen in the perinatal period of life has no parallel in the prenatal studies. While modest increases in resorptions were seen in the 2-generation studies, in the main, pup development until the time of birth was fairly normal. Gross appearance notwithstanding, the incidence of pup mortality was severe (at the higher doses) on the day of birth and in the immediate perinatal period. The study design did not permit insight as to the factor(s) that contribute to the lethal response. Results of the 2-generation studies indicate that fertility and reproductive performance are not impaired at doses that cause adverse body weight effects on males and females. It is not clear whether the resorptions are due to direct effects on the fetus or secondary to altered maternal physiology associated with decreased food consumption and weight gain.

A PFOS cross-foster study was performed to ascertain the role of pre-natal, post-natal, or combined exposure on pup mortality and health (RS48). A single gavage dose, 1.6 mg/kg PFOS, was used. Female Sprague Dawley rats were treated with 0 or 1.6 mg/kg daily during a 42 days pre-mating, mating, pregnancy, and a lactation period of 21 days. At birth, 25 litters from control or treated dams were cross-fostered with 12 –13 control or PFOS treated dams. Thus four groups were established. The results of the study that ended on post-natal day 21 is summarized in Table 4-12.

Table 4-12. Cross-foster PFOS Study
Post-natal Pup Effects During 21 Day Lactation Period^a

PFOS Exposure ^b		Number Dead	Total Pups	Percent Mortality	Litters Affected	Pup Weight ^c
Gestation	Lactation					
0	0	3	191	1.6	3	29.0
0	1.6	2	181	2.0	2	26.2
1.6	0	16	166	9.6	10	26.7
1.6	1.6	34	177	19.2	8	24.6

^a extracted from RS48

^b refers to daily female dose of 0 or 1.6 mg/kg PFOS.

^c mean weight in Grams on LD 14

Mortality was increased (9.6%) in pup litters whose exposure was solely *in utero*. Mortality was greatest (19.2%) in pup litters exposed *in utero* who also nursed treated females. There was no increase in mortality in pup litters not exposed *in utero* that nursed treated females; body weight gain was reduced. The greatest reduction in weight gain was in pup litters who had *in utero* and lactation exposure. PFOS serum levels were determined in litters and dams from this study.

These data are summarized in Table 4-13.

Table 4-13. PFOS Serum Values at Time of Necropsy (LD 21/22) in ug/mL (ppm)

PFOS Exposure ^a		Nursing Dams	Litters
Gestation	Lactation	Mean	Pooled Mean
0	0	0.05* (12)**	0.05* (6)
0	1.6	82.96 (13)	22.35 (6)
1.6	0	2.02 (13)	53.88 (6)
1.6	1.6	88.97 (12)	89.71 (6)

* 0.05 uG/mL is Lower Limit of Quantitation.

** Number in parentheses is number of samples

The data clearly indicate that treatment of a pregnant dam can result in significant levels of *in utero* exposure to PFOS. This is demonstrated by serum values of about 54 ppm in 21 day old pups who had only *in utero* exposure. PFOS also appears to be readily secreted in milk, as evidenced by pups with no gestational exposure having serum levels of about 22 ppm after nursing treated dams. Drawing upon results from a pharmacokinetic study discussed in 4.3.1 (RS52) it appears that a PFOS fetal serum level of ~117 ppm just preceding birth is associated with perinatal toxicity and death, e.g., fetuses from dams with exposure to 1.6 mg/kg PFOS. The preparting sera concentration of dams in this dose group averaged 185 ppm.

In summary, *in utero* exposure to 1.6 mg/kg PFOS via the dam lead to perinatal mortality and reduced growth. In a separate study, maternal exposure to 1.6 mg/kg led to serum levels of 117 uG/mL in fetuses just prior to birth. *In utero* and peri-natal exposure to 1.6 mg/kg appear to be additive with respect to toxic effects and perinatal death in pups. Finally, exposure via milk from mothers receiving 1.6 mg/kg did not cause death although a decrease in pup weight was observed. The serum levels support a hypothesis that the degree and severity of developmental and peri-natal toxicity is directly associated with PFOS concentration.

Ongoing Study

A study is planned that will assess the role of reduced cholesterol metabolism as a cause of perinatal mortality in rats.

4.3.5 Genetic Toxicity

PFOS. PFOS has been tested for genotoxic activity in a battery of microbial and mammalian systems. These included assays for induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli*, (RS14, RS15, RS16, RS17, RS18) a test for gene conversion in the D4 strain of *Saccharomyces cerevisiae* (RS18); an *in vitro* assay for chromosomal aberrations in human whole blood lymphocytes (RS21), the mouse micronucleus assay (RS19), and an assay for

unscheduled DNA synthesis (UDS) in primary rat liver cell cultures (RS20). PFOS was negative in all assays in which it was tested.

Potassium perfluorooctylsulfonate did not induce reverse mutation at the histidine locus of *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, or at the tryptophan locus of *E. coli* WP2uvrA, and did not induce gene conversion at the try locus in the D4 strain of *S. cerevisiae* when tested with or without metabolic activation from Aroclor-induced rat liver microsomes at doses up to 5,000 µg/plate (RS15, RS18).

The diethanolamine salt of PFOS was likewise without genotoxic activity in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 when tested at up to 5,000 µg/plate, with and without metabolic activation, and in the D3 strain of *Saccharomyces cerevisiae* gene recombination assay at up to 5% (RS14).

Potassium perfluorooctylsulfonate did not induce chromosomal aberrations in human lymphocytes when tested at up to cytotoxic concentrations, with or without metabolic activation by Aroclor-induced rat liver microsomes (RS21). Nor did it induce UDS in primary cultures of rat hepatocytes when tested at up to cytotoxic levels (RS20).

In the *in vivo* mouse micronucleus assay, potassium perfluorooctylsulfonate did not induce micronuclei in the bone marrow of Crl:CD-1 BR mice given a single gavage dose of 237.5, 450, or 950 mg/kg (RS19).

N-EtFOSE. Negative results have been obtained in batteries of genotoxicity tests of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE).

N-EtFOSE gave negative results in micronucleus assays in rats (RS12) and mice (RS11) at doses up to lethal levels by oral gavage. N-EtFOSE, did not induce UDS in liver cells of rats that had received a single dose of 203, 405, or 810 mg/kg by gavage 2-3 hours earlier, or 15-16 hours earlier (RS13). A mammalian cell gene mutation assay in mouse lymphoma cells (RS10), which the authors considered as providing evidence of mutagenicity in the presence of metabolic activation, suffers from a number of methodological and interpretative problems that render it uninterpretable. These deficiencies include:

- Inadequate identification of mouse lymphoma test strain;
- Use of excessive, potentially toxic levels of S9 mix;
- Use of an inappropriate positive control chemical for the non-activation assay;
- Poor detection of small colony mutants;
- Use of excessively high, toxic concentrations of test chemical in the mutation assays;
- Use of an excessively long mutant expression period (3 days);
- Use of insufficiently large numbers of cells for mutation assays; and
- Over-interpretation of study results.

N-MeFOSE. N-methyl perfluorooctane sulfonamidoethanol (N-MeFOSE) gave uniformly negative results in an Ames assay in *Salmonella typhimurium* (RS22), a mammalian cell gene

mutation assay in L5178Y mouse lymphoma cells (RS24), a human lymphocyte chromosome aberration assay (RS23), a rat liver cell UDS assay (RS26), and a rat bone marrow micronucleus assay (RS25).

4.3.6 Any other Human Health Relevant Information

Ocular Irritation

PFOS was found to be mildly irritating to the eyes of albino rabbits when as powder. The ocular irritation was limited to the conjunctivae in the six test rabbits. Irritation was noted at the 1, 24, and 48 hour post-instillation reading times. The maximum irritation score was 9.3 out of a highest possible score of 110 at the one-hour reading. By 72 hours post-instillation the score subsided to 0.0 (RS1).

Dermal Irritation

PFOS was found to be non-irritating to the skin of albino rabbits when tested under conventional Draize procedures. No signs of dermal irritation were observed in any of the test animals at any time during the study period. The primary skin irritation score was 0.0 out of a highest possible score of 8.0 (RS4).

Sensitization

No reports on the sensitization potential of PFOS are available.

Human Data

There are no known cases of irritation or sensitization associated with human exposure.

Conclusions

PFOS is potentially a mild irritant on contact with eyes and is not expected to irritate skin.

4.4 Data from Studies in Humans

4.4.1 Background and Early Medical Surveillance

There have been two major types of initiatives to examine the health of the 3M fluorochemical production workforce: periodic medical surveillance examinations and a retrospective cohort mortality study. There have been no epidemiological studies of the general (non-occupational) population nor have any other occupational cohorts studies been published.

Following reports of the finding of organic fluorine in sera samples, a fluorochemical medical surveillance program began at 3M's Decatur (Alabama) manufacturing facility in the late 1970's. The voluntary program has generally consisted of biennial tests of clinical chemistries, pulmonary function, blood counts, accompanied by biomonitoring of fluorochemical exposure. A total organic fluorine measurement was routinely done until the mid-1990's. A total organic

fluorine assay measures the amount of fluorine that was covalently bound to carbon in the serum sample and is not specific for PFOS. When test data were available, a company physician reviewed each employee's results. These physicians did not, and have not, found abnormalities in individuals that they believed were related to fluorochemical exposure. That is, medical conditions, medications and lifestyle factors adequately explained any observed laboratory abnormalities.

4.4.2 Medical Surveillance Studies

Beginning in the mid-1990's, the 3M medical surveillance programs at the Decatur and Antwerp (Belgium) plants incorporated serum measurements of PFOS and perfluorooctanoate (PFOA) rather than total organic fluorine (RS8). High performance liquid chromatography-mass spectrometry was the analytical method used to detect and quantify these chemicals. An aggregate analyses was conducted of the Decatur and Antwerp male employees participating in 1994/1995 (n = 178) and 1997 (n = 149). (There were too few female employees to afford data analysis.) Sixty-one employees participated in the program during both time periods. Results from hematological, standard clinical chemistry tests and several hormone assays (cortisol, dehydroepiandrosterone sulfate, estradiol, follicle stimulating hormone, 17-alpha hydroxyprogesterone, luteinizing hormone, prolactin, sex hormone binding globulin, free testosterone, bound testosterone, and thyroid stimulating hormone) were analyzed in relation to serum PFOS levels.

During both time periods, serum PFOS levels in 95 percent of the employees were below 6 ppm. The two plant populations differed by age, body mass index, and alcohol consumption, resulting in differences between the populations in several clinical chemistry parameters. Multivariable analyses adjusted for these potential confounders. When analyzed in aggregate, no consistent significant associations were observed between the employees' serum PFOS levels and the clinical chemistries or hematology parameters for either time period. (Total bilirubin levels appeared to trend downwards; further analysis found that this was restricted to Decatur employees and the values were all within the reference range.) Multivariable regression models were fitted with PFOS level (analyzed as a continuous variable) using linear, as well as non-linear, transformations in order to maximize the possibility of finding associations between PFOS and the parameters of interest while adjusting for potential confounders.

No consistent associations were observed by plant, by year, or by both. As discussed in the toxicology section (4.3), the most sensitive clinical chemistry endpoint in rats and monkeys with increasing exposure to PFOS or N-EtFOSE appears to be a reduction in serum cholesterol levels. It is of note, therefore, that mean serum cholesterol levels in these production workers remained constant or increased with increasing serum PFOS levels. An aggregate analysis of both plants' HDL levels appeared to show negative association with increasing PFOS level; however, this was confounded by the fact that all the workers with PFOS levels of 6 ppm or greater were older than workers in the lowest PFOS category, had higher body mass indices (BMIs), and, in 1997, were only employed at the Decatur plant. Multivariable analyses and stratification by plant found no consistent associations between HDL and PFOS levels. In 1995, hormone values were also obtained from a subsample of employees with the higher PFOS measurements. After adjusting for age and body mass index, no significant associations were observed between these hormones and serum PFOS levels, with the exception of estradiol. The latter quadratic

association was highly influenced by one employee with high PFOS measurement (12.83 ppm) and a large BMI; removal of this employee from the analysis resulted in no significant association with estradiol.

The results from these analyses suggested that, among these Decatur and Antwerp male fluorochemical production employees, significant hematological, clinical chemistry, and hormonal abnormalities were not associated with serum PFOS levels up to 6 ppm. (It was not possible to draw conclusions regarding the small number of employees with serum PFOS levels > 6 ppm.) Limitations of these surveillance analyses included its cross-sectional design, the voluntary participation rates of less than 50 percent, the small number of subjects exposed at the highest levels and a single hormone measurement, rather than multiple hormone measurements.

4.4.3 Mortality Studies

A retrospective cohort mortality study of employees who worked at least one year (1961-1990) at the 3M Decatur manufacturing site was conducted to determine whether the mortality experience of these production workforce was significantly different from that which would be expected [RS5]. A total of 1,957 employees (1,639 males and 318 females) constituted the cohort, which represented 37,915 person-years of follow-up. Only six employees (0.3%) were lost to follow-up. Vital status was searched through 1991 using company records, credit bureaus, Social Security Administration and the National Death Index. A total of 74 deaths were reported and 72 (97%) death certificates were obtained.

Observed deaths were compared to an expected number calculated by using indirect standardization techniques with three comparison populations: United States; Alabama; and regional Alabama counties. Analyses were also stratified by whether male employees ever and only worked in the chemical and film plants at the Decatur site.

No statistically significant elevations in Standardized Mortality Ratios (SMRs) were found for any specific cause of death or for any of the comparisons. Table 4-14 provides the data for the most common causes of death.

Table 4-14. Retrospective Cohort Mortality Analysis for Male Employees of the Decatur Chemical Plant (n = 1,050)

Cause of Death*	Observed	SMR	95% Confidence Interval
All Causes	57	70.0	53.0 – 96.0
All Malignant Neoplasms	13	76.9	40.9 – 131.5
Cancer of the Bronchus, Trachea, or Lung	7	120.7	48.5 – 248.7
Cardiovascular Heart Disease	11	48.8	24.4 – 87.4
External Causes	20	90.2	55.1 – 139.3
* Three or fewer deaths were observed for all other categories.			

Despite the excellent follow-up of the cohort, there were three important limitations to this study: 1) the few person-years of follow-up; 2) the short latency period; and 3) the lack of a PFOS-specific job exposure matrix.

4.4.4 Work In Progress

3M, in conjunction with epidemiologists from the University of Minnesota Division of Occupational and Environmental Health, is in the midst of completing an update of the cohort mortality study. Several methodological improvements have occurred since the original study, including the computerization of the work history record for all past and present employees, which, in conjunction with information regarding serum fluorochemical levels acquired from medical surveillance exams and the random sample assessment (described above), will allow for the construction of a PFOS-specific job exposure matrix. Estimated date of completion of this updated study is November, 2000. (A comparable retrospective cohort mortality study cannot be done among the Antwerp employee population due to the confidential nature of death certificate registration in Belgium.)

An additional health-related research effort that is scheduled for completion in 2000 is the analysis of health claims data from January 1, 1993, through December 31, 1998, of the Decatur chemical and film plant employees. Health claims data are not available for analysis purposes prior to 1993. Clinical Care Groups™ methodology will be used to group all visits (inpatient and outpatient), procedures, ancillary services, and prescription drugs considered in the diagnosis, treatment, and management of approximately 400 diseases or conditions. An episode will be considered a constellation of one or more claims data records representing an occurrence of a disease or condition for a particular condition. The observed group claims data will be compared to an expected number calculated by indirect standardization methods to adjust for age and gender. Corrected for their different age structures, the ratio of the observed to expected chemical plant claims experience will be compared to the film plant's [Ederer and Mantel, 1974].

4.5 Initial Assessment for Human Health

4.5.1 Approach to Assessment

PFOS has been identified in serum samples from both occupationally and non-occupationally exposed populations. This initial assessment is focused on the question of whether and to what extent the levels of PFOS found in serum samples from those populations pose a human health risk. Both epidemiological and animal toxicology data are available for use in the assessment.

PFOS comes to be present in human serum from a variety of sources and there is as yet no clear understanding of the relative importance of those different sources. Some may arise from exposure to precursor molecules that degrade and metabolize to PFOS (N-EthylFOSE Alcohol, for example), while other exposure may be to PFOS itself through underdetermined environmental pathways. It is for this reason that the typical approach to risk assessment, involving comparisons of external (administered) doses known to be associated with adverse health effects with doses experienced by the populations under evaluation, is not appropriate or even possible for PFOS. The approach to be taken here involves, instead, comparisons of serum levels that have been the subject of epidemiological and toxicological studies with serum levels found in exposed populations. This approach is, for several reasons, likely to provide greater scientific certainty than the more traditional approach.

One reason for this conclusion has already been noted: PFOS reaches and accumulates in blood from several different sources, so that the serum findings reflect total human exposure. Moreover, because PFOS is relatively persistent, with an elimination half-life in humans currently estimated to be 300 days, sera levels integrate exposure over time. External doses, especially for substances having multiple sources, are highly variable over time, and it is often difficult to obtain information on the degree of variability. Serum levels are likely to be a far more stable estimate of long-term exposure.

Uncertainty is reduced when risk assessments are based on direct comparisons of serum levels. In the traditional assessment, based on intra- and interspecies comparisons of external doses, ADME differences are often unknown and are accounted for by the introduction of uncertainty factors that are general, not chemical-specific, in nature. The PFOS assessment described herein does not suffer from this important source of uncertainty and interspecies extrapolations have greater reliability than they do when external dose is used.

Most of the recently produced epidemiology and toxicology data have involved the development of health effects information in relation to serum levels; current and future studies have been designed to acquire similar type of data.

4.5.2 Health Effects of PFOS and Dose-Response Relationships

Data from studies of 3M fluorochemical production workers have thus far revealed no adverse effects from the PFOS serum levels identified in those populations. The data derive from medical surveillance investigations and from a retrospective cohort mortality study.

Beginning in the late 1970's, biennial hematological and clinical chemistry tests, along with tests of pulmonary function, were performed on workers at 3M's Decatur manufacturing facility. Until the mid-1990's, fluorochemical exposure was based only on the level of serum organic fluorine and was not PFOS-specific. In none of the years of testing did occupational physicians report abnormalities they believed to be related to organic fluorine exposures.

In 1994/1995 and again in 1997, the health of male employees at 3M's Decatur and Antwerp production facilities was assessed in relationship to serum levels of PFOS. In addition to the standard sets of clinical chemistry and hematological tests, assays for 11 different hormones were performed on a subset of the workers' blood. In the 1994/95 sampling 178 employees were evaluated and in 1997, 149 were tested; all were volunteers. No significant hematological, clinical chemistry, or hormonal abnormalities were associated with serum levels up to 6 ppm in either of the study periods. Serum cholesterol levels were not affected; this finding is significant because of the experimental observations that PFOS at higher doses cause declines in this parameter.

A 1995 retrospective cohort mortality study of 1,957 employees who had worked at least one year at the 3M Decatur production facility revealed no statistically significant elevations in risk for any specific cause of death, including cancer. This study did not include PFOS serum measurements.

These studies of production workers, although limited in several ways, provide important information for risk assessment. First, they involve direct attempts to identify adverse health effects in the most highly exposed human populations. Second, they involve not only an examination of cancer and other causes of mortality, but also a wide range of clinical parameters that are likely to represent sensitive indicators of adverse effects based on experimental data. At the same time, it must be recognized that the studied populations are not representative of the general population in that they do not include children, the elderly, or those suffering from ill-health, and represent women in only a very limited way. Studies now underway may address some of these limitations.

More extensive data on the effects of PFOS are available from studies in monkeys, rats, and rabbits (teratology only). As discussed in the introduction to this section (4.1), repeat-dose animal studies on N-EtFOSE and N-MeFOSE have been included because both compounds undergo metabolism leading to PFOS. Thus, comparative toxicology studies of these compounds and PFOS could reveal whether any of the effects of the former two PFOS precursors are due entirely to the end product of their metabolism (PFOS), or may also involve one or more of the intermediary breakdown products or metabolites. The substantial database now available on PFOS, N-EtFOSE, and N-MeFOSE demonstrates a consistency of effects, and some data suggest that these effects may be due to PFOS. Additional study will be necessary to

confirm this initial impression.

In recent repeat-dose studies in rats, PFOS and these precursor compounds reduce serum cholesterol levels, cause reductions in body weight gain, and cause liver enlargement and vacuolization. The same pattern of effects is observed in PFOS exposure studies involving monkeys. At the highest doses tested, PFOS has caused unexplained deaths in monkeys and rats. Similar effects were observed in earlier repeat-dose studies of PFOS.

Both PFOS and N-EtFOSE have been evaluated for teratologic effects in rats and rabbits and for adverse reproductive and developmental effects in a two-generation rat study. Neither compound has been demonstrated to produce teratogenic effects. In the two-generation studies, however, both compounds affected fetal survival and body weight gains in dams and fetuses.

The dose-response relationships for some effects, as identified in PFOS dosing studies, are presented in Table 3-5, which is reproduced below as Table 4-15. At this time, the N-MeFOSE and N-EtFOSE data, as summarized in Sections 4.2.3 and 4.2.4, are used to confirm the PFOS results, but are not sufficiently complete to be used for quantitative analysis.

Table 4-15. PFOS Toxicity Data for Mammals: Observed Effects, Serum and Liver PFOS Concentrations, and Cumulative Dose

Group ¹	Observed Effect	Serum PFOS Concentration (ppm)	Liver PFOS Concentration (ppm)	Cumulative Dose (mg/kg)
26-week Capsule-Dosing Study in Cynomolgus Monkeys				
0.15 mg/kg/d	NOEL	85	80	27.3
0.75 mg/kg/d	Hepatomegaly; hepatocyte enlargement	> 100, < 300	415 (average)	> 27.3, < 137
0.75 mg/kg/d	Decreased cholesterol in females	> 134 ± 25	415 (average)	46.5
0.75 mg/kg/d	Decreased cholesterol in males	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Decreased T3	> 152 ± 30	415 (average)	67.7
0.75 mg/kg/d	Death or early sacrifice for 2/6 males	> 150, < 300	415 (average)	> 100, < 137
14-Week Dietary Study in Sprague Dawley Rats				
2.0 ppm Males	NOEL	17.9	76.8	~ 17
2.0 ppm Females	NOEL	26.9	68.25	~ 13
5.0 ppm Males	Hepatocellular hypertrophy and vacuolization	45.6	386.53	24.4
5.0 ppm Females	NOEL	62.9	362.45	38.2
20 ppm Males	Hepatocellular hypertrophy and vacuolization; decreased cholesterol; increased AAT	134	599.94	106
20 ppm Females	Hepatocellular hypertrophy and vacuolization	216	617.52	141
Reproduction PK Dosing 6 weeks prior to mating and 21 days of gestation				
0.4 mg/kg/d Dam PM	NOEL	47.1 ± 5.00 (n = 16)	--	16.8
0.4 mg/kg/d Fetus EG	NOEL	39.7 ± 5.90 (n = 5)	--	N/A
0.4 mg/kg/d Dam EG	NOEL	30.3 ± 17.0 (n = 6)	--	28
1.6 mg/kg/d Dam PM	Slight body weight	185 ± 14.0 (n = 16)	--	67.2
1.6 mg/kg/d Fetus EG	Survival, body weight	117 ± 14.5 (n=2)	--	N/A

Table 4-15. PFOS Toxicity Data for Mammals: Observed Effects, Serum and Liver PFOS Concentrations, and Cumulative Dose

Group¹	Observed Effect	Serum PFOS Concentration (ppm)	Liver PFOS Concentration (ppm)	Cumulative Dose (mg/kg)
1.6 mg/kg/d Dam EG	Slight body weight	158 ± 86.6 (n = 4)	--	112
3.2 mg/kg/d Dam PM	Body weight	368 ± 23.6 (n = 16)	--	134
3.2 mg/kg/d Fetus EG	Stillbirth, survival	191 ± 26.4 (n = 6)	--	N/A
3.2 mg/kg/d Dam EG	Body weight	180 ± 41.5 (n = 6)	--	224

¹ PM = Pre-Mating, after 42 days of dosing; and EG = End of Gestation, day 21 of gestation

Examination of Table 4-15 reveals that the effects of PFOS occurring at serum levels of 45.6 ppm (hypertrophy and vacuolization in the livers of male rats in the 14-week study) represents the minimum effect level for this compound. The No Observed Effect Level (NOEL) for these liver effects in male rats is 17.9 ppm. The NOEL for this effect in female rats is 62.9 ppm. It is noteworthy that the liver effects are not observed in monkeys until serum levels exceed 100 ppm; declines in cholesterol levels are also observed in monkeys at serum levels over 100 ppm. Recovery studies in monkeys have shown that the hepatic enlargement resolves upon cessation of dosing and serum cholesterol levels return to normal. The NOEL from the 6-month monkey study is 85 ppm (serum PFOS).

Pup survival is affected adversely when dam serum levels reach 185 ppm, measured after 42 days of dosing but pre-mating. The NOEL for this effect, measured in dams at the same pre-mating time, is 47.1 ppm. Thus, although the serum levels in dams declines during gestation, the fetal effects are not observed unless the dam enters pregnancy with a serum PFOS level of *ca.* 185 ppm; the pups of a dam having a serum level of up to 47.1 ppm are not at risk. Thus, 47.1 ppm in the rat dam will be taken as the relevant NOEL for assessing perinatal effects.

The NOELs from the animal studies useful for assessing human risk are shown in Table 4-15.

Table 4-16. NOELs from Animal Studies to be Used for Initial Assessment of Human Risk

NOEL (PFOS Serum Level)	Source	Effect at Next Higher Serum Level	Estimated Cumulative Dose
17.9 ppm	14-week, repeat-dose, male rats	Liver enlargement, vacuolization	~17 mg/kg
47.1 ppm	2-generation reproduction, rats	Reduced fetal survival as measured in dams, pre-mating	16.8 mg/kg
85 ppm	6 month, repeat-dose, monkey	Liver enlargement; decreased cholesterol levels	27.3 mg/kg

4.5.3 Other Experimental Data Related to Health Effects

PFOS, N-EtFOSE, and N-MeFOSE have been subjected to an extensive battery of *in vitro* and *in vivo* tests for genotoxic potential. There is no evidence of any such potential for these compounds.

These substances are also relatively weak inducers of peroxisomes in rodents. It is not known whether this effect has any relationship to the observed toxic properties of these compounds. At this time, the mechanisms underlying toxicity are not understood. 3M has under consideration several types of exploratory investigations that may begin to shed light on how these compounds produce toxicity.

4.5.4 Initial Assessment of Risk

Occupational Exposures and Risks

Serum levels of PFOS in 3M production workers average less than 2 ppm, and some workers have exhibited levels in the range of 10–13 ppm. Direct studies of production workers have revealed no evidence of excess mortality and no evidence of effects as measured by standard hematological and clinical chemistry tests and by assays of 11 different hormones, at serum PFOS levels up to 6 ppm. Serum cholesterol levels are not affected in this range of serum PFOS levels. Within the limitations of these studies, this information suggests that workers are not at risk at the serum levels reported. Animal studies of PFOS, N-EtFOSE, and N-MeFOSE reveal that PFOS is associated with some effects that would not be expected to be found in the occupational health studies because of lower serum PFOS concentrations. The NOELs from the animal studies, as shown in Table 4-16, range from serum levels of 17.9 ppm to 85 ppm. Assuming the non-human primate is taken as the best animal model for effects in humans, then the most highly exposed workers bear serum levels approximately 15 percent of the NOEL and average worker levels are 50 times lower than the NOEL in monkeys. On the other hand, using the results from the 14-week rat study, production worker exposures are, at the upper end, more than one-half of the NOEL and, on average, are about 10 times lower than the NOEL.

As explained earlier, the direct interspecies comparisons of serum levels rather than external dose reduces many of the usual uncertainties associated with interspecies extrapolation, most

especially those associated with ADME differences. This, in turn, reduces the need for a significant fraction of the uncertainty factors typically used to extrapolate from animals to humans. Moreover, uncertainty factors used for the general (non-occupational) population (see below) are generally larger than those used for worker populations, because the latter are not expected to exhibit the same high degree of variability in sensitivity to toxic effects.

Little serum PFOS data are available for occupational exposures in various user facilities. 3M production workers are exposed to unreacted, concentrated starting materials. The majority of downstream workers are exposed to fluorochemical products that typically contain less than 1% residual unreacted starting material that could be absorbed and metabolized to PFOS. Although it seems likely that such workers exhibit lower exposure levels, and therefore would demonstrate larger safety margins than do 3M production workers, this conclusion can not be fully documented. The study of non-production workers in 3M's Japanese facility, where PFOS precursors are handled, seems to support it.

General (Non-Occupational) Exposures

The currently available data from sampling of blood from selected human populations and from blood banks reveals that non-occupational exposures to PFOS range from *ca.* 0.01 to 0.1 ppm. The upper end of this range is about 60 times lower than the level (6 ppm) found to be without associated adverse effects in the occupational studies. It is also about 180 to 850 times lower than the NOELs from the rat and non-human primate studies, respectively. Again, the direct interspecies comparisons of serum levels reduces uncertainties related to ADME differences. Taken together, the data from human and animal studies suggest that PFOS serum levels in members of the general population are substantially below levels associated with adverse effects.

4.4.5 Uncertainties in Assessment and Work in Progress

The initial assessment presented above is based on a substantial body of health effects data. It is based on an interspecies comparison of serum PFOS levels and is more certain than typical assessments, which are based on cross-species comparisons of external doses. This reduced uncertainty translates to a need for smaller uncertainty factors for cross-species extrapolations. In light of this conclusion, the margins separating NOELs from human exposure levels described above should not be compared with the larger margins observed or used in the more typical assessment.

The limitations associated with the use of human data have already been identified, and will be reduced when additional epidemiological investigations are completed. Results from chronic rodent studies of PFOS and N-EtFOSE are still under evaluation. Definitive conclusions regarding the effects of chronic exposures will become available in the future. Mechanistic understanding of the underlying causes of toxicity, particularly those relating to reduced survival of offspring in the two-generation study is under study.

Additional data are forthcoming pertaining to the blood levels and distribution of PFOS in human sera. The distribution of PFOS levels according to age, gender, and geographic location will be available for future assessments. As detailed in Section 4.2.4, several additional studies will add substantially to understanding this area.

5.0 CONCLUSIONS AND RECOMMENDATIONS

This report summarizes the information that is available as of July 20, 2000. There is a substantial body of data relating human and environmental exposures to PFOS and the possible biological effects of these exposures. This information suggests that human serum PFOS levels found in occupational and non-occupational populations are not associated with adverse health effects. Similarly, levels found in the environment and in wildlife are not associated with adverse effects. Additional research now underway will be used to refine this initial assessment.

Ongoing studies in several areas will improve this initial assessment of risk. Areas under study include environmental source assessment, human exposure (through sera measurement), chronic studies in laboratory animals and additional tissue measurements of PFOS from previous studies. Much of this information will be available in the next year.

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APPENDIX I
SUMMARY REPORTS FOR PHYSICAL/CHEMICAL PROPERTIES
CONTENTS

	Boiling Point
Reference 55	Melting Point
Reference 57	Vapor Pressure
Reference 58	Octanol/Water Coefficient
Reference 59	Air/Water Partition Coefficient
Reference 60	Water Solubility

BOILING POINT

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: Testing was not conducted. Boiling point would be in excess of 400°C.

MELTING POINT
(Reference No. 55)

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-hepta-decafluoro-, 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-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 102

GLP: Yes

Year completed: 1998

Remarks: Study utilized a Büchi Melting Point B-540 instrument, calibrated and inspected just prior to use using anthraquinone and 1,8-naphthalimide.

RESULTS

Melting point value in °C: >400°C (No melting observed).

Decomposition (yes-temperature °C/ no /ambiguous): No

Sublimation (yes/no/ambiguous): No

Remarks: Measurements of the melting point / melting range were limited to □400□C, the maximum specification for the instrument used. Fine droplets were not observed to adhere uniformly or otherwise to the walls of the melting point tubes.

CONCLUSIONS

The melting point/melting range was not observed and therefore could not be determined.

Remarks: While no melting of the test substance was evident, discoloration of the test samples was observed. The white powder turned to a light brown and eventually black as temperatures rose.

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

OTHER

Last changed: 5/3/00

VAPOR PRESSURE

(Reference No. 57)

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 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 104, U.S. EPA OPPTS 830.7950

GLP (Y/N): Yes

Year completed: 1999

Remarks: Determination of the vapour pressure was done by using the Spinning Rotor Gauge method.

RESULTS

Vapor Pressure: 3.31×10^{-4} Pa

Temperature °C: 20°C

Decomposition (yes/no/ambiguous): No

Remarks: The measured vapour pressure was repeatable.

CONCLUSIONS

Remarks: The vapor pressure of the test substance was determined to be 3.31×10^{-4} Pa at 20°C using the spinning rotor gauge method.

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

OTHER

Last changed: 5/3/00

Draft Initial Assessment Report PFOS - Appendix I

OCTANOL/WATER PARTITION COEFFICIENT (Reference No. 58)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 107

GLP (Y/N): See Remarks

Year completed: Study completed 1999. Report completed 2000

Remarks: A feasibility test was conducted to determine if the physical properties of the test substance were compatible with shake flask methodology proposed for use in an n-octanol/water partition coefficient determination.

Upon completion of the test procedure, a definitive partition interface was not obtained. Instead, a beige/white emulsion was observed throughout the sample.

RESULTS

Log P_{ow}: Not determined.

Remarks: The observation of an inseparable emulsion in the preliminary test precluded conduct of a definitive test, as indicated in the protocol (No 454/120298/107F/SUB454, 3M Lab Request U2723). Therefore, a study cancellation report was generated by the laboratory conducting the testing after consultation with 3M.

CONCLUSIONS

The study substance exhibits physical/chemical characteristics that make determination of the n-octanol/water partition coefficient infeasible by the Shake Flask Method.

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

DATA QUALITY

Reliability: Klimisch ranking NA. Study not feasible.

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

OTHER

Last changed: 5/3/00

AIR/WATER PARTITION COEFFICIENT

(Reference No. 59)

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. No information was recorded on the purity.

METHOD

Method: There is no standardized methodology used to determine this value for regulatory purposes. The experiment was designed by Dr. Richard Purdy of 3M's Environmental Laboratory and Don Mackay of D. Mackay Environmental Research Limited.

GLP (Y/N): No

Year completed: 1999

Remarks: The following method was devised and used:

- Weigh approximately 0.01 gram of the test substance directly into a tared 250-mL Pyrex[®] beaker. Record weight.
- Transfer 200 mL of NANOpure[®] water into the beaker using a Class A glass volumetric pipet.
- Prepare solvent blank sample. Using a gas-tight syringe, transfer a 250 µL aliquot of NANOpure[®] water into a 25-mL Class A glass volumetric flask partially filled with 50% methanol / 50% ammonium acetate buffer reagent. Bring to volume with 50% methanol / 50% ammonium acetate buffer reagent. Ampulate in an amber glass autosampler vial.
- Mix and sonicate the test substance in water sample (50 µg test substance/mL target nominal concentration) for approximately 10 minutes to ensure dissolution of the test material.
- Prepare the control sample. Transfer a 250 µL aliquot of the test substance in water sample into a 25-mL Class A glass volumetric flask partially filled with 50% methanol / 50% ammonium acetate buffer reagent. Bring to volume with 50% methanol / 50% ammonium acetate buffer reagent. Ampulate in an amber glass autosampler vial.
- Place the test substance in water sample beaker on a hotplate and bring solution to a boil. After approximately 10 mLs (5%) of water has evaporated, remove beaker from hotplate and cool to room temperature in an ice-water bath. Transfer contents of sample into a graduated cylinder and record actual volume.
- Process a 250 µL aliquot of sample as described above for solvent blank and control samples.
- Return sample to original beaker and bring sample to boil.
- Repeat steps 6-8 until sample has evaporated to 100 mLs. Submit all ampulated samples for LCMS analysis.

RESULTS

K_{aw}: 0*

Temperature °C: Not recorded.

Remarks: *Don Mackay provided the following interpretation of the analytical results:

“As I interpret the lab results they established an initial concentration of 50 mg/L in 200 mL water then distilled off 10 mL aliquots and analyzed the residue. They then calculated the percentage of the original test substance present which remained in the beaker unevaporated. These ‘percentage recoveries’; ranged from 136 to 105 with no real trend. I conclude that the test substance did not evaporate to any measurable extent. This is a very sensitive method of measuring low air-water partition coefficients. It can be shown that if water and the solute evaporate equally (e.g., the contents do not change in composition as would occur with an azeotrope) then K_{aw} or H is identical for water and the solute. For water, H is approximately 2400 Pa (approximately 20°C) divided by 55000 mol/m³ or 0.044 Pa m³/mol or a K_{aw} of about 2 x 10⁻⁵. The test substance must thus have a K_{aw} considerably less than this, i.e. less than 2 x 10⁻⁶. It is thus essentially non-volatile from aqueous solution. This is probably because of its ionic nature. The simple expedient is to assign it a K_{aw} of zero, i.e. is a type 2 involatile chemical in our nomenclature.”

