ENVIRONMENTAL AND HEALTH ASSESSMENT OF PERFLUOROOCTANE SULFONIC ACID AND ITS SALTS

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3M

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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 or metabolism of other fluorochemical products produced by electrochemical fluorination. As a fully fluorinated organic molecule, PFOS does not degrade except by combustion. 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 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 June 30, 2003, 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 Organisation for Economic and Cooperative Development Investigation of High Production Volume Chemicals (OECD, 1997).

In sum, the observed levels of PFOS are not expected to cause adverse effects on human health, wildlife, or the environment. However, as a matter of product stewardship in light of the persistence of this compound, the primary manufacturer of PFOS and its precursor molecules (3M Company) announced on May 16, 2000 that it would voluntarily cease manufacturing perfluorooctanyl-based chemistry. The company has cooperated with the U.S. Environmental Protection Agency and its customers in implementing the phase-out of production. 3M has also made available in EPA's public docket all of the epidemiologic, toxicologic, and environmental studies of PFOS and several related molecules.

Analytical Methodology

Analysis of the Perfluorooctanesulfonate (PFOS) anion has presented many analytical challenges. Unique physical properties of PFOS adversely affecting analysis include extremely low volatility, absence of chromophores, resistance to derivitization, and its ability to adhere to suspended particulates and container surfaces making it unavailable for analysis. Because of the aforementioned physical properties of PFOS, the selection of appropriate analytical methodologies to accurately quantify a wide range of concentrations in a broad array of environmental and biological matrices becomes critical. Analytical techniques employing liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) were developed and validated for a number of sample matrices including biological tissues, soil, and water. In each case, the validation activity included the determination of matrix spike recoveries within prescribed acceptance criteria of +/- 30%. Adherence to specific data quality objectives assured that the type, quantity, and quality of the analytical data generated was technically sound and defensible. These same standards are also applied to all studies conducted for 3M by contract laboratory facilities.

Environmental Risk Assessment

3M has supported extensive research into the fate, exposure, effects, and ecological risk of PFOS. The research was designed to answer the following questions: (1) What is the concentration and global distribution of PFOS in organisms? (2) What are the ecotoxicological effects of PFOS, as determined by laboratory testing? (3) Based on this information, what is the potential risk of PFOS to exposed species?

Much direct evidence is available to assess the exposure concentrations and effects concentrations of PFOS. Those data have been used to evaluate the potential risk to a wide variety of species -- including fish, invertebrates, piscivorous (i.e., fish-eating) mammals, and aquatic plants-- from exposure to PFOS found in freshwater and marine environments. The sections below briefly summarize the information on environmental fate, exposure concentrations, ecotoxicity studies and ecological risk characterization.

Environmental Properties

Releases of PFOS and its precursor molecules (molecules that through degradation or metabolism can result in the formation of PFOS) can occur during 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 indicate that most of the waste generated at the 3M Decatur, Alabama manufacturing site and throughout the supply chain was in the form of solid waste that was either incinerated or disposed of in landfills. Smaller amounts were released in wastewater or to air.

In the environment, PFOS is resistant to chemical and biological changes and does not degrade under any observed conditions except for combustion. Numerous studies on the environmental fate mechanisms of PFOS have been completed. These include studies of biodegradability, hydrolysis, and photolysis of PFOS and PFOS precursors.

PFOS precursors enter the environment though factory releases, as manufacturing residuals in products, or in products themselves. The actual method by which PFOS is dispersed throughout the environment is unknown. Possible methods include: 1) transport through surface water; 2) dispersion in air; 3) adsorption onto particles present in surface water, sediments, and air; and, 4) uptake by aquatic, avian or terrestrial organisms.

Environmental Exposure Data

Three major studies were conducted to generate exposure data. The first was the Global Biosphere Monitoring Program, conducted by scientists from Michigan State University. Liver and serum concentrations of PFOS were obtained from many archived samples of species from the Northern Hemisphere. (No animals were sacrificed.) The resulting data base was unusually large. For example, 595 liver samples were available from 33 species of piscivorous birds, piscivorous mammals, and fish. In addition, 378 serum samples were available from 17 species. In total, over 1,200 samples were analyzed.

A second source of data was the Multi-City Monitoring Study, in which water, sediment, and other samples were collected from six U.S. cities and analyzed for PFOS.

Finally, samples were collected in the Tennessee River in the vicinity of the 3M manufacturing facility in Decatur, Alabama, including Tennessee River water, mollusks, and whole-body fish representing four key species of fish.

Together, these exposure studies provide significant insights into the expected concentrations of PFOS in aquatic, avian and terrestrial communities. In addition, 3M has made great strides in the ability to detect and quantify PFOS at very low levels in the environment and in tissues of aquatic and terrestrial organisms. Therefore, the exposure data used in the risk assessment include measurements of PFOS at very low (ppb-ppt) concentrations.

Ecotoxicity Data

Numerous acute and chronic toxicity laboratory 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 organisms. A total of 26 ecotoxicological studies were conducted, utilizing freshwater (14 studies), marine (7 studies) and terrestrial (5 studies) organisms. Of these 26 studies, 17 utilized short-term acute exposure regimes and 9 were longer term (subchronic) exposures, where reproduction and/or growth were monitored to determine a No Observed Effect Concentration (NOEC). In addition, NOEC values for rat liver and serum were obtained from a series of mammalian toxicity tests, including a two-generation study.

Assessment of Ecological Risk

A risk assessment was performed for: (1) potential risk to aquatic biota from exposure to PFOS in surface water, (2) potential risk to aquatic biota from exposure to PFOS in sediments, (3) potential risk to fish and mollusks from exposure to PFOS in the Tennessee River, and (4) potential risk to piscivorous mammals using liver and serum PFOS concentrations. An ecological assessment of avian wildlife was not conducted at this time because avian chronic studies are still underway.

The risk assessment compared the available exposure and effects data and, considering a margin of safety, generated measures of potential risk due to PFOS exposure. Exposure-to-effect ratios were generated for multiple species, using appropriate tissue-specific NOECs or aquatic toxicity test endpoints. A safety factor of ten applied to the chronic laboratory NOEC data was used to derive a Predicted No Effect Concentration, or PNEC, which could be compared to the observed water concentration or to the serum or liver PFOS concentrations found in the various species. Ratios of the exposure concentration to the PNEC above 1.0 are indicative of potential risk (i.e., warrant further screening to evaluate whether an actual risk is presented). An aspect that strengthens this ecological risk assessment is the reliance on direct measurements of PFOS exposure and effects, rather than modeled values.

The risk assessment is presented in detail in the text of this document. One example will be summarized here, the assessment of potential risk to piscivorous mammals using liver concentration data. The liver is the target organ for PFOS toxicity, and the highest levels of PFOS were found in piscivorous birds and mammals. Data from the Global Biosphere Monitoring Program provided a large information base on PFOS liver concentrations from multiple species collected from the Northern Hemisphere. The data were compiled into an

electronic data base, and the species-specific mean and maximum liver concentrations were calculated. The highest mean liver concentrations were found in mink (based on a data set of 77 liver samples), with an average concentration of 1.22 ug/g (ppm) wet weight. The remaining 32 species had average liver concentrations that ranged from <0.008 ppm to 0.94 ppm (wet weight), well below the mink liver values.

The risk assessment for mink used reproductive endpoints from toxicological studies in rats as the most appropriate effects measure for assessing ecological effects. This follows the OECD protocol for ecological risk assessment. Examination of existing toxicological data showed that reproductive endpoints from a 2-generation rat study provided a No Observed Effect Concentration (NOEC) for PFOS in liver of 107 ug/g (wet wt.). A safety factor of 10 was applied, resulting in a Predicted No-Effect Concentration (PNEC) of 10.7 ug/g (tissue wet wt.). This value was used as a conservative measure of effects, for comparison to the mean (or maximum) liver exposure concentrations. The comparison of mean exposure to effects (i.e., 1.22 / 10.7) results in a ratio of 0.114. This is well below the screening level of 1.0 deemed to be indicative of potential risk. The average liver concentrations would need to be almost 10 times higher for the ratio to approach 1.0, indicating a wide margin of safety in the risk calculations. In fact, because a safety factor of 10 was previously applied in the calculation, the true margin of safety may be as high as almost 100 for mink. In addition, the calculated hazard ratio is still less than 1.0 even using the maximum value for a single animal (ratio 0.455). The mink is the worst-case species. Data from the remaining 32 species had much larger margins of safety.