DATA QUALITY

Reliability: Klimisch ranking 2. Data has limited reliability. Sample purity was not noted. Study temperature was not recorded.

CONCLUSIONS

Testing indicates this substance is essentially non-volatile from aqueous solution.

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

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

Study review by Don Mackay of D. Mackay Environmental Research Limited,

OTHER

Last changed: 5/1/00

WATER SOLUBILITY

(Reference No. 60)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 105, OPPTS 830.7840, and 40 CFR 796.1840.

GLP (Y/N): Yes

Year completed: Study completed 1999. Report completed 2000

Remarks: The definitive test consisted of equilibration of an excess amount of test substance with NANOpure® water at 30°C followed by equilibration at 20°C and analyzing subsamples by high performance liquid chromatography with mass spectrometric detection (LCMS).

NANOpure® water is equivalent to ASTM Type II Designation D1193-91.

RESULTS

Value (mg/L) at temperature °C: 519 mg/L at 20 ± 0.5°C.

Description of solubility: Slightly soluble.

Remarks: Triplicate subsamples were removed from the appropriate bottles after one, two and three days of shaking in a water bath maintained at 30 ± 1.0°C and following one day of a 20 ± 0.5°C equilibration period. Analysis of aqueous subsamples after one day had a mean analytical result of 459 mg/L (SD = 8.96, CV = 1.95%). For subsamples collected after two and three days, the mean concentration were 537 mg/L (SD = 27.6, CV = 5.14%) and 501 mg/L (SD = 64.2, CV = 12.8%), respectively.

CONCLUSIONS

The Day 2 and Day 3 mean solubility concentration were within 15% of each other and were averaged to obtain the overall mean solubility concentration. The overall mean solubility concentration of the test substance in NANOpure® water was 519 mg/L (SD = 48.3; CV = 9.31%; N = 6).

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

Study conducted at the request of 3M Company by Wildlife International, Ltd. of Easton, Maryland.

OTHER

Last changed: 5/3/00

APPENDIX II

SUMMARY FOR AQUATIC TOXICOLOGY STUDIES (PNECs)

CONTENTS

Reference 71	Acute Toxicity to Fish, 1999
Reference 73	Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia), 1999
Reference 75	Acute Toxicity to Aquatic Invertebrates (Freshwater Mussel), 1999
Reference 77	Acute Toxicity to the Saltwater Mysid, 1999
Reference 78	Chronic Toxicity to Early life Stage of Fish, 1999
Reference 79	Chronic Toxicity to Freshwater Invertebrates (Daphnia Magna), 1999
Reference 80	Chronic Toxicity to the Saltwater Mysid, 1999
Reference 84	Chronic Toxicity to Early Life Stage of Fish, 1978
Reference 86	Acute Toxicity to Fish, 1994
Reference 87	Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia), 1994
Reference 90	Acute Toxicity to Fish, 1979
Reference 93	Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia), 1991
Reference 184	Acute Toxicity to Fish, 1974
Reference 185	Acute Toxicity to Fish, 1974
Reference 503	Acute Toxicity to Fish, 1996
Reference 504	Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia), 1996

ACUTE TOXICITY TO FISH (Reference No. 71)

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 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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

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: 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 mm filtered well water

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

Hardness: 131 (128-136) mg/L as CaCO₃

Alkalinity: 177 (176-178) mg/L as CaCO₃

pH: 8.3

TOC: < 1.0 mg/L

Conductivity: 311 (310-315) mmhos/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

Nominal concentrations: Bk 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: 24-hour $LC_{50} = > 28$ mg/L (C.I. not calculable)

48-hour $LC_{50} = > 28$ mg/L (C.I. not calculable)

72-hour $LC_{50} = 27$ (22 – 41) mg/L

96-hour $LC_{50} = 9.5$ (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_{50} value is questionable because a concentration-effect relationship was not demonstrated over a reasonable range of percent dead. The 24 and 48-hour LC_{50} values were determined by visual interpretation. Probit was used to calculate the 72-hour LC_{50} and Moving Average for the 96-hour LC_{50} .

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

The potassium perfluorooctanesulfonate 96-hour LC_{50} 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

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

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)
(Reference No. 73)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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: EC₅₀ 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.

Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: 0.45 mm filtered well water

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

Hardness: 132 (128-136) mg/L as CaCO₃

Alkalinity: 178 (176-178) mg/L as CaCO₃

pH: 8.3 (8.2-8.3)

TOC: < 1.0 mg/L

Conductivity: 313 (310-315) mmhos/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: Bk control, 12, 20, 33, 55, 91 mg/L

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

Element value: 24-hour EC_{10} = 82 (81-83) mg/L

24-hour EC_{50} = >91 mg/L (C.I. not calculable)

24-hour EC_{90} = >91 mg/L (C.I. not calculable)

48-hour EC_{10} = 53 (<11->91) mg/L

48-hour EC_{50} = 61 (33-91) mg/L

48-hour EC_{90} = 63 (<11->91) mg/L

All element values based on mean measured concentrations

Statistical evaluation: The EC_{50} 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_{10} and EC_{90} 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

The potassium perfluorooctanesulfonate 48-hour EC50 for *Daphnia magna* was determined to be 61 mg/L with a 95% confidence interval of 33-91 mg/L. The 48-hour noimmobilization and no observed effect concentration was 33 mg/L.

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

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

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (FRESHWATER MUSSEL)
(Reference No. 75)

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 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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 complanatus*

Analytical monitoring: Test substance concentrations measured by LCMS at 0, 48, 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 organism source: Obtained from Carolina Biological Supply Company, Burlington, North Carolina. Carolina collected from the wild.

Test organism age at study initiation: Unknown

Test Conditions

Dilution water: 0.45 mm filtered well water

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

Hardness: 126 (120-132) mg/L as CaCO₃

Alkalinity: 174 (170-178) mg/L as CaCO₃

pH: 8.3 (8.1-8.5)

TOC: < 1.0 mg/L

Conductivity: 321 (310-330) mmhos/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: 96-hour LC_{50} = 59 mg/L (51-68 mg/L)

Statistical evaluation: The LC_{50} 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

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC50 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

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

ACUTE TOXICITY TO THE SALTWATER MYSID (Reference No. 77)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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

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: < 24-hours old

Pretreatment: None

Test Conditions

Dilution water: Natural seawater diluted to 20‰ with well water, 0.45mm 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: Bk 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: 24-hour $LC_{50} = > 5.4$ mg/L (CI not calculable)

48-hour $LC_{50} = > 5.4$ mg/L C.I. not calculable)

72-hour $LC_{50} = 4.4$ (3.6-6.2) mg/L

96-hour $LC_{50} = 3.6$ (3.0-4.6) mg/L

All element values based on mean measured concentrations

Statistical evaluation of mortality: LC_{50} 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

The potassium perfluorooctanesulfonate 96-hour LC_{50} 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

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

CHRONIC TOXICITY TO EARLY LIFE STAGE OF FISH (Reference No. 78)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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

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 mm filtered well water

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

Hardness: 126 (124-128) mg/L as CaCO₃

Alkalinity: 172 (170-172) mg/L as CaCO₃

pH: 8.2 (8.2-8.3)

TOC: < 1.0 mg/L

Conductivity: 321 (315-330) mmhos/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 mm 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

Nominal concentrations: Bk 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 $\pm$ SD, mm	Wet Weight Mean $\pm$ SD, mg	Dry Weight Mean $\pm$ SD, mg
Negative Control	65	26.5 $\pm$ 0.721	158 $\pm$ 9.10	32.5 $\pm$ 1.20
0.15	60	26.6 $\pm$ 0.208	160 $\pm$ 3.10	33.3 $\pm$ 0.900
0.30	58	26.6 $\pm$ 0.813	167 $\pm$ 11.9	34.2 $\pm$ 2.70
0.60	50	26.5 $\pm$ 0.399	166 $\pm$ 11.3	33.5 $\pm$ 2.70
1.2	4	26.7 $\pm$ 2.02	185 $\pm$ 33.8	35.4 $\pm$ 6.66
2.4	0	-	-	-
4.6	0	-	-	-

CONCLUSIONS

Fathead minnows exposed to potassium perfluorooctanesulfonate at concentrations ≤ 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

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

CHRONIC TOXICITY TO FRESHWATER INVERTABRATES (*Daphnia magna*) (Reference No. 79)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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

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 mm 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 CaCO₃

Alkalinity: 169 (164-172) mg/L as CaCO₃

pH: 8.2 (8.0-8.3)

TOC: < 1.0 mg/L

Conductivity: 329 (315-340) mmhos/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* (3.5×10^7 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 ≤ 12 mg/L started producing neonates on Day 9. The Bonferroni t-test showed that reproduction was not significantly reduced in any treatment group ≤ 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 ≤ 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 $\pm$ SD, mm	Dry Weight, Mean $\pm$ SD, mg
Negative Control	10	4.65 $\pm$ 0.111	0.695 $\pm$ 0.100
1.5	9	4.66 $\pm$ 0.118	0.669 $\pm$ 0.0623
2.9	9	4.62 $\pm$ 0.100	0.724 $\pm$ 0.110
5.6	9	4.61 $\pm$ 0.124	0.727 $\pm$ 0.0665
12	9	4.59 $\pm$ 0.102	0.723 $\pm$ 0.0661
24	0	--	--
48	0	--	--

Reproduction

Mean Measured Concentration, mg/L	Number of Surviving Daphnids	Mean Live Young/ Surviving Adult Daphnid ($\pm$ SD)	First Day of Reproduction	Total Number of Dead / Immobile Neonates	Total Number of Aborted Eggs
Negative Control	10	122 $\pm$ 19.2	9	0	0
1.5	9	142 $\pm$ 24.7	9	0	0
2.9	9	136 $\pm$ 17.9	9	0	0
5.6	9	132 $\pm$ 19.5	9	0	0
12	9	119 $\pm$ 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 ≤ 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

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

CHRONIC TOXICITY TO THE SALTWATER MYSID (Reference No. 80)

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 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

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

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, 0.45mm 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: Bk 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 pre-test 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, 0.0509, 0.0515, 0.0569, 0.0580, 0.0514	0.057	66
.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 ($p \leq 0.05$). 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 ($p \leq 0.05$).

Second Generation Acute Exposure: Survival in all PFOS treatment groups was $\geq 95\%$ 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, Day 35
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	
0.057	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	

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/ Reproductive Day	Overall Mean $\pm$ SD
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 ≤ 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.

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

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

CHRONIC TOXICITY TO EARLY LIFE STAGE OF FISH

(Reference No. 84)

TEST SUBSTANCE

Identity: 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-heptadecafluoro-, 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.

METHOD

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

30-Day NOEC: 1 mg/L

30-Day LOEC: 1.9 mg/L

30-Day MATC: >1 mg/L and <1.9 mg/L

DATA QUALITY

Reliability: Klimisch ranking = 2. This study satisfied criteria for quality testing at the time performed, but the analytical methodology was questionable.

REFERENCES

This study was conducted at E G & G, Bionomics, Aquatic Toxicology Laboratory in Wareham, Massachusetts at the request of the 3M Company.

OTHER

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

Last changed: 5/3/00

ACUTE TOXICITY TO FISH
(Reference No. 86)

TEST SUBSTANCE

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-heptadecafluoro-, 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

Analytical monitoring: pH and DO content

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated by Trimmed Spearman - Karber.

Test fish age: 79 days.

Length and weight: Average length = 2.1 ± 0.3 cm
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

RESULTS

Nominal concentrations: Bk control, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0 mg/L

Element value: 96-hour LC_{50} = 19 mg/L (95% C.I.: 16-24 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

The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 96-hour LC_{50} for fathead minnow of 19 mg/L.

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

DATA QUALITY

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

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 3/25/94.

OTHER

Last changed: 5/2/00

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)
(Reference No. 87)

TEST SUBSTANCE

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: EC₅₀ values calculated using Trimmed Spearman-Kärber method

Test daphnid source: Obtained from U.S. EPA-NETAC, Duluth, Minnesota.

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: Bk control, 100, 180, 320, 560, 1000 mg/L

Element value: 24-hour EC_{50} = 330 (290-370) mg/L

48-hour EC_{50} = 210 (190-230) mg/L

48-hour NOEC = 100 mg/L

Statistical Evaluation: The EC_{50} 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

The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 48-hour EC_{50} for *Daphnia magna* of 210 mg/L.

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

DATA QUALITY

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

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 2/10/94.

OTHER

Last changed: 5/2/00

ACUTE TOXICITY TO FISH
(Reference No. 90)

TEST SUBSTANCE

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-heptafluoro-, 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

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.

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 ± 2.17 mm.
Average weight = 0.60 ± 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 CaCO₃

Alkalinity: 368 mg/L as CaCO₃

pH: 7.8

Conductivity: 50 $\square$ mhos/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

Nominal concentrations: Bk control, 18, 37, 75, 160, 320, 650 mg/L

Element value: 24-hour LC_{50} = 460 (370-580) mg/L

48-hour LC_{50} = 370 (290-470) mg/L

96-hour LC_{50} = 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_{50} 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_{50} 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

The test sample 96-hour LC₅₀ 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.

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

DATA QUALITY

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

This study was conducted by Analytical BioChemistry Laboratories, Inc. of Columbia, Missouri on behalf of the 3M Company.

OTHER

Last changed: 5/2/00

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)
(Reference No. 93)

TEST SUBSTANCE

Identity: 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-heptafluoro-, 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: EC₅₀ 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.

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 umhos/cm*

TOC: <2.0 mg/L

* Values measured at time of test.

Lighting: Cool white fluorescent lights, intensity 23 uE/s/m². 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 umhos/cm (control exposure)

1200 – 1300 umhos/cm (150 mg/L exposure)

Element basis: mortality

RESULTS

Nominal concentrations: Bk control, 25, 40, 60, 100, 150 mg/L

Element values: 24-hour EC_{50} = >150 mg/L (C.I. not calculable)

48-hour EC_{50} = 49 (43-56) mg/L

Perfluorooctanesulfonate concentration adjusted element value:

24-hour EC_{50} = >42 mg/L

48-hour EC_{50} = 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 sublethal 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

The test substance 48-hour EC_{50} 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_{50} value is 14 mg/L (49 mg/L X 0.28).

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

DATA QUALITY

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 Perfluorooctanesulfonate 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

This study was conducted at EnviroSystems Division, Resource Analysts, Incorporated, Hampton, NH at the request of the 3M Company.

OTHER

Last changed: 5/3/00

ACUTE TOXICITY TO FISH
(Reference No. 184)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH_4^+ salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, 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

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_{50}

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)

7.0-7.1 (100 mg/L exposure)

Test temperature range (0 – 96 hours)

21 – 22°C (70-72°F)

RESULTS

Nominal concentrations: Bk 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

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

DATA QUALITY

Reliability: Klimisch ranking 3.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 7/29/74 to 8/2/74.

OTHER

Last changed: 5/3/00

ACUTE TOXICITY TO FISH
(Reference No. 185)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH_4^+ salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, 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

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_{50} .

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)

RESULTS

Nominal concentrations: Bk control, 50, 75, 100, 125, 150 mg/L

Element value: 96-hour LC_{50} = 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

The test sample containing 25% Perfluorooctanesulfonate, ammonium salt exhibited a 96-hour LC_{50} for fathead minnow of 100 mg/L

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

DATA QUALITY

Reliability: Klimisch ranking 3.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 10/15/74 to 10/19/74.

OTHER

Last changed: 5/3/00

ACUTE TOXICITY TO FISH
(Reference No. 503)

TEST SUBSTANCE

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_{50} 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 $CaCO_3$

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 – 20.9C

Conductivity range (0 – 96 hours):

128 – 142 $\square$ mhos/cm (control exposure)

118 – 154 $\square$ mhos/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

Nominal loading concentrations: Bk control, 400, 700, 1,000 mg/L.

Element value: 24-hour LL_{50} = 618 (568 - 673) mg/L

48-hour LL_{50} = 607 (554 - 664) mg/L

72-hour LL_{50} = 595 (551 - 643) mg/L

96-hour LL_{50} = 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

The test sample 96-hour LL_{50} 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

DATA QUALITY

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

This study was conducted at AScI Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

Last changed: 5/24/00

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)
(Reference No. 504)

TEST SUBSTANCE

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 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_{50} 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 $CaCO_3$

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 – 21.0C

Conductivity range (0 – 48 hours):

142 – 155 μ mhos/cm (control exposure)

120 – 124 μ mhos/cm (50 mg/L exposure)

Element basis: mortality and immobilization

RESULTS

Nominal loading concentrations: Bk control, 3.13, 6.25, 12.5, 25, 50 mg/L

Element value: 24-hour EL_{50} = 27.0 (18.7-39.0) mg/L

48-hour EL_{50} = 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_{50} 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 EL50 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

DATA QUALITY

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

This study was conducted at AScI Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

Last changed: 5/24/00

APPENDIX III
AQUATIC EXPOSURE DATA (MULTI-CITY STUDY)
CONTENTS

Analytical reports for water column samples from the following cities:

Cleveland, TN
Decatur, AL
Mobile, AL
Pensacola, FL
Columbus, GA
Port St. Lucie, FL

Analytical Report

Fluorochemical Characterization of Surface Water Samples

Cleveland, Tennessee (W1973)

Centre Analytical Laboratory Report No. 023-014A

Testing Laboratory

Centre Analytical Laboratory, Inc.
3048 Research Drive
State College, PA 16801

3M Environmental Laboratory Contact

Kent R. Lindstrom
Bldg. 2-3E-09
P.O. Box 33331
St. Paul, MN 55133-3331
Phone: (651) 778-5352

Requester

Kris J. Hansen, Ph.D.
3M Environmental Technology & Safety Services
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P.O. Box 33331
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Introduction

Results are reported for the analysis of a series of surface water samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Cleveland, Tennessee. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) was requested for all samples. A total of 8 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical method used here was validated by Centre. The validation protocol and results are on file with Centre. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Eight individual sample containers were received. Samples were received on 05/03/00. The sample collection dates were not supplied. Chain-of-custody information is presented in Attachment A.

Sample MC-102H was not analyzed as per client request. (E-mail communication from Kris Hansen on 5/16/00).

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. The stability of the analytes of interest for longer periods has not been determined.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 ml portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

4.1.2 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate

means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Samples were extracted on 5/16/00 and analyzed by MS/MS on 5/18/00. Sample MC-108H was extracted on 5/22/00 and analyzed on 5/23/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in attachment D.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt). The response of the quantitation ion versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical method.

5.4 Matrix Spikes

Matrix spikes were prepared for every field sample (excluding blanks) at a concentration of 100 ng/L using all compounds of interest. Matrix spike recoveries are given in Attachment

C. All laboratory matrix spikes showed recoveries of all compounds between 70-130%.

Field spikes were prepared on sample MC-101H at a concentration of 100 ng/L using all compounds of interest. Field spike recoveries are also given in Attachment C. PFOSA showed low recovery in the field spike. All other compounds showed recoveries between 70-130%.

5.5 Duplicates

All field samples (excluding blanks) were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.6 Laboratory Control Samples

Milliq water was spiked with all compound of interest at 25 and 250 ng/L. All recoveries for all compounds were between 70-130% in each LCS. Recoveries are given with the raw data in Attachment D.

5.7 Sample Related Comments

Field blank samples consisted of empty containers. Forty milliliters of type I water filtered through a hypercarb cartridge was added to the empty container and analyzed in the same manner as the other samples.

Data Summary

Please see Attachment B for a detailed listing of the analytical results.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified.
All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.1 Attachment A: Chain-of-Custody

1.2 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries (Field and Laboratory Spikes)

8.4 Attachment D: LC/MS/MS Raw Analytical Data

9 Signatures

John M. Flaherty, Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Enaksha Wickremesinhe

Karen Smith

David Bell

**Analytical Results W1973 Cleveland,
Tennessee**

3M Sample Identification	Sample Description	PFOS (ng/L)	PFOSA (ng/L)	POAA (ng/L)
MC-101H	Site 1 P/N Surface Water	14.7 J	5.6 J	16.7 J
MC-103H	Site 1 P/N Surface Water Duplicate	< 25	< 25	< 25
MC-106H	Site 2 P/N Surface Water	< 25	< 25	< 25
NA	Site 2 P/N Surface Water Duplicate	< 25	< 25	< 25
MC-107H	Site 3 P/N Surface Water	< 25	< 25	< 25
NA	Site 3 P/N Surface Water Duplicate	< 25	< 25	< 25
MC-108H	Field Blank P/N Empty	< 25	< 25	< 25

J – Compound is present, but below the reporting limit of 25 ng/L. The result is an estimated value.

Method Detection Limits are approximately 2.5 ng/L for PFOS and PFOSA and 7.5 ng/L for POAA.

Analytical Report

Fluorochemical Characterization of Surface Water Samples

Decatur, Alabama (W1979)

Centre Analytical Laboratory Report No. 023-014B

Testing Laboratory

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Requester

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Introduction

Results are reported for the analysis of a series of surface water samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Decatur, Alabama. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) was requested for all samples. A total of 9 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical method used was validated by Centre. The validation protocol and results are on file with Centre. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Nine individual sample containers were received. Sample MC-202H was not analyzed as per client request. (E-mail communication from Kris Hansen on 5/16/00). Samples were received on 05/03/00. The sample collection dates were not supplied. Chain-of-custody information is presented in Attachment A.

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. The stability of the analytes of interest for longer periods has not been determined.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 ml portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

4.1.3 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Samples were extracted on 5/16/00 and analyzed by MS/MS between 5/19/00 and 5/20/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in attachment D.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt). The response of the quantitation ion versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical method.

5.5 Matrix Spikes

Matrix spikes were prepared for every field sample (excluding blanks) at a concentration of 100 ng/L using all compounds of interest. Matrix spike recoveries are given in Attachment C. All laboratory matrix spikes showed recoveries of all compounds between 70-130%.

Field spikes were prepared on sample MC-201H at a concentration of 100 ng/L using all compounds of interest. Field spike recoveries are also given in Attachment C. PFOS showed high recovery in the field spike. All other compounds showed recoveries between 70-130%.

5.7 Duplicates

All field samples (excluding blanks) were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.8 Laboratory Control Samples

Milliq water was spiked with all compound of interest at 25 and 250 ng/L. All recoveries for all compounds were between 70-130% in each LCS. Recoveries are given with the raw data in Attachment D.

5.7 Sample Related Comments

Field blank samples consisted of empty containers. Forty milliliters of type I water filtered through a hypercarb cartridge was added to the empty container and analyzed in the same manner as the other samples.

Data Summary

Please see Attachment B for a detailed listing of the analytical results.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.3 Attachment A: Chain-of-Custody

1.4 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries (Field and Laboratory Spikes)

8.4 Attachment D: LC/MS/MS Raw Analytical Data

9 Signatures

John M. Flaherty, Manager-Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Enaksha Wickremesinhe
Karen Smith
David Bell

**Analytical Results W1979 Decatur,
Alabama**

3M Sample Identification	Sample Description	PFOS (ng/L)	PFOSA (ng/L)	POAA (ng/L)
MC-201H	Site 1 P/N Surface Water	23.1 J	< 25	9.2 J
MC-203H	Site 1 P/N Surface Water Duplicate	8.3 J	< 25	< 25
MC-206H	Site 2 P/N Surface Water	14.5 J	< 25	< 25
NA	Site 2 P/N Surface Water Duplicate	22.0 J	< 25	11.1 J
MC-207H	Site 3 P/N Surface Water	< 25	< 25	< 25
NA	Site 3 P/N Surface Water Duplicate	< 25	< 25	< 25
MC-208H	Field Blank P/N Empty	< 25	< 25	< 25
MC-284H	Quiet P/N Surface Water	108	18.9 J	63.1
NA	Quiet P/N Surface Water Duplicate	114	18.1 J	57.3

J – Compound is present, but below the reporting limit of 25 ng/L. The result is an estimated value.

Method Detection Limits are approximately 2.5 ng/L for PFOS and PFOSA and 7.5 ng/L for POAA.

Analytical Report

Fluorochemical Characterization of Surface Water Samples

Pensacola, Fl (W2176)

Centre Analytical Laboratory Report No. 023-014C

Testing Laboratory

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Kris J. Hansen, Ph.D.
3M Environmental Technology & Safety Services
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P.O. Box 33331
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Introduction

Results are reported for the analysis of a series of surface water samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Pensacola, Fl. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) was requested for all samples. A total of 8 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical method used was validated by Centre. The validation protocol and results are on file with Centre. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Sixteen individual sample containers were received. Samples were received on 05/03/00. The sample collection dates were not supplied. Chain-of-custody information is presented in Attachment A.

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. The stability of the analytes of interest for longer periods has not been determined.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 ml portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

4.1.4 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Samples were extracted on 5/17/00 and analyzed by MS/MS on 5/19/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in attachment D.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt). The response of the quantitation ion versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical method.

5.6 Matrix Spikes

Matrix spikes were prepared for every field sample (excluding blanks) at a concentration of 100 ng/L using all compounds of interest. Matrix spike recoveries are given in Attachment C. All laboratory matrix spikes showed recoveries of all compounds between 70-130%.

Field spikes were prepared on sample MC-301H at a concentration of 100 ng/L using all compounds of interest. Field spike recoveries are also given in Attachment C. All field spikes showed recoveries of all compounds between 70-130%.

5.9 Duplicates

All field samples (excluding blanks) were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.10 Laboratory Control Samples

Milliq water was spiked with all compound of interest at 25 and 250 ng/L. All recoveries for all compounds were between 70-130% in each LCS. Recoveries are given with the raw data in Attachment D.

5.7 Sample Related Comments

Field blank samples consisted of empty containers. Forty milliliters of type I water filtered through a hypercarb cartridge was added to the empty container and analyzed in the same manner as the other samples.

Data Summary

Please see Attachment B for a detailed listing of the analytical results.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.5 Attachment A: Chain-of-Custody

1.6 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries (Field and Laboratory Spikes)

8.4 Attachment D: LC/MS/MS Raw Analytical Data

9 Signatures

John M. Flaherty, Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Enaksha Wickremesinhe

Karen Smith

David Bell

**Analytical Results W1979 Decatur,
Alabama**

3M Sample Identification	Sample Description	PFOS (ng/L)	PFOSA (ng/L)	POAA (ng/L)
MC-201H	Site 1 P/N Surface Water	23.1 J	< 25	9.2 J
MC-203H	Site 1 P/N Surface Water Duplicate	8.3 J	< 25	< 25
MC-206H	Site 2 P/N Surface Water	14.5 J	< 25	< 25
NA	Site 2 P/N Surface Water Duplicate	22.0 J	< 25	11.1 J
MC-207H	Site 3 P/N Surface Water	< 25	< 25	< 25
NA	Site 3 P/N Surface Water Duplicate	< 25	< 25	< 25
MC-208H	Field Blank P/N Empty	< 25	< 25	< 25
MC-284H	Quiet P/N Surface Water	108	18.9 J	63.1
NA	Quiet P/N Surface Water Duplicate	114	18.1 J	57.3

J - Compound is present, but below the reporting limit of 25 ng/L. The result is an estimated value.

Method Detection Limits are approximately 2.5 ng/L for PFOS and PFOSA and 7.5 ng/L for POAA.

Analytical Report

Fluorochemical Characterization of Surface Water Samples

Mobile, Alabama (W2151)

Centre Analytical Laboratory Report No. 023-014D

Testing Laboratory

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Phone: (651) 778-5352

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Kris J. Hansen, Ph.D.
3M Environmental Technology & Safety Services
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St. Paul, MN 55133-3331

Introduction

Results are reported for the analysis of a series of surface water samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Mobile, Alabama. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) was requested for all samples. A total of 8 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical method used was validated by Centre. The validation protocol and results are on file with Centre. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Eight individual sample containers were received. Samples were received on 05/03/00. The sample collection dates were not supplied. Chain-of-custody information is presented in Attachment A.

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. The stability of the analytes of interest for longer periods has not been determined.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 ml portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

4.1.5 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Samples were extracted on 5/17/00 and analyzed by MS/MS on 5/20/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in attachment D.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt) The response of the quantitation ion versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical method.

5.7 Matrix Spikes

Matrix spikes were prepared for every field sample (excluding blanks) at a concentration of 100 ng/L using all compounds of interest. Matrix spike recoveries are given in Attachment C. POAA showed low matrix spike recoveries in samples MC-407H and MC-484H. All other compounds showed matrix spikes recoveries between 70-130% in all samples.

Field spikes were prepared on sample MC-401H at a concentration of 100 ng/L using all compounds of interest. Field spike recoveries are also given in Attachment C. All compounds showed recoveries between 70-130%.

5.11 Duplicates

All field samples (excluding blanks) were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.12 Laboratory Control Samples

Milliq water was spiked with all compound of interest at 25 and 250 ng/L. All recoveries for all compounds were between 70-130% in each LCS. Recoveries are given with the raw data in Attachment D.

5.7 Sample Related Comments

Field blank samples consisted of empty containers. Forty milliliters of type I water filtered through a hypercarb cartridge was added to the empty container and analyzed in the same manner as the other samples.

Data Summary

Please see Attachment B for a detailed listing of the analytical results.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.7 Attachment A: Chain-of-Custody

1.8 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries (Field and Laboratory Spikes)

8.4 Attachment D: LC/MS/MS Raw Analytical Data

9 Signatures

John M. Flaherty, Manager-Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Enaksha Wickremesinha

Karen Smith

David Bell

Analytical Report

Fluorochemical Characterization of Surface Water Samples

Columbus, GA (W2336)

Centre Analytical Laboratory Report No. 023-014E

Testing Laboratory

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Kris J. Hansen, Ph.D.
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Introduction

Results are reported for the analysis of a series of surface water samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Columbus, GA. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) was requested for all samples. A total of 8 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical method used was validated by Centre. The validation protocol and results are on file with Centre. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Eight individual sample containers were received. Samples were received on 05/03/00. The sample collection dates were not supplied. Chain-of-custody information is presented in Attachment A.

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. The stability of the analytes of interest for longer periods has not been determined.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 ml portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

4.1.6 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Samples were extracted on 5/18/00 and analyzed by MS/MS between 5/20/00 and 5/21/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in attachment D.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt) The response of the quantitation ion versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical method.

5.8 Matrix Spikes

Matrix spikes were prepared for every field sample (excluding blanks) at a concentration of 100 ng/L using all compounds of interest. Matrix spike recoveries are given in Attachment C. All compounds showed matrix spike recoveries between 70-130% in all samples.

Field spikes were prepared on sample MC-501H at a concentration of 100 ng/L using all compounds of interest. Field spike recoveries are also given in Attachment C. The field spike results showed low recovery for PFOSA. All other compounds showed matrix spike recoveries between 70-130%.

5.13 Duplicates

All samples (excluding blanks) were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.14 Laboratory Control Samples

Milliq water was spiked with all compound of interest at 25 and 250 ng/L. All recoveries for all compounds were between 70-130% in each LCS. Results are given along with the raw data in Attachment D.

5.7 Sample Related Comments

Field blank samples consisted of empty containers. Forty milliliters of type I water filtered through a hypercarb cartridge was added to the empty container and analyzed in the same manner as the other samples.

Data Summary

Please see Attachment B for a detailed listing of the analytical results.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.9 Attachment A: Chain-of-Custody

1.10 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries (Field and Laboratory Spikes)

8.4 Attachment D: LC/MS/MS Raw Analytical Data

9 Signatures

John M. Flaherty, Manager-Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Enaksha Wickremesinha
Karen Smith
David Bell

**Analytical Results W2336
Columbus, GA**

3M Sample Identification	Sample Description	PFOS (ng/L)	PFOSA (ng/L)	POAA (ng/L)
MC-501H	Site 1 P/N Surface Water	63.8	11.7 J	26.1
MC-503H	Site 1 P/N Surface Water	59.9	13.2 J	25.6
	Duplicate			
MC-506H	Site 2 P/N Surface Water	76.6	16.5 J	26.1
NA	Site 2 P/N Surface Water	83.3	17.6 J	26.7
	Duplicate			
MC-507H	Site 3 P/N Surface Water	55.4	12.0 J	23.6 J
NA	Site 3 P/N Surface Water	55.4	14.9 J	24.3 J
	Duplicate			
MC-508H	Field Blank-P/N	< 25	< 25	< 25
	Empty			
MC-584H	Quiet Surface Water	< 25	< 25	< 25
NA	Quiet Surface Water	< 25	< 25	< 25
	Duplicate			

J – Compound is present, but below the reporting limit of 25 ng/L. The result is an estimated value.

Method Detection Limits are approximately 2.5 ng/L for PFOS and PFOSA and 7.5 ng/L for POAA.

Analytical Report

Fluorochemical Characterization of Water and Sediment Samples

Port St. Lucie Resampling (E00-2003)

Centre Analytical Laboratory Report No. 023-014P

Testing Laboratory

Centre Analytical Laboratory, Inc.
3048 Research Drive
State College, PA 16801

3M Environmental Laboratory Contact

Kent R. Lindstrom
Bldg. 2-3E-09
P.O. Box 33331
St. Paul, MN 55133-3331
Phone: (651) 778-5352

Requester

Dale Bacon Ph.D.
3M Environmental Technology & Safety Services
Bldg. 2-3E-09
P.O. Box 33331
St. Paul, MN 55133-3331

Introduction

Results are reported for the analysis of a series of water and sediment samples received by Centre Analytical Laboratories, Inc. (Centre) from the 3M Environmental Laboratory. The samples were collected from Port St. Lucie, Florida and are part of 3M Project E00-2003. The Centre study number assigned to the project is 023-014.

Specific fluorochemical characterization by liquid chromatography / tandem mass spectrometry (LC/MS/MS) and ion chromatography was requested for all samples. A total of 45 samples were received for analysis.

The samples were prepared and analyzed by LC/MS/MS for the following list of fluorochemicals:

- Table 1: Target Analysis

Compound Name	
Perfluorooctane Sulfonate	
Perfluorooctane Sulfonylamide	
Perfluorooctanoate	

The analytical methods used for water samples were validated by Centre. The validation protocol and results are on file with Centre. The methods were modified for the sediment samples, however the procedures have not been fully validated for this matrix. Data presented here is the highest quality data available at this time.

Sample Receipt

The samples were submitted in individual plastic containers and were not preserved. Forty-five individual sample containers were received. Samples were received on 7/25/00. The samples were collected between 7/18/00 and 7/19/00. Chain-of-custody information is presented in Attachment A.

Holding Times

The analytical method used was validated against a maximum holding time of 14 days. Stability after this time period has not been validated, however it should be noted that field fortifications have shown acceptable recoveries at the 100 ng/L for periods longer than 14 days.

Methods - Analytical and Preparatory

4.1 LC/MS/MS

4.1.1 Sample Preparation for LC/MS/MS Analysis

Water samples were initially treated with 200 uL of 250 mg/L sodium thiosulfate solution to remove residual chlorine. Solid phase extraction (SPE) was used to prepare the samples for LC/MS/MS analysis. A forty-milliliter portion of sample was transferred to a C₁₈ SPE cartridge. The cartridge was first eluted with 5 mL of 40% methanol in water solution. The eluate was discarded and the SPE column was then eluted with 100% methanol. A 5 mL portion of methanol was collected for analysis by LC/MS/MS. This treatment resulted in an eight-fold concentration of the samples prior to analysis.

For the sediment and sludge samples, a representative portion of sample (5 grams) was first extracted into 5 ml of methanol. The extracts were filtered and diluted to a final volume of 40 mL with Type I water. The diluted extracts were then treated in the same manner as the water samples, beginning with the solid phase extraction.

4.1.7 Sample Analysis by LC/MS/MS

In HPLC, an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in

the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray is generally operated at relatively mild temperatures; molecules are ionized, fragmented, and detected. Ions characteristic of known fluorochemicals are observed and quantitated against standards.

A Hewlett-Packard HP1100 HPLC system coupled to a Micromass Ultima MS/MS was used to analyze the sample extracts. Analysis was performed using selected reaction monitoring (SRM). Water samples were extracted between 8/15/00 and 8/17/00 and analyzed by MS/MS between 8/18/00 and 8/23/00. Sediment and sludge samples were extracted on 8/18/00 and were analyzed by MS/MS on 8/21/00. The HPLC and MS/MS methods used for analysis and instrument parameters can be found in Attachments D and E.

Analysis

5.1 Calibration

A 7-point calibration curve was analyzed at the beginning and end of the analytical sequence for the compounds of interest. The calibration points were prepared at 0, 25, 50, 100, 250, 500, and 1000 ng/L (ppt) for LC/MS/MS analysis. The instrument response versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and correlation coefficient (r) and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r \geq 0.985$ ($r^2 \geq 0.970$).

Calibration standards are prepared using the same SPE procedure used for samples.

Calibration check standards were analyzed periodically (every three to five sample injections) throughout the analysis sequence. Compliance is obtained if the standard analyte concentrations are within +/-20% of the actual value.

For the results reported here, calibration criteria were met.

5.2 Blanks

Extraction blanks were prepared and analyzed with every extraction batch of samples. The extraction blanks should not have any target analytes present at or above the concentration of the low-level calibration standard. For these samples, the extraction blanks were compliant.

Instrument blanks in the form of clean methanol solvent were also analyzed after every high-level calibration standard, and after known high-level samples. Again, the blanks should not have any target analytes present at or above the low-level calibration standard. For the samples presented here the instrument blanks are compliant.

5.3 Surrogates

Surrogate spikes are not a component of the LC/MS/MS analytical methods.

5.9 Matrix Spikes

Matrix spikes were prepared for every field sample using all compounds of interest. Matrix spike recoveries are given in Attachment C.

Field spikes were submitted with the water samples. Field spike recoveries are also included in Attachment C. The results from the 200 ppt field spike for sample QSW Palm City-C-FMS-200ppt A indicate that this sample was spiked at 1000 ppt rather than 200 ppt. The results for QSW Palm City-C-FMS-200ppt B indicate a 200 ppt spiking concentration.

5.15 Duplicates

All field samples were analyzed in duplicate. Results are given along with the sample results in Attachment B.

5.16 Laboratory Control Samples

For LC/MS/MS analyses, MilliQ water was spiked with all compounds of interest at 25 and 250 ng/L during each extraction set. All recoveries for all compounds were between 70-130% in each LCS. Results are given along with the raw data in Attachments D and E.

5.7 Sample Related Comments

There are no other sample related comments for this data set.

Data Summary

Please see Attachment B for a detailed listing of the analytical results. Surface water results are reported in parts per trillion (ppt) (ng/L). Sediment and sludge sample results are reported in parts per billion (ppb) (ng/g) on both an as-received and dry-weight basis.

Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Centre.

Attachments

1.11 Attachment A: Chain-of-Custody

1.12 Attachment B: Results.

8.3 Attachment C: Matrix Spike Recoveries

8.4 Attachment D: LC/MS/MS Raw Analytical Data (Surface Water Samples)

8.5 Attachment E: LC/MS/MS Raw Analytical Data (Sediment and Sludge Samples)

9 Signatures

John M. Flaherty, Manager- Operations Manager

Date

Kevin J Lloyd, Vice President

Date

Other Lab Members Contributing to Data

Karen Smith

**Analytical Results E00-2003 Port St. Lucie, FL Resampling
(solids)**

3M Sample Identification	PFOS (ug/Kg) (as received)	PFOSA (ug/Kg) (as received)	POAA (ug/Kg) (as received)
NPWWTP Sludge-C	1.36	< 0.200	< 0.200
NPWWTP Sludge-C dup	1.30	< 0.200	< 0.200
SED #3 SH-C	< 0.200	< 0.200	< 0.200
SED #3 SH-C dup	< 0.200	< 0.200	< 0.200
SED#1 Penpark- C	< 0.200	< 0.200	< 0.200
SED#1 Penpark-C dup	< 0.200	< 0.200	< 0.200
SED#2 MCM-C	< 0.200	< 0.200	< 0.200
SED#2 MCM-C dup	< 0.200	< 0.200	< 0.200

3M Sample Identification	PFOS (ug/Kg) (dry weight)	PFOSA (ug/Kg) (dry weight)	POAA (ug/Kg) (dry weight)
NPWWTP Sludge-C	62.9	< 9.3	< 9.3
NPWWTP Sludge-C dup	60.2	< 9.3	< 9.3
SED #3 SH-C	< 0.261	< 0.261	< 0.261
SED #3 SH-C dup	< 0.261	< 0.261	< 0.261
SED#1 Penpark- C	< 0.263	< 0.263	< 0.263
SED#1 Penpark-C dup	< 0.263	< 0.263	< 0.263
SED#2 MCM-C	< 0.304	< 0.304	< 0.304
SED#2 MCM-C dup	< 0.304	< 0.304	< 0.304

J - Compound is present, but below the reporting limit. The result is an estimated value.

APPENDIX IV

**PLANNED ENVIRONMENTAL STUDIES ON
PERFLUOROOCTANE SULFONATES (PFOS)**

PLANNED ENVIRONMENTAL STUDIES ON PERFLUOROOCTANE SULFONATES

The 3M Company is planning to conduct several studies in three subject areas: (1) environmental fate and transport, (2) ecotoxicity studies, and (3) environmental monitoring (environmental sampling and release estimation). These studies are listed in Table IV-1.

Table IV-1. Planned Environmental Studies on Perfluorooctane Sulfonates (PFOS)

Title	Conducted By	Status
<i>Environmental Fate and Transport</i>		
Soil and sediment adsorption/desorption	3M Environmental Laboratory	Underway
Multi-media modeling of PFOS distribution	D. Mackay, Trent University	Underway
Bioconcentration in bluegill sunfish	Wildlife International, Ltd.	Underway
Biodegradability - aerobic, acclimated closed bottle	Springborn Laboratories, Inc.	Underway
Biodegradability - aerobic soil/sediment	Springborn Laboratories, Inc.	Underway
Biodegradability - aerobic, pure culture (4 species)	Springborn Laboratories, Inc.	Underway
Biodegradability - anaerobic	Springborn Laboratories, Inc.	Underway
Hydrolysis	3M Environmental Laboratory	Underway
Hydrolysis on soils	3M Environmental Laboratory	Underway
Photolysis	3M Environmental Laboratory	Underway
Photolysis with humic matter	3M Environmental Laboratory	Underway
Photolysis with metals	3M Environmental Laboratory	Underway
Photolysis with soils	3M Environmental Laboratory	Underway
Solubility - Confirmation Tests	3M Environmental Laboratory	Underway
Activated sludge sorption isotherm	3M Environmental Laboratory	Underway

Table IV-1. Planned Environmental Studies on Perfluorooctane Sulfonates (PFOS)

Title	Conducted By	Status
Photolysis - atmospheric	3M Environmental Laboratory	Underway
<i>Ecotoxicity Studies</i>		
96-Hr <i>Anabaena flos-aquae</i> growth inhibition	Wildlife International, Ltd.	Underway
96-Hr <i>Navicula pelliculosa</i> growth inhibition	Wildlife International, Ltd.	Underway
96-Hr <i>Skeletonema costatum</i> growth inhibition	Wildlife International, Ltd.	Underway
7-day <i>Lemna gibba</i> growth inhibition	Wildlife International, Ltd.	Underway
Phytotox - seedling emergence and plant uptake	Wildlife International, Ltd.	Underway
FETAX (frog embryo teratogenesis)	Wildlife International, Ltd./ University of Maryland	Underway
Histo-pathological exam of tissues from mallard duck pilot reproduction	EPI Laboratory	Underway
Histo-pathological exam of tissues from bobwhite quail pilot reproduction	EPI Laboratory	Underway
Pilot study on mallard duck reproduction - final comprehensive report	Wildlife International, Ltd.	Underway
Pilot study on bobwhite quail reproduction - final comprehensive report	Wildlife International, Ltd.	Underway
Evaluation of PFOS protein binding using mallard and quail pilot study specimens	Michigan State University	Underway
Validation and determination of PFOS in tissues of mallard and quail	Centre Analytical	Underway
Acute toxicity to bluegill sunfish	Wildlife International, Inc.	Underway
Acute toxicity to sheepshead minnow	Wildlife International, Inc.	Underway
Acute toxicity to rainbow trout	Wildlife International, Inc.	Underway
Acute toxicity to honeybees - oral & contact exposure	Wildlife International, Inc.	Underway
Acute toxicity to earthworm (<i>Eisenia foetida</i>)	Wildlife International, Inc.	Underway
Mallard duck reproduction (dietary)	Wildlife International, Inc.	Underway

Table IV-1. Planned Environmental Studies on Perfluorooctane Sulfonates (PFOS)

Title	Conducted By	Status
Bobwhite quail reproduction (dietary)	Wildlife International, Inc.	Underway
<i>Environmental Monitoring (Environmental Sampling and Release Estimation)</i>		
Multi-city Study - sediments	Centre Analytical	Underway
Multi-city Study - POTW sludges and effluent	Centre Analytical	Underway
Multi-city Study - landfill leachates	Centre Analytical	Underway
Multi-city Study - market baskets	Centre Analytical	Underway
Watershed description for Multi-city sample points	Battelle Memorial Institute	Underway
Decatur environmental sampling	Michigan State University	Underway
Comprehensive report - interpretation of Multi-city sample results	Battelle Memorial Institute	Underway
Comprehensive report interpretation of biosphere sampling	Michigan State University	Underway

APPENDIX V

ROBUST SUMMARIES

<u>ROBUST SUMMARY #</u>	<u>TITLE</u>
RS1	Eye Irritation Report on Sample T-1117, 1974
RS2	An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. 1979
RS3	Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD ₅₀) Study in Rats. 1978.
RS4	Skin Irritation Report on Sample T-1117, 1974.
RS5	Retrospective Cohort Mortality Study of the 3M Decatur Plant, 1995
RS6	Random Sample Assessment of Fluorochemical Serum Levels in Decatur Production Employees, 1998
RS7	Analysis for Fluorochemicals in 39 Individual Swedish Blood Blood Samples, 1999
RS8	Clinical Chemistries, Hematology and Hormones from Voluntary Medical Surveillance of Male Fluorochemical Production Workers, 1995 and 1997
RS9	Serum Fluorochemical Levels in Sumitomo employees, 1999
RS10	Evaluation of the Mutagenic Activity of T-6906 [N-EtFOSE alcohol] in an <i>In Vitro</i> Mammalian Call Gene Mutation Test With L5187Y Mouse Lymphoma Cells (With Independent Repeat), 1998
RS11	Mutagenicity Test on T-6292 [N-EtFOSE alcohol] in an <i>In Vivo</i> Mouse Micronucleus Assay, 1996
RS12	Mutagenicity Test on T-5710 [N-EtFOSE alcohol] in an <i>In Vivo</i> Rat Micronucleus Assay, 1993
RS13	Genotoxicity Test on T-5710.1 [N-EtFOSE alcohol] in the <i>In Vivo/In Vitro</i> Unscheduled DNA Synthesis and Cell Proliferation Assay in Rat Liver Cells, 1993
RS14	<u>In Vitro</u> Microbiological Mutagenicity Assays of 3M Company Compounds T-2247 CoC [perfluorooctylsulfonate DEA salt] and T-2248 CoC, 1978
RS15	<i>Salmonella - Escherichia Coli</i> /Mammalian-Microsome Reverse Mutation Assay with PFOS, 1999
RS16	Salmonella Typhmuri Spot Test on FC-95 [PFOS], 1977
RS17	Bacterial Reverse Mutation Test of (τ-1, 1996

RS18	Mutagenicity Evaluation of T-2014 CoC [PFOS] in the Ames Salmonella/Microsome Plate Test, 1978
RS19	Mutagenicity Test on T-6295 [PFOS] in an In Vivo Mouse Micronucleus Assay, 1996
RS20	Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS, 1999
RS21	Chromosomal Aberrations in Human Whole Blood Lymphocytes with PFOS, 1999
RS22	Evaluation of the Mutagenic Activity of T-5874 [N-MeFOSE alcohol] in the Ames Salmonella/microsome test (with independent repeat), 1994
RS23	Evaluation of the ability of T-5874 [N-MeFOSE alcohol] to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes, 1994
RS24	Evaluation of the Mutagenic Activity of T-5874 [MeFOSE alcohol] in an <i>In Vitro</i> Mammalian Call Gene Mutation Test With L5187Y Mouse Lymphoma, 1994
RS25	Mutagenicity Test on T-5711 [N-MeFOSE alcohol] in an <i>In Vivo</i> Rat Micronucleus Assay, 1993
RS26	Genotoxicity Test on T-5711.1 [N-MeFOSE alcohol] in the <u>In Vivo/In Vitro</u> Unscheduled DNA Synthesis and Cell Proliferation Assays in Rat Liver Cells, 1993
RS27	Detection of Fluorochemicals in 35 Lots of Commercial Sera, 1999
RS28	Analysis of Pooled Blood Samples from 3 European Blood Banks
RS29	Analysis of Pooled Blood Samples from 18 U.S. Blood Banks
RS30	Analysis of PFOS from Pooled Serum of Two Commercial Laboratories, 1998
RS31	A Pilot Study to Identify Fluorochemicals in Children With Limited Sera for Analysis, 1999
RS32	26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys. Unaudited Draft Final Report
RS33	Two Year Oral (Diet) Toxicity Carcinogenicity Study of Fluorochemical FM-3924 [N-EtFOSE alcohol] in Rats
RS34	4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys. Unaudited Draft. 1998.
RS35	First ninety-day rhesus monkey toxicity study [PFOS], 1979
RS36	Second ninety-day rhesus monkey toxicity study [PFOS], 1978
RS37	Ninety day study in rats, [PFOS], 1978
RS38	13-Week Dietary Toxicity Study with N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE, T-6314) in Rats

RS39	104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS: T-6295) in Rats. Summary Report Week 53
RS40	Oral Developmental Toxicity Study of N-EtFOSE in Rabbits
RS41	Oral Teratology Study of FM3422 in Rats - Experiment No. 0680TR0010 [N-EtFOSE]
RS42	Oral (GAVAGE) Developmental Toxicity Study of N-EtFOSE in Rats
RS43	Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats 3M 418-009; Argus Research Laboratories Study Number: 6295.9, 1999
RS44	Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits - 3M T-6295.10, Argus Research Laboratories Study Number: 418-012, 1999
RS45	Oral Teratology Study of FC-95 in Rats - Experiment No. 0680TR0008
RS46	Rat Teratology Study T-3351 Final Report – Project No. 154-160
RS47	Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats - Argus Research Laboratories Study Number: 6295.9, 1999. Protocol 418-008
RS48	Oral (Gavage) Cross-Fostering Study of PFOS in Rats
RS49	Ninety day subacute rat toxicity study on FM-3422 [N-EtFOSE alcohol]
RS50	Ninety Day Subacute Rhesus Monkey Toxicity Study on FM-3422 [N-EtFOSE alcohol]
RS51	104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl-Perfluorooctanesulfonamido Ethanol in Rats. SUMMARY REORT – WEEK 53
RS52	Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats (1999)
RS53	Determination of Serum Half-Lives of Perfluorooctanesulfonate and Other Fluorochemicals
RS54	28-Day Percutaneous Absorption Study with FC-95 in Albino Rabbits (1981)

RS55	Oral Teratology Study of T-2999CoC in Rabbits
RS56	Absorption and biotransformation of N-EtFOSE and tissue distribution and elimination of carbon-14 after administration of N-EtFOSE-¹⁴C in feed (1983)
RS57	Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-14C (1979)
RS58	Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium [14C]Perfluorooctanesulfonate (1984)
RS59	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 (1999)
RS60	Absorption of FC-95-14C in Rats after a Single Oral Dose (1979)

ROBUST SUMMARY - RS1

Title: Eye Irritation Report on Sample T-1117. 1974

TEST SUBSTANCE

Identity: Potassium perfluorooctanesulfonate

Remarks: 3M Sample T-1117, FC-95

METHOD

Note pH of test material: Not specified

Method/Guideline followed: Not specified

Test Type: in vivo

Species/strain/cell type or line: Rabbit/Albino

Sex (males/females/both): Not specified

Number of animals/sex/dose: 6/single dose

Total dose: It is assumed to be 0.1 gram per eye (the method specifies 0.1 ml for liquids and 0.1 gram for solids., and FC-95 is a solid as sold)

Length of time test material is in contact with animal/cell: Based on the description of the method, it is assumed that the material was in contact for one hour, although this is not specified.

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. Either 0.1 ml or 0.1 gram of the test substance was instilled in one eye, the other eye was left untreated as a control. Since FC-95 is a solid material, it is assumed that 0.1 gram was instilled. If 0.1 ml was instilled, the concentration of the solution is not specified. The method calls for removing any residue of the test material at each observation period. Since the first observation period is at one hour, it is assumed that the contact period was one hour; although contact period is not otherwise specified. 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. It appears that scores reached a maximum of 9.33 at 24 hours after treatment then decreased over the rest of the study to zero at the 72 hour observation. The authors conclude only T-1117 is irritating to the eyes.

RESULTS

Corrosive: no

Irritation score: Only total scores provided.

One hr: 8.00; 24 hr: 9.33; 48 hr: 3.33; 72 hr: 0.0

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.

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. Biesemeier and D.L. Harris. 1974. Eye and Skin Irritation Report on Sample T-1117. Project No. 4102871, WARF Institute Inc.

ROBUST SUMMARY - RS2

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)

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

LC50 = 5.2 (4.4 – 6.4) mg/l; 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 LC₅₀ 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 LC₅₀ 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.

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. 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.

OTHER – Summary modified 8/11/00

ROBUST SUMMARY - RS3

Title: Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD₅₀) Study in Rats. 1978.

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: Fluorad Fluorochemical Surfactant, FC-95, White powder

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, 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

LD50 female rats: 271 (200-369) mg/kg

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 (LD₅₀) 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.) .

OTHER – Summary modified 8/11/00

ROBUST SUMMARY - RS4

Title: Skin Irritation Report on Sample T-1117.

Identity: Potassium perfluorooctanesulfonate

Remarks: 3M Sample T-1117; FC-95

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: 0.5 grams per each of two test sites per rabbit (intact-wet and abraded-wet)

Vehicle: None

Length of test material is in contact with animal/cell: 72 hr

Grading scale: Scales of 1 to 4, increasing in severity for erythema and eschar (combined) formation and edema formation are used and scores for each endpoint are summed such that the score equals the sum of erythema and edema scores. 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 ml) of test material was placed on abraded or intact prepared test sites (intact-wet and abraded-wet), then covered with gauze patches and taped. After 24 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale. An additional observation and scoring was performed at 72 hours.

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. Biesemeier and D.L. Harris. 1975. Eye and Skin Irritation Report on Sample T-1117. Project No. 4102871, WARF Institute, Inc.

ROBUST SUMMARY - RS5

Title: Retrospective Cohort Mortality Study of the 3M Decatur Plant, 1995

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Retrospective cohort mortality study.

Manufacturing/Processing/Use: 3M chemical and film manufacturing facility in Decatur, Alabama.

Hypothesis tested: To determine whether the mortality experience of employees at the 3M Decatur plant was significantly different from that which would be expected.

Study period: The cohort consisted of employees who had worked at least one year at the Decatur plant from March 1961 to December 31, 1991.

Setting: Occupational. Plants located in Decatur, Alabama. The chemical plant and film plant were physically distinct entities (approximately 300 yards apart).

Total population: 1957 employees were eligible for the cohort

Subject selection criteria: Employed at least one year at the 3M Decatur plant and at least 1 day after March 1, 1961.

Total # of subjects in study: 1639 males (70 deaths), 318 females (4 deaths)
1050 men ever employed in the chemical plant
1116 men ever employed in the film plant

Comparison population: U.S. population, Alabama population, and the population in counties in Alabama where more than one-half of the county was within 100 miles of Decatur, excluding counties in which there was a city with greater than 100,000 persons.

Participation rate: Vital status was determined for 99.7% of the cohort. Only 6 employees were lost to follow-up (all male).

Subject description:

	<u>Males</u>	<u>Females</u>
Total number of person-years	33,108	4,807
Number of deaths	70	4
Average age started work	25	26
Average year of entry	1971	1977
Average age at death	47	28
Average year of death	1984	1980
Still employed at plant	810/1639	141/318

Data collection methods: Mortality data derived from: National Death Index, death certificates from state vital statistics offices, Equifax Death Search, and TRW FAC+ Summary. Work histories (employee records) were used to verify that the employee worked at the Decatur plant for at least one year.

Exposure period: Potential exposure period was March 1, 1961 to December 31, 1991.

Description/delineation of

exposure groups/categories: 1639 males, 70 deaths; 318 females, 4 deaths.

Measured or estimated exposure: 1) ever employed in the chemical department(s); 2) only employed in the chemical department(s); 3) ever employed in the film department(s); 4) only employed in the film department(s).

Statistical methods: SMRs calculated using the Occupational Cohort Mortality Analysis Program (OCMAP). 95% confidence intervals provided.

Other methodological information: SMRs calculated using observed to expected number of deaths specific for the cause of death, race, sex, age, and time (based on ICD-8). The expected number of deaths was calculated by applying cause-, race-, sex-, age-, and time-specific rates for the comparison population to the person-years at risk. Cohort members did not contribute person-years until they had met the minimum length of work criterion. Person-years of follow-up were contributed until death, loss to follow-up, or the end of the study. Employees were assumed to be white because no information was available on race. Deceased study members for whom a death certificate could not be obtained only were included in the "all causes of death" and "unknown cause of death" categories.

SMRs computed using the Occupational Cohort Mortality Analysis Program and compared to the US Death Rates program. Results were virtually identical. Mortality rates for whites were used to calculate the expected numbers for all men and women.

RESULTS

Describe results: SMRs based on the Alabama and Alabama counties comparison populations were similar to, but lower than, those based on the US comparison population. Using the US as the comparison population, SMRs for men for all causes of death, heart disease, and respiratory disease (nonmalignant) were significantly less than 100 (62.9, 59.1, and 0, respectively). The SMR for all cancers was 68.4, although not significant. Most of the specific cancer SMRs were less than 100 except for cancer of the bladder and other urinary organs and cancer of other lymphatic and hematopoietic tissue. For these 2 causes, the SMRs were based on only 1 or 2 deaths and were not statistically significant.

There were only 4 deaths for women. When compared to the US population, no cause of death had an SMR significantly different from 100. Three of the 4 deaths were from non-work-related external causes.

For men ever employed in the chemical department(s), the SMR for all causes was significantly less than 100 (48.8; 95% CI 24.4, 87.4). SMRs for all cancer were 76.9 (95%CI 40.9, 131.5). Although not statistically significant, SMRs > 100 were reported for men ever employed in the chemical department(s) (n = 1,050) for cancer of the bronchus, trachea and lung [SMR = 120.7; 95% CI 48.5-248.7], cancer of the bladder and other urinary organs [SMR = 415.5, 95% CI 10.4 - 2,315.3], cancer of the brain and other CHS [SMR = 117.2, 9% CI 2.9 - 653.0], leukemia and aleukemia [SMR = 120.0, 95% CI 3.0-668.8], and cancer of other lymphatic and hematopoietic tissue [SMR = 137.2, 95% CI 3.4-764.5]. All of these SMRs > 100, except for cancer of the bronchus, trachea and lung, were based on just one observed death. Comparable nonsignificant findings were reported when the analyses were restricted to men who only worked in the chemical plant (n = 485). All of the above SMRs were based on the US comparison population.

There were 37 deaths among men ever employed in the film department(s). A statistically significant deficit was observed for all causes of death combined (58.6, 95% CI 41.3, 80.8). Eleven deaths were observed for men only employed in the film department(s). There were no statistically significant increases in SMRs for any of the causes of death.

Study strengths and weaknesses: Few deaths 74/1951, only approximately 45% of the study subjects were older than 45 years of age at the end of study, few women could be studied, length of employment was not measured, PFOS serum levels were not examined in relation to mortality (does not address latency, turnover, etc.). Only half of the employees are still employed at the plant.

This study has a very high rate of follow up for employees (97.3%), and will be updated in 2000.

Research sponsors: University of Minnesota, School of Public Health, Division of Environmental and Occupational Health.

Consistency of results: There are no other mortality worker studies on PFOS.

CONCLUSIONS

Among males, SMRs were below the null value for all major causes of death regardless of the comparison population used to calculate the expected values. Given that the worker population was so young, it is difficult to draw many conclusions. An update of the study will provide a small additional amount of information; however, only half of the employees are still employed at the plant, and PFOS-related products will soon be removed from the manufacturing site.

REFERENCE

Mandel JS and Johnson RA. March 13, 1995. Mortality study of employees at 3M plant in Decatur, Alabama. University of Minnesota, School of Public Health.

ROBUST SUMMARY - RS6

Title: Random Sample Assessment of Fluorochemical Serum Levels in Decatur Production Employees, 1998

TEST SUBSTANCE

Identity: PFOS

Remarks: Other fluorochemicals were assayed in this study. However, only PFOS is reported here.

METHOD

Study design: Random, cross-sectional occupational study.

Manufacturing/Processing/Use: Comparison of chemical plant employees to film plant employees.

Hypothesis tested: To randomly sample employees from the Decatur chemical plant to determine the distribution of employee serum fluorochemical levels according to demographics, current and longest held jobs, years worked, and building locations. This was done since the voluntary nature of the medical surveillance program did not provide for a complete understanding of the distribution of fluorochemical serum levels in the Decatur workforce.

Study period: October – November 1998

Setting: Occupational--Decatur, Alabama.

Total population: 232 employees randomly chosen + 76 “volunteers” who requested that they participate (total # of workers at plant not provided)

Subject selection criteria: Current employment at the film or chemical plant in Decatur, Alabama.

Total # of subjects in study: 186 employees out of the random sample of 232, plus 76 employees who volunteered to participate.

Comparison population: N/A

Participation rate: 80% of the random sample participated

	<i>Chemical plant employees</i>	<i>Film plant employees</i>
Average age	42 years old	46 years old
Avg. length of employment	16 years	19 years
Gender	mostly male	mostly male

Data collection methods: Work history questionnaire and blood sera samples

Details on data collection: Sera samples were extracted using an ion-pairing extraction procedure, and PFOS was measured using high-pressure liquid chromatography/electrospray tandem mass spectrometry evaluated versus an extracted curve.

Exposure period: N/A

Description/delineation of exposure groups/categories: On questionnaire, employees recorded current and longest-held jobs, age, BMI, hand-to-mouth activity.

Measured or estimated exposure: PFOS serum level is surrogate for exposure

Exposure levels: N/A

Statistical methods: Used SAS and JMP to calculate student's t-test, chi square, ANOVA, single and multivariable regression using linear and nonlinear analyses. Geometric means calculated (log normal distributions). For serum values less than the LLOQ, a midpoint between the LLOQ and 0 was used.

Other methodological information: Data analyses were conducted to: 1) compare responders and nonresponders in random sample by demographic characteristics; 2) compare mean serum fluorochemical levels within the chemical plant; 3) compare mean serum fluorochemical levels within the film plant by similar factors.

RESULTS

Describe results: Chemical plant employees (n = 126): arithmetic mean PFOS level was 1.505 ppm (95% CI, 0.091 – 10.600); geometric mean PFOS level was 0.941 ppm (95% CI, 0.785 – 1.128). Male (0.897 ppm) and female (0.459 ppm) geometric means were significantly different from each other. On average, serum fluorochemical levels were an order of magnitude higher among chemical plant employees than other workers. Film plant employees (n = 60): arithmetic mean PFOS level = 0.172 ppm (95% CI, 0.015 – 0.946); geometric mean PFOS level = 0.136 ppm (95% CI, 0.114 – 0.162).

Results were comparable to the employees' self-reported longest-held jobs. Current chemical plant job categories were strongly associated with production building assignments. Arithmetic

mean serum PFOS levels (range in parenthesis) for the eight chemical plant current job categories (range) were: cell operators 2.903 ppm (0.325-6.840); waste operators 2.649 ppm (0.254-7.880); chemical operators 1.781 ppm (0.471-7.260); maintenance workers 1.672 (0.291-4.060); supervisors/management 1.879 (0.091-10.600); mill operators 0.718 (0.230-2.040); engineer/laboratory workers 0.634 (0.095-1.740); and secretaries 0.497 (0.220-1.140).

PFOS was modestly positively associated ($r^2 = 0.11$) with years worked in the chemical plant.

Study strengths and weaknesses: This study addressed the possible volunteer bias that could have occurred in previous Decatur studies in which participation was voluntary. This study also measured other perfluorinated compounds in blood serum.

This study did not provide exposure information, but did provide additional information on job categories.

Research sponsors: 3M Environmental Lab

Consistency of results: The distribution of serum levels are consistent with those reported in the voluntary medical surveillance programs.

CONCLUSIONS

Based on a random sample of employees, the data obtained from this biological assessment allowed for a better understanding of the distribution of seven fluorochemicals in the chemical and film plant employee populations (only PFOS reported here). Distribution of serum samples observed were comparable to those reported in the voluntary medical surveillance programs.

REFERENCE

Olsen G, Logan PW, Simpson CA, Burris JM, Burlew JM, Schumpert JC, Mandel JH. August 11, 1999. Fluorochemical exposure assessment of Decatur chemical and film plant employees. 3M Medical Department, St. Paul, MN. FYI-0500-01378.

OTHER

ROBUST SUMMARY - RS7

Title: Analysis for Fluorochemicals in 39 Individual Swedish Blood Blood Samples, 1999

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional data on PFOS detected in individual serum samples from a Swedish disease laboratory.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the presence of PFOS in the serum of the general population.

Study period: 1998

Setting: N/A

Total population: Provided 39 individual Swedish samples. Age and gender demographic information was also provided.

Subject selection criteria: At discretion of the Swedish disease laboratory.

Total # of subjects in study: 39

Comparison population: N/A

Participation rate: N/A

Subject description: There were 16 males and 23 females. Age ranged from 5 through 86 years of age. Only 3 samples were under the age of 17 (ages 5, 5 and 12). Mean age was 42.

Health effects studied: PFOS levels in blood

Data collection methods: Blood sera samples

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: Means calculated.

Other methodological information:

RESULTS

Describe results: A total of 28 (72%) of the 39 individuals had serum PFOS levels below the Lower Limit of Quantitation (LLOQ) which was 31.4 ppb. The mean serum PFOS level for the 11 individuals whose serum levels were above the LLOQ was 48.0 ppb (range 31.6-85.4 ppb). There were no significant demographic differences between individuals who had LLOQ versus those whose serum PFOS values were > LLOQ. Average ages were 40 and 43, respectively. Percent male were 39 and 45 percent, respectively. Among those individuals who had serum PFOS values greater than the LLOQ, there was no association with age and/or gender.

Study strengths and weaknesses: These data are cross-sectional data used to determine an initial PFOS level in the general population--no other descriptive information about the subjects was collected. Limited number of samples and fewer yet that had serum PFOS levels greater than LLOQ. Blood donors cannot be considered representative of the general population.

Research sponsors: 3M Medical Department

Consistency of results: N/A

CONCLUSIONS

N/A

ROBUST SUMMARY - RS8

Title: Clinical Chemistries, Hematology and Hormones from Voluntary Medical Surveillance of Male Fluorochemical Production Workers, 1995 and 1997

TEST SUBSTANCE

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 in relation to serum PFOS levels as measured in the medical surveillance examinations of Antwerp and Decatur employees in 1995 and 1997.

Study period: Fall 1994 to Spring 1995, and 1997

Setting: Occupational. Plants located in Antwerp, Belgium and Decatur, Alabama.

Total population: Approximately 300 Decatur employees and 200 Antwerp production employees were eligible for the study. The total plant populations were not provided.

Subject selection criteria: Voluntary participation in medical surveillance program 1995 and 1997—only males were analyzed because of small number of female workers.

Total # of subjects in study: In 1995, 90 Decatur and 88 Antwerp employees participated. There was also a subset of employees for whom hormonal parameters were studied: 50/88 Antwerp and 38/90 Decatur employees.

In 1997, 84 Decatur and 65 Antwerp employees volunteered in the medical surveillance program.

Comparison population: N/A

Participation rate: In both years, less than 50% of the eligible production employees participated in these voluntary medical surveillance examinations. Only 61 employees were common to both time periods of the study.

Subject description: Decatur employees were significantly older and had a higher Body Mass Index than the Antwerp employees in both 1995 and 1997. Antwerp employees also smoked less (1995) than Decatur employees, consumed more alcohol, and had significantly different (p

<.001) mean measurements of alkaline phosphatase (lower), total bilirubin (higher), glucose (lower), HDL (higher), triglycerides (lower), and MCHC across both time periods.

Health effects studied: To determine if there were differences in the following parameters based on increasing PFOS 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 hormonal parameters (cortisol, dehydroepiandrosterone sulfate, estradiol, follicle stimulating hormone, 17-alpha hydroxyprogesterone, luteinizing hormone, prolactin, sex hormone binding globulin, free testosterone, bound testosterone, and thyroid stimulating

Data collection methods: Medical questionnaire, blood sera samples, measurements of height, weight, and blood pressure, and standard clinical chemistry and hematology tests, and pulmonary

Details on data collection: Questionnaire content, design, administration, etc. was not provided in report. Data on blood collection (amount, etc.) not provided.

Serum PFOS measured in 1995 by 3M's Environmental Technology Services in St. Paul, Minnesota using high-performance liquid chromatography thermospray mass spectrometry. In 1997, the serum samples were analyzed by Advanced Bioanalytical Services, Inc. using high-performance liquid chromatography electrospray mass spectrometry

Exposure period: Unknown. PFOS serum levels are surrogates for exposure.

Description/delineation of exposure groups/categories: The PFOS levels detected in workers' sera were grouped for each plant as: 0 - <1 ppm; 1- < 3 ppm; 3 - < 6 ppm; >= 6 ppm PFOS.

Distribution of Employees by Year and PFOS levels

1995: Antwerp (n = 88)

39% 0 - <1 ppm PFOS
36% 1- < 3 ppm PFOS
22% 3 - < 6 ppm
3% >= 6 ppm

1995: Decatur (n = 90)

12% 0 - <1 ppm PFOS
66% 1- < 3 ppm PFOS
18% 3 - < 6 ppm
4% >= 6 ppm

1997: Antwerp (n = 65)

48% 0 - <1 ppm PFOS
38% 1- < 3 ppm PFOS
14% 3 - < 6 ppm
0 >= 6 ppm

1997: Decatur (n = 84)

35% 0 - <1 ppm PFOS
45% 1- < 3 ppm PFOS
14% 3 - < 6 ppm
6% >= 6 ppm

Measured or estimated exposure: Serum PFOS levels are surrogates for exposure. No worker exposure data are available.

Statistical methods: Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance, and multivariable regression used to evaluate associations between PFOS and each hematological and clinical chemistry test and hormonal assay. Adjusted for age, BMI, alcohol consumption, and cigarette use as potential confounders.

Other methodological information:

RESULTS

Describe results:

	Distribution of Serum PFOS			
	1995		1997	
	Antwerp	Decatur	Antwerp	Decatur
0 - < 1 ppm	34	11	31	29
1 - < 3 ppm	32	59	25	38
3 - <6 ppm	19	16	21	12
≥ 6 ppm	3	4	0	5

In 1995 for both locations combined, PFOS was significantly ($p < .05$) correlated with HDL (negative association), total bilirubin (negative), WBC (positive) and platelets (negative). In 1997 for both locations combined, PFOS was significantly correlated with age (positive), BMI (positive), alanine aminotransferase (positive), direct bilirubin (negative), cholesterol (positive), LDL (positive) and hematocrit (negative).

The means of the 4 PFOS categories combined across both plants were all significantly different from each other. The youngest employees had the lowest PFOS levels. Of all of the clinical chemistries and hematological parameters, only total bilirubin had significant ($p < .05$) differences in means from the lowest exposure category (0 - <1 ppm) for both years. The lowest mean platelet count was observed at the highest PFOS exposure category in both years, although they were not significantly different across categories.

Using linear regression and adjusting for potential confounders, PFOS was significantly ($p < .10$) associated in both years only for total bilirubin. In one of the 2 years, PFOS was associated with direct bilirubin creatinine, cholesterol, LDL, HDL, hematocrit, hemoglobin, and platelet count. These variables were then separated by plant location and year. Only total bilirubin and HDL were significantly (negatively) associated with PFOS for at least one plant location for both time periods. Total bilirubin had a significant negative association with PFOS for the Decatur plant in both years and no significant association at the Antwerp plant for either year. HDL was significantly negatively associated with PFOS in Antwerp in both years but not significantly associated with PFOS in Decatur in either year.

There was no evidence of a decline in serum cholesterol associated with an increase in PFOS at the serum levels measured. The overall levels between plants and over both years were not statistically significant. Cholesterol levels by PFOS serum levels are presented below.

Serum Cholesterol Levels by Plant and Year

	1995	
	Antwerp	Decatur
0 - <1 ppm	220	215
1 - < 3 ppm	206	221
3 - <6 ppm	217	209
≥ 6 ppm	223	206
	F = 0.6	F = 0.5
	p = 0.6	p = 0.7

	1997	
	Antwerp	Decatur
0 - <1 ppm	192	204
1 - < 3 ppm	213	218
3 - <6 ppm	228	230
≥ 6 ppm	228	229
	F = 2.9	F = 2.0
	p = 0.1	p = 0.1

Sixty-one employees participated in biomonitoring for both years. The mean age of these employees was lower than that of the 1995 employees and higher than that of the 1997 employees. Cholesterol and LDL were significantly higher in the 61 participants in 1997. Twenty-seven of the 61 employees were from the Antwerp plant. These employees had significantly higher mean PFOS exposures, were significantly older, had greater BMIs and higher cholesterol values than the Antwerp participants in 1997. Regardless of plant location, mean PFOS levels were higher for those employees who were selected for hormone measurements in 1995.