Risk characterization of aquatic organisms exposed to PFOS in surface water proceeded in much the same way as the assessment for mammals described above. Exposure concentrations were available from the Multi-City Study and the assessment of the Tennessee River near Decatur, Alabama. Site-specific average and maximum PFOS concentrations were calculated. Effects data were derived from standardized aquatic toxicity tests. The lowest NOEC, from a growth and reproduction test using mysid shrimp, was used in combination with a safety factor to derive the PNEC. After application of a safety factor of 10, the PNEC was found to be 0.025 ppm. Comparison of the PNEC to the average site-specific exposure values (which generally are parts per trillion or parts per billion) indicated that most of the sites in the database had margins of safety of over 1000. In the area immediately adjacent to the manufacturing site outfall in Decatur, Alabama, the ratio is 2.44 based on the average exposure concentration. However, this is limited to the immediate vicinity of the outfall. Ratios at downstream sites are well below 1.0.

Similarly, potential risk to benthic organisms in sediment in the Tennessee River or elsewhere in the Multi-City Study is limited to the area around the plant outfall; all other locations had ratios well below 1.0. Ratios for fish and mollusks were all less than 1.0, using mean or maximum concentrations.

In sum, the observed levels of PFOS from a wide variety of environmental samples would not be expected to result in adverse health effects to aquatic organisms or wildlife. Calculated ratios comparing actual exposure levels to no-effect concentrations from laboratory toxicity studies for a large number of species indicate a wide margin of safety. This assessment applies a well-accepted methodology. While uncertainty exists in this analysis, use of serum and liver data as a measure of internal dose reduces some of the uncertainty in inter-species extrapolation. The use

of actual rather than modeled concentration data, and the substantial size of the exposure and toxicity databases, add further confidence to the assessment.

Human Health Risk Assessment

Human Exposures

Although organically bound fluroine had been identified in human blood for some time, in the 1990's, improvements in analytical methodology allowed for the routine measurement of specific organofluorine molecules. 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 1-2 ppm, with the highest measured levels at approximately 13 ppm. In non-occupational populations, 95 percent of individual serum PFOS concentrations from studies of children, adult blood donors and an elderly cohort were less than 0.10 ppm. The average serum PFOS concentrations ranged between 0.030 and 0.040 ppm.

Health Effects Data

The database for human health risk characterization includes a large number of toxicology studies as well as medical surveillance and epidemiological investigations of exposed workers. The toxicology studies include: subchronic studies in rodents and monkeys; a two-year cancer bioassay in rats; an extensive array of genotoxicity tests; reproduction/ developmental studies in rats and mice, including a multigeneration reproduction study in rats; fetal developmental studies in rats and rabbits; toxicokinetic data; and, various investigations into the mode of action of PFOS. In addition, medical surveillance and epidemiological investigations in exposed workers are available. The studies provide a comprehensive database for use in hazard evaluation and risk assessment.

Human Studies

It is reasonable to assume 3M fluorochemical production workers have had the highest level of human exposure to PFOS. 3M has conducted medical surveillance of fluorochemical production workers for over 25 years. A battery of clinical tests (including lipids, hematological parameters, enzymes and 11 different hormone assays) showed no association between these measurements and PFOS levels in workers. Medical surveillance data include voluntary participation studies in 1994, 1995, 1997, 2000, and 2002, and a randomized study conducted in 1998. A longitudinal analysis is also available.