The mean age of the lowest PFOS exposure category (0- < 1) was 10 years less than that of the highest exposure category; therefore, mean DHEAS, 17-HP, free testosterone and bound testosterone levels at this exposure level were greater than the means of the higher PFOS exposure categorizations. Adjusting for the differences in age (a confounder for male testosterone hormone levels) and other confounders in the regression models resulted in no significant associations between PFOS and the hormones analyzed, except for estradiol. However, when one employee with 12.83 ppm PFOS serum level, confounded by a high BMI (33 kg/m²) was excluded from the analysis, the finding was no longer significant.

Study strengths and weaknesses: Cross-sectional design, voluntary participation, small number of employees with PFOS levels above 6 ppm, low participation rate in both plants in both time periods. There was a large turnover rate between the 2 study years (only 61 employees common to both study periods). The data were combined across 2 sites that were very different, and they also cannot be considered independent populations. The serum levels of PFOS may be below the no-effect level in lab animals. Serum PFOS may not accurately reflect body burden. No occupational exposure data were collected at either plant.

This study provides data on PFOS serum levels and biological parameters not studied before, and provides a comparison of these data across plants.

Research sponsors: 3M

Consistency of results: There are no other studies of this kind on PFOS; however, the results of the hepatic and lipid clinical chemistry tests have been published. The reference is: Olsen GW, Burris JM, Mandel JH, Zobel LR. Serum Perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. JOEM. Sept. 1999;41:799-806.

CONCLUSIONS

The authors concluded that among Antwerp and Decatur male employees, significant hematological, clinical chemistry and hormonal abnormalities were not associated with serum PFOS concentrations less than 6 ppm.

REFERENCE Olsen GW, Burris JM, Mandel JH, Zobel LR. April 22, 1998. An epidemiologic investigation of clinical chemistries. Hematology and hormones in relation to serum levels of perfluorooctane sulfonate in male fluorochemical production employees. 3M Medical Department. FYI-0500-01378.

OTHER There are several methodological issues that should be noted. They are:

- 1) Cross-sectional design does not allow for a direct analysis of the temporality of an association.
- 2) The voluntary participation rates were low as both production sites had less than 50% participation.
- 3) Given the suspected long half-life of PFOS, it may be conceivable that there may be some biological accommodation to the effects of PFOS which would minimize the possibility of finding an association.
- 4) Serum PFOS measurements may reflect body burden. In the cynomolgus primate, liver tissue concentrations approximated serum PFOS levels up to 100 ppm (low- and mid-dose groups). However, in the rat, this ratio was in the range of 3:1 to 6:1.
- 5) The 2 cross-sectional analyses cannot be viewed as independent populations as 61 employees were studied in both years (due to large turnover between study years).
- 6) There could be measurement error in important confounding variables.
- 7) The pulsatile nature of some of the hormones studied has resulted in prior recommendations that mean hormone measurements should be the result of pooled blood from multiple samples taken at short intervals; however, this was not feasible in this study.

ROBUST SUMMARY - RS9

Title: Serum Fluorochemical Levels in Sumitomo employees, 1999

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional.

Manufacturing/Processing/Use: Processing and formulation of fluorochemicals into products.

Hypothesis tested: To determine PFOS serum levels in employees at the Sumitomo, Japan 3M Plant.

Study period: 3 weeks in March 1999

Setting: Occupational—Sumitomo 3M employees, Sagamihara Plant, Japan.

Total population: Total number of employees working at this plant was not provided in the report.

Subject selection criteria: Voluntary participation in medical surveillance program at Sumitomo 3M Plant.

Total # of subjects in study: 94 volunteers (managerial and production employees)

Comparison population: Sagamihara plant management employees (n = 32) and management employees from the Tokyo Head Office (n = 30)

Participation rate: Not provided.

Subject description: Across all 3 “exposure groups”, the age range was 31 – 67 years old. No other information was provided.

Health effects studied: PFOS levels in blood

Data collection methods: Blood sera samples

Details on data collection: Sera was analyzed for fluorochemicals using high-pressure, liquid chromatography/electrospray tandem mass spectrometry. The LLOQ for PFOS was 0.0314 ppm.

Exposure period: N/A

Description/delineation of exposure groups/categories: Sagamihara Plant production employees (n = 32); Sagamihara plant management employees (n = 32); Management employees from Tokyo Head Office (n = 30), 40 km from Sagamihara Plant

Measured or estimated exposure: PFOS serum level is surrogate for exposure

Statistical methods: Descriptive statistics and t-tests (calculated using the LLOQ value for those employees with values less than the LLOQ)

Other methodological information:

RESULTS

Describe results: 24 of 94 employees had serum levels <LLOQ. 38% of the plant management employees had serum levels <LLOQ, and 40% of Tokyo office. PFOS was quantifiable in all production employees.

Sagamihara Plant production employees: arithmetic mean PFOS level = 0.135 ppm, range 0.0475-0.628 ppm.

Sagamihara Plant management employees: arithmetic mean PFOS 40.3 ppb, range 31.9- 56.6 ppb

Management employees from Tokyo Head Office: arithmetic mean PFOS 52.3 ppb, range 33 – 96.7 ppb

The arithmetic mean (0.135 ppm) was significantly different ($p < .05$) than the mean PFOS value for both managers' groups.

Study strengths and weaknesses: No information on exposure in the workplace, no descriptive information on the employees.

Research sponsors: 3M

Consistency of results: No other processing employees have been sampled.

CONCLUSIONS

The authors concluded that serum PFOS levels of Sagamihara Plant production employees were below those of workers at the 3M Antwerp and Decatur plants.

REFERENCE

Burris J, Olsen GW, Mandel JH, Schumpert JC. September 3, 1999. Determination of Serum Fluorochemical Levels in Sumitomo 3M Employees, Final Report, 3M Medical Department, Epidemiology, 220-3W-05. FYI-0500-01378.

OTHER

ROBUST SUMMARY - RS 10

Title: Evaluation of the Mutagenic Activity of T-6906 in an *In Vitro* Mammalian Cell Gene Mutation Test With L5187Y Mouse Lymphoma Cells (With Independent Repeat), 1998

TEST SUBSTANCE

Identity: T-6906; N-EtFOSE alcohol

Remarks: "Creme coloured solid;" purity 97%; Identification: T-6906 batch 040016.

METHOD

Method/guideline: OECD Guideline 476; EEC Directive 67/548/EEC, EEC Publication No. L133 & L248

Test type: Reverse mutation assay

Test System: Mammalian cell line

GLP: Yes (OECD Principles of Good Laboratory Practice).

Year study performed: 1998

Species/Strain: L5187Y mouse lymphoma cells.

Metabolic activation: S9 from Aroclor-induced adult male Wistar rat liver added at 8% v/v.

Statistical methods: None

Remarks:

Study Design:

Concentration: Rangefinding test: 3, 10, 33, 100, 333, and 1000 µg/ml with and without S9; Experiment 1: 10, 18, 33, 42, 56, 75 & 100 µg/ml without S9; 33, 100, 333 & 1000 µg/ml with S9; Experiment 2: 10, 20, 30, 40, 45, 50 & 55 µg/ml without S9; 10, 33, 100, 175, 250, 375, 500 & 750 µg/ml with S9

Number of replicates: 2

Plates/test: two 96-well plates/concentration for cloning efficiency (~1 cell/well); three 96-well plates/concentration for mutation frequency (~2000 cells/well).

Solvent: Dimethylsulfoxide (final concentration in culture medium not specified).

Positive controls: Without S9: ethylmethanesulfonate (1 mM in Hank's balanced salt solution, without calcium and magnesium); with S9: dimethylnitrosamine (0.5 mM in Hank's balanced salt solution, without calcium and magnesium).

RESULTS

Cytotoxic concentration: In rangefinding test with S9, no reduction was seen in cell number or cloning efficiency at any dose (test material precipitated at highest dose). Without S9, cell count and cloning efficiency immediately after treatment were reduced significantly at 333 and 1,000 µg/ml. In Experiment 1, without S9, concentrations of 56 µg/ml and higher were "too toxic for further testing." In the presence of S9, no severe toxicity was seen and all dose levels were

evaluated for mutagenicity. In experiment 2, without S9, concentrations of 50 and 55 µg/ml were "too toxic" and in the presence of S9, concentrations of 375 and 500 µg/ml were "too toxic" and were not evaluated for mutagenicity.

Genotoxic effects: No increase in mutant frequency seen without S9; an apparent increase in mutant frequency with S9 was seen only at the highest dose in experiment 1, but this was confounded by precipitation of test material (i.e., unphysiological conditions). In experiment 2, no significant increase in mutant frequency was seen without S9. Slight increases in mutant frequency at 750 and 250 µg/ml (not dose-proportional) with S9 were also confounded by test material precipitation at 750 µg/ml and an aberrant dose-response curve for survival (see table).

Remarks: A summary of the results of the two experiments is presented in the table below. Although the study authors concluded that the test material "is considered mutagenic in the presence of S9-mix," this is a highly questionable conclusion. Deviations from standard procedures for this assay, confounding by test material precipitation, and aberrations in dose-response relationships for toxicity and putative mutagenicity render the results questionable, and no valid conclusions can be drawn from them. In experiment 2, with S9, dose levels of 375 and 500 µg/ml were discarded as being too toxic, but the higher level of 750 µg/ml, at which the test material precipitated in the treatment medium, was (inappropriately) included in the evaluation of mutagenicity.

Summary of Cytotoxic and Mutagenic Response of L5178Y Cells to N-EtFOSE Alcohol			
Concentration (µg/ml)	Relative Cell Survival (% Control)	CE at Day 3 (%)	Mutation Frequency x10 ⁵
Experiment 1, Without S9			
Solvent Control	100	133	6.5
10	90	125	5.8
18	137	135	4.9
33	62	152	3.9
42	78	111	4.3
Positive Control (EMS)	82	79	55.9
Experiment 1, With S9			
Solvent Control	100	93	7.2
33	87	95	4.0
100	117	111	3.2
333	31	94	6.3
1000	127	19	160
Positive Control (DMN)	66	31	75.8
Experiment 2, Without S9			
Solvent Control	100	95	7.0
20	74	74	7.7
30	54	88	4.3
40	55	61	7.4
45	10	49	12.1
Positive Control (EMS)	8	18	109.8
Experiment 2, With S9			
Solvent Control	100	147	4.7
33	98	182	8.5
100	32	162	4.4
175	17	165	4.4
250	57	147	14.9
750	100	157	11.1
Positive Control (DMN)	33	32	54.6

CONCLUSIONS

Methodological problems prevent reaching definitive conclusions. Dr. Brian Myhr, Associate Director of Genetic and Cellular Toxicology at Covance Laboratories, Vienna, VA, has prepared a detailed nine-point critique of this study¹, and concludes that the results are "not believable without confirmation under better assay conditions." His criticisms include:

- Inadequate identification of mouse lymphoma test strain;
- Use of excessive, potentially toxic levels of S9 mix;
- Use of an inappropriate positive control chemical for the non-activation assay;

¹ Letter from S.R. Haworth, Covance Laboratories to P. Lieder, 3M, July 1, 1999
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Poor detection of small colony mutants;
Use of excessively high concentrations of test chemical in mutation assays;
Use of an excessively long mutant expression period (3 days);
Use of insufficiently large numbers of cells for mutation assays; and
Over-interpretation of study results.

REFERENCE

NOTOX. 1998. Evaluation of the Mutagenic Activity of T-6906 in an *In Vitro* Mammalian Cell Gene Mutation Test With L5187Y Mouse Lymphoma Cells (With Independent Repeat).
NOTOX Project 223458

ROBUST SUMMARY - RS11

Title: MUTAGENICITY TEST ON T-6292 IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY, 1996

TEST SUBSTANCE

Identity: T-6292, N-EtFOSE alcohol

Remarks: Amber waxy solid; purity not reported; Identification: T-6292

METHOD

Method/guideline: No regulatory guideline

Test type: micronucleus assay

GLP: Yes

Year study performed: 1996

Species/Strain: Mouse; Crl:CD-1 (ICR) BR

Sex: Male and female

No. of animals: 5/sex/dose

Route of administration: Oral gavage in acetone:corn oil (40:60 v/v) emulsion.

Doses/concentration levels: 550, 1100, and 2200 mg/kg

Frequency of treatment: Single dose; subgroups examined after 24, 48, and 72 hours.

Statistical methods: Analysis of variance on untransformed (or rank transformed if variance heterogeneous) proportions of cells with micronuclei, followed by Dunnett's test if significant.

Remarks: Adult mice (age 49 days at time of dosing) were quarantined for 7 days prior to study. A rangefinding study (3/sex/dose) were conducted with doses of 1000, 1400, 1800, 2200, and 2600 mg/kg, administered by oral gavage in acetone:corn oil suspension. Dose levels selected for main study based on hypoactivity seen at doses of 1400 mg/kg or more, and one death at 2200 mg/kg. The main study used groups of 5/sex/dose/harvest time. A secondary group of 5/sex receiving the high dose level was used to replace primary high-dose animals that died. Five males and five females receiving vehicle (acetone:corn oil, 40:60, 10 ml/kg) and positive control (cyclophosphamide, 80 mg/kg) were examined for micronuclei at 24 hours after dosing. All animals were observed immediately after dosing and periodically throughout the study for signs of toxicity. Femoral bone marrow was recovered at the designated harvest time and spread on slides, fixed in methanol, and stained with May-Grunwald solution followed by Geimsa. Coded slides were analyzed for PCE/NCE ratio and 1,000 PCE per animal were scored for micronuclei.

RESULTS

Toxicity: Significant bone marrow toxicity was evidenced by reduced PCE/NCE ratio seen in females at all dose levels at the 72 hour harvest, and in positive control females.

Genotoxic effects: Negative -- no increase in micronucleated polychromatic erythrocytes at any dose. The positive control produced the expected significant increase in micronuclei compared to vehicle control ($3.36 \pm 0.97\%$ v. $0.02 \pm 0.2\%$ in males and $4.52 \pm 0.72\%$ v. 0.14 ± 0.06 in females).

Remarks: Animals in all treatment groups receiving N-EtFOSE alcohol showed signs of toxicity, as evidenced by hypoactivity (some also with excessive salivation) shortly after dosing. The low-dose animals and some mid-dose males had recovered by 20.4 hours after dosing; others remained hypoactive. High-dose animals developed dyspnea, rough hair coats, hunched posture, squinted eyes, and chromodacryorrhea, and one high-dose female was found dead at 47.5 hours after dosing. One mid-dose female was also found dead at 68.4 hours after dosing.

CONCLUSIONS

N-EtFOSE alcohol was negative for inducing micronuclei in mouse bone marrow polychromatic erythrocytes.

REFERENCE

Murli, H. 1996. Mutagenicity Test on T-6292 in an *In Vivo* Mouse Micronucleus Assay. Corning Hazleton Inc., Vienna, VA 22182. Final Report, May 2, 1996. CHV Study No. 17384-0-455. Submitted to 3M, St Paul, Minnesota, 55144-1000.

ROBUST SUMMARY - RS12

Title: MUTAGENICITY TEST ON T-5710 IN AN *IN VIVO* RAT MICRONUCLEUS ASSAY, 1993

TEST SUBSTANCE

Identity: T-5710, N-EtFOSE alcohol

Remarks: Cream-colored granular material; purity not reported; Identification: L-10059 (T-5710)

METHOD

Method/guideline: FIFRA Guideline 84-2

Test type: *In vivo* rat micronucleus assay

GLP: Yes

Year study performed: 1993

Species/Strain: Charles River Sprague-Dawley rats.

Sex: Male and female

No. Animals: 5/sex/dose

Route of administration: Oral gavage in corn oil suspension.

Doses/concentration levels: 1250, 2500, and 5000 mg/kg in corn oil (20 ml/kg), Trial I; 312.5, 625, 1250 mg/kg in corn oil (10 ml/kg), Trial II

Frequency of treatment: Single dose; subgroups examined after 24, 48, and 72 hours.

Statistical methods: Analysis of variance on square root arcsine-transformed proportions of cells with micronuclei, followed by Tukey's Studentized range test with adjustment for multiple comparisons if significant.

Remarks: Adult rats (approximately 8 weeks old) were quarantined for 7 days prior to study. Animals were randomized and weighed prior to dosing. Groups of 5/sex/dose/harvest time were used, plus an additional 8/sex (Trial I) or 10/sex (Trial II) that received the high dose of test compound to be used, if needed, as replacements for animals that died prior to cell harvest. Five males and five females receiving vehicle (corn oil, 20 ml/kg in Trial I, 10 ml/kg in Trial II) and positive control (cyclophosphamide, 60 mg/kg in sterile deionized water, 10 ml/kg) were examined at 24 hours after dosing. All animals were observed immediately after dosing and periodically throughout the study for signs of toxicity. Many animals in Trial I died and all became moribund prior to the end of the study. Too few animals survived to provide a valid analysis, so the trial was aborted and Trial II was conducted. Tibial bone marrow was recovered

at the designated harvest time and spread on slides, fixed in methanol, and stained with May-Grunwald solution followed by Geimsa. Coded slides were analyzed for PCE/NCE ratio and 1,000 PCE per animal were scored for micronuclei.

RESULTS

Toxicity: No significant bone marrow toxicity was seen, based on the PCE/NCE ratio.

Genotoxic effects: Negative -- no increase in micronucleated polychromatic erythrocytes at any dose. The positive control produced the expected significant increase in micronuclei compared to controls ($4.64 \pm 0.64\%$ v. $0.12 \pm 0.05\%$ in males and $3.12 \pm 0.70\%$ v. $0.12 \pm 0.04\%$ in females).

Remarks: Trial I was aborted due to excessive mortality and moribundity. In Trial II, evidence of toxicity (ataxia) was seen at all doses at about 5 hours after dosing. Low- and mid-dose groups recovered by ~23 hours; high-dose animals remained ataxic through the end of the study and two males were found dead at ~71 hours.

CONCLUSIONS

N-EtFOSE alcohol was negative for inducing micronuclei in rat bone marrow polychromatic erythrocytes.

REFERENCE

Murli, H. 1993. Mutagenicity Test on T-5710 in an *In Vivo* Rat Micronucleus Assay. Hazleton Washington, Vienna, VA 22182. Final Report, April 23, 1993. HWA Study No. 15516-0-454.

ROBUST SUMMARY - RS13

Title: GENOTOXICITY TEST ON T-5710.1 IN THE IN VIVO/IN VITRO UNSCHEDULED DNA SYNTHESIS AND CELL PROLIFERATION ASSAY IN RAT LIVER CELLS, 1993

TEST SUBSTANCE

Identity: T-5710, N-EtFOSE alcohol

Remarks: Cream-colored granular material; purity not reported; Identification: T-5710.1

METHOD

Method/guideline: Williams 1980, Mirsalis et al. 1982; Butterworth 1987

Test type: *In Vivo/In Vitro* Unscheduled DNA synthesis and cell proliferation in rat liver cells.

Test System: Primary cells from animals dosed *in vivo*

GLP: Yes

Year study performed: 1993

Species/Strain/cell type/cell line: Primary hepatocytes from male Sprague-Dawley rat (CRL:CD BR).

Metabolic activation: NA

Statistical methods: For UDS assay: Procedure of Casciano and Gaylor (1983). For cell proliferation assay: one-way analysis of variance followed by Dunnet's t-test (rank transformation of data was performed if variances were heterogeneous).

REMARKS

Study Design:

UDS Assay:

Groups of 3 rats/dose/timepoint received a single oral gavage dose of one of three doses of test material. Additional groups of three received the vehicle or positive control. Timepoints were 2-3 hours and 15-16 hours after dosing when hepatocytes were harvested. Hepatocytes were obtained by perfusion of rat liver in situ with calcium and magnesium free Hanks balanced salts containing 0.5 mM ethyleneglycol-bis(β -aminoethyl ether)-N,N-tetraacetic acid (EGTA) and Hepes buffer, pH 7.2, followed by 50-100 units/ml of collagenase in Williams Medium E (WME). Cells were cultured in WME with 10% fetal bovine serum (FBS) in culture dishes containing plastic coverslips. After incubation for 2 hours at about 37°C to allow attachment, unattached cells were removed and culture medium replaced with WME containing 10 μ Ci/ml tritiated thymidine (47 Ci/mmol). After a labeling period of 4 hours, cells were refed with WME + 10% FBS containing 0.25 mM thymidine and incubated for 18-19 hours. Nuclei were then swollen by addition of 1% sodium citrate for 7-10 minutes, cells were fixed in acetic acid:

ethanol (1:3), and dried. Fixed coverslips were processed for autoradiography. UDS was assessed by counting nuclear grains and subtracting the average number of grains in nucleus-sized areas of cytoplasm. The net nuclear grain counts on 50 randomly selected cells per coverslip (3 coverslips/animal) were measured and averaged for each treatment condition.

Doses: 203, 405, 810 mg/kg

Number of replicates: One

Animals/dose: Three per timepoint (2 timepoints).

Solvent: Corn oil (10 ml/kg)

Positive controls: Dimethylnitrosamine, 10 mg/kg.

Cell Proliferation Assay: Groups of five young male adult Crl:CDBR Sprague-Dawley rats (4 for positive control) were dosed as for the UDS assay 72 hours before sacrifice. At about the same time "three days prior to sacrifice," these animals were implanted subcutaneously (dorsal surface) with an Alzet 2ML1 osmotic pump loaded with 2 ml of BrdU solution (20 mg/ml). 72 Hours after dosing, liver and a cross-section of duodenum (labeling control) were removed and fixed in neutral buffered formalin. Paraffin-embedded sections (5 μ m) from the left lateral, right median, and right anterior lobes and from duodenum were prepared. Cell proliferation was detected immunohistochemically with DAB stain. Once appropriate staining of duodenum sections was confirmed, and staining was confirmed as similar on all three liver lobes examined, counting of labeled cells was performed using the left lateral lobe. At least 2000 nuclei per animal were examined from a minimum of three sections, covering 8 microscope fields (selected at random by computer) per section.

RESULTS

Genotoxic Effects: No increase in net nuclear grains or % cells with ≥ 5 net nuclear grains at any dose..

Cell Proliferation Effects: No increase in proportion of labeled liver cells at any dose compared to controls.

Remarks: There were no test-specific confounding factors.

In Vivo/In Vitro UDS and Liver Cell Proliferation in Rats Dosed with N-EtFOSE alcohol			
Dose	Mean Net Nuclear Grains	% Cells with ≥ 5 Net Nuclear Grains	Percent of Labeled Hepatocytes
Control	0.04 $\pm$ 0.37 -0.73 $\pm$ 0.68 -0.73 $\pm$ 0.40	4.00 2.67 1.33	0.61 $\pm$ 0.18
101 mg/kg	--	--	0.49 $\pm$ 0.46
203 mg/kg	0.43 $\pm$ 0.40 0.63 $\pm$ 0.41 0.05 $\pm$ 0.54	7.33 7.33 7.33	0.26 $\pm$ 0.26
405 mg/kg	-0.10 $\pm$ 0.23 0.75 $\pm$ 0.20 -0.40 $\pm$ 0.12	3.33 7.33 2.67	0.50 $\pm$ 0.38
810 mg/kg	-0.29 $\pm$ 1.40 -0.40 $\pm$ 0.48 -0.24 $\pm$ 1.07	9.33 1.33 6.67	0.34 $\pm$ 0.25
Positive Control (DMN 15 mg/kg)	3.95 $\pm$ 2.86 4.05 $\pm$ 3.38 5.01 $\pm$ 2.62	38.67 38.00 48.00	31.51 $\pm$ 15.86

CONCLUSIONS

N-EtFOSE alcohol was negative for inducing Unscheduled DNA synthesis.

REFERENCE

Cifone, M.A. 1993. Genotoxicity Test On T-5710.1 in the In Vivo/In Vitro Unscheduled DNA Synthesis and Cell Proliferation Assay in Rat Liver Cells. Hazleton Washington, Vienna VA 22182. Final Report, September 14. HWA Study No. 15516-0-494 Submitted to 3M Corporation, St Paul, MN 55144-1000.

ROBUST SUMMARY - RS14

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:

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.

OTHER

None

ROBUST SUMMARY - RS15

Title: *SALMONELLA – ESCHERICHIA COLI*/MAMMALIAN-MICROSOME REVERSE MUTATION ASSAY WITH PFOS, 1999

TEST SUBSTANCE

Identity: PFOS; CAS #2795-39-3; FC-95; potassium perfluorooctylsulfonate, T-6295

Remarks: Lot 217; White crystalline powder; Stored at room temperature

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*uvrA*

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 5,000 µg/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, 333 µg/plate, 1,000 µg/plate and 5,000 µg/plate without activation.

E. coli: 33.3 µg/plate, 100 µg/plate, 333 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*uvrA*, 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 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

OTHER

None

ROBUST SUMMARY - RS16

Title: SALMONELLA TYPHIMURIUM SPOT TEST ON FC-95, 1977

TEST SUBSTANCE

Identity: FC-95 (CAS #2795-39-3; potassium perfluorooctylsulfonate, T-6295, PFOS)

Remarks:

METHOD

Method/Guideline followed: Spot test

Test type: Reverse mutation

Test system: Bacterial

GLP: N

Year study performed: 1977

Species/Strain/cell-type/cell line: Salmonella typhimurium TA1535, TA98, TA100

Metabolic activation: Aroclor 1254 rat liver S-9

Concentrations tested: 10 mcg

Statistical methods used: None.

Remarks: Paper discs saturated with the equivalent of 10 mcg of FC-95 were placed in the center of plates of S. typhimurium either with or without rat liver S-9. The solvent was DMSO. The positive control was 2-aminofluorene at 10 mcg and 1 mcg. 2-AF requires activation and was positive in this test. No repeats were performed. A positive response is judged by the formation of a ring of mutant colonies around the disc with the test agent.

RESULTS

Overall results: positive, negative, ambiguous: Cannot be judged.

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation):
Unconfirmed because of test and reporting conditions.

Cytotoxic concentration: Concentrations cannot be judged from the spot test because the test agent diffuses into the agar from the disc and cannot be quantitated.

Statistical results: None

Remarks: The spot test is regarded as highly insensitive and while positive results may serve as a qualitative indicator of mutagenicity, negative results must be confirmed in a plate incorporation assay.

CONCLUSIONS

This is a one-page memo report. In addition to being an insensitive assay, there is insufficient information presented to evaluate the results.

REFERENCE

Rohlfing, S.R. 1977. Salmonella/Mammalian-Microsome Mutagenicity Testing. 3M Riker Laboratories Inc. Interoffice Correspondence to A.N. Welter, Environmental Engineering & Control.

OTHER

None

ROBUST SUMMARY - RS17

Title: BACTERIAL REVERSE MUTATION TEST OF 9-1, 1996

TEST SUBSTANCE

Identity: 9-1; Reaction product of perfluorodimethylcyclohexylsulfonyl fluoride, perfluoroalkyl (C=0-2) cyclohexylsulfonyl fluoride, potassium carbonate and sulfuric acid; CAS #67584-42-3 [the main component A(n=2)]

Remarks: Lot 293, White powder, MW ~500, 100% w/w% pure, Stored at room temperature

METHOD

Method/Guideline followed: Standards for Toxicity Investigations (Japan's MOL, No. 77, September 1, 1988)

Test type: Reverse mutation

Test system: Bacterial

GLP: Y

Year study performed: 1996

Species/Strain/cell-type/cell line: Salmonella typhimurium TA100, TA98, TA1535 and TA1537
Escherichia coli WP2uvrA

Metabolic activation: S9 homogenate from male SD male rats pretreated with phenobarbital and 5,6-benzoflavone

Concentrations tested: For S. typhimurium TA100, TA1535, TA1537 and E. coli WP2uvrA without activation: 39.1 µg/plate, 78.1 µg/plate, 156 µg/plate, 313 µg/plate, 625 µg/plate, and 1250 µg/plate.

For S. typhimurium TA98 without activation and for all strains with activation 156 µg/plate, 315 µg/plate, 625 µg/plate, 1250 µg/plate, 2500 µg/plate, and 5000 µg/plate.

Statistical methods used: None

Remarks: There were no significant protocol deviations. (1) Each test concentration and positive control was tested in duplicate; the negative control was tested in triplicate; (2) the solvent and negative control was DMSO; (3) the positive controls were chosen according to strain and activation system and included: 2-AF; sodium azide, ICR-191, and 2-aminoanthracene; (4) the test was not repeated; (5) The test substance was judged to positive when the number of revertant colonies was twice or more the negative control, there was a dose-response relationship and the results were reproducible.

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/plate in *S. typhimurium* TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA* and 5000 µg/plate with *S. typhimurium* TA98 without activation. 5000 µg/plate with *S. typhimurium* TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA* with activation.

Statistical results: Statistics were not used to evaluate the results.

Remarks: There was no increase in the number of revertants with any strain at any concentration tested. The positive controls gave the expected response. Negative controls were within the historical range for the assay.

CONCLUSIONS

The authors conclude that the test substance is negative in this assay. This is correct.

REFERENCE

BACTERIAL REVERSE MUTATION TEST OF 9-1. 1996. Hita Research Laboratories, Hita, Oita 877 Japan, Final Report. For Sumitomo 3M Limited, Kanagawa, 229 Japan

OTHER

Nothing

ROBUST SUMMARY - RS18

Title: MUTAGENICITY EVALUATION OF T-2014 CoC IN THE AMES SALMONELLA/MICROSOME PLATE TEST, 1978

TEST SUBSTANCE

Identity: T-2014 CoC, CAS #2795-39-3, potassium perfluorooctylsulfonate FC-95, PFOS

Remarks: White Powder

METHOD

Method/Guideline followed: Ames, 1975

Test type: Reverse mutation

Test system: Bacteria; Yeast

GLP: N

Year study performed: 1977/1978

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: 0.1 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, quinacrine mustard, nitrofluorene, 2-anthramine; and 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 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

OTHER

None

ROBUST SUMMARY - RS19

Title: MUTAGENICITY TEST ON T-6295 IN AN IN VIVO MOUSE MICRONUCLEUS ASSAY, 1996

TEST SUBSTANCE

Identity: T-6295, CAS #2795-39-3, potassium perfluorooctylsulfonate, FC-95, PFOS

Remarks: Off-white mixture of powder and flakes

METHOD

Method/Guideline followed: Heddle, 1983

Test type: Micronucleus

GLP: Y

Year study performed: 1996

Species/Strain: Mouse; CrI:CD-1[®](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 H₂O; 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 ± 0.11 ; females 0.52 ± 0.10

48 hours: males 0.48 ± 0.04 ; females 0.80 ± 0.10

72 hours: males 0.39 ± 0.11 ; females 0.42 ± 0.14

450 mg/kg

24 hours: males 0.75 ± 0.11 ; females 0.59 ± 0.08

48 hours: males 0.71 ± 0.05 ; females 0.37 ± 0.07

72 hours: males 0.29 ± 0.06 ; females 0.40 ± 0.12

950 mg/kg

24 hours: males 0.56 ± 0.13 ; females 0.59 ± 0.08

48 hours: males 0.54 ± 0.08 ; females 0.44 ± 0.11

72 hours: males 0.17 ± 0.05 ; females 0.17 ± 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 Inc. (CHV), Vienna, Virginia 22182. Final Report. CHV Study No.: 17403-0-455. Submitted to 3M St. Paul, Minnesota 55144-1000

OTHER: None

ROBUST SUMMARY - RS20

Title: Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS, 1999

TEST SUBSTANCE

Identity: PFOS; CAS #2795-39-3; potassium perfluorooctylsulfonate; T-6295

Remarks: Lot 217, White crystalline powder; Stored at ambient temperature

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 ³H-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 above 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

OTHER

None

ROBUST SUMMARY - RS21

Title: CHROMOSOMAL ABERRATIONS IN HUMAN WHOLE BLOOD LYMPHOCYTES WITH PFOS, 1999

TEST SUBSTANCE

Identity: PFOS; FC-95; CAS #2795-39-3; potassium perfluorooctylsulfonate; T-6295

Remarks: Lot #217, White crystalline powder, stored at room temperature

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.

OTHER: None

ROBUST SUMMARY - RS22

Title: Evaluation of the mutagenic activity of T-5874 in the Ames Salmonella/microsome test (with independent repeat), 1994.

TEST SUBSTANCE

Identity: N-Methyl perfluorooctanesulfonamido ethanol (N-MeFOSE)

Remarks: Cream solid; Identification T-5874, batch 2334, purity "100%"

METHOD

Method/guideline: OECD Guideline No. 471

Test type: *Salmonella*- mammalian microsome reverse mutation assay

GLP: Yes (OECD)

Year: 1994

Species/Strain: *Salmonella typhimurium* TA-1535, TA-1537, TA-98, TA-100.

Metabolic activation: S9 from Aroclor-induced rat liver.

Statistical methods: None

REMARKS

Study Design:

Concentration (with and without S9): 100, 333, 1000, 3330, 5000 µg/plate with all strains, plus 3, 10, 33 µg/plate for TA100.

Number of replicates: Two

Plates/test: Three per dose.

Procedure: Aliquots of 100 µl of cells of each strain from overnight cultures were mixed with molten agar supplemented with biotin and a trace of histidine (for *Salmonella*) or with tryptophan (for *E. Coli*). An appropriate quantity of test material (in DMSO) was added to each tube, and poured over the surface of selective agar plates. For activation assays, 0.5 ml of S9 mix was added before mixing and pouring. *Salmonella* plates were incubated for 48 hours at 37°C and scored for numbers of revertants. Yeast D4 plates were incubated at 30°C (nonactivation) or 37°C (with S9) for 3-5 days and scored for revertants.

Solvent: DMSO (100 µl/plate)

Positive controls: Nonactivation: Sodium azide, 1 µg/plate for TA-1535;

methylnmethanesulfonate, 650 µg/plate for TA-100; 9-aminoacridine, 60 µg/plate for TA-1537; daunomycine 4 µg/plate for TA-98. Activation: 2-aminoanthracene, 5 µg/plate for TA-1535, TA-1537, and 0.5 µg/plate for TA-98 and TA-100.

RESULTS

Cytotoxic concentration: No toxicity seen with or without S9 in TA100 at any dose. Some precipitation of test material at two highest doses.

Genotoxic effects: No increase in mutants seen at any dose, with any strain, with or without metabolic activation.

REMARKS

Some precipitation of test material at two highest doses.

CONCLUSIONS

N-MeFOSE was not mutagenic in the Ames assay.

REFERENCE

NOTOX 1994. Evaluation of the mutagenic activity of T-5874 in the Ames Salmonella/microsome test (with independent repeat). NOTOX Project 115932; NOTOX Substance 38187

ROBUST SUMMARY - RS23

Title: Evaluation of the ability of T-5874 to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat)., 1994

TEST SUBSTANCE

Identity: N-Methyl perfluorooctanesulfonamido ethanol (N-MeFOSE)

Remarks: Cream solid; Identification T-5874, batch 2334, purity "100%"

METHOD

Method/guideline: OECD Guideline No. 473

Test type: Chromosomal aberrations in human whole blood lymphocytes

GLP: Yes (OECD)

Year: 1999

Species/Strain: Venous blood from healthy adult male humans.

Metabolic activation: S9 from Aroclor-induced rat liver (method of Ames et al. 1975).

Statistical methods: Chi-square test.

REMARKS FIELD FOR TEST CONDITIONS

Study Design:

Whole blood cultures in F10 culture medium, supplemented with 9 mg/ml phytohemagglutinin were incubated for 2 days prior to addition of test material with S9 mix for 3 hours, or without S9 for 24 or 48 hours. Fresh medium was added and the cells incubated with S9 for an additional 20-22 or 44-46 hours, with 0.5 µg/ml Colcemid added during the last three hours prior to harvest. Cells were centrifuged, treated with hypotonic KCl and fixed in methanol: acetic acid (3:1). Slides were prepared, air dried, and stained with 5% Giemsa solution for analysis of mitotic index and chromosome aberrations. A pilot study with concentrations of 3, 10, 33, 100, and 333 µg/ml was used to set the concentrations for the aberration test

Concentration: -S9: 10, 33, 56, 100, 178, & 333 µg/ml (24 hr exposure); 33, 100, 178, & 333 µg/ml (48 hour exposure); Only 56, 100 and 178 µg/ml (24 hr exposure) and 178 µg/ml (48 hour exposure) used for scoring chromosome aberrations.

+S9: 10, 33, 100, & 333 µg/ml (24 hr & 48 hr exposure); 33, 100, and 333 µg/ml (24 hr exposure) and 100 & 333 µg/ml (48 hour exposure) used for scoring chromosome aberrations.

(Repeat trial used doses for 24-hour exposure only, and scored slides at 33, 100, & 178 µg/ml - S9, and 33, 100, & 333 µg/ml +S9.

Number of replicates: Two

Cultures/test: Two.

Solvent: DMSO (0.9%)

Positive controls: Nonactivation: Mitomycin C, 0.2 µg/ml in HBSS for 24-hour treatment; , 0.1 µg/ml in HBSS for 48-hour treatment. Activation: cyclophosphamide, 15 µg/ml in HBSS.

RESULTS

Cytotoxic concentration: Some reduction in mitotic index compared to control seen at 178 and 333 µg/ml .