A mortality study of workers at the Decatur plant showed no significant excess for any cause of death, with the exception of urinary bladder cancer. Bladder effects have not been observed in any of the animal studies, and worker urinalysis results have not shown abnormalities. Although 3M continues to investigate the apparent increase in bladder cancer mortality in fluorochemical production workers with an incidence study, PFOS does not appear to have the properties of known bladder carcinogens with respect to genotoxicity and insolubility.

An analysis of health claims data showed that the overall episodes of care experience was comparable between fluorochemical production workers and non-fluorochemical production

workers (film plant) at the 3M Decatur production facility. This study included neonatal diagnoses and other endpoints related to reproductive outcomes.

Toxicological Data

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 cells, increased liver size and weight, and apparent alterations in metabolic processes. Other effects that may relate to the liver response have also been noted, e.g., effects on body weight. Liver cell hypertrophy and reduction in serum cholesterol are early responses to PFOS. These effects occur in rats as well as monkeys. Both species display an apparent threshold for the toxic effects of PFOS that can be expressed in terms of cumulative dose or body burden, with no observable response at lower cumulative doses or body burdens.

In a two-year dietary cancer bioassay in rats, male and female rats at the highest dietary dose (20 ppm PFOS in diet) had small but significant elevations in benign liver tumors (hepatocellular adenoma) that likely are related to PFOS treatment. Since PFOS has not produced genotoxicity in a variety of test systems, these tumors are considered to originate through a threshold-mediated, non-genotoxic mechanism.

Reproduction/developmental studies in rats, including a multi-generation study in rats, have demonstrated that PFOS does not alter fertility. In the multi-generation study, there were no effects on developmental milestones, including post-natal neurological development, or on fertility and estrous cycling in offspring. However, at top study doses, PFOS adversely affected growth and survival of rat pups in the neonatal period of life as a result of transfer of PFOS to the fetus *in utero*. The effect appears to be strongly related to maternal (and fetal) body burden of PFOS at the end of gestation and is associated with a clearly defined threshold within a narrow range of doses/body burdens. PFOS treatment of dams has produced decreased weight gain in pups during lactation in reproduction studies.

PFOS is not a selective developmental toxicant (teratogen), although reduced fetal weight, abortions, resorptions, or structural anomolies are seen in developmental studies in mice, rats, and rabbits at maternally toxic doses.

The mechanism of toxicity of PFOS is not well understood. The liver appears to be the target organ in rodents and non-human primates. The hepatocellular hypertrophy and vacuolation noted in subchronic studies cannot be fully ascribed to peroxisome proliferation or increased mitochondrial biogenesis. The observed hypolipidemia and extensive vacuolation of liver cells suggests alterations in metabolism. There is a suggestion that myopathy or increased protein catabolism may be present during PFOS-induced terminal illness in laboratory animals. The liver and clinical effects noted are reversible on cessation of exposure. It is likely that PFOS, which resembles, in some respects, a fatty acid, may insert itself in binding sites and in processes normally involving fatty acid transport, storage and metabolism. However, PFOS does not concentrate in fatty tissue.

Dose-Response Relationships and Body Burden

PFOS is well-absorbed orally and very slowly eliminated from the body, and these combined properties can result in the accumulation of PFOS body burden from various sources and pathways of exposure. Serum elimination half-lives in rats, monkeys and humans are currently estimated to be ~100 days, ~200 days, and several years, respectively. In all three species, small and frequent external doses of PFOS or precursor chemicals above a threshold would be expected to result in an accumulation of PFOS body burden, as reflected by serum PFOS concentration. PFOS is not metabolized in any of the multiple species studied, although it can be formed metabolically from perfluorooctanesulfonyl-based precursors. Unlike many chemicals of environmental consequence, PFOS does not preferentially distribute to fatty tissue, preferring instead to associate with proteins in blood and liver. PFOS crosses the placenta in rats, and there is evidence for distribution in rat breast milk.

Serum and liver PFOS concentration measurements made in connection with toxicology studies have demonstrated that PFOS concentrations in liver and serum are linearly proportional to cumulative dose over a wide range of cumulative exposure and provide a measure that relates directly to body burden. This proportionality is expected to exist within the measured concentration range of occupational and non-occupational human exposure. Serum PFOS concentration can also be used to develop exposure-effect (dose-response) relationships from toxicology studies. Therefore, serum PFOS concentration can be used as an integrated measure of exposure over time and related to the probability of toxic response, regardless of source or pathway of exposure. It should be noted that the method variation in measuring PFOS in serum in most of the human and toxicology studies can be as high as ± 30%. Even so, the overall potential variability in using serum PFOS concentrations in risk analysis is likely to be much less than attempting to estimate external exposures to humans from various sources.

Using serum or liver PFOS concentrations as a measure related to integrated exposure to PFOS for risk characterization has several distinct advantages. Foremost of these is overcoming the uncertainty involved in attempting quantitative estimates of external PFOS exposure from a variety of sources, routes of exposure and exposure pathways. Another important advantage is the ability to compare no-observed-adverse-effect levels (NOAELs) or calculated benchmark doses (BMDs), both expressed as serum or liver PFOS concentration, between studies and species, thus reducing uncertainty in interspecies extrapolation. This methodology can therefore be used to assess risk using a margin-of-exposure approach, where human exposure represented by serum or liver PFOS concentration is compared to animal no-effect or benchmark doses and the margin between the two is evaluated.

Assessment of Human Health Risk

After consideration of serum and liver PFOS concentrations associated with NOAEL and those associated with the BMD (benchmark internal concentration, or BMIC) values from various toxicology studies, protective values were chosen as "points of departure" for risk assessment. For serum comparisons, a PFOS concentration of 31 ppm was chosen, which represents the lower 95% CL of the BMIC (LBMIC) for a 5% response in reduced pup weight gain during

lactation. While pup weight gain is the most sensitive endpoint, comparisons can also be made for other endpoints. For liver response, the male rat NOAEL for liver toxicity of 44 ppm PFOS in serum was chosen as most appropriate. For liver tumors in male and female rats, the LBMIC (10% response) is associated with a serum value of 62 ppm. Thus, the value for pup weight gain in lactation would also be protective against liver toxicity and liver tumors.

For liver values, the liver PFOS concentration of 59 ppm associated with the study NOAEL for male cynomolgus monkeys in a six-month oral toxicity study of PFOS was chosen for risk assessment.

These values were used as points of departure for a margin-of-exposure (MOE) analysis. The ratios of the point of departure value for serum or liver from the animal studies to measured human serum PFOS concentrations (general population mean and upper bound) or estimated human liver concentrations were determined. The use of direct serum or liver comparisons reduces the uncertainty in inter-species extrapolation.

Table ES-1. Human-Health Risk Assessment for PFOS Body Burden: Margin-of-Exposure Analysis based on Human Serum and Estimated Human Liver PFOS Concentrations

PFOS Concentration in Humans (C), ppm	Point-of-Departure (POD), ppm	Endpoint	Margin of Exposure (Ratio POD:C)
Serum:			
0.040 (mean)	31	Pup weight gain	775
0.040 (mean)	44	Liver effects, rats	1100
0.040 (mean)	62	Liver tumors, rats	1550
0.100 (upper bound)[a]	31	Pup weight gain	310
0.100 (upper bound)[a]	44	Liver effects, rats	440
0.100 (upper bound)[a]	62	Liver tumors, rats	620
Liver: Human liver concentrations estimated from serum assuming a liver-to-serum ratio of 1.7:1.			
0.068[b] (mean)	59	Liver effects, monkeys	868
0.170 (upper bound)[a,b]	59	Liver effects, monkeys	341

[a] Upper 95% confidence limit at 95% tolerance limit.
[b] Conservative estimate of liver concentration assuming a liver-to-serum PFOS concentration ratio of 1.7:1.

Margins of exposure for non-occupational human populations based on serum PFOS range from 310 to 1550. For the most sensitive endpoint, pup weight gain during lactation, the MOE based on mean population serum PFOS values was 775, and this decreased to 310 using the upper-bound estimate for population serum PFOS concentration (95th percent bound of the 95th percentile of individual serum PFOS concentrations measured in studies of children, adult blood donors, and an elderly cohort (0.1 ppm)). The LBMIC (10% response) for liver tumors gave a MOE of 1550 when compared to the mean population value of 0.040 ppm PFOS in serum.