Genotoxic effects: No increase in chromosome aberrations with or without metabolic activation.

REMARKS

CONCLUSIONS

N-MeFOSE was negative for inducing chromosome aberrations.

REFERENCE

NOTOX 1994. Evaluation of the ability of T-5874 to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). NOTOX Project 115919; NOTOX Substance 38187

ROBUST SUMMARY - RS24

Title: Evaluation of the Mutagenic Activity of T-5874 in an *In Vitro* Mammalian Cell Gene Mutation Test With L5187Y Mouse Lymphoma Cells (With Independent Repeat), 1994

TEST SUBSTANCE

Identity: N-Methyl perfluorooctanesulfonamido ethanol (N-MeFOSE)

Remarks: Cream solid; purity "97%"; Identification: T-5874 batch 2334.

METHOD

Method/guideline: OECD Guideline 476; EEC Directive 67/548/EEC, EEC Publication No. L133 & L248

Test type: Reverse mutation assay

Test System: Mammalian cell line

GLP: Yes (OECD).

Year study performed: 1994

Species/Strain: L5187Y mouse lymphoma cells.

Metabolic activation: S9 from Aroclor-induced adult male Wistar rat liver added at 20% v/v.

Statistical methods: None

Remarks:

Study Design:

Concentration: Rangefinding test: 1, 3.3, 10, 33, 100, and 333 µg/ml with and without S9; Experiment 1 & 2: 10, 33 100 and 333 µg/ml with and without S9.

Number of replicates: 2

Plates/test: Three P90 plates/concentration for cloning efficiency (200 cells/plate); ten P90 plates/concentration for mutation frequency (~150000 cells/plate).

Solvent: Dimethylsulfoxide (final concentration in culture medium, 0.8%).

Positive controls: Without S9: ethylmethanesulfonate (2 mM in Hank's balanced salt solution, without calcium and magnesium); with S9: dimethylnitrosamine (0.5 mM in Hank's balanced salt solution, without calcium and magnesium).

RESULTS

Cytotoxic concentration: In rangefinding test some reduction in cell growth and cloning efficiency was seen at the higher concentrations (test material precipitated at highest concentration)

Genotoxic effects: No consistent evidence of genotoxicity seen with or without S9

Summary of Cytotoxic and Mutagenic Response of L5178Y Cells to N-MeFOSE			
Concentration (µg/ml)	CE at Day 0 (% Control)	CE at Day 3 (%)	Mutation Frequency x10 ⁵
Experiment 1, Without S9			
Solvent Control	100	60	2.3
10	102	61	2.6
33	103	60	3.8
100	90	62	5.2
333	70	77	1.6
Positive Control (EMS)	129	60	15.9
Experiment 1, With S9			
Solvent Control	100	58	2.1
10	83	57	1.5
33	94	59	1.0
100	92	59	0.6
333	61	83	2.1
Positive Control (DMN)	30	38	19.8
Experiment 2, Without S9			
Solvent Control	100	85	3.3
10	95	98	4.0
33	106	99	2.5
100	59	94	4.3
333	65	97	3.6
Positive Control (EMS)	94	91	17.1
Experiment 2, With S9			
Solvent Control	100	103	2.2
10	82	78	2.8
33	92	100	1.6
100	96	73	1.2
333	91	101	2.6
Positive Control (DMN)	41	46	21.6

CONCLUSIONS

N-MeFOSE was not mutagenic in the L5178Y mouse lymphoma gene mutation assay.

REMARKS

The protocol used for this study differed in several ways from normal practice. These differences include: use of rather high levels of S9 mix (20%); use of a positive control chemical (EMS) for the non-activation assay that induces few "small-colony" mutants; no differentiation between small- and large-colony mutants; and use of an unusually long mutant expression period (3 days).

REFERENCE

NOTOX. 1994. Evaluation of the Mutagenic Activity of T-5874 in an *In Vitro* Mammalian Cell Gene Mutation Test With L5187Y Mouse Lymphoma Cells (With Independent Repeat).

NOTOX Project 115921; NOTOX Substance 38187

ROBUST SUMMARY - RS25

Title: MUTAGENICITY TEST ON T-5711 IN AN *IN VIVO* RAT MICRONUCLEUS ASSAY, 1993

TEST SUBSTANCE

Identity: N-Methyl perfluorooctanesulfonamido ethanol (N-MeFOSE)

Remarks: Cream-colored granular material; purity not reported; Identification: L-1276 (T-5711). A separate document (Memo from B.C. Copley to R.D. Howells, September 14, 1995, Key to FC alcohol Tox Samples) identifies this as "wide range" MeFOSE, containing 83.88% N-MeFOSE and small amounts (~5% or less) of related fluorochemicals, particularly shorter chain (C₆, C₇, C₅, C₄, C₃, and C₂) analogs.

METHOD

Method/guideline: FIFRA Guideline 84-2

Test type: *In vivo* rat micronucleus assay

GLP: Yes

Year study performed: 1993

Species/Strain: Charles River Sprague-Dawley rats.

Sex: Male and female

No. Animals: 5/sex/dose

Route of administration: Oral gavage in 0.5% carboxymethylcellulose suspension.

Doses/concentration levels: 1250, 2500, and 5000 mg/kg in 0.5% carboxymethylcellulose (10 ml/kg).

Frequency of treatment: Single dose; subgroups examined after 24, 48, and 72 hours.

Statistical methods: Analysis of variance on square root arcsine-transformed proportions of cells with micronuclei, followed by Tukey's Studentized range test with adjustment for multiple comparisons if significant.

Remarks: Adult rats (approximately 8 weeks old) were quarantined for 7 days prior to study. Animals were randomized and weighed prior to dosing. Groups of 5/sex/dose/harvest time were used, plus an additional 5/sex that received the high dose of test compound to be used, if needed, as replacements for animals that died prior to cell harvest. Five males and five females receiving vehicle and positive control (cyclophosphamide, 60 mg/kg in sterile deionized water, 10 ml/kg) were examined at 24 hours after dosing. All animals were observed immediately after dosing and periodically throughout the study for signs of toxicity. Tibial bone marrow was recovered at

the designated harvest time and spread on slides, fixed in methanol, and stained with May-Grunwald solution followed by Geimsa. Coded slides were analyzed for PCE/NCE ratio and 1,000 PCE per animal were scored for micronuclei.

RESULTS

Toxicity: No significant bone marrow toxicity was seen, based on the PCE/NCE ratio.

Genotoxic effects: Negative -- no increase in micronucleated polychromatic erythrocytes at any dose. The positive control produced the expected significant increase in micronuclei compared to controls ($2.24 \pm 0.64\%$ v. $0.12 \pm 0.05\%$ in males and $2.22 \pm 0.33\%$ v. $0.02 \pm 0.02\%$ in females).

Remarks: Toxicity in the high-dose group was evidenced by a languid appearance of the animals from 47 hours after dosing to the end of the study and by the deaths of a total of four males and two females among the primary and secondary high dose groups. Also one mid-dose female was found dead at 47 hours after dosing.

CONCLUSIONS

N-MeFOSE alcohol was negative for inducing micronuclei in rat bone marrow polychromatic erythrocytes.

REFERENCE

Murli, H. 1993. Mutagenicity Test on T-5711 in an *In Vivo* Rat Micronucleus Assay. Hazleton Washington, Vienna, VA 22182. Final Report, April 30, 1993. HWA Study No. 15515-0-454.

ROBUST SUMMARY -RS26

Title: GENOTOXICITY TEST ON T-5711.1 IN THE IN VIVO/IN VITRO UNSCHEDULED DNA SYNTHESIS AND CELL PROLIFERATION ASSAYS IN RAT LIVER CELLS, 1993

TEST SUBSTANCE

Identity: N-Methyl perfluorooctanesulfonamido ethanol (N-MeFOSE)

Remarks: Cream-colored granular material; Identification: T-5711.1, L-1276. A separate document (Memo from B.C. Copley to R.D. Howells, September 14, 1995, Key to FC alcohol Tox Samples) identifies this as "wide range" MeFOSE, containing 83.88% N-MeFOSE and small amounts (~5% or less) of related fluorochemicals, particularly shorter chain (C₆, C₇, C₅, C₄, C₃, and C₂) analogs.

METHOD

Method/guideline: Williams 1980, Mirsalis et al. 1982; Butterworth 1987

Test type: *In Vivo/In Vitro* Unscheduled DNA synthesis and cell proliferation in rat liver cells.

Test System: Primary cells from animals dosed *in vivo*

GLP: Yes

Year study performed: 1993

Species/Strain/cell type/cell line: Primary hepatocytes from male Sprague-Dawley rat (CRL:CD BR).

Metabolic activation: NA

Statistical methods: For UDS assay: Procedure of Casciano and Gaylor (1983). For cell proliferation assay: one-way analysis of variance followed by Dunnet's t-test (rank transformation of data was performed if variances were heterogeneous).

REMARKS

Study Design:

UDS Assay:

Groups of 3 rats/dose/timepoint received a single oral gavage dose of one of three doses of test material. Additional groups of three received the vehicle or positive control. Timepoints were 2-3 hours and 15-16 hours after dosing when hepatocytes were harvested. Hepatocytes were obtained by perfusion of rat liver *in situ* with calcium and magnesium free Hanks balanced salts containing 0.5 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N-tetraacetic acid (EGTA) and Hepes buffer, pH 7.2, followed by 50-100 units/ml of collagenase in Williams Medium E (WME). Cells were cultured in WME with 10% fetal bovine serum (FBS) in culture dishes containing plastic coverslips. After incubation for 2 hours at about 37°C to allow attachment,

unattached cells were removed and culture medium replaced with WME containing 10 μ Ci/ml tritiated thymidine (47 Ci/mmol). After a labeling period of 4 hours, cells were refed with WME + 10% FBS containing 0.25 mM thymidine and incubated for 18-19 hours. Nuclei were then swollen by addition of 1% sodium citrate for 7-10 minutes, cells were fixed in acetic acid: ethanol (1:3), and dried. Fixed coverslips were processed for autoradiography. UDS was assessed by counting nuclear grains and subtracting the average number of grains in nucleus-sized areas of cytoplasm. The net nuclear grain counts on 50 randomly selected cells per coverslip (3 coverslips/animal) were measured and averaged for each treatment condition.

Doses: 250, 500, 1000 mg/kg

Number of replicates: One

Animals/dose: Three per timepoint (2 timepoints).

Solvent: Corn oil (10 ml/kg)

Positive controls: Dimethylnitrosamine, 10 mg/kg.

Cell Proliferation Assay: Groups of five young male adult Crl:CDBR Sprague-Dawley rats (4 for highest dose and positive control) were dosed as for the UDS assay 72 hours before sacrifice.

At about the same time "three days prior to sacrifice," these animals were implanted subcutaneously (dorsal surface) with an Alzet 2ML1 osmotic pump loaded with 2 ml of BrdU solution (20 mg/ml). 72 Hours after dosing, liver and a cross-section of duodenum (labeling control) were removed and fixed in neutral buffered formalin. Paraffin-embedded sections (5 μ m) from the left lateral, right median, and right anterior lobes and from duodenum were prepared. Cell proliferation was detected immunohistochemically with DAB stain. Once appropriate staining of duodenum sections was confirmed, and staining was confirmed as similar on all three liver lobes examined, counting of labeled cells was performed using the left lateral lobe. At least 2000 nuclei per animal were examined from a minimum of three sections, covering 8 microscope fields (selected at random by computer) per section.

Doses: 625, 1250, 2500, 5000 mg/kg

Number of replicates: One

Animals/dose: Five.

Solvent: Corn oil (10 ml/kg)

Positive controls: Dimethylnitrosamine, 15 mg/kg.

RESULTS

Genotoxic Effects: No increase in net nuclear grains or % cells with ≥ 5 net nuclear grains at any dose (see Table 1).

Cell Proliferation Effects: A dose-related increase in labeling index (statistically significant at the two highest doses) was observed (see Table 2).

Remarks: There were no test-specific confounding factors.

Table 1 In Vivo/In Vitro UDS in Rats Dosed with N-MeFOSE alcohol				
Dose	Mean Net Nuclear Grains		% Cells with ≥ 5 Net Nuclear Grains	
	2-4 Hr	15-16 Hr	2-4 Hr	15-16 Hr
Control	-0.92 $\pm$ 0.41	-0.17 $\pm$ 0.15	4.00	1.34
	0.11 $\pm$ 0.20	-0.79 $\pm$ 0.57	4.00	0.67
	-0.29 $\pm$ 0.53	-0.49 $\pm$ 0.41	2.67	0.00
250 mg/kg	-0.15 $\pm$ 0.35	-0.17 $\pm$ 0.15	2.67	1.33
	0.03 $\pm$ 0.35	-0.89 $\pm$ 0.01	3.33	0.00
	0.04 $\pm$ 0.20	-0.37 $\pm$ 0.48	1.00	0.67
500 mg/kg	0.23 $\pm$ 0.20	-0.52 $\pm$ 0.05	3.33	0.00
	-0.61 $\pm$ 0.58	-0.91 $\pm$ 0.22	2.00	0.00
	0.06 $\pm$ 0.45	-1.21 $\pm$ 0.10	3.00	0.00
1000 mg/kg	-0.36 $\pm$ 0.51	-0.58 $\pm$ 0.36	2.00	0.00
	0.06 $\pm$ 0.23	0.02 $\pm$ 0.14	1.00	1.00
	-0.49 $\pm$ 0.39	-0.98 $\pm$ 0.64	0.67	0.00
Positive Control (DMN 10 mg/kg)	17.98 $\pm$ 2.06	3.95 $\pm$ 2.86	95.33	38.67
	8.09 $\pm$ 7.98	4.05 $\pm$ 3.38	56.00	38.00
	10.45 $\pm$ 0.60	5.01 $\pm$ 2.62	89.33	48.00

Table 2 In Vivo/In Vitro Liver Cell Proliferation in Rats Dosed with N-MeFOSE alcohol			
Dose	Labeling Index (%)	Liver Weight (g)	Terminal Body Weight (g)
Control	0.952 $\pm$ 0.73	18.95 $\pm$ 2.64	491.8 $\pm$ 41.8
625 mg/kg	1.06 $\pm$ 0.56	19.70 $\pm$ 4.49	458.4 $\pm$ 22.1
1250 mg/kg	2.39 $\pm$ 2.25	21.82 $\pm$ 1.55	477.9 $\pm$ 16.8
2500 mg/kg	9.06 $\pm$ 3.13*	25.73 $\pm$ 2.93	455.1 $\pm$ 23.1
5000 mg/kg	8.13 $\pm$ 3.15*	25.73 $\pm$ 2.93*	425.4 $\pm$ 23.9*
Positive Control (DMN 15 mg/kg)	17.98 $\pm$ 2.06*	19.48 $\pm$ 2.06	459.8 $\pm$ 30.1
* Significantly different from control mean ($p \leq 0.01$)			

CONCLUSIONS

N-MeFOSE alcohol was negative for inducing unscheduled DNA synthesis, but did cause cell proliferation.

REFERENCE

Cifone, M.A. 1993. Genotoxicity Test On T-5711.1 in the In Vivo/In Vitro Unscheduled DNA Synthesis and Cell Proliferation Assay in Rat Liver Cells. Hazleton Washington, Vienna VA 22182. Final Report, September 14. HWA Study No. 15515-0-494 Submitted to 3M Corporation, St Paul, MN 55144-1000.

ROBUST SUMMARY - RS27

Title: Detection of Fluorochemicals in 35 Lots of Commercial Sera, 1999

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional data on PFOS detected in 35 lots of individual or pooled human sera samples from US chemical or biological supply companies.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the levels of PFOS in the serum of the general population.

Study period: 1999

Setting: N/A

Total population: 35 lots of individual or pooled human sera samples from US chemical or biological supply companies. No other information provided.

Subject selection criteria: Not provided in report.

Total # of subjects in study: Approximated—see “total population” above

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

Details on data collection: No information was provided as to how the blood was drawn, stored, analyzed, 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

Other methodological information:

RESULTS

Describe results: The mean PFOS serum level was 35 ppb, with a range of 5 to 85 ppb.

Study strengths and weaknesses: These data are only preliminary cross-sectional data used to determine PFOS levels in the general population--no other descriptive information about the subjects was collected. The blood serum collected is from a small pool. Geographic regions were not specified. Other demographic information was not available. Blood donors cannot be considered representative of the general population of the US.

Research sponsors: 3M Environmental Lab

Consistency of results: N/A

CONCLUSIONS

N/A

REFERENCE

Supplemental Notice on Sulfonate-based and Carboxylic-based fluorochemicals. Analyses of blood sera samples from the general population. May 26, 1999. 3M Company. 8EHQ-0699-373.

OTHER

ROBUST SUMMARY - RS28

Title: Analysis of Pooled Blood Samples from 3 European Blood Banks

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional data on PFOS detected in pooled serum from blood banks in three European countries: Belgium, Netherlands and Germany.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the presence of PFOS in the serum of the general population.

Study period: 1998

Setting: N/A

Total population: Serum pooled from 3 regional blood banks in three European countries. The Belgium blood bank provided 5 pooled samples (10 donors per sample). The Netherlands sample provided 6 pooled samples (10 donors per sample). The Germany blood bank provided 6 pooled samples (30 donors per sample). Altogether, these 16 pooled samples represented 290 donors.

Subject selection criteria: Telephone request. Other European country blood banks were requested but permission was not granted.

Total # of subjects in study: Approximated—see “total population” above

Comparison population: N/A

Participation rate: 50% response

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

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: Means calculated.

Other methodological information:

RESULTS

Describe results: The mean serum PFOS level for the 5 Belgium pooled samples was 17 ppb (range 4.9-22.2 ppb). The mean serum PFOS level for the 5 Netherland pooled samples was 53 ppb (range 39-61 ppb). The mean serum PFOS level for the 6 Germany pooled samples was 37 ppb (range 32-45.6 ppb).

Study strengths and weaknesses: These data are cross-sectional data used to determine an initial PFOS level in the general population--no other descriptive information about the subjects was collected. Blood serum was collected from only 3 blood banks— other European blood banks contacted did not participate. PFOS levels varied by geographic region; however whether this is to geographical differences or to the demographic characteristics of the pooled donors is not known as the latter was not available. Blood donors cannot be considered representative of the general population.

Research sponsors: 3M Medical Department

Consistency of results: N/A

CONCLUSIONS

N/A

REFERENCE

OTHER

ROBUST SUMMARY - RS29

Title: Analysis of Pooled Blood Samples from 18 U.S. Blood Banks

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional data on PFOS detected in pooled serum from blood banks in different regions of the US.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the presence of PFOS in the serum of the general population.

Study period: 1998

Setting: N/A

Total population: Serum pooled from 18 regional blood banks in various geographic regions in the US. There were 68 pools and an estimated 340-680 donors.

Subject selection criteria: Many blood banks were approached to participate, but many of them refused.

Total # of subjects in study: Approximated—see “total population” above

Comparison population: N/A

Participation rate: 50% response

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

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: Means calculated.

Other methodological information:

RESULTS

Describe results: PFOS serum levels varied by geographic location. The overall mean PFOS serum level across pools was 29.7 ppb. The range across geographic regions was 9 ppb (Omaha, NE) to 56 ppb (Greenville, SC). The range of the averages was 14 to 52 ppb.

Study strengths and weaknesses: These data are cross-sectional data used to determine an initial PFOS level in the general population--no other descriptive information about the subjects was collected. Blood serum was collected from only 18 blood banks—half of the blood banks contacted did not participate. PFOS levels varied by geographic region; however whether this is to geographical differences or to the demographic characteristics of the pooled donors is not known as the latter was not available. Blood donors cannot be considered representative of the general population of the US.

Research sponsors: 3M Environmental Lab

Consistency of results: N/A

CONCLUSIONS

N/A

REFERENCE

Perfluorooctane sulfonate: Current Summary of Human Sera, Health and Toxicology Data, Jan. 21, 1999, 3M Company. 8EHQ-0299-373

Buxton B, Struass W, Chang O. Working Memorandum on Data Quality Assessment. Columbus (OH): Battelle Laboratory, September 22, 1998.

OTHER

ROBUST SUMMARY - RS30

Title: Analysis of PFOS from Pooled Serum of Two Commercial Laboratories, 1998

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional data on PFOS detected in pooled serum from commercial sources.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine levels of PFOS in the serum of the general population.

Study period: 1998

Setting: N/A

Total population: Six pooled sera samples, obtained from 2 commercial sources (Intergen and Sigma). There were approximately 500 individuals in the donor pools from Intergen and a minimum of 200 donors in the pools from Sigma.

Subject selection criteria: It was not reported how these sources of blood were identified to participate.

Total # of subjects in study: Approximated—see “total population” above

Comparison population: N/A

Participation rate: The total number of commercial sources approached to participate in this study was not provided.

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 obtained from 2 commercial sources of blood.

Details on data collection: No information was provided as to how the blood was drawn, stored, analyzed, 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: Means calculated.

Other methodological information:

RESULTS

Describe results: PFOS serum levels were 43, 44, and 44 ppb (mean = 44 ppb) from the 3 Intergen Pools. The 3 pools from Sigma contained PFOS at levels of 26, 28, and 45 ppb (mean = 33 ppb).

Study strengths and weaknesses: These data cannot be considered representative of the PFOS levels in the general population of the US. It is a small sample and no other descriptive information about the subjects was collected. Geographic and other demographic information were not available. These data should only be used as a preliminary analysis of general levels of PFOS levels in blood in a specified human population.

Research sponsors: 3M Environmental Lab

Consistency of results: N/A

CONCLUSIONS

N/A

REFERENCE

Perfluorooctane sulfonate: Current Summary of Human Sera, Health and Toxicology Data, January 21, 1999, 3M Company. 8EHQ-0299-373.

OTHER

ROBUST SUMMARY - RS31

Title: A Pilot Study to Identify Fluorochemicals in Children With Limited Sera for Analysis, 1999

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Cross-sectional pilot data.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine that the analytic technique used to test PFOS in human serum could be used on a small volume of serum.

The objective of the study to be completed is 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: n = 10

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 = 10

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 levels in blood

Data collection methods: Blood sera samples

Details on data collection: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS using high-pressure liquid chromatography/electrospray tandem mass spectrometry and evaluated versus an extracted curve. Qualitative analysis was conducted by comparing peak response in the samples to that obtained from standards, when possible. If standard material was not available, compound identification was based on reasonable HPLC-retention time and predicted mass spectrometer response. Less than 100 uL of sera were available for analysis. The limit of detection was 3 ppb.

Exposure period: N/A

Description/delineation of exposure groups/categories: Blood sera samples were collected from children 6 – 12 years old.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Means calculated.

Other methodological information: The small sample volume posed analytical restrictions. The method detection limits are significantly higher than reported in earlier studies.

RESULTS

Describe results: PFOS serum levels in these 10 children ranged from 31 to 116 ppb. The average level was 54 ppb. These individual levels are higher than those reported in pooled samples in adults (29-44 ppb) in the general population. A study analyzing over 600 pediatric samples is ongoing.

Study strengths and weaknesses: Very small number of samples, no descriptive information about the subjects.

Research sponsors: 3M Environmental Lab

Consistency of results: To date, no other data have been collected on PFOS serum levels in children.

CONCLUSIONS

The authors conclude that PFOS can be detected in very small volumes of serum, but that no other conclusions can be drawn about the levels detected in this small sample of children.

REFERENCE

Laboratory Report, Analysis of FCs in Samples of Children's Sera, May 21, 1999, 3M Environmental Laboratory. Report No. FACT-GEN-011.

OTHER

The samples analyzed in this pilot project were collected to verify the analytic technique used on small volumes of serum.

ROBUST SUMMARY - RS32

Title: 26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys, 2000

TEST SUBSTANCE

Identity: PFOS; T-6295, FC-95, CAS #2795-39-3

Remarks: Lot 217, white to off-white powder, stored at room temperature.

METHOD

Method/guideline followed: Twenty-six week treatment period, followed by 52 weeks recovery.

Study duration: 78 weeks

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

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 ratios 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 high density lipoprotein cholesterol lower than control at the two time points it was measured during treatment. HDL cholesterol was measured only on days 153 and 182, and no predosing values are available for comparison for each dose group. However, only the effect in the 0.75 mg/kg group was statistically significant. 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. 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. Additionally, neither males nor females in the 0.15 dose group had triiodothyronine values that differed from predosing values at any time during the study, and variations in triiodothyronine

levels were not accompanied by other indications of thyroid effects (TSH or T4 level, or histopathologic changes).

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 which 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 reversible.

This is a draft report that will be finalized after electron microscopy and light microscopy have been completed.

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 within 4 weeks for total cholesterol, 9 weeks for HDL cholesterol, and within a 27 week recovery period for microscopic liver effects.

Remarks: None

REFERENCE

Thomford, P.J. (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.

OTHER

ROBUST SUMMARY - RS33

Identity: N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE alcohol)

Remarks: Fluorochemical FM-3924, Lot No. 547

METHOD

Method/guideline: None

Test type: 104 week oral toxicity study

GLP: No

Year: 1987

Species/Strain: Rat; Sprague-Dawley [CrI:COBS⁷ CD(SD)BR]

Route of administration: Oral (in chow)

Doses/concentration levels: 0, 10, 30, 100 ppm N-EtFOSE alcohol

Sex: Male & Female

Exposure period: 104 weeks

Frequency of treatment: ad libitum

Control group and treatment: Concurrent vehicle

Post exposure observation period: None

Duration of test: 104 weeks

Statistical methods: Bartlett=s test for variance homogeneity was used for body weights, feed consumption, absolute and relative organ weights, and other laboratory data; if the data were not significant at $p=0.001$, the data were further analyzed using a two-tailed Dunnett=s test ($p \neq 0.05$). If, however, Bartlett=s test was significant at $p = 0.001$, the data were ranked and a two-tailed Dunnett=s test was performed on the ranks. A two-tailed Fisher=s Exact Test was used for each organ/lesion classification. A $p = 0.05$ significance level with Bonferroni=s adjustment for multiple comparisons was used within each organ/lesion/sex category. If the expected value of each cell was greater than 20, then Yates= corrected Chi-Square test was used.

Remarks:

Test Subjects:

Age at study initiation: 39 to 41 days

Weight at study initiation: (Mean)

Male: 165.3 g for control group, 165.6 g for 10 ppm dose group, 169.1 g for 30 ppm dose group, 168.2 g for 100 ppm dose group

Female: 138.2 g for control group, 135.6 g for 10 ppm dose group, 135.8 g for 30 ppm dose group, 138.7 g for 100 ppm dose group

No. of animals per sex per dose: 65 per sex in control group, 50 per sex in 10 ppm dose group, 50 per sex in 30 ppm dose group, 65 per sex in 100 ppm dose group

Study Design:

Vehicle: Chow

Satellite groups and reasons they were added: 60 untreated sentinel rats (30 housed with the treated and 30 with the control rats). During the first week, at 1 month and at 3 months, 5 animals per sex were sacrificed and plasma samples were analyzed for organic fluorine.

Clinical observations performed and frequency: Observed for morbidity/moribundity daily. Each animal was removed from cage and examined once weekly. Food consumption data were collected for Weeks 1 through 16 and once every 4 weeks thereafter. Body weights were determined weekly for the first 6 months of treatment and once every 2 weeks thereafter. Eye examinations using indirect ophthalmoscopy and/or slit lamp biomicroscopy were performed on control and high dose rats prior to treatment, at approximately one year, and 2 to 3 weeks prior to termination of the study. Blood and urine samples for hematology, clinical chemistry, and urinalysis were collected from 15 animals per sex per dose group at 3, 6, 12, 18, and 24 months.

Termination schedule: An interim termination at 1 year involved 15 animals per sex from the control and high dose groups.

Organs examined at necropsy:

Organ weight: Adrenal glands, brain, heart, kidney, liver, uterus and testes for 15 animals per sex per group.

Macroscopic examination: External surface of body, all orifices, and contents of abdomen, thorax, and cranium.

Microscopic examination: The following organs were examined for animals from the control and high dose groups: adrenal, aorta, brain, eyes, heart, small intestine, large intestine, kidney, liver, lung, lymph node (mesenteric), mammary gland, ovaries, pancreas, pituitary, salivary gland, spinal cord/bone marrow (vertebrae), spleen, stomach, thyroid/ parathyroid/trachea/esophagus, testis/epididymides, urinary bladder, uterus or prostate, any tissue masses (suspected tumors), and any gross lesion. All of the above tissues were examined for animals in the low and middle dose groups except: aorta, brain, eyes, small and large intestines, lymph node(s), and spinal cord/bone marrow.

Additional Endpoints:

Metabolism examination: Overnight urine and fecal samples were collected at 2, 5, 11, and 23 months and samples of liver, kidney, spleen, lung, and bone marrow were taken from 5 animals per sex per group at the 1 and 2 year scheduled sacrifices for total organic fluoride analysis for the presence of N-EtFOSE alcohol and PFOS.

RESULTS

NOAEL (NOEL): The investigators considered a dose of 30 ppm to be the NOAEL. It should be noted that incidence of ovarian tubular hyperplasia was significantly increased for all treated groups and apparently dose-related.

LOAEL (LOEL): The investigators considered 100 ppm to be the LOAEL based on liver effects, including hypertrophy and vacuolation. Again, it should be noted that incidence of ovarian tubular hyperplasia was significantly increased for all treated groups and apparently

dose-related.

Remarks:

Body weight: Body weight gains were depressed in the treated males when compared to controls for the first 6 weeks of the study and this lower body weight (approximately 14% decrease for high dose and 2 to 6% decrease for low and mid-dose) was maintained for the duration of the study. This effect was also observed in treated females, although the onset was slower and the difference from controls was greater (21% decrease for high dose, 15% for mid, and 3.5% for low dose at the end of the study).

Food/water consumption: Daily feed consumption per kilogram mean body weight was generally increased in treated animals, gradually increasing when compared to control group feed intake. This effect was pronounced in treated males and less consistent for treated females until the last 10 study weeks.

Test Material Consumption: The following table summarizes the amount of test material consumed by animals on a mg/kg/day basis:

Test Material Consumption		
Target Dose Levels	Achieved Dose Levels - Mean (mg/kg/day)	
	Males	Females
10 ppm	0.4	0.5
30 ppm	1.3	1.6
100 ppm	4.5	5.5

Clinical signs (description, severity, time of onset and duration): Clonic convulsions, which occurred at slightly elevated rates in high dose males, were the only clinical sign occurring more frequently in treated animals. The treated population experienced a suspected outbreak of sialodacryoadenitis viral infection, with clinical signs including swollen submandibular salivary glands and occasional ocular manifestations, between the first and second month. The swelling resolved within 10 days and the incidence of residual ocular changes was extremely low. The control population had comparable signs during Month 16.

Hematology: Red blood cell counts were decreased in high dose females throughout the study; the difference from controls was statistically significant at 6, 12, and 18 months. Morphological changes in red blood cells were observed in high and mid-dose females at 24 months; the degree of change was generally noted as slight. Hematocrit values for high dose females were significantly decreased at the Month 3 evaluation and hemoglobin values were significantly decreased in high dose females from Month 3 to Month 24. Slight differences in white blood cell parameters were not considered treatment-related or biologically meaningful.

Clinical Chemistry: At Month 3, slight increases in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and blood urea nitrogen were observed in treated males. High dose (and occasionally mid-dose) males had more pronounced increases in alanine aminotransferase and aspartate aminotransferase from Month 6 until the study conclusion.

Ophthalmologic findings: No treatment-related effect.

Mortality and time to death: The following table summarizes the survival data:

Survival After 104 Weeks of Treatment		
Dose Group	Percent Survival	
	Males	Females
Control	70%	50%
10 ppm	78%	44%
30 ppm	70%	51%
100 ppm	64%	68%

Gross pathology incidence and severity: At the Year 1 sacrifice, high dose males had an increased incidence of pale and/or tan livers (other dose groups were not examined). Other organs from treated animals were not notably different from controls. For scheduled study termination sacrifices and unscheduled deaths of animals in the high dose group, gross findings were primarily limited to the liver, including an increased incidence of hepatic masses, nodules and raised lesions, mottled livers, and yellow or pale focal lesions. No treatment-related effects were observed for animals in the low and mid-dose groups.

Organ weight changes: Year 1 Sacrifice: Animals in the high dose group had statistically significant increased absolute and relative liver weights and significantly decreased absolute heart and spleen weights. Relative kidney and brain weights were also observed to be significantly increased in these animals. High dose males also had significantly lower adrenal (absolute) and pituitary (absolute and relative) weights and significantly increased relative testicular weights. High dose females had significantly increased relative uterine and adrenal weights. End of Study: Only high dose animals had statistically significant increased absolute liver weights; high and mid-dose animals had significantly higher relative liver weights. Relative kidney weights were significantly increased in high dose animals. Absolute spleen weights were significantly decreased in both high and mid-dose males; however, only high dose males had significant decreases in relative spleen weights.

Histopathology: Year 1 Sacrifice: Hepatocellular hypertrophy and vacuolation was common for animals in the high dose group and hepatocellular necrosis was found in 6 of 15 high dose males examined.

End of Study: Hepatocellular hypertrophy and vacuolation were consistently observed for animals in the high dose group and found at lower incidence rates in the male low and mid-dose groups. Incidence of hepatocellular cystoid degeneration appeared to be associated with dose in males, while this affect was observed only in high dose females. The incidence of nodular hyperplasia of the adrenal cortex was significantly increased in the high dose males. There was a statistically significant and dose dependent increase in tubular hyperplasia of the ovarian stroma of females (0/50 in controls; 13/50 at 10 ppm; 23/50 at 30 ppm; 31/50 at 100 ppm; $p < 0.001$ for all doses by Fisher's exact test). The incidence of nodular hyperplasia of the adrenal cortex was

significantly increased in high dose males. A statistically significant increased incidence of foamy macrophage accumulation in the lung of high dose animals was observed. Other non-neoplastic changes were not considered treatment-related.

Sporadic incidences of various neoplasms were noted, including pituitary adenomas and mammary gland adenocarcinomas; however, these did not appear to have a relationship to dose. Hepatocellular carcinomas and adenomas were observed with the following incidences:

Incidence of Hepatocellular Carcinomas and Adenomas*			
Dose Group	Type of Neoplasm	No. of Animals Affected (%)	
		Males	Females
Control	Carcinoma	3 (6%)	0 (0%)
	Adenoma	0 (0%)	0 (0%)
10 ppm	Carcinoma	1 (2%)	0 (0%)
	Adenoma	0 (0%)	0 (0%)
30 ppm	Carcinoma	1 (2%)	0 (0%)
	Adenoma	0 (0%)	0 (0%)
100 ppm	Carcinoma	2 (4%)	3 (6%)
	Adenoma	0 (0%)	4 (8%)
*The incidence of carcinoma and adenoma did not overlap (i.e., both neoplasms did not occur in any one animal)			

The investigators noted that while the incidence of hepatocellular adenomas and carcinomas in high dose females was outside of historical control limits, the difference did not achieve statistical significance.

Additional Endpoints: Metabolism examination: Results not provided (analysis performed by Sponsor)

CONCLUSIONS

The most pronounced treatment-related effects were seen in the liver and occurred significantly more frequently in high dose animals than in controls. A statistically significant increased incidence of foamy macrophage accumulation in the lung of high dose animals was observed and thought to be possibly related to treatment. In addition, there was a statistically significant and dose-dependent increase in tubular hyperplasia of the ovarian stroma of female rats. Based on tumor incidence, types of tumors, onset time of tumor appearance, malignancy patterns of tumors and the final mortality values at 2 years, the investigators concluded that N-EtFOSE alcohol was not carcinogenic in the rat under the design and conditions of the study.

REFERENCE

Riker Laboratories, Inc. 1989. TWO YEAR ORAL (DIET) TOXICITY CARCINOGENICITY STUDY OF FLUOROCHEMICAL FM-3924 IN RATS. (RIKER Experiment No. 0281CROO12)

ROBUST SUMMARY - RS34

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: PFOS; T-6295

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: 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 thyroxine. 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

NOEL (dose and effect): 0.02 mg/kg/day

LOAEL (dose and effect): 2.0 mg/kg/day. Dramatic reduction in serum cholesterol both male and female

Toxic response/effects by dose level: Only high-dose effect, 2.0 mg/kg/day. Dramatic reduction in serum cholesterol both male and female

Statistical results: None, high-dose too few animals (1 male, 1 female)

CONCLUSIONS

Dramatic reduction in serum cholesterol in the high dose animals. Estradiol 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: Draft report does not discuss drop in serum cholesterol

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.

OTHER

Summary revised 8/11/00

ROBUST SUMMARY - RS35

Title: First ninety-day rhesus monkey toxicity study, 1979

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95

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.

OTHER

ROBUST SUMMARY - RS36

Title: Second ninety-day rhesus monkey toxicity study, 1978

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95

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 (Reviewer disagrees, see remarks)

LOAEL (dose and effect): 0.5 mg/kg/day (Reviewer disagrees, see remarks)

Toxic response/effects reported 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), slight reduction in serum alkaline phosphatase

Statistical results:

Statistical results are generally reported as a comparison to the controls, and males and females are treated separately. With the small numbers (2/sex/group) and the fact that these were wild-caught rhesus monkeys, this type of comparison may not be as appropriate for many of the endpoints as a comparison of the actual change in individual values for various parameters from pre-study individual values. A case in point is the analysis of alkaline phosphatase, as discussed at length below.

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. After 90 days of treatment, the females had a significant reduction in serum alkaline phosphatase activity and serum potassium levels were noted in the report. 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. After 90 days of treatment, a slight decrease in serum alkaline phosphatase was noted in the report. Necropsy revealed no treatment-related lesions.

The study reports significant decreases in alkaline phosphatase in all dose groups. The test for significance appears to be a comparison of male and female group means with controls. Due to the small numbers of animals and wide potential variation in wild-caught rhesus monkeys, a comparison of individual values obtained after study initiation with pre-study values would seem more appropriate. The table below presents all the individual data for alkaline phosphatase. Examination of the individual values and the magnitude of change in these values from the pre-study to the term value within the control and dosed groups suggests that no significant changes occurred in the 0.5 mg/kg and 0.15 mg/kg dosed groups. A reported significant "decrease" in alkaline phosphatase in the 0.5 mg/kg dosed group is the primary basis for representing this dose level as a LOEL. The report tables actually show a significant difference at the $p < 0.05$ level for alkaline phosphatase in the males, and this is taken as a decrease. This may be correct based on the statistical comparison against the control; however, in reality, it is clearly in error, since both males actually showed approximately 10 % increases in alkaline phosphatase. Their pre-study values just happened to be considerably lower than their two male control colleagues. Based on this analysis, it would appear that a NOAEL for the study does exist, and that this NOAEL is 0.5 mg/kg.

Table. Individual and group mean alkaline phosphatase values.

Dose Group	Animal	Individual and Mean Alkaline Phosphatase (IU/L) by Time Period and Dose Group			Absolute Change, Pre-Study Through Term	Percent Change, Pre-Study Through Term
		Pre-Study	1 Month	3 Months		
Control Monkeys	M 7355	1050	1146	1110	60	5.71
	M 7358	1090	1080	1080	-10	-0.92
	F 7368	1170	1158	876	-294	-25.13
	F 7372	1175	1026	786	-389	-33.11
	Mean	1121	1103	963	-158	-14.10
0.5 mg/kg Dose Group Monkeys	M 7463	635	924	690	55	8.66
	M 7483	725	870	804	79	10.90
	F 7466	1250	1164	978	-272	-21.76
	F 7504	775	720	744	-31	-4.00
	Mean	846	920	804	-42	-4.97
1.5 mg/kg Dose Group Monkeys	M 7462	800	870	816	16	2.00
	M 7486	1270	1350	966	-304	-23.94
	F 7500	1080	816	690	-390	-36.11
	F 7501	460	690	546	86	18.70
	Mean	903	932	755	-148	-16.39
4.5 mg/kg Dose Group Monkeys	M 7484	985	312	---	-673	-68.33
	M 7485	1615	816	---	-799	-49.47
	F 7502	965	564	---	-401	-41.55
	F 7503	785	666	---	-119	-15.16
	Mean	1088	590	---	-498	-45.77

CONCLUSIONS

As reported, the study does not have a NOAEL, since a reduction in alkaline phosphatase is noted in the 0.5 mg/kg dose group (the low dose). In actuality, this reviewer believes that a NOAEL of 0.5 was obtained in the study as a result of inappropriate analysis of alkaline phosphatase values.

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.

ROBUST SUMMARY - RS37

Title: Ninety day study in rats, 1978

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95

METHOD

Method/guideline followed: None

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1978

Species/strain: Charles River CD (Sprague-Dawley) 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, liver, adrenals, spleen, pituitary, kidneys, 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, , eyes, , heart (with coronary vessels), duodenum, ileum,

jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, , mammary gland, nerve spleen, pancreas, prostate/uterus, bone/bone marrow (sternum), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, and urinary bladder.

RESULTS

NOAEL (dose and effect): None

LOAEL (dose and effect): 30 ppm (2 mg/kg/day)

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 (females) and relative liver weight (males and 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 but the lowest dose group 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 γ -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.

OTHER

ROBUST SUMMARY - RS38

Title: 13-Week Dietary Toxicity Study with N-Methyl Perflurooctanesulfonamido Ethanol (N-MeFOSE) in Rats.

TEST SUBSTANCE

Identity: N-Methyl Perflurooctanesulfonamido Ethanol (N-MeFOSE)

Remarks:

METHOD

Method/guideline: None

Test type: 14 week dietary toxicity study

GLP: Yes

Year: Not specified (Study start date: August 27, 1998)

Species: Rat

Strain: CrI:CD⁷(SD)IGS BR

Route of administration: Oral (in chow)

Doses/concentration levels: 0, 3, 30, 100 ppm N-MeFOSE

Sex: Male & Female

Exposure period: 14 weeks

Frequency of treatment: ad libitum

Control group and treatment: Concurrent vehicle

Post exposure observation period: Not specified

Duration of test: 14 weeks

Statistical methods: Levine's test for variance homogeneity; in case of heterogeneity of variance ($p \leq 0.05$), transformations were used to stabilize the variance. Comparison tests took variance heterogeneity into consideration. One-way analysis of variance (ANOVA), if applicable: analyzed body weights and body weight changes, food consumption, organ weights, and continuous pathology values. If significant, Dunnett's test performed for control vs. treated group comparisons. Group comparisons were evaluated at the 5.0%, two-tailed probability level.

Test Subjects:

Age at study initiation: 39 to 45 days

Weight at study initiation: 184 to 236 g (males); 140 to 179 g (females)

No. of animals per sex per dose: 20 per sex in each dose group

Study Design:

Vehicle: Chow

Satellite groups and reasons they were added: Not specified

Clinical observations performed and frequency: Observed for morbidity/moribundity twice daily (am and pm). Each animal was removed from cage and examined at least once weekly. Food consumption data were collected weekly. Body weights were determined on the first day of treatment and weekly thereafter. Blood samples for hematology and clinical chemistry evaluation were collected from 10 animals/sex/group during Weeks 5 and 14. Urine samples for urinalysis were collected from 10 animals/sex/group during Week 14.

Termination schedule: 5 animals/sex/group were sacrificed at Week 5 and Week 14. The livers were collected for palmitoyl CoA oxidase and PFOS analysis; blood samples were collected for serum PFOS determination. These animals were not necropsied. The remaining animals were sacrificed at Week 14 and necropsied.

Organs examined at necropsy:

Macroscopic examination: External surface of body, all orifices, thoracic, abdominal, and cranial cavities, organs, tissues.

Microscopic examination: Livers and macroscopic lesions were examined for low-dose animals; the liver, stomach, and macroscopic lesions were examined for mid-dose animals. Tissues listed under *Tissues preserved* were examined for the control and high dose groups and for animals undergoing unscheduled sacrifice.

Organ weights: Adrenal, brain, kidney, liver, ovary, spleen, testis, thymus, thyroid with parathyroid

Tissues preserved: Adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis, esophagus, eyes, femur with bone marrow, Harderian gland, heart, ileum, jejunum, kidney, lesions, liver, lung with mainstem bronchi, lymph nodes (mesenteric and mandibular), mammary gland, ovary, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testis, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus, vagina, Zymbal's gland

Additional Endpoints:

Serum PFOS determination: 5 animals/sex/group were sacrificed at Week 5 and Week 14 and blood samples were collected.

Palmitoyl-CoA oxidase analysis: Liver samples were collected from each animal at sacrifice.

Liver PFOS determination: Liver samples were collected from each animal at sacrifice.

Proliferation Cell Nuclear Antigen (PCNA) evaluation: Liver samples were collected from 5 animals/sex/group at terminal sacrifice.

RESULTS

NOAEL: 3 ppm (reduced weight gain, but no significant effect on absolute body weight).

LOAEL: 30 ppm (reduced body weight, increased liver weight, centrilobular hepatocellular hypertrophy and vacuolation, decreased serum cholesterol and triglycerides)

Remarks:

Body weight: Test material-related lower body weights were noted for males given 3 and 30 ppm and for males and females given 100 ppm. Statistically significantly lower mean body weights were noted beginning at Week 4 and continuing through the end of the study for animals in the 100 ppm dose group. Significantly lower mean body weights were noted beginning at Week 7 and continuing through the end of the study for males in the 30 ppm dose group. Body weight changes were sporadically significant for males in the 30 ppm dose group and for animals given 100 ppm. Overall body weight changes (Weeks 1 - 14) were statistically significantly lower for all treated groups.

Food/water consumption: Although statistically significant only at Week 13 for males in the 100 ppm dose group, food consumption was lower for males given 30 and 100 ppm when compared to controls for most of the study. Females in the 100 ppm dose group had significantly lower food consumption than controls at Weeks 3, 4, 6, 7, 11, and 12.

Test Material Consumption: The following table summarizes the amount of test material consumed by animals on a mg/kg/day basis:

Test Material Consumption		
Target Dose Levels	Mean Achieved Dose Levels (Range) [mg/kg/day]	
	Males	Females
3 ppm	0.203 (0.148 - 0.304)	0.240 (0.184 - 0.319)
30 ppm	2.041 (1.390 - 3.094)	2.588 (2.204 - 3.395)
100 ppm	6.844 (5.054 - 10.291)	7.702 (6.494 - 10.378)

Clinical signs (description, severity, time of onset and duration): There were no apparent test material-related observations noted.

Hematology & Clinical Chemistry: Treatment was associated with:

- lower hematocrit (100 ppm animals),
- higher albumin (100 ppm males),
- lower globulin (30 or 100 ppm males),
- lower cholesterol (30 or 100 ppm males & 100 ppm females),
- lower triglycerides (30 or 100 ppm males & 100 ppm females),
- higher alanine aminotransferase (100 ppm males), and
- higher urea nitrogen (100 ppm animals).

Effects were mild except those on cholesterol and triglycerides for high-dose males, which were moderate. All findings were more prominent at Week 14 than at Week 5. Mean corpuscular volume was significantly lower for high-dose females; this finding was of uncertain relationship to treatment. Other statistically significant differences were considered incidental and not treatment-related.

Ophthalmologic findings: No treatment-related effects

Mortality and time to death: One female in the 3 ppm dose group was sacrificed on Day 59 due to severe laceration of the tail. There were no macroscopic or microscopic changes to indicate that this condition was test material-related. All other animals survived to scheduled sacrifice.

Gross pathology, incidence and severity: Large and diffusely dark livers were observed in some high-dose animals. Dark foci or areas were observed in the glandular stomach of high-dose animals. Test material-related findings were not observed for animals in other dose groups.

Organ weight changes: Liver weights (absolute and/or relative ratios) were significantly increased for animals in the 30 and 100 ppm dose groups.

Histopathology: One or more of the following treatment-related liver effects were observed in high-dose animals: slight-to-moderate centrilobular or diffuse hepatocellular hypertrophy, minimal-to-slight coagulative necrosis, minimal hepatocellular pigment, minimal-to-slight centrilobular to midzonal or diffuse hepatocellular vacuolation. Centrilobular hepatocellular hypertrophy was also observed in 30 ppm animals, although the incidence and severity was decreased at this dose. A low incidence of minimal hepatocellular vacuolation occurred in males in the 30 ppm dose group. The liver of one 30 ppm female had minimal coagulative necrosis. A relatively low incidence of mucosal erosions in the glandular stomach was observed in the 100 ppm dose groups and was considered to be possibly related to treatment. No test material-related changes were seen in animals in the 3 ppm dose group.

Additional Endpoints:

Serum PFOS determination: Analyses reported separately by Sponsor.

Palmitoyl-CoA oxidase analysis: Treatment was associated with higher hepatic palmitoyl CoA oxidase for animals in the 100 ppm dose group.

Liver PFOS determination: Analyses reported separately by Sponsor.

Proliferation Cell Nuclear Antigen (PCNA) evaluation: Analyses will be included in the final report.

CONCLUSIONS

Chronic dietary exposure to N-MeFOSE was associated with lower body weights at all treatment doses examined. Hepatocellular hypertrophy and other hepatocellular effects were observed in animals from the 30 and 100 ppm dose groups. Changes in various clinical chemistry and hematology parameters, particularly decreased cholesterol and triglyceride levels, were also observed for animals in the 30 and 100 ppm dose groups.

REFERENCE

Covance Laboratories, Inc. 1999. 13-Week Dietary Toxicity Study with N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE, T-6314) in Rats. AUDITED DRAFT May 25, 1999.

ROBUST SUMMARY - RS39

Title: 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS: T-6295) in Rats. Summary Report Week 53.

TEST SUBSTANCE

Identity: PFOS: T-6295

Remarks: No details in interim report

METHOD

Method/guideline: Not specified; presumably standard 2-year chronic toxicity and carcinogenicity trial

Test type: Chronic Toxicity and Carcinogenicity with Satellite Analyses of Serum PFOS levels; Hepatocellular proliferation rate; Palmitoyl-CoA oxidation (measure of peroxisome proliferation); Mitochondrial activity; Interim histopathology

GLP: Yes

Year: Interim report issued 1999

Species/Strain: Rat; Crl:CD⁷ (SD) IGS BR

Route of administration: Oral (dietary)

Doses/concentration levels: 0, 0.5, 2.0, 5.0 20.0 ppm T-6295 in diet.

Sex: Male and Female

Exposure period: 104 weeks, but this is 53 week interim report

Frequency of treatment: Daily *ad libitum*

Control group and treatment: Basal diet

Post exposure observation period: NA, one recovery group (see table below)

Duration of test: 104 weeks, but this is a 53-week interim report.

Statistical methods: Unknown for bioassay. Student's T-test for Palmitoyl Co-A oxidation analysis.

Remarks:

Test Subjects:

Age at study initiation: 6-8 weeks

Weight at study initiation: 100-300 grams

No. of animals per sex per dose: 360 males, 360 females. See table below.

Number of Animals per Sex per Dose					
Dietary Level (ppm)	Males (n)	Females (n)	Week 4 sac* (n) □/□	Week 14 sac* (n) □/□	Week 53 sac** (n) □/□
0	70	70	5/5	5/5	10/10
0.5	60	60	5/5	5/5	10/10
2.0	60	60	5/5	5/5	10/10
5.0	60	70	5/5	5/5	10/10
20.0	70	70	5/5	5/5	10/10
20.0	40	40			
* Sacrifices at week 4 and 14 were performed to determine hepatocellular proliferation rates (via measurement of proliferation cell nuclear antigen (PCNA), biochemical analyses (palmitoyl-CoA oxidation), mitochondrial activity analyses, and histopathology (Week 14 only). ** Intermediate sacrifice after at least 52 weeks of treatment. Hepatocellular proliferation rates were determined via BrDU incorporation.					

Study Design:

Vehicle: Basal diet

Satellite groups and reasons they were added:

Hepatocellular proliferation rates, performed by Pathology Associates International. See separate write-up.

Determination of cyanide insensitive Palmitoyl-CoA oxidation . Performed by Centre for Xenobiotic Research, University of Dundee, Study Number XR0108. See separate write-up.

Mitochondrial activity analyses

Histopathology

Recovery

Clinical observations performed and frequency:

Hematology, clinical chemistry, urinalyses, urine chemistry during weeks 4, 14, 27, and 53

Organs examined at (interim) necropsy:

Week 14: adrenals, brain, eyes, kidneys, liver, mesenteric lymph node, pancreas, spleen, testes, and ovaries.

Week 53: All tissues from Control and 20.0 ppm interim group members

RESULTS

NOAEL (NOEL) None identified by testing lab.

LOAEL (LOEL) None identified by testing lab.

REMARKS All remarks apply for weeks 4, 14, 27, and 53, unless otherwise noted.

Body weight: Males: Animals at the highest dose (20.0 ppm) had significantly lower mean body weights compared to control during weeks 9-53. All other treated groups showed no significant differences in body weights relative to control.

Females: Animals at the highest dose (20.0 ppm) had significantly lower mean body weights compared to control during weeks 3-53. All other treated groups showed no significant differences in body weights relative to control.

Food/water consumption:

Males: Although not consistently statistically significant, animals receiving the highest dose (20.0 ppm) tended to consume less food during weeks 1-24; no significant difference in food consumption relative to controls (or other treated groups) observed in weeks 28-52.

Females: At highest dose (20.0 ppm) animals showed a statistically significant decrease in food consumption relative to controls during weeks 2-44.

Clinical signs (description, severity, time of onset and duration):

Test lab reports no apparent test material-related clinical observations noted through Week 53. Unscheduled deaths occurred in 3 males and one female fed 20.0 ppm PFOS. 2/3 males and 1/1 females exhibited large, mottled, or diffusely dark livers. No other gross changes that could be attributed to the administration of the test material were observed.

Hematology:

No remarkable findings except for a statistically significant increase in the absolute number of segmented neutrophils in males treated at 20.0 ppm at the 14-week sampling. The effect did not persist, and may have been spurious.

Biochem:

Urea nitrogen: mildly higher (statistically significant) for males and females fed 5.0 or 20.0 ppm. Not considered adverse.

Cholesterol: mildly to moderately lower for males and females fed 20.0 ppm. Not considered adverse.

Alanine aminotransferase: mildly higher for males fed 20.0 ppm. Not considered adverse, and test labs opines that findings likely associated with histopathological findings of hepatocellular hypertrophy and vacuolation.

Glucose: mildly lower for males and females fed 20.0 ppm; statistically lower for males fed 2.0 or 5.0 ppm at week 53.

Satellite Study: Determination of cyanide insensitive Palmitoyl-CoA oxidation

Ophthalmologic findings: Nothing remarkable.

Mortality and time to death:

Males: no significant changes in survival up to interim (53 week) sacrifice

Females: slight decrease (unknown statistical significance) in treated groups (100% vs. ~94-98%, no dose-response)

Gross pathology incidence and severity:

Males and Females. No clear or consistent gross observations at Week 53 sacrifice attributable to administration of test material.

Organ weight changes:

Males: absolute and relative liver weights increased in animals treated at 20.0 ppm. Absolute and relative spleen weights decreased in males given 20 ppm.

Females: liver to body weight ratio statistically significantly increased in 20.0 ppm group. Absolute spleen weight significantly decreased at 20.0 ppm, as was absolute lung weight at 2.0, 5.0, and 20.0 ppm. Spurious, significant increases in left thyroid/parathyroid to body weight percentages observed at 5.0 or 20.0 ppm.

Histopathology:

Males: Centrilobular hepatocyte hypertrophy and midzonal to centrilobular vacuolation were increased in incidence and severity at 5.0 and 20.0 ppm.

Females: Centrilobular hypertrophy, without vacuolation, and with less severe effects than in males, and increased centrilobular hepatocyte pigment were observed in the 20.0 ppm group.

CONCLUSIONS

Consistent with other studies, the liver is the primary target of PFOS in rats.

REFERENCE

Covance Laboratories. 1999. 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS: T-6295) in Rats. Summary Report Week 53. Covance Study Number: 6329-183.

ROBUST SUMMARY - RS40

Title: Oral Developmental Toxicity Study of N-EtFOSE in Rabbits (Argus 1999)

TEST SUBSTANCE

Identity: N- Ethyl FOSE. Lot FM 3929

Remarks:

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): ICH Harmonized Tripartite Guideline, stages C & D

GLP (Y/N): Yes

Year study performed: 1999

Species/Strain: Rabbit Hra:(NZW) SPF

Number of animals per dose: Twenty-two

Route of administration: Oral (intubation)

Dosing regimen (list all with units): Does were dosed at a volume of 5 ml/kg, once daily, on gestation days (GD) 7-20 (day 0). The vehicle was 2% Tween 180 with reverse osmosis deionized water

Doses: 0, 0.1, 1.0, 2.5, or 3.75 mg/kg bodyweight.

Statistical methods used: Clinical data by variance test for homogeneity. Continuous data by Bartlett's test and ANOVA. If Bartlett significant @ 0.05 then Kruskal-Wallis used followed by either Fischer's Exact or Dunn's Method of Multiple Comparisons. If ANOVA significant at 0.05 then Dunnett's Test.

Remarks – Detail and discuss any significant protocol parameters and deviations: There were extra animals for a satellite study where they were dosed similar to main groups and killed on GD 21. Samples collected were serum, liver, pooled fetuses and placentas, from each doe, that were frozen and shipped to sponsor (3M). Numbers of does totalled 19 with 3, 5, 3, 3, and 5 in 0, 0.1, 1.0, 2.5, and 3.75 mg/kg groups, respectively.

There were extra animals for a satellite study where they were dosed similar to main groups and killed on GD 21. Samples collected were serum, liver, pooled fetuses and placentas, from each

doe, that were frozen and shipped to sponsor (3M). Numbers of does totalled 19 with 3, 5, 3, 3, and 5 in 0, 0.1, 1.0, 2.5, and 3.75 mg/kg groups, respectively.

RESULTS

NOAEL (dose and effect) – maternal and developmental:

Maternal 0.1 mg/kg.

Developmental 1.0 mg/kg.

LOAEL (dose and effect) – maternal and developmental:

Maternal. 1.0 mg/kg – reduced weight gain on GD 7-10 only.

Developmental 2.5 mg/kg - abortions

Toxic response/effects by dose level - maternal: Maternal weight: Mean weight gain reduced during GD 7-10 in 1.0 mg/kg dose. There was an actual weight decrease in the mean BW in the 2.5 and 3.75 mg/kg dose groups at the GD 7-10 and GD 10-14 periods. Food consumption was also significantly decreased during these time periods at these two doses.

Toxic response/effects by dose level - developmental. There were 1, 2 and 5 abortions in the 0, 2.5 and 3.75 mg/kg dose groups.

Statistical results: Results discussed above were statistically significant

Remarks – Additional information to adequately assess the data: The report states there was a statistically higher incidence of late resorptions in the 3.75 mg/kg group; however, the incidence of total resorptions was similar to controls.

CONCLUSIONS

Comment on author's conclusions and whether you agree: The reduced body weight gain in the GD 7-10 time period at the 1.0 mg/kg dose is the sole basis for assuming this dose is a LOAEL. While plausible, it is very weak data upon which to establish a LOAEL.

The study authors attribute the 2 abortions at 2.5 mg/kg to be related to EtFOSE exposure. This, in the face of one abortion in the controls. There were 5 abortions at the next higher dose that is likely chemically related. Abortions are listed by the study authors as a developmental effect. Given the body weight loss and reduced food intake in the does it seems more plausible to be associated with maternal toxicity.

REFERENCE

Final Argus Report, protocol 418-010, Oral Developmental Toxicity Study of N-EtFOSE in Rabbits. 3M 6316.8. CD#1 00012621.pdf

OTHER

Need to establish status of tissues taken for possible analytical work.

ROBUST SUMMARY - RS41

TITLE: Oral Teratology Study of FM3422 in Rats - Experiment No. 0680TR0010 [N-EtFOSE]

TEST SUBSTANCE

Identity: N-EtFOSE (FM3422)

Remarks: Material identified as FM 3422, Lot 784. FM 3422 identified as 96% narrow range N-EtFOSE, with 3% primarily a C6 FOSE alcohol per 3M memo of D.R.Ricker dated December 10, 1980.

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: Charles River 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 test material in corn oil by gavage on gestation days 6-15. Doses were adjusted according to the most recent recorded body weight.

Doses: 0, 25, 37.5, and 75 mg/kg/day

Statistical methods used: The animals were 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:
FM 3422 was administered in corn oil by gavage to four groups of 22 time-mated Sprague-Dawley rats weighing 175-261g, at doses of 0, 25, 37.5, and 75 mg/kg/day on days 6-15 of gestation (Gortner, 1980). The animals were dosed according at 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 was 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.

RESULTS

NOAEL (dose and effect) – maternal and developmental:

A NOAEL of 25 mg/kg/day for maternal toxicity was indicated.

A NOAEL for developmental toxicity could not be established when lens effects are considered. If lens effects are considered an artifact, there is a NOAEL of 25 mg/kg.

LOAEL (dose and effect) – maternal and developmental:

Maternal: 37.5 based on significant reductions in mean body weights during gestation. Death occurred in 3 rats at the 75 mg/kg dose.

Developmental: 37.5 based on cleft palate reduced fetal body weight and skeletal changes particularly sternebrae. Incidence of blood in the kidney parenchyma was significantly elevated at the 75 mg/kg dose.

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 FM-3422 groups.

Statistical results: 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 –Three rats in the high dose group died. Clinical signs in these animals included thin appearance, lethargy, ataxia, bloody stools and encrusted blood around the nares. Signs of maternal toxicity were not seen in other rats. Significant reductions in mean body weights the gestation period in rats from the 37,5 and 75 mg/kg groups. There were no effects seen on

maternal ovary, mean number of implantation sites, corpora lutea, resorption sites and the mean numbers of viable male, female, and total fetuses at any dose.

Mean fetal weight was significantly lower than control values in all treated groups. There was a statistically significant increase in cleft palate in the mid and high dose whereas no cleft palates were seen at the control or low dose. The study authors described an increase in severity and sometimes incidence in sternal malformations at all treatment doses; stated as related to exposure to FM 3422. Other skeletal aberrations were also stated as being associated with exposure to the test substance. A notable sign of developmental toxicity observed in all FM 3422 groups consisted of abnormalities of the lens of the eye. 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 manner and frequency in which the lens cleft artifact was created by freehand sectioning and the limitations inherent in visualizing the embryonal nucleus. A subsequent study was not able to repeat this finding.

CONCLUSIONS

Comment on author's conclusions and whether you agree: Reviewer generally agrees with the study authors. However it is believed that the lens defect is an artifact associated with the method of free hand sectioning. This lens effect was also observed in this laboratory with studies with PFOS. Other studies in other laboratories have never reported lens effects. A summary of the lens issue in the Riker Laboratories is contained in a memorandum to the file written by the Study Director, EG Gortner, dated November 6, 1981.

REFERENCE

Gortner EG, Lanprecht EG, Case MT. Riker Laboratories, Inc. "Oral Teratology Study of FM-3422 in Rats", January 22, 1981. Experiment Number: 0680TR0010. CD ROM 00012291.pdf

Gortner EG. Memo to Study Files titled "Fetal Rat Lens Artifact – Summary of Developments to Date", 3pp, November 6, 1981.

Ricker DR. 3M memo to WC McCormick. Dated December 10, 1980. CD ROM 00012992.pdf

OTHER

Any other information deemed appropriate: None

ROBUST SUMMARY - RS42

Title: Oral (GAVAGE) Developmental Toxicity Study of N-EtFOSE in Rats (Argus 1998)

TEST SUBSTANCE

Identity: N- Ethyl FOSE. Lot FM 3929

Remarks: Material, described as a waxy solid, was received on May 28, 1998 and stored at room temperature.

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): The requirements of the ICH Harmonized Tripartite Guidelines stages C & D were used as the basis for the study design. The purpose of the study was to detect adverse effects to N-EtFOSE in pregnant CR:CD (Sprague Dawley) rats and development of embryo and fetus consequent to exposure of the dam from the period of implantation to hard palate closure.

GLP (Y/N): Yes The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the US 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 has been documented.

Year study performed: 1998

Species/Strain: Rat. Sprague Dawley [CrI:CDBR VAF Plus]

Number of animals per dose: Twenty-five in main study, 3 or 5 in a satellite study where tissues were to be collected for possible future analyses of N-EtFOSE or metabolites

Route of administration: Oral (intubation)

Dosing regimen (list all with units): Rats were dosed at a volume of 5 ml/kg, once daily, on gestation days (GD) 6-17 (day 0). The vehicle was 2% Tween 180 with reverse osmosis deionized water

Doses: 0, 1, 5, 10, or 20 mg/kg body weight. Based on dose-range study where bodyweight effects were observed at 10 mg/kg bw/day and higher.

Statistical methods used: Clinical data by variance test for homogeneity. Continuous data by Bartlett's, test and ANOVA. If Bartlett significant @ 0.05 then Kruskal- Wallis used followed by either Fischer's Exact or Dunn's Method of Multiple Comparisons. If ANOVA significant at 0.05 then Dunnett's Test.

Remarks –There were extra animals for a satellite study where they were dosed similar to main groups and killed on GD 18. Samples collected were serum, liver, pooled fetuses and placentas, from each dam, that were frozen and shipped to sponsor (3M). Numbers of dams totalled 19 with 3, 5, 3, 3, and 5 in 0, 1, 5, 10, and 20 mg/kg groups, respectively.

Female rats were observed twice daily for viability. Body weights were recorded weekly during acclimation period, on GD 0 and GD 4 and daily during the dosage and post dosage period (GD 6-20). Food consumption was recorded on GD 0, 4, and on alternate days thereafter through GD 20.

RESULTS

NOAEL (dose and effect) – maternal and developmental:

Maternal 5 mg/kg.

Developmental 5 mg/kg.

LOAEL (dose and effect) – maternal and developmental:

Maternal. 10 mg/kg – reduced weight gain.

Developmental 10 mg/kg – reduced fetal body weight and delays in caudal vertebra ossification.

Toxic response/effects by dose level - maternal: Maternal weight: Mean weight gain reduced during GD 8-12 in 10 mg/kg dose. Reduced fetal weight gain during the GD 6-20 period at the 20 mg/kg dose group. Food consumption was also significantly decreased during this time period in the 20 mg/kg group.

Toxic response/effects by dose level - developmental. Reduced fetal body weight at 10 and 20 mg/kg and delayed ossification in caudal vertebrae. Increased incidence of wavy ribs in 20 mg/kg group.

Statistical results: Results discussed above were statistically significant

Remarks –No deaths, premature deliveries or abortions were observed during the study and there were no clinical signs attributed to effects of the test material. Minimal and transient (GD 8-10) decreases in maternal body weight gain were seen at doses of 5mg/kg. Effects were more persistent at 10 mg/kg dose where there was a significant decrease during the GD 8-12 period followed by and increased weight gain during GD 14-16. There was actual weight loss or reduced weight gain in the 20 mg/kg group throughout the GD 6-20 period. Food consumption decreases were observed in the 10 and 20 mg/kg dose groups in a pattern similar to the effects observed on body weight.

Fetal body weight was significantly reduced in litters from dams in the 10 and 20 mg/kg dose groups. Fertility and fetal viability parameter were comparable across the control and N-EthylFOSE groups. Significant delays in ossification of caudal vertebrae were noted in fetuses from the 10 and 20 mg/kg groups; an increase in the incidence of wavy ribs was also seen in the 20 mg/kg fetuses. All other gross, visceral or skeletal observations (malformation and

variations) were judged to be unrelated to exposure to N-EtFOSE.

CONCLUSIONS

Comment on author's conclusions and whether you agree: Agree with the study authors' conclusions.

REFERENCE

Final report, Protocol 418-011, Oral (Gavage) Developmental Toxicity Study of N-EtFOSE in Rats, December 17, 1998. 3M 6316.7. CD#1 00012345.pdf

OTHER

Need to establish status of tissues taken for possible analytical work.

ROBUST SUMMARY - RS43

TITLE: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF N-EtFOSE IN RATS 3M 418-009; ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.9, 1999.

TEST SUBSTANCE

Identity: N-EtFOSE alcohol

Remarks: Lot number FM-3929 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: A modification of the requirements of the US Food and Drug Administration (FDA) were used as a basis for the study design.

Type of study: Two-generation reproductive toxicity.

GLP (Y/N): Yes. The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the US 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 has been documented.

Year study performed: 1999

Species/Strain: Rat. Sprague Dawley [CrI:CDBR VAF Plus]

Sex (males/females/both): Both.

Number of animals per dose: F_0 = 35 [25 females for full evaluation of F_1 generation; 10 females for determination of reproductive status at DG 10]. F_1 had 25 each sex, per dose.

Route of administration: Oral (gavage).

Dosing regimen (list all with units): Five groups of 35 rats per sex per dose group were administered EtFOSE by gavage for six days prior to cohabitation and during 14 days of mating. Treatment in F_0 male rats continued until one day before sacrifice (approximately 63 days total); female rats were treated daily throughout gestation, parturition, and lactation. F_1 rats received daily gavage commencing on LD 22 and each day thereafter until sacrificed.

Doses: 0, 1, 5, 10, 15 mg/kg bw/day

Premating exposure period for males/females F_0 , F_1 : F_0 males were dosed daily beginning 28 days before cohabitation, during mating, and until the day of sacrifice for a total of ~60 days dosing. F_0 females were dosed for 28 days before cohabitation and daily thereafter until sacrificed. F_1 rats exposed in utero and during lactation; direct dosing commenced at weaning and continued until their litters were weaned.

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, pre-weaning reflex/physical developmental data and post-weaning 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:

See Table [Schema on page 1-4 of the Report to be reproduced] for schematic description of study.

F_0 Generation:

Parental rats (F_0) 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 that were dosed until gestation day (GD) 10, sacrificed, and necropsied to determine the number of corpora lutea, implantations, and number of viable and non-viable embryos. 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 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. The final results of these analyses were not available at the time of this review.

F₁ Generation:

Since F₁ generation pup viability was significantly reduced in the 10 and 15 mg/kg/day dosages groups, only the 1 and 5 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 1, and 5 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, female evaluations commenced to determine the age of vaginal patency and on LD 34, male rat evaluation commenced to determine the age of preputial separation. One rat per sex per litter was evaluated in a water-filled M-maze on LD 70. Assignment to cohabitation within each dosage group began on approximately 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 and raise litters until LD 21. 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 – F₀, F₁, and F₂:

F₀ 1mg/kg bw/day; reproductive NOAEL 5 mg/kg.

F₁ < 1 mg/kg bw/day based on body weight effects; reproductive 5mg/kg .

F₂ 1 mg/kg bw/day.

LOAEL (dose and effect) – F₀, F₁, and F₂:

F₀ 5 mg/kg based on body weight effects; reproductive 10 mg/kg based on stillbirths.

F₁ 1 mg/kg based on body weight effects, greater than 5 mg/kg, the highest dose tested;

F₂ 5 mg/kg. Based on effects on viability & growth.

Toxic response/effects by dose level:

F₀/F₁: F₀ had reductions in food consumption and body weight gain at 10 and 15 mg/kg bw/day.

F₁ had reductions in body weight gain at 5 mg/kg; at 10 and 15 mg/kg there was pre-implantation loss, reduced litter size, pup viability, growth and survival.

F₁/F₂: In the F₁ there were reductions in feed consumption and body weight gain at the 1 and 5 mg/kg doses. In the F₂ there were stillbirths and reductions in litter size, pup viability, growth, and survival in the 5 mg/kg dose group.

Statistical results: In the main, results summarized above were statistically significant or evidenced dose response characteristics.

Remarks – Additional information to adequately assess the data:

F₀ Generation.

General toxicity. No treatment related deaths occurred in either sex. In male rats at the 15 mg/kg dose there was an impaired righting reflex, there were no treatment-related clinical signs of toxicity. Significant increases in localized alopecia during pre-mating, gestation, and lactation were observed in females in the 5, 10, and 15 mg/kg/day dosage groups. Reductions in male and female body weight gains occurred in the 5, 10 and 15 mg/kg/day dosage groups throughout all phases of the study. Reductions in food consumption were consistently seen in the 10 and 15 mg/kg groups and frequently seen in the 5 mg/kg group throughout the study. Terminal body weights were reduced in males from the 5, 10 and 15 mg/kg groups. Observations found at necropsy in both sexes were unrelated to treatment.

Reproductive Toxicity. Male-related effects on mating or any fertility parameters evaluated were not seen in any dose group. Rats from the 15 mg/kg group were had statistically significant reduction in absolute seminal vesicle and prostate weight that were interpreted by the study authors to be chemically related. While a decrease in prostate and seminal vesicle weight was seen on an absolute basis, when expressed as an organ to body weight ratio it was not significantly different from controls. Evaluation of sperm number, motility or morphology was not part of the study protocol.

Estrous cycling, mating and fertility were similar among all groups. Statistically significant reductions in litter averages for implantations and live embryos were observed in the 15 mg/kg females examined on DG 10. A reduced number of implantation sites and litter size was also noted in the 15 mg/kg dose group in the main portion of the study, where females birthed their litters. The gestation duration was significantly reduced in the 5, 10, and 15 mg/kg dose.

F₁ Generation

Developmental Toxicity. Four females in the 15 mg/kg group had no live born pups and the others had reduced litter size and poor pup viability; all pups were dead by DL 5. The 10 mg/kg group had an increased incidence of stillborn pups or pups that died or were presumed cannibalized on DL 1; additional pups died in the 2-4, 4-7 and 8-14 day lactation periods. Viability index values demonstrate the steep nature of the dose-response for pup mortality; values are 92.8, 99.1, 92.0, 30.2 and 1.2.0% for the 0, 1, 5, 10 and 15 mg/kg dose, respectively.