Liver concentrations for non-occupational populations were conservatively estimated by multiplying the measured serum concentration by a factor of 1.7, the upper 95% confidence limit of human liver-to-serum PFOS concentration ratios. MOE values comparing the liver value

selected for risk assessment (the 59 ppm NOAEL from the monkey study) with estimated liver values in humans range from 341 for the upper bound of exposure to 868 for the representative mean exposure.

Occupationally-exposed populations have higher exposure levels and hence narrower margins of exposure compared to the animal no-effect levels, but these populations have been monitored for over 25 years without evidence of adverse health effects attributable to PFOS.

In summary, the observed levels of PFOS in human serum demonstrate adequate margins of exposure and should not be associated with increased health risk.

Conclusion

This report summarizes the information that is available as of June 30, 2003. There is a substantial body of data addressing human and wildlife exposures to PFOS and the potential environmental and health effects of PFOS. These data, when taken in the context of ecological and health risk, demonstrate adequate margins of exposure in wildlife and human populations. Therefore, adverse effects are not expected in either wildlife or humans.

INTRODUCTION

Background

The human health and ecological risk assessment 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 and Cooperative 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. This assessment exceeds the normal content of a SIDS Initial Assessment Report, as extensive data are available and risk characterization is possible. The environmental portion of this report is effectively a Tier I Screening-Level Risk Assessment as envisioned by U.S. EPA. This revised assessment updates 3M's earlier draft initial assessment dated October 2000, but has been re-titled to reflect the substantially expanded database and risk characterization addressed here.

Overall, this assessment presents information on PFOS exposure and effects and integrates that information into an assessment of potential risk. In sum, the observed levels of PFOS are not expected to result in adverse effects on humans, wildlife, or the environment. The information in this report is based on a substantial body of data and represents our current knowledge.

Section 1 of this report provides the PFOS acid and salt CAS numbers and molecular formula of the acid; 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.

Section 3 describes ecological exposure, effects on aquatic and terrestrial ecosystems, and other ecological effects.

Section 4 evaluates human health exposures, potential hazards, and risks.

Section 5 presents the conclusions and recommendations of this assessment.

Section 6 lists the literature and data sources upon which this document is based.

The Appendices contain supporting documentation including Robust Summaries of the underlying studies. Appendix I provides Robust Summaries on physical/chemical properties, and environmental fate studies. Appendix II presents summaries of ecotoxicological studies. Appendix III reports toxicological, epidemiological, and health studies. Appendix IV provides reports of benchmark dose calculations.

Analytical Methodology

The ability to detect and quantify PFOS and its precursor compounds at very low levels in the environment has been improved over the past few years, 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. A significant level of research has been completed to assess the presence of PFOS in the environment, its atypical partitioning behavior, and fate mechanisms. However, existing fate-and-transport models are not applicable due to the unique properties of PFOS, and gaps in physicochemical and environmental data also complicate the characterization of the environmental fate of PFOS.

Analysis of the Perfluorooctanesulfonate (PFOS) anion has presented many analytical challenges. The unique physical properties of PFOS that affect analysis include:

1) Extremely low volatility: The non-volatility of PFOS eliminates the possibility of using conventional methodology such as gas chromatography/mass spectrometry (GC/MS) for analysis.
2) No chromophores: The absence of chromophores for PFOS as compared to the many strong chromophores contained in biological sample matrices nullifies using conventional methodology such as UV-Vis spectroscopy or liquid chromatography/UV-Vis spectroscopy (LC/UV).
3) Not easily derivatized: PFOS is difficult to derivatize, thereby eliminating the use of conventional methodology such as GC/MS for analysis.
4) Strong anion: In aqueous solution, PFOS forms a strong anion that adheres to container surfaces and suspended particulates, making it unavailable for analysis.

Because of these aforementioned