Dose dependent and statistically significant reduced body weights were seen in the 5, 10 and 15 mg groups. There was also a tendency for reduced pup weight in the 1 mg/kg group. These weight effects persisted throughout the lactation period. A number of biological measures consistently revealed delays in pup development during the lactation period. In litters born of mothers that received 5 or 10 mg/kg EtFOSE (all 15 mg/kg pups were dead by DL 5). These included surface righting reflex, pinna unfolding, eye opening, acoustic startle response, and air righting. A transient delay in age of pinna unfolding was also seen in the 1 mg/kg group. Normal development was ultimately achieved for each of these indices in all groups. Due to severe pup mortality during lactation in the 10 mg/kg group it was determined that no further studies involving the surviving rats would occur and they were euthanized. Twenty five each, male and female F₁ rats commenced receiving daily gavage doses of 0, 1, and 5 mg/kg EtFOSE at weaning. This dosing regimen continued through the growth and mating period in both sexes and in females through gestation and 21 days of nursing and lactation.

In the post-weaning period, age of preputial separation in the 1 mg/kg group and attainment of vaginal patency in the 1 and 5 mg/kg dose groups was similar to control pups. Preputial separation was significantly delayed in the 5 mg/kg by approximately one day; this was considered not to be toxicologically important by the study authors. Two neurological tests were performed on one rat per sex per litter; passive avoidance at 28 days of age and a water-filled M maze at 70 days of age. No chemically related effects were observed.

Reproductive Performance. Body weight was significantly reduced at time of weaning and at necropsy in males from the 5 mg/kg group. Males in the 1 mg/kg group commonly had reduced body weight gains during the first month of the post-weaning period. Food consumption was reduced in the 5, but not the 1, mg/kg group. In females from the 5 mg/kg group, weight was reduced at weaning and throughout the pre-cohabitation period. Body weight gain during gestation generally was significantly reduced in the 1 and 5 mg/kg groups. This reduction in weight gain persisted throughout lactation. Reduced food consumption was consistent with body weight effects. At 90 days of age males and females were mated. After mating males were killed and necropsied. Females were allowed to litter and nurse their young until DL 21 at which time they were killed and necropsied. No effects on mating and fertility were observed in either sex. No toxicological findings were noted at necropsy.

F₂ Generation

No significant effect on litter size, number of live or stillborn pups was noted in the 1 or 5 mg/kg group. However, the number of dams with stillborn pups was significantly increased at the 5 mg/kg dose. The number of pup deaths or presumed cannibalized was significantly elevated during the DL 1 through 14 periods. Viability index was 98.8, 97.1, and 85.1 % in 0, 1, and 5 mg/kg dose groups, respectively; the 5 mg was significantly reduced. Pup weight was also significantly reduced at birth and throughout the lactation period in the 5 mg/kg group. Clinical and necropsy findings in the F₂ pups were unremarkable.

CONCLUSIONS

Comment on author's conclusions and whether you agree: Generally agree. Do not

understand the basis for determining that the slight but significant delay in age of preputial separation is not related to chemical exposure at 5 mg/kg. While such a delay should not be considered a reproductive effect, it is entirely consistent with reduced body weight and other maturational delays seen at this dose.

REFERENCE

Christian M, Hoberman A & York R., Argus Research Laboratories. COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF N-EtFOSE IN RATS. Final Report, 30 June, 1999. Protocol 418-009; STUDY NUMBER: 6316.5.

ROBUST SUMMARY - RS44

Title: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS – 3M T-6295.10, ARGUS RESEARCH LABORATORIES STUDY NUMBER: 418-012, 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, 1994, 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

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

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 Cesarean-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 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.

RESULTS

NOAEL – 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 all doses 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 all doses; reductions in mean body weight and body weight gain at all doses; reductions in 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 ($p \leq 0.01$). 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. Feed 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 – Additional information to adequately assess the data:

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. 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.

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

Comment on author's conclusions and whether you agree: Conclusions are summarized above and this reviewer agrees.

REFERENCE

Provide full citation of study reviewed: 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".

OTHER

None

ROBUST SUMMARY - RS45

Title: Oral Teratology Study of FC-95 in Rats - Experiment No. 0680TR0008

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

Remarks: Test and/or control article characterization for FC-95, Lot 640. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances were determined and documented as of May 8, 1980.

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 were 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). 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. The remaining fetuses were subjected to a skeletal examination using alizarin red.

RESULTS

NOAEL – maternal and developmental: A NOAEL of 5 mg/kg/day for maternal toxicity was indicated. A NOAEL for developmental toxicity could not be established when lens effects are considered. If lens effects are considered an artifact, there is a NOAEL of 10 mg/kg.

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 for developmental toxicity was not established as highest dose tested was a NOAEL.

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: 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 concluded 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 manner and frequency in which the lens cleft artifact was created by freehand sectioning and the limitations inherent in visualizing the embryonal nucleus. A subsequent study was not able to repeat this finding.

CONCLUSIONS

Comment on author's conclusions and whether you agree: The lens defect is not considered chemically related based on subsequent studies. A summary of the lens issue in the Riker Laboratories is contained in a memorandum to the file written by the Study Director, EG Gortner, dated November 6, 1981.

REFERENCE

Provide full citation of study reviewed: Gortner, E.G. 1980. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0680TR0008, December, 1980. "Oral Teratology Study of FC-95 in Rats".

Gortner EG. Memo to Study Files titled "Fetal Rat Lens Artifact – Summary of Developments to Date", 3pp, November 6, 1981.

OTHER

Any other information deemed appropriate: None

ROBUST SUMMARY - RS46

TITLE: Rat Teratology Study T-3351 Final Report – Project No. 154-160 (Hazelton 1983)

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

Remarks: The test material, T-3351 (Lot No. 80275), a white powder, was received from the sponsor on January 5, 1983 and was stored at room temperature. The test material was assumed to be 100% active compound. Information on the method of synthesis, stability, as well as data on composition, or other characteristics which define the test material, are on file with the sponsor.

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. 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 CO₂ 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 – maternal and developmental: The NOAEL for maternal toxicity is 1 mg/kg/day. The NOAEL for developmental toxicity is 1 mg/kg/day.

LOAEL – 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 hair coat, 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 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 cryptorchidism 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.

CONCLUSIONS

Comment on author's conclusions and whether you agree: 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.

REFERENCE

Wetzel, L.T. 1983. Hazelton Laboratories America, Inc. Project Number: 154-160, December 19, 1983. "Rat Teratology Study, T-3351, Final Report".

OTHER

None

ROBUST SUMMARY - RS47

Title: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.9, 1999. Protocol 418-008

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate (PFOS), 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: 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: Two-generation reproductive toxicity

GLP (Y/N): Yes. 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 were documented.

Year study performed: 1999

Species/Strain: Rat Crl:CDBR VAF Plus (Sprague-Dawley)

Sex (males/females/both): Both

Number of animals per dose: F_0 = 35. Twenty-five females for full evaluation of F_1 generation; 10 females for determination of reproductive status at DG 10. F_1 had 25 each sex, per dose.

Route of administration: Oral (gavage)

Dosing regimen: Five groups of 35 rats per sex per dose group were administered PFOS by gavage for six weeks prior to cohabitation and during 14 days of mating. Treatment in F_0 male rats continued until one day before sacrifice (approximately 63 days total); female rats were treated daily throughout gestation, parturition, and lactation. F_1 rats selected for mating and rearing of the F_2 generation received daily (gavage) doses of PFOS at 22 days of age and thereafter.

Doses: 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day

Premating exposure period for males/females: Six weeks for P (F_0); in utero and lactation, direct dosing starting at day continuing until LD 21 of F_1 litters.

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 that 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:

See Table [Schema on page 1-4 of the Report to be reproduced] for schematic description of study.

F_0 Generation:

Parental animals (F_0) 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 that were dosed until gestation day (GD) 10, sacrificed, and necropsied to determine the number of corpora lutea, implantations, and number of viable and non-viable embryos. The remaining females comprised the second set, which delivered naturally. After the 21-day gestation period, the dams were evaluated for clinical signs during parturition and length of gestation. During parturition 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 F_0 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 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. The final results of these analyses were not available at the time of this review.

F₁ Generation:

Since F₁ generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosage 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, female evaluations commenced to determine the age of vaginal patency and on LD 34, male rat evaluation commenced to determine the age of preputial separation. One rat per sex per litter was 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 and raise litters until LD 21. 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

The results incorporate the findings of the original report and Final Report Amendment I dated 13 April, 2000 submitted by the study director (RG York) and cosigned by the quality assurance manager (NJ Gongliewski).

NOAEL for F₀, F₁, and F₂: The NOAEL for the F₀ generation males and females = 0.1 mg/kg/day, the lowest dose tested. The NOAEL for the F₁ generation = 0.4 mg/kg/day, the highest dose tested. The NOAEL for the F₂ generation = 0.4 mg/kg/day, the highest dose tested.

LOAEL (dose and effect) – for F₀, F₁, and F₂: The LOAEL for the F₀ generation males and females = 0.4 mg/kg/day, based on reductions in body weight gain and food consumption. The LOAEL for the F₁ generation = 1.6 mg/kg/day, based on significant reductions in the number of implantation sites, litter size, pup viability, growth and survival. A LOAEL for the F₂ generation was not established as 0.4 mg/kg/day, the highest dose tested, was the NOAEL.

Toxic response/effects by dose level and generation:

F₀/F₁. In F₀ generation rats reductions in both body weight gains and in absolute and relative food consumption occurred 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. There was no reproductive toxicity in the F₀ generation males. While significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate were seen at the highest dose group of 3.2 mg/kg/day organ to body weight ratios were not significantly different from controls. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was 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. Significant reductions ($p \leq 0.01$) in gestation length, implantation sites, and litter size were observed at 3.2 mg/kg/day. Interestingly, reduction in implantation sites was not seen in the 10 rats sacrificed at DG 10.

In 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.

F₁/F₂: 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 F₁ generation pups consisted of reduced pup viability at the two highest dose groups (1.6 and 3.2 mg/kg/day). In the 3.2 mg/kg/day dose group 45% (71/156) of the pups were found dead on LD1; no pups survived beyond LD 4. In the 1.6 mg/kg/day dose group, 10.6% (27/254) of pups were dead on LD1; and an additional 26% (59/227) died between LD 2-4. 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.

Toxic effects in the F₂ generation pups consisted of transient 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 dosage groups. Significant reductions in 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 premating 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 was 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. At the doses 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. 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 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 – Additional information to adequately assess the data:

F₀ Generation.

General toxicity. In male rats, there were no treatment-related clinical signs of toxicity. Localized alopecia during pre-mating, gestation, and lactation were the only findings reported in some females in the 0.4, 1.6, and 3.2 mg/kg/day dosage groups. Mortality was not seen in either sex. Reductions in male and female body weight gains and in absolute and relative food consumption occurred in the 1.6 and 3.2 mg/kg/day dosage groups throughout all phases of the study. Following mating, food consumption was significantly reduced in the male 0.4 mg/day dosage group. However, terminal body weight was different than control values only at the 1.6 and 3.2 mg/kg doses. Observations found at necropsy in females were unrelated to treatment. In males a brown discoloration of the liver was seen in some rats gavaged with 3.2 mg/kg body weight PFOS.

Reproductive Toxicity. Male-related effects on mating or any fertility parameters evaluated were not seen in any dose group. While a decrease in prostate and seminal vesicle weight was seen on an absolute basis at 3.2 mg/kg, when expressed as an organ to body weight ratio it was not significantly different from controls. This was interpreted as a generalized effect rather than a

specific effect on a reproductive organ. Evaluation of sperm number, motility or morphology was not part of the study protocol.

Estrous cycling, mating and fertility were similar among all groups. No statistically significant or biologically important differences in litter averages for corpora lutea, implantations, live and dead embryos were observed in the females examined on DG 10. However, a reduced number of implantation sites and litter sizes was noted, along with an increase in stillborn pups, in the 3.2 mg/kg dose group that was part of the main portion of the study. The gestation duration was also significantly reduced at this dose.

F₁ Generation

Developmental Toxicity. At the dose of 3.2 mg/kg, 71/156 (45.5%) pups were found dead or presumed cannibalized on DL 1; the remaining pups died during DL 2-4. At the 1.6 mg/kg dose, 27/254 (10.6%) were found dead or presumed cannibalized on DL 1; an additional 59/227 (26%) died during DL 2-4. Viability index values demonstrate the steep nature of the dose-response for pup mortality; values are 98.7, 98.3, 98.3, 66.1 and 0.0% for the 0, 0.1, 0.4, 1.6 and 3.2 mg/kg dose, respectively.

A statistically significant ($p \leq 0.01$) reduced pup body weight was seen in the 1.6 and 3.2 mg/kg/day dosage groups at birth. This effect persisted throughout the lactation period in the 1.6 mg/kg group. There was a tendency for slight reductions in body weight at the lower doses; these differences did not achieve statistical significance. A number of biological measures revealed delays in pup development during the lactation period in litters born of mothers that received 1.6 mg/kg PFOS. These included surface righting reflex, pinna unfolding, eye opening, acoustic startle response, and air righting. The mean age at eye opening was also delayed at the 0.4 mg/kg dose; a transient delay in age of pinna unfolding was also seen at this dose. Normal development was ultimately achieved for each of these indices. At weaning the pups in the 1.6 mg/kg dose group had gained 20% less weight than the controls and for humane considerations, it was determined that no further studies involving these rats would occur and they were euthanized. Twenty five each, male and female F₁ rats commenced receiving daily gavage doses of 0, 0.1, 0.4 mg/kg PFOS at weaning. This dosing regimen continued through the growth and mating period in both sexes and in females through gestation and 21 days of nursing and lactation.

In the post-weaning period, age of preputial separation in males and attainment of vaginal patency in females was similar in control and treated pups in the 0.1 and 0.4 mg/kg dose groups. Two neurological tests were performed on one rat per sex per litter; passive avoidance at 28 days of age and a water-filled M maze at 70 days of age. No chemically related effects were observed.

Reproductive Performance. Body weight gain during the pre-cohabitation period was not significantly different from the controls. At 90 days of age males and females were mated. After mating males were killed and necropsied. Females were allowed to litter and nurse their young until DL 21 at which time they were killed and necropsied. No effects on mating and fertility were observed. Body weight during gestation similar to that of controls except for a transient decrease at DG 1-4 in the 0.4 mg/kg group. No toxicological findings were noted at necropsy.

F₂ Generation

No effect on litter size, number of live or stillborn pups were noted. Viability index was 97.1, 98.6, and 96.7 % in 0, 0.1, and 0.4 mg/kg dose groups, respectively. Evidence of treatment related effects were confined to a statistically significant reduced mean body weight in the 0.4 mg/kg group during the DL 7-14 period. Clinical and necropsy findings in the F₂ pups were unremarkable.

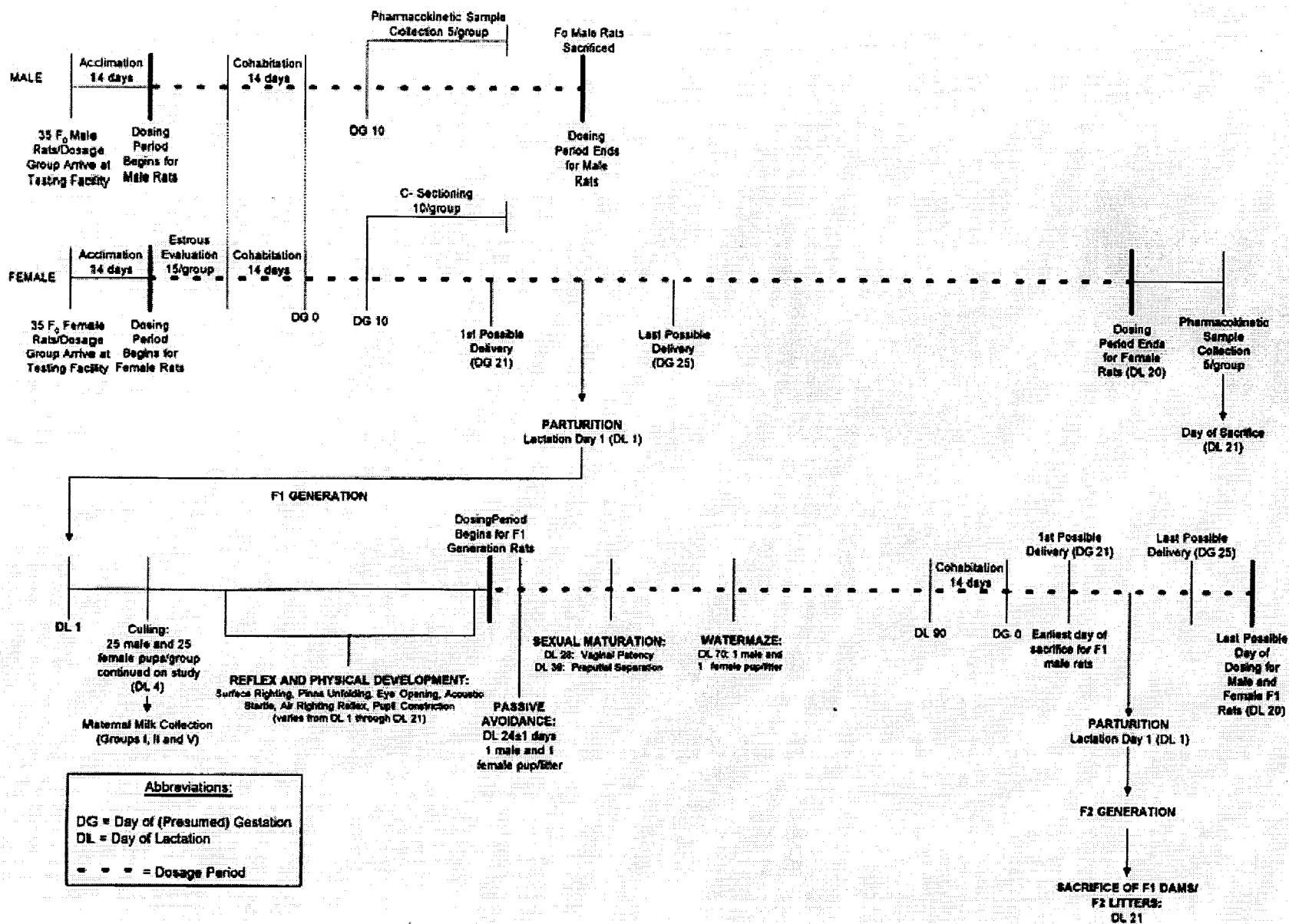
CONCLUSIONS

Comment on author's conclusions and whether you agree: 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.

Final Report Amendment I dated 13 April, 2000. "Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats".



ROBUST SUMMARY - RS48

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

TEST SUBSTANCE

Identity: Perflourooctanesulfonate (PFOS) (FC-95)

Remarks: The test material was received on 21 October 1998 and stored at room temperature.

METHOD

Method/Guideline followed: The general guidelines of the FDA were followed. The objective of the study was to evaluate the survival of F1 generation pups following PFOS treatment of F0 female during pre-mating, gestation, and lactation. F1 pups were cross fostered during the lactation period to differentiate effects on pups exposed to PFOS *in utero* and pups exposed to PFOS via maternal milk. Selected tissues were collected from the F0 female rats and F1 pups and analyzed to determine the presence and amount of PFOS.

Females treated for 42 days with either 0 or 1.6 mg/kg bw/day PFOS were cohabitated with untreated males. All pups in a litter were removed from their dams as soon as parturition was completed and placed with a dam treated with either 0 or 1.6 mg/kg PFOS. The cross-fostering procedure resulted in four groups of either 12 or 13 dams & pups, i.e., pups from a control dam fostered on a control dam (CC), pups from a control dam fostered on a PFOS treated dam (CT), pups from a PFOS treated dam fostered on a control dam (TC) or, pups from a PFOS treated dam fostered on a PFOS treated dam (TT). Litters were normalized to 10 pups on DL 4.

GLP (Y/N): Yes. The cross-foster study was conducted in compliance with Good Laboratory Practice Regulations of the US Food and Drug Administration, Japanese Ministry of Health and Welfare, and the European Economic Community. The analytical analyses for PFOS were also conducted in compliance with FDA Good Laboratory Practices. There were no deviations from the GLP regulations that affected the quality or integrity of either study.

Year study performed: 1998-1999

Species/Strain: Rat. Crl:CD BR VAF (Sprague-Dawley)

Number of animals per dose: Twenty five litters from control or treated dams were cross-fostered with 12 or 13 control or treated dams; thus four treatment groups were established. In addition, 2 and 8 rats from the 0 and 1.6 mg/kg groups were allowed to keep their litters and were used as a source of biological specimens for determination of PFOS levels in dams and pups on DL 14. Maternal serum, liver and mammary gland and pup serum and liver (pooled per litter) were collected.

Route of administration: Oral (gavage)

Dosing regimen: Female rats were administered appropriate test material daily during a 42 day pre-cohabitation period and continuing through mating, gestation and a 21 day lactation period. Doses were selected based on the results of a 2-generation reproductive toxicity study performed using the same route and rat strain in the same laboratory.

Suspensions of the test material were prepared daily at concentrations of 0 and 0.32mg/mL using 0.5% Tween 80 in reverse osmosis membrane processed deionized water.

Doses: 0 , 1.6 mg/kg bw/day

Statistical methods used: Averages and percentages were calculated. Litter values were used where appropriate.

Remarks – Detail and discuss any significant protocol parameters: Day of birth was designated as lactation day 1. Each litter was evaluated for viability twice each day. Pup count and physical signs were recorded daily. Pup weights were recorded on DL 1, 4, 7, 14 and 21. The lungs and livers were collected from the first 10 culled pups on DL 4 and preserved. On DL 21 six litters from each of the four cross-foster subsets had pooled litter samples of liver and serum collected for possible analysis for PFOS.

RESULTS

Toxic responses - maternal: All F0 females survived to scheduled sacrifice. There were no clinical or necropsy observations attributed to PFOS administration. Absolute body weights were slightly reduced in the 1.6 mg/kg groups at the end of cohabitation and continued through gestation and lactation. Feed consumption was also reduced in the group throughout the study. Litter size at birth was slightly reduced in the 1.6 mg/kg group, 16.2 and 14.8 in the 0 and 1.6 mg/kg groups, respectively. Number of implantation sites in the 1.6 mg/kg group was also reduced, 16.0 vs. 17.7 in control.

Toxic response – pups: Pup mortality is summarised in the table below. It was increased in pups from treated dams that foster nursed control dams. Mortality was greatest in pups from treated dams that foster nursed treated dams. There were no differences in mortality in pups from control dams that foster-nursed treated dams when compared with pups from control dams that foster nursed control dams. PFOS exposure via maternal milk appeared to reduce pup body weight gain regardless of *in utero* exposure to the chemical. For example, mean pup weight per litter was 29.0 (CC), 26.2 (CT), 26.7 (TC) and 24.6 (TT) grams on DL 14. The greatest reduction in weight gain was in those pups exposed *in utero* and during lactation.

Evaluation of livers from LD1 pups from dams in the 1.6 mg/kg group revealed increased (2X) numbers of peroxisomes when examined by electron microscopy. Differences in mitochondrial cell membranes were not observed. Type II pneumocytes were increased in number as were the number of lamellar bodies in samples of lung.

Cross-foster PFOS Study
Post-natal Pup Effects During 21 Day Lactation Period

PFOS Exposure ^a		Number Dead	Total Pups	Percent Mortality	Litters Affected	Pup Weight
Gestation	Lactation					
0	0	3	191	1.6	3(12)	29.0 ^b
0	1.6	2	181	2.0	2(11)	26.2
1.6	0	16	166	9.6	10(11)	26.7
1.6	1.6	34	177	19.2	8(12)	24.6

^a refers to daily female dose of 0 or 1.6 mg/kg PFOS.

^b mean weight in Grams on LD 14

Additional information to adequately assess the data: Serum samples collected from dams and litters on scheduled day of necropsy (LD 21) have been analyzed for PFOS content and the results are presented below. Other samples have not been analyzed. Pooled sera from litters born of control dams that were cross-fostered on control dams had values below the lower limit of detection as did their foster mothers. In contrast, pooled sera from litters born of treated dams who also foster nursed treated dams had values of 89.71 uG/mL, similar to their foster mothers and other treated mothers. Pooled sera from litters born of treated dams but who foster nursed control dams had values that were 60% (53.88 ug/mL) of the TT litters. Interestingly, their control foster mothers developed low sera values probably as a result of grooming their litters and coprophagy. Pooled sera from litters born of control dams that foster-nursed treated dams had PFOS values of 22.35 ug/mL indicating significant transfer of PFOS through milk. Considering pup viability data it is suggested that maternal sera levels in the range of 82-89 ug/mL are associated with pup mortality.

PFOS Values in Serum Collected From Dams and Pups at Time of Necropsy

<u>Pup Exposure Regimen</u>		<u>Nursing Dams</u>	<u>Litters</u>
<u>Gestation</u>	<u>Lactation</u>	<u>Mean</u>	<u>Pooled Mean</u>
Control	Control	0.05* (12)**	0.05* (6)
Control	Treated	82.96 (13)	22.35 (6)
Treated	Control	2.02 (13)	53.88 (6)
Treated	Treated	88.97 (12)	89.71 (6)

* 0.05 uG/mL is Lower Limit of Quantitation.

** Number in parentheses is number of samples

CONCLUSIONS

In utero exposure to 1.6 mg/kg PFOS can cause postnatal pup mortality. Continued PFOS exposure in the postnatal period appears to have an additive effect on the incidence of postnatal pup mortality. At 1.6 mg/kg effects on maternal weight, implantations and litter size were similar to those observed in a prior 2 generation study. Significant quantities of PFOS appear to be secreted in milk. The quantities secreted in milk can plausibly account for the additive mortality in pups exposed *in utero* and through lactation compared to mortality levels in pups solely exposed *in utero*. Sera levels in pups exposed to PFOS during gestation and lactation are equivalent to maternal values at time of weaning

Comment on author's conclusions and whether you agree: Agree

REFERENCE

Christian M, Hoberman AM & York RG. Argus Research Laboratories, July 23, 1999. Oral (Gavage) Cross-Fostering Study of PFOS in Rats. Protocol 418-014 Sponsor Study T-6295.13 CD 00001108.pdf

Hansen KJ, Perkins JR. 3M Environmental Laboratory, June 28, 1999. Analytical Laboratory Report on Determination of the Presence of PFOS in the Serum of Sprague-Dawley Rats. Lab Report Number U2779. Sponsor Study T-6295.13

Nold JM. Pathology Associates International, April 5, 1999.

ROBUST SUMMARY - RS49

Title: Ninety day subacute rat toxicity study

TEST SUBSTANCE

Identity: N-EtFOSE alcohol

Remarks: Lot FM-03422 41d-2700-3422-0 Lot 784/ Net-35 DR-1

METHOD

Method/guideline: Not listed

Study duration: 90 days

GLP: No, prior to start of GLP

Year study performed: 1978

Species/Strain: Rat; Charles River CD

Route of administration: Oral, diet

Doses tested and frequency: 100, 300, 1,000, 3,000 and 10,000 ppm in initial (11/4/1977) trial, an addition 30 ppm group initiated 11/25/1977 in accordance with an approved protocol modification. Treated feed available ad lib.

Sex: Male and female

Control group and treatment: Purina Laboratory Chow (ground basal diet)

Post exposure observation period: none

Statistical methods: Analysis of variance (1-way classification), Bartlett=s test for homogeneity of variances, and the Aappropriate@ t-test (for equal or unequal variances) as described by Steel and Torrie, using Dunnett=s multiple comparison tables to judge significance of differences.

Remarks:

Test Subjects:

Age at study initiation: not specified

Weight at study initiation: 240-306 g (male); 180-226 g (female)

No. of animals per sex per dose: 5

Study Design:

Vehicle: Purina lab chow

Satellite groups and reasons they were added: none

Clinical observations performed and frequency: Animals observed twice daily for duration of trial. Body weight, food consumption, gross behavior, eyes, nose, mouth.

Organs examined at necropsy: Adrenals, aorta, bone, brain (with segment of cervical cord) eyes, heart (with coronary vessels). Duodenum, ileum, jejunum, colon, kidneys, liver, lung, thymus, mammary gland, nerve (with muscle) spleen pancreas, prostate/uterus, bone marrow

(sternum) salivary gland, spinal cord (lumbar) pituitary, stomach, testes/ovaries, thyroid, parathyroid, urinary bladder, mesenteric lymph node, and any other tissues with abnormalities.

RESULTS

NOAEL (NOEL) 30 ppm NOAEL/LOEL (because of lack of statistical significance and addition of 30 ppm group *post hoc*)

LOAEL (LOEL) 100 ppm LOAEL

Remarks: *Body weight:*

Body Weight Changes at Conclusion of 90-day Study		
Dose (ppm)	Group mean body weight (g) (% difference from Control)	
	Male	Female
Control	501	286
30	491 (-2.0)	269 (-5.9)
100	482 (-3.8)	248 (-13.3)*
300	392 (-21.8)**	227 (-20.6)**
All animals in 1,000, 3,000, and 10,000 ppm died prior to scheduled sacrifice. * p<0.05; ** p<0.01		

Food/water consumption:

Food Consumption		
Dose (ppm)	Average Food Consumption (g/rat/day)	
	Male	Female
Control	27.6	19.9
30	27.2	19.3
100	27.4	18.6
300	24.2*	15.1*
*Statistically significant relative to control at p <0.01.		

Clinical signs (description, severity, time of onset and duration):

Clinical Signs During 90 Day Study	
Observation	Number of Rats/Observation/Dosage Level

Dose (ppm)	0	30	100	300	1,000	3,000	10,000
Emaciation					6	10	10
Altered posture					4	8	10
Reduced motor activity					1	1	7
Convulsions					3	5	
Increased sensitivity					2		
Red material, eyes	1	1		3		1	
Red material, nose, mouth				1	2	2	1
Yellow material					2		2
Pupillary dilation			1				
Excessive salivation		1					
Death usually followed manifestation of symptoms.							

Haematology: For rats at the 30 and 100 ppm dosage at 1 and 3 months of study, and for rats treated at 300 ppm at 1 month, all hematological levels were within the expected range. 3-month erythrocyte counts, hemoglobin and hematocrit values for male and female rats at 300 pp were statistically significantly lower relative to controls. All other hematologic values were not significantly different from controls at the conclusion of the study.

Biochemistry: Statistically significant responses (see table): Glucose elevated in female rats at 300 ppm; BUN elevated in males at 300 ppm; Alkaline phosphatase elevated in males and females at 300 ppm; PGOT decreased in males at 100 ppm, but not at 300 ppm. PGPT elevated in males at 300 ppm. No compound related changes noted in urinalysis

Summary of Biochemistry Results from 90 Day Study									
Parameter	Study Month	0 ppm		30 ppm		100 ppm		300ppm	
		%	&	%	&	%	&	%	&
Glucose mg/100ml	Pretest	74	89	62	67	82	89	76	97
	1	100	118	101	109	107	109	98	108
	3	126	128	123	132	120	115	119	108*
BUN mg/100ml	Pretest	14.2	16.9	14.0	12.9	12.7	15.5	12.6	13.3
	1	14.6	15.6	21.6	16.7	14.5	16.9	19.3	28.9
	3	16.3	18.2	15.2	17.2	18.8	20.6	22.7**	21.2

Summary of Biochemistry Results from 90 Day Study									
Parameter	Study Month	0 ppm		30 ppm		100 ppm		300ppm	
		%	&	%	&	%	&	%	&
γ GTP sigma units/ml	Pretest	1	1	2	1	6	1	1	1
	1	1	1	5	2**	1	0	7	1
	3	0	1	6*	1	0	1	0	2
Creatine phosphokinase sigma units/ml	Pretest	25	6	16	13	5	6	10	5
	1	20	8	11	15	9	10	13	13
	3	9	7	5*	10	10	6	9	9
Alkaline phosphatase IU/L	Pretest	na	+	216	140	na	+	na	+
	1	172	91	170	116	179	83	128	249**
	3	126	69	112	82	148	68	214**	148
PGOT IU/L	Pretest	113	114	107	88	116	115	112	106
	1	109	91	83	121	101	79	98	196
	3	104	101	140	98	101	85*	93	115
PGPT IU/L	Pretest	113	103	74	68	95	97	95	95
	1	50	41	42	49	41	39	61	160
	3	3	28	66	32	40	33	116*	83
Calcium meq/L	Pretest	10.2	9.8	7.5	7.7	10.7	9.7	9.7	9.6
	1	9.0	8.9	9.7	10.0	8.6	8.7	8.4	9.0
	3	11.4	11.3	10.9	11.2	11.0	11.2	11.2	11.4
na = not available γ GTP = gamma glutamyl transpeptidase PGPT = plasma glutamic pyruvic transaminase PGOT = plasma glutamic oxalacetic transaminase Statistical analyses not conducted on the pretest values. * Significantly different from Control group mean p<0.05. **Significantly different from Control group mean p<0.01. + not measured due to anticoagulant interference.									

Ophthalmologic findings: (see clinical observation table)

Mortality and time to death: (see table below. Time to death not reported).

Survival at 90 days		
Dose (ppm)	Survivors/Initiated	
	Male	Female
0	5/5	5/5

30	5/5	5/5
100	5/5	5/5
300	5/5	5/5
1,000	0/5	0/5
3,000	0/5	0/5
10,000	0/5	0/5

Gross pathology incidence and severity:

Liver Lesions: All rats treated at 300 ppm exhibited compound-related gross liver lesions, occurring singly or in combination. Changes included liver enlargement, accentuated lobulations, diffuse brown discoloration and gray/yellow/white areas of discoloration. Males appears to be more severely affected than females. Similar but less severe liver lesion observed in animals from the 100, 1,000, and 3,000 ppm groups.

Stomach: Compound related gross lesions including hyperemia/congestion, red/brown foci/hemorrhage in the some animals receiving 1,000, 3,000, or 10,000 ppm.

Organ weight changes:

Liver: Statistically significant increase in absolute and relative liver weights in 100 and 300 ppm groups, with males more severely affected than females.

Other organs: statistically significant variations observed at 100 and 300 ppm, without morphological changes (see table).

Statistically Significant Organ Weight Changes					
Organ	Dosage (ppm)	Sex	Weight	Change	p <
Kidney	100	M	relative	increase	0.05
Brain	300	M	relative	increase	0.05
		F	relative	increase	0.05
Thyroid/Parathyroid	300	M	relative	increase	0.01
Pituitary	300	M	relative	increase	0.05

Histopathology:

Liver: Microscopic alteration observed in livers from all treated groups consisting of very slight to marked centrilobular to pan-lobular hepatocytic hypertrophy in 30, 100, 300, and 1,000 ppm groups. Hepatocytic hyperplasia observed in the 3,000 and 10,000 ppm groups. Very slight to moderate increase in lipid-containing cytoplasmic vacuoles at 100 and 300 ppm. Focal to multifocal coagulative centrilobular to midzonal hepatocellular necrosis of very slight to marked severity observed in 300, 1,000, 3,000 and 10,000 groups. Minimal amount of associated inflammatory infiltrate observed. Increased intracytoplasmic acculation of brown pigments in hepatocytes and Kupffer cells observed primarily at 300 ppm. Kupffer cells stained positive for iron; hepatocytes did not.

Kidneys: Most animals in the 300 ppm group exhibited very slight to marked tubular nephrosis with associated proteinaceous cast formation, and intracellular accumulation of brown pigment (staining positive for iron) and reddish-brown hyaline droplets in tubular epithelial cells. Mineralization of luminal tubular debris observed in some rats.

Other tissues: Lesions in other tissues were not considered compound-related.

CONCLUSIONS

30 ppm Slightly depressed weight gain compared to controls. Significant increase in γ -GTP in males and females, but no dose-dependence.

100 ppm Reduction in body weight for both sexes; depressed food consumption in females, one female rat demonstrated behavioral change. Statistically significant liver weight increase.

300 ppm 2 premature deaths in females following blood collection. Group mean body weights and food consumption were significantly lower. Hematologic values (erythrocyte count, hemoglobin and hematocrit were slightly but statistically significantly lower than controls. Changes observed in biochemical values (e.g., alkaline phosphatase, PGPT, PGOT, γ -GTP and BUN). Gross liver lesions observed. Statistically significant liver weight increase; changes in kidneys observed.

Remarks: Although old, study appears to have been well conducted and results well reported. It clearly would have benefited from having a range-finding study. Some concern about *post hoc* addition of 30 ppm group, and the lack of an additional control group entering at the same time, which makes it difficult to rely wholeheartedly on the effects noted at 30 ppm. Given that the dose spacing to the next exposure level was 3-fold and not 10-fold, it is not inappropriate to

consider 30 ppm a NOEL, and 100 ppm a LOAEL.

REFERENCE

International Research and Development Corporation. 1978. Ninety day subacute rat toxicity study on FM-3422. IRDC Study No. 137-086. Final Report, November 10, 1978.

ROBUST SUMMARY - RS50

Title: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TEST SUBSTANCE

Identity: N-EtFOSE alcohol

Remarks: FM-3422 41-2700-3422-) Lot 784/Net-35 DR-1

METHOD

Method/guideline: None specified

Test type: 90 day gavage

GLP: None specified

Year: Study performed in 1978; final report 1979

Species: Monkey

Strain: Rhesus

Route of administration: oral gavage

Doses/concentration levels: 1, 3, 10, 30 mg/kg/day

Sex: Male and female

Exposure period: 90 days

Frequency of treatment: daily

Control group and treatment: propylene glycol

Post exposure observation period: none

Duration of test: 90 days

Statistical methods: Analysis of variance (1-way classification), Bartlett=s test for homogeneity of variances, and the Appropriate@ t-test (for equal or unequal variances) as described by Steel and Torrie, using Dunnett=s multiple comparison tables to judge significance of differences.

Remarks: On days 1 and 2 of study, test material was dissolved in 5 ml propylene glycol; on all days thereafter, 2 ml was used. Controls mirrored treatment groups.

Test Subjects:

Age at study initiation: Not specified

Weight at study initiation: males: 3.0-4.35 kg; females 3.05-4.20 kg

No. of animals per sex per dose: 2

Study Design:

Vehicle: propylene glycol

Satellite groups and reasons they were added: none

Clinical observations performed and frequency: twice daily for general physical appearance, behavior and pharmacotoxic signs. Individual body weights recorded weekly; general physical

examinations monthly during study. Hematological, biochemical and urinalysis were conducted once prior to the beginning of the experiment, and at 1 and 3 months of study.

Organs examined at necropsy: heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries, brain, eyes, thyroid/parathyroid.

RESULTS

NOAEL (NOEL): 10 mg/kg/day

LOAEL (LOEL): 30 mg/kg/day

Remarks:

Body weight: During the first month, all monkeys including controls lost weight. At 12 weeks of study all the monkeys were at or near the original body weight except for the groups receiving 10 and 30 mg/kg/day. Although one monkey showed a weight loss, no statistically significant weight loss was observed for any group at the conclusion of the experiment.

Food/water consumption: Food consumption was estimated.

Clinical signs (description, severity, time of onset and duration): On and after the second day the study, all the monkeys, including controls, showed slight to marked decrease in activity, ataxia, and anorexia. The ataxia disappeared from all the monkeys after the amount of propylene glycol was reduced from 5 to 2 ml/kg on study day 3. Several weeks into the study, stool changes were observed in the monkeys, with black stool, mucus and bloody mucus in the stool observed in most of the monkeys from each group. Two of the monkeys receiving 30 mg/kg/day had these symptoms persist for most of the treatment period.

Haematology: No unusual changes observed for blood. One monkey receiving 10 mg/kg/day exhibited a slight decrease in hematocrit, hemoglobin and erythrocyte counts at 1 month of study that was not observed at 3 months.

Biochemistry: *Glucose:* Most of the monkeys exhibited blood glucose values in the lower range of expected values.

Cholesterol: 1 monkey receiving 10 mg/kg/day (showed a low cholesterol level (84 mg/100ml); 2 monkeys receiving 30 ppm showed low cholesterol values (56 and 67 mg/100ml, also with low potassium (3.5 meq/L)).

Ophthalmologic findings: None reported.

Mortality and time to death: One monkey receiving 30 mg/kg/day died during the last week of the study. Death was preceded by a marked loss of body weight (1.25 kg) with ataxia and a marked decrease in activity. All other monkeys survived until sacrifice.

Gross pathology incidence and severity: No gross lesions considered compound-related were observed at the end of the study.

Organ weight changes: Statistically significant variations in sex group mean weights of liver and brain in animals treated at 10 and 30 mg/kg/day. No morphological changes were observed in these organs relative to control.

Organ Weight Changes

Organ	Dose (mg/kg/day)	Sex	Weight	Change	p <
liver	10	M	relative	increase	0.05
	30	M	absolute and relative	increase, increase	0.05, 0.01
Brain	10	M	Absolute	decrease	0.01
	30	M	Absolute	decrease	0.05

Histopathology:

1, 3, or 10 mg/kg/day: No changes were observed in tissues.

30 mg/kg/day: One male and two females exhibited slight to severe lipid depletion in adrenal glands. Two females exhibited moderate atrophy of the pancreatic exocrine cells, with decrease cell size and loss of zymogen granules. One pancreas also had several eosinophilic intranuclear inclusions in the exocrine cells. No changes in tissues other than the adrenal glands and pancreas were considered compound-related.

CONCLUSIONS Clear NOEL and LOEL can be derived from this study.

Remarks: Old, but generally well-conducted study. Some concerns about effect of vehicle, due to GI symptoms in all animals, although there does appear to be a compound-related exacerbation of symptoms.

REFERENCE

International Research and Development Corporation. 1979. Ninety Day Subacute Rhesus Monkey Toxicity Study, on FM-3422. IRDC Study No. 137-088. Final Report, January 16, 1979.

ROBUST SUMMARY - RS51

Title: 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl-Perfluorooctanesulfonamido Ethanol in Rats. SUMMARY REPORT - WEEK 53

TEST SUBSTANCE

Identity: N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE alcohol)

Remarks: Test substance is Anarrow range, consisting of 98.1% N-EtFOSE plus other related compounds

METHOD

Method/guideline: None

Test type: 104 week oral toxicity study (Week 53 Interim Report)

GLP: Yes

Year: Not specified (Study start date: January 26, 1998)

Species/Strain: Rat; Crl:CD⁷(SD)IGS BR

Route of administration: Oral (in chow)

Doses/concentration levels: 0, 3, 30,100, 300 ppm N-EtFOSE alcohol (Additional doses of 0 and 1 ppm N-EtFOSE alcohol added at a later date)

Sex: Male & Female

Exposure period: 104 weeks (53 week interim)

Frequency of treatment: ad libitum

Control group and treatment: Concurrent vehicle

Post exposure observation period: At least 52 weeks following 52 weeks of exposure

Duration of test: 104 weeks (53 week interim)

Statistical methods: Not specified in Week 53 Summary Report

Remarks:

Test Subjects: Age at study initiation: 6-8 weeks

Weight at study initiation: 100 -300 g

No. of animals per sex per dose: 70 per sex in control group, 60 per sex in 3 ppm dose group, 60 per sex in 30 ppm dose group, 70 per sex in 100 ppm dose group, 70 per sex in 300 ppm dose group, 40 per sex in 100 ppm recovery group, 40 per sex in 300 ppm recovery group, 70 per sex in 1 ppm dose group, 70 per sex in additional controls for the 1 ppm dose group.

Study Design:

Vehicle: Chow

Satellite groups and reasons they were added: Not specified in Week 53 Summary Report

Clinical observations performed and frequency: Observed for morbidity/moribundity twice daily (am and pm). Each animal was removed from cage and examined once prior to treatment

and once weekly thereafter. Food consumption data were collected for Weeks 1 through 16 and once every 4 weeks thereafter. Body weights were determined weekly for Weeks 1 through 17 and once every 4 weeks thereafter. Blood and urine samples for hematology, clinical chemistry, urine chemistry, and urinalysis were collected from 5 animals per sex in the control and high dose groups during Week 8 and from 10 animals per sex in the 1 ppm dose and second control group during Weeks 14, 27, and 53. Blood and urine samples for hematology, clinical chemistry, urine chemistry, urinalysis, and serum sampling were collected from 10 animals per sex in the control, 3, 30, and 100 ppm dose groups during Weeks 14, 27, and 53. Blood samples for serum analyses were collected from 5 animals per sex in all but the recovery groups during Week 4; from 5 animals per sex in the control, 3, 30, 100, and 300 ppm dose groups during Week 8; from 5 animals per sex in all but the high dose and recovery groups during Week 14; and from 5 animals per sex in the both control groups, the 1, and the 100 ppm dose groups during Week 53.

Termination schedule: 5 animals per sex were necropsied for the following groups at the following time intervals: Week 4: All groups but the recovery groups; Week 8: Control and 300 ppm dose groups; Week 14: Both control groups, 1, 3, 30, and 100 ppm dose groups.

During Week 53, five animals per sex from the initial control group and 10 animals per sex from the additional control, the 1, and the 100 ppm dose groups were necropsied.

NOTE: During Week 8, the 300 ppm dose groups were removed from the study because of toxic effects seen for body weight, food consumption, and clinical pathology and the remaining high dose non-recovery animals were discarded without necropsy. The 1 ppm dose group and its concurrent control group were subsequently added.

Organs examined at necropsy:

Organ weight: At Week 4, only the liver was evaluated. Week 8: Adrenal, brain, kidneys, liver, lung, ovary, spleen, testes, thyroid/parathyroid, and ovaries. Week 14: For control, 3, 30, and 100 ppm dose groups, adrenals, brain, kidneys, liver, lung, ovary, spleen, testes, thyroid with parathyroid, and ovaries were examined. For the additional control and 1 ppm dose groups, only the liver was evaluated. Week 53: Adrenal, brain, kidneys, liver, lung, ovary, spleen, testes, thyroid/parathyroid, and ovaries.

Microscopic examination: At Week 4, only the liver was evaluated. Week 8: Eye, brain, spinal cord, kidney, liver, thymus, lymph nodes (mesenteric, mandibular, mediastinal), spleen, pituitary, adrenal, stomach, duodenum, jejunum, pancreas, epididymides, testes, ovaries, vagina, uterus. Week 14: For control, 3, 30, and 100 ppm dose groups, eye, brain, kidney, liver, mesenteric lymph nodes, spleen, adrenal, pancreas, testes, ovaries, vagina, uterus. For additional control and 1 ppm dose groups, liver was examined. Week 53: Eye, brain, spinal cord, kidney, liver, lung, heart, femer bone with marrow, sternum bone with marrow, skeletal muscle, sciatic nerve, thyroid with parathyroid, trachea, esophagus, salivary glands, harderian gland, thymus, lymph nodes (mesenteric, madibular, mediastinal, lumbar/iliac), spleen, pituitary, adrenal, cecum, stomach, colon, rectum, duodenum, jejunum, ileum, pancreas, bladder, skin, mammary gland, prostate, seminal vesicles, epididymides, testes, ovaries, cervix, vagina, uterus, tail.

Collected organs were also examined macroscopically. Unscheduled deaths were necropsied, but organ weights were not recorded and liver samples were not collected.

Additional Endpoints:

Proliferation Cell Nuclear Antigen (PCNA) evaluation

Mitochondrial analyses: Liver samples collected from animals sacrificed during Week 4 from the control, 3, 30, 100, and 300 ppm dose groups.

Palmitoyl-CoA oxidase analyses

RESULTS

NOAEL (NOEL): Week 53 Interim Report: 3 ppm

LOAEL (LOEL): Week 53 Interim Report: 30 ppm (decreased cholesterol levels).

Remarks: *Body weight:* After the first week of treatment, animals given doses of 100 or 300 ppm had significantly lower body weights compared to controls. Animals in the 30 ppm dose group had lower body weights compared to controls, but the difference was not generally statistically significant until approximately Week 8. Body weights of animals in the 1 or 3 ppm dose groups were not significantly different from controls. Body weight gain was significantly lower for animals in the 300 ppm dose groups when compared to controls beginning in the first week of treatment. Animals in the 100 ppm dose groups frequently had significantly lower body weight gain; for the 30 ppm dose group, changes were sporadically significant. Overall change in body weight (Week 1 to Week 53) was significantly lower than controls for the 100 and the 300 ppm dose groups. Body weight gain of animals in the 1 or 3 ppm dose groups was not significantly different from controls.

Food/water consumption: Animals in the 100 and 300 ppm dose groups had significantly lower food consumption compared to controls starting in the first week of treatment. Starting in Week 3, males in the 30 ppm dose group had a number of significantly lower food consumption intervals when compared to control animals. This effect was not observed for the females in the same dose group, nor for the animals in the 1 and 3 ppm dose groups.

Test Material Consumption: The following table summarizes the amount of test material consumed by animals on a mg/kg/day basis:

Test Material Consumption		
Target Dose Levels	Achieved Dose Levels - Range (mg/kg/day)	
	Males	Females
1 ppm	0.036 - 0.103	0.049 - 0.108
3 ppm	0.108 - 0.259	0.147 - 0.307
30 ppm	1.126 - 2.618	1.454 - 0.307
100 ppm	3.847 - 8.724	5.686 - 10.166
300 ppm	16.058 - 25.408	19.568 - 28.712
100 ppm recovery	3.841 - 8.873	5.355 - 10.135
300 ppm recovery	15.803 - 25.193	20.004 - 29.270

Clinical signs (description, severity, time of onset and duration): There were no apparent test material-related observation noted through Week 53.

Hematology: At a dose level of 300 ppm for approximately 8 weeks, administration of N-EtFOSE alcohol was associated with hematological effects, including lower red blood cell count, hemoglobin, and hematocrit. Most of the effects were moderate to marked in magnitude.

Dietary administration of N-EtFOSE alcohol was associated with mildly decreased red blood cell count, hemoglobin, and hematocrit for females in the 100 ppm dose groups. No effects were seen for the 1 and 3 ppm dose groups.

Clinical Chemistry: At a dose level of 300 ppm for approximately 8 weeks, administration of N-EtFOSE was associated with many effects on clinical chemistry results, including lower glucose, globulin, and cholesterol, and higher urea nitrogen, albumin, total bilirubin (males only), aspartate aminotransferase (males only), and alanine aminotransferase. Most of the effects were moderate to marked in magnitude.

Mildly lower glucose and mildly higher urea nitrogen was observed for animals given 100 ppm (predominantly at Weeks 14 and 27). Males given 100 ppm were observed to have moderately higher albumin and moderately lower globulin (predominantly at Weeks 27 and 53). Males given 30 or 100 ppm and females given 100 ppm had moderately to markedly lower cholesterol. No effects were seen for the 1 and 3 ppm dose groups.

Ophthalmologic findings: Not performed

Mortality and time to death: Two males and 2 females given 1 ppm, 2 males given 3 ppm, one male and one female given 30 ppm, 6 males and one female given 100 ppm, 4 males given 300 ppm, and a total of 8 control animals died on test or were sacrificed moribund. One of the males given 3 ppm was sacrificed due to a fracture of the hard palate. The cause of death was undetermined for one male given 100 ppm and one male given 300 ppm. Histopathology was not performed on animals from the 1 ppm dose groups; the macroscopic findings were typical of those observed in rats in long term studies. All of the other treated animals that died or were

sacrificed at unscheduled intervals had hepatocellular hypertrophy or hepatocellular necrosis due to treatment with the test material, or both. Of these animals, the 3 ppm dose group male was sacrificed because of a large subcutaneous undifferentiated sarcoma, one 300 ppm dose group male and one 30 ppm dose group female were sacrificed moribund with pituitary adenomas, hepatocellular necrosis was the cause of death for 2 males given 100 ppm and 3 males given 300 ppm, and one 100 ppm dose group male died with necrosis of the musculature of the anus.

The following table summarizes the survival data for the 53 week interim report:

Survival After 53 Weeks of Treatment		
Dose Group	Percent Survival	
	Males	Females
Control	98.0%	98.0%
3 ppm	96.0%	100%
30 ppm	98.0%	98.0%
100 ppm	96.0%	96.0%
100 ppm recovery	90.0%	100%
Additional Control	96.0%	92%
1 ppm	96.0%	96%

Gross pathology incidence and severity:

Week 4: No gross findings.

Week 8: Enlarged livers were found in all of the males and 2 of the females and mottling of the liver was observed in one female (300 ppm dose group). There were no other abnormal macroscopic findings.

Week 14: No gross findings.

Week 53: No gross findings.

Organ weight changes:

Week 4: Absolute and relative liver weights were higher for animals in the 100 and 300 ppm dose groups.

Week 8: Absolute and relative liver weights were significantly higher for animals in 300 ppm dose group (only treatment group examined). There were no other test material-related changes for absolute or relative organ weights.

Week 14: Absolute and relative liver weights were significantly higher for animals from the 100 ppm dose group, but not for animals from the 1, 3, or 30 ppm dose group. There were no other test material-related changes for absolute or relative organ weights.

Week 53: Absolute and relative liver weights were significantly higher for animals from the 100 ppm dose group, but not for animals from the 1 ppm dose groups. There were no other test

material-related changes for absolute or relative organ weights.

Histopathology:

Week 8: Hepatocellular hypertrophy, hepatocellular necrosis, and hemorrhage were observed for the livers of animals from the 300 ppm dose group. Other findings unremarkable.

Week 14: Hepatocellular hypertrophy was observed for males given 30 or 100 ppm and females given 100 ppm. Other findings unremarkable.

Week 53: Hepatocellular hypertrophy and vacuolation were observed for animals from the 100 ppm dose group. Hepatocellular necrosis was observed for one male and 2 females from the 100 ppm dose groups and one male and 2 females from the 100 ppm dose group exhibited bile stasis.

One 100 ppm dose group female had a hepatocellular adenoma, which are occasionally observed as an incidental finding in 1 year old rats. Other findings unremarkable.

Additional Endpoints:

Proliferation Cell Nuclear Antigen (PCNA) evaluation: Results not provided in Week 53 Summary Report (analysis performed by Sponsor)

Mitochondrial analyses: Results not provided in Week 53 Summary Report (analysis performed by Sponsor)

Palmitoyl-CoA oxidase analyses: At the Week 4 and Week 8 sacrifices, administration of N-EtFOSE alcohol was associated with mildly, but statistically significant higher hepatic palmitoyl-CoA oxidase activity in the animals given 300 ppm.

CONCLUSIONS

Chronic dietary exposure to N-EtFOSE alcohol was associated with decreased levels of cholesterol in rats at a dose level of 30 ppm and above. Hepatocellular hypertrophy and other hepatocellular effects were observed in animals from the 30 and 100 ppm dose groups. No treatment-related effects were observed at an exposure level of 3 ppm.

REFERENCE

Covance Laboratories Inc. No Date. 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl-Perfluorooctanesulfonamido Ethanol in Rats. SUMMARY REPORT - WEEK 53. Prepared for 3M. COVANCE STUDY NUMBERS: 6329-212 and -228.

ROBUST SUMMARY - RS52

Title:

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats (1999)

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, potassium salt (FC-95), CAS 2795-39-3

Remarks: Purity 98.9%, Lot # 217

METHOD

Method/guideline followed: GLP, FDA, EEC

Test type: in vivo

Species/strain/cell type or line: rat/Sprague-Dawley/pregnant Crl:CD(R)BR VAF/Plus(R)

Sex: Fo: female, F1: both

Age and body weight range of animals used: 65 days, 192-231 g

Number of animals/sex/dose: Fo: 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, once daily beginning 43 days prior to cohabitation until confirmed evidence of mating. Only the Fo females were dosed.

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

Fo urine, feces, serum, liver. F1 liver and serum.

Statistical methods used: averages and percentages

Method remarks: 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 (DGs 6 to 7, 14 to 15, and 20 to 21), and days of lactation (DLS) 21 to 22. Blood samples were collected from each of the maternal rats on the day cohabitation was initiated (prior to cohabitation), DGs 7, 15 and 21, and DLs 14 and 22. All surviving rats assigned to the study were sacrificed on DL 22. A liver section was collected from each dam.

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. On DL 21, all remaining pups were sacrificed. 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 Sponser for analysis.

On days 1 to 4 of the 43-day pre mating period, Fo female rats received 25% greater dose due to an incorrect calculated amount of test substance in vehicle.

RESULTS

Detailed results: PK results not final.

Metabolites measured: none

CONCLUSIONS

REFERENCE

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, Final Report. Argus Research Laboratories, Inc. Protocol 418-015, 3M Reference No. T-6295.14, Advanced Bioanalytical Services Study No. FACT-TOX-111, July 23, 1999.

Report Author: RG York et al.

OTHER

ROBUST SUMMARY - RS53

Title: Determination of Serum Half-Lives of Perfluorooctanesulfonate and Other Fluorochemicals

TEST SUBSTANCE

Identity: PFOS

Remarks:

METHOD

Study design: Collect and analyze serum every six months for up to 5 years (beginning November 1998) from 27 retired, fluorochemical production employees in order to determine the serum half-life of PFOS and other fluorochemicals

Manufacturing/Processing/Use: N/A

Hypothesis tested: Determine serum half-life of PFOS and other fluorochemicals.

Study period: 1998-2003

Setting: N/A

Total population: 27 retirees from 3M Decatur (n = 24) and Cottage Grove (n = 3) manufacturing sites.

Subject selection criteria: Not provided in report.

Total # of subjects in study: N = 24

Comparison population: N/A

Participation rate: 100 percent, to date

Subject description: 25 male and 2 female retirees. As of beginning of study, average age of retiree was 60 years (range 55 - 74 years). Mean number of months from retirement to the start of the study was 30 months (range 5 - 130 months).

Health effects studied: None

Data collection methods: Blood sera samples

Details on data collection: Beginning in November, 1998 blood is collected every six months. Three collections have occurred, to date, according to the 1st interim report. No information was provided as to how the blood was drawn, stored, analyzed, etc.

Exposure period: Unknown.

Description/delineation of exposure groups/categories: Retirees with prior occupational exposure to fluorochemicals

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Serum half-life calculated based on three data points (approximately six month intervals) assuming a one compartmental model.

Other methodological information:

RESULTS

Describe results: Initial serum PFOS level ranged from 0.1 - 3.1 ppm. Median serum half life of PFOS was 344 days for 20 male and female retirees whose log linear regression model had an $r^2 > 0.6$. Neither age nor the number of months retired was associated with the serum half-life calculation for PFOS.

Study strengths and weaknesses: This is the first interim report of this serum PFOS half-life study. Interim reports will be issued every six months (after each new data collection). Results should be interpreted cautiously due to the paucity of data available to date. Seven of the 27 subjects were not reported; some because they did not yet have their third data point and others because of the lack of fit of the linear model.

Research sponsors: 3M Medical Department

Consistency of results: N/A

CONCLUSIONS

N/A

REFERENCE

Interim Report #1. Determination of Serum Half-Lives of Several Fluorochemicals. 3M Company: St. Paul, June 8, 2000.

OTHER

ROBUST SUMMARY - RS54

Title: 28-Day Percutaneous Absorption Study with FC-95 in Albino Rabbits (1981)

TEST SUBSTANCE

Identity: Potassium perfluorooctanoic acid, CAS 2795-39-3

Remarks: FC-95

METHOD

Method/guideline followed: NA

Test type: in vivo

Species/strain/cell type or line: rabbit, New Zealand White

Sex: male and female

Age and body weight range of animals used: 1.82 – 2.37 kg

Number of animals/sex/dose: 2 male and 2 female (range-finding); 10 male and 10 female (definitive)

Route of administration: dermal (approximately 40% of body surface area)

Vehicle: None specified

Doses: Single doses of 1,000 and 5,000 mg/kg (range-finding); Single doses of 5,000 mg/kg (definitive)

Excretion routes, body fluids, and tissues monitored and/or sampled during study: blood for serum taken from retro-orbital sinus prior to dosing and on days 1, 7, 14 and 28 after dosing.

Statistical methods used: Not specified

Method remarks: The trunk of each rabbit was clipped free of hair and the test article placed on the intact skin to cover approximately 40% of the skin surface area. Impervious plastic sheeting was used to occlude the test article. A flexible plastic collar was used to minimize the possibility that animals may disturb the application. After 24 hours of contact, the test material was removed from the skin. Animals were observed for pharmacotoxic signs immediately after administration, at one and two hours post-dose, after removal of the test article and daily thereafter until termination (14 days for the range-finding study and 28 days for the definitive study). In the definitive study, blood was obtained from the retro-orbital sinus for serum analysis prior to application and on days 1, 7, 14 and 28 post-dosing. Serum was frozen for analysis. In the definitive study, body weights were recorded initially and on days 7, 14 and 28. Gross

necropsy was performed on day 28.

RESULTS

Detailed results: No deaths were observed at any dose in both the range-finding study and the definitive study. Hyperactivity was noted among 5 of 10 males on days 6 and 7 post-dosing. No visible lesions were noted at necropsy. One male had weight loss noted at the end of the study. Only serum from the day 1 and day 28 blood draws from one male and one female rabbit were analyzed. Female day 1 and day 28 serum total fluorine levels were 0.9 and 128.0 ppm, respectively. Male day 1 and day 28 serum total fluorine levels were 10.3 and 130.2 ppm, respectively.

Metabolites measured: Total fluorine (assumed to represent perfluorooctane sulfonate)

CONCLUSIONS

The dermal LD₅₀ is greater than 5,000 mg/kg.

No definitive conclusions can be drawn from the pharmacokinetic phase of this study other than the fact that absorption appears to occur.

REFERENCE

O'Malley, K. D. and Ebbens, K. L. (1981) 28 Day Percutaneous Absorption Study with FC-95 in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Experiment No. 0979AB0632.

OTHER

This was not a GLP study; however, it was audited by the QAU function. The study is adequate to establish the lack of significant dermal toxicity from single application of a large dose. The study is not adequate in providing useful information on the dermal absorption of PFOS.

ROBUST SUMMARY - RS55

Title: Oral Teratology Study of T-2999CoC in Rabbits

TEST SUBSTANCE

Identity: Lot FM 3924.

Remarks: Identified as "wide-range" N- Ethyl FOSE alcohol in an internal 3M memo from DR Ricker to WC McCormick dated December 10, 1980. Wide range was given to be:

88% C₈F₁₇SO₂N(CH₂CH₃)CH₂CH₂OH
10% C₂, C₄ and C₆-F_{2n+1} SO₂N(CH₂CH₃)CH₂CH₂OH,
1% N-Ethyl amide and
1% high boiler.

METHOD

Method/Guideline followed: Complied with general recommendation of the US FDA issued in January, 1966 . "Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Usage."

GLP: Yes. Stated to be conducted in compliance with the FDA 1978 Good Laboratory Practice Regulations.

Year study performed: 1981

Species/Strain: Rabbit, New Zealand White/Miniken from Dutchland Laboratories.

Number of animals per dose: Rangefinder study, six. Principal study, eighteen

Route of administration: Oral (intubation)

Dosing regimen (list all with units): Does were dosed at a constant volume of 1 ml/kg, once daily, on gestation days (GD) 6-18 (day 0). The vehicle was corn oil.

Doses: Rangefinder: 0, 6, 9, 12, 15, 25, 50, 75, or 100 mg/kg bodyweight.

Principal study: 0, 1.5, 5, 15 mg/kg bodyweight.

Statistical methods used: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corporea lutea. Chi square for percent abnormalities.

RESULTS

NOAEL:

Maternal 5 mg/kg.

Developmental 5.0 mg/kg.

LOAEL (dose and effect) – maternal and developmental:

Maternal. 15 mg/kg – reduced mean body weight gain commencing with dosing through termination of the study.

Developmental 15 mg/kg – abortions, total resorptions, poor survival during 24 hour incubation period.

Toxic response/effects by dose level - maternal:

Rangefinder. Doses of 100, 75, 50, or 25 mg/kg resulted in compound and dose related deaths and signs of abortion. Over 50% of females died at the 2 highest dose levels, deaths also occurred at the lower levels. All dams either aborted or were in the process of absorbing their litters when the study was terminated at GD 22. There was also a mean body weight loss at all doses. In a 2nd rangefinder study there were no compound related deaths and a majority of the conceptuses at the high dose (15 mg/kg) were maintained during pregnancy.

Principal study. The 15 mg/kg dose had a reduced mean weight gain compared to control throughout the dosing period until termination of the study. The reduction was statistically different during the GD 6-9 period. There were no compound related maternal effects seen at the 5 or 1.5 mg/kg dose.

Toxic response/effects by dose level - developmental. Embryotoxicity was observed at 15 mg/kg as evidenced by statistically significant increases in resorption and decreased viable fetuses. The number of implantation sites, corpora lutea and fetal weight was not affected. The fetuses experienced poor survival during the 24 hour incubation period, 14/28 vs 16/81 in control ($p < 0.05$). No gross, internal, or skeletal malformations were considered to be compound related.

There were no compound related fetal effects seen at the 5 or 1.5 mg/kg dose.

Statistical results: Statistically significant results discussed above.

Remarks – Additional information to adequately assess the data: None.

CONCLUSIONS

Comment on author's conclusions and whether you agree: Agree with the study author's conclusions.

REFERENCES

Report: Oral Teratology Study of T-2999CoC in Rabbits, January 7, 1982. Riker Laboratories, St. Paul MN. Experiment Number 0681TB0212.

Report: Oral Rangefinder Study of T-2999CoC in Pregnant Rabbits, June 25, 1981. Riker Laboratories, St. Paul MN. Experiment number 0680RB0019

ROBUST SUMMARY - RS56

Title: Absorption and biotransformation of N-EtFOSE and tissue distribution and elimination of carbon-14 after administration of N-EtFOSE-¹⁴C in feed (1983)

TEST SUBSTANCE

Identity:

2-N-ethyl perfluorooctanesulfonamido ethanol labelled with carbon-14 at the carbon alpha to the sulfur atom (Riker Isotope Inventory Number 468, 0.483 ± 0.020 μ Ci/mg)

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 277 g (range 220-329)

Number of animals/sex/dose: 3 per each time-point experimental group (only one dose used) for time points 1,2,4,8,16 and 32 days post-dose.

Route of administration: dietary, mixed in feed, given to fasted rats for two hours (time-point groups for 1,2, and 4 days) or 12 hours (time-point groups for 8,16 and 32 days)

Vehicle: feed only

Doses: 0.531 mg/g feed (531 ppm in diet) given as a single "dose" to fasted rats with a mean dose of 10.13 mg/kg as calculated from the weight of feed consumed.

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

Urine and feces (continuous 24-hour collections for time-points 1,2,4 and 8 days and pooled collections for each animal in time-point groups for 16 and 32 days). Tissues and fluids taken at terminal sacrifice of each group were blood/plasma, liver, kidney, lung, spleen, bone marrow, sub-cutaneous and abdominal fat, and muscle (all animals), as well as digestive tract (esophagus, stomach and intestines) and remaining carcass (time-point groups for 1 and 2 days post-dose).

Statistical methods used: mean, standard deviation

Method remarks:

Rats were fasted with free access to water for 24 hours prior to dosing. Groups of three rats for each time point were assembled such that their individual body weights did not differ by more than 21 grams. The diet/test compound mixture was provided for two hours to animals in the groups for 1, 2 and 4 days. All feed was consumed in this period. For animals in time-point groups 8, 16 and 32 days, feeding was allowed for 12 hours; however, most of the feed was immediately consumed. Dose was verified by analysis of the feed/test compound mixture and by weighing the amount of feed consumed. The authors note that very little feed was spilled.

RESULTS

Detailed results:

The authors conclude that at least 70 % of the dose administered in feed was absorbed. Elimination in urine was concluded to be poor, with less than 3.0 % of the dose being eliminated in urine by 32 days post-dose. Fecal elimination was 20-30 times more extensive than urinary elimination, with approximately 60 % of the dose being eliminated in 32 days. Total recovery of radioactivity over 48 hours was 86 %. A mean of 9.5 % of the dose was in the liver after 32 days. After 32 days, the mean liver/plasma, spleen/plasma and bone marrow/plasma ratios were 11.8, 0.4 and 0.4, respectively. Liver to plasma ratios increased rapidly to plateau after 16 days. The serum elimination half-life was found to be 7.5 days over the first 16 days; however, there was practically no change in the serum concentration from day 16 to day 32 (2.2 µg equivalents versus 2.1 µg equivalents at day 32). Perfluorooctanesulfonate (PFOS) was identified as a metabolite in the liver extracts of rats sacrificed at 48 hours post-dose. PFOS represented at least 22 % of the radioactivity found in the liver at 48 hours. Perfluorooctanesulfonamide was tentatively identified as another metabolite in the 48-hour liver extracts. This metabolite, assumed to be perfluorooctanesulfonamide, represented at least 32 % of the radioactivity found in the liver at 48 hours post-dose. Other metabolites were present but not identified.

The distribution of carbon-14 in tissues over time is represented by the following table. Data are expressed as a percent of dose in tissue.

Table. Carbon-14 content of tissues after an oral dose of N-EtFOSE-¹⁴C in feed to male rats (mean dose, 10.13 mg/kg)

Day Post Dose	Percent of Dose in Tissue/Fluid							
	Liver	Spleen	Kidneys	Lungs	RBC ^(a)	Plasma ^(a)	GI Tract	Carcasses
1	17.26	0.13	0.88	0.47	7.00	2.82	16.61	19.88
2	20.04	0.13	0.81	0.45	5.09	2.52	11.41	15.74
4	19.25	0.13	0.83	0.41	5.68	2.78	--- ^(b)	--- ^(b)
8	15.52	0.06	0.38	0.22	3.00	1.46	--- ^(b)	--- ^(b)
16	10.65	0.02	0.20	0.11	0.95	0.91	--- ^(b)	--- ^(b)
32	9.53	0.02	0.22	0.09	0.48	0.85	--- ^(b)	--- ^(b)

(a) Estimate

(b) Sample not taken

Metabolites measured: Perfluorooctanesulfonate identified and quantitated.
Perfluorooctanesulfonamide tentatively identified.

CONCLUSIONS

The study is well-conducted and thorough. Agree with the conclusions of the authors.

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.

ROBUST SUMMARY - RS57

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:

FC-95-14C, Carbon-14 labeled 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%. Radioactive content in feces was too low to measure after 64 days. 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 (Days)	Percent Dose 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 (Days)	Percent Dose 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

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.

OTHER

ROBUST SUMMARY - RS58

Title: Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium [14C]Perfluorooctanesulfonate (1984)

TEST SUBSTANCE

Identity:

Potassium [14C]Perfluorooctanesulfonate (14C-PFOS)

Ammonium [14C]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 [14C]Perfluorooctanesulfonate (PFOS): 3.4 mg/kg mean, single dose, 0.56 mg/ml

PFOS control animals: 3.5 mg/kg mean

Ammonium [14C]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

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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
¹⁴C-PFOS			
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
¹⁴C-PFO			
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.

OTHER

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.

ROBUST SUMMARY - RS59

Title: 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 (1999)

TEST SUBSTANCE

Identity:

Potassium Perfluorooctanesulfonate (PFOS), CAS Number: 2795-39-3

Remarks:

PFOS purity 99.28%, 3M Chemical Lot Number 193

METHOD

Method/guideline followed: US FDA GLP Final Rule 21 CFR 58

Test type: in vivo

Species/strain/cell type or line: rat, Sprague-Dawley

Sex: both

Age and body weight range of animals used: 60 days, males 300-325 g, females 200-225 g

Number of animals/sex/dose: F0 35, F1 25

Route of administration: oral

Vehicle: 0.5% Tween 80, 5 ml/kg

Doses: 0.1, 0.4, 1.6, or 3.2 mg PFOS per kg/day in 0.5% Tween 80. These doses correspond to concentrations of 0.2, 0.8, 0.32, and 0.64 mg/ml. Groups of vehicle control F0 rats were administered only Tween 80. Male F0 animals were treated 42 days prior to mating and through mating period; female F0 animals were administered PFOS daily 42 days prior to mating, through gestation, and up to 20 days following litter delivery. F1 male and female rats were exposed to the chemical in utero and during lactation. Following weaning at 21 days of age, selected F1 animals were treated during development and production of F2 animals.

Excretion routes, body fluids, and tissues monitored and/or sampled during study:
liver and serum

Statistical methods used:

Means and standard deviations were calculated using Microsoft Excel, and relative standard deviations were calculated manually.

Method remarks:

Liver and sera samples collected from the initial population of dosed animals (F0) and their offspring (F1) were analyzed for the presence of PFOS. Liver samples were homogenized, and liver and sera samples were extracted by an ion-pairing extraction procedure. The extracts were quantitatively analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry, and PFOS levels were evaluated against extracted standards. Some minor deviations from US FDA GLP Final Rule 21 CFR 58 are listed in the report.

RESULTS**Detailed results:**

F0 results by dose group:

Dose group (mg/kg/day)	Average PFOS conc. in serum (ug/ml)	Average PFOS conc. in liver (ug/g)
0.0:	female 0.0307 male 0.0244	female 0.171 male 0.665
0.1:	female 5.28 male 10.5	female 14.8 male 84.9
0.4:	female 18.9 male 45.4	female 58.0 male 176
1.6:	female 82 male 152	female 184 male 323
3.2:	female NR* male 273	female NR* male 1360

*samples not received

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. No samples collected from F1 males or females that received 3.2 mg/kg/day were submitted for analysis.

Metabolites measured:

Analyses were performed to determine the presence of EtFOSE, PFOSA, POAA<PFOSEA, PFOSAA, and the monoester; however, these data were collected for informational purposes only, and were not reported.

CONCLUSIONS: agree**REFERENCE**

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. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Report No. U2006, Requestor Project No. 3M TOX 6295.9, 3M Environmental Laboratory Report No. FACT Tox-012. York, RG, Hansen, K, and Clemen, L., October 27, 1999.

OTHER

ROBUST SUMMARY - RS60

Title: Absorption of FC-95-14C in Rats after a Single Oral Dose (1979)

TEST SUBSTANCE

Identity:

FC-95-14C, Carbon-14 labeled 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.

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.

OTHER

This oral dosing experiment (FC-Experiment 4) was paired with an iv dosing experiment (FC-Experiment 3) which was designed to provide data on the route and extent of total C-14 excretion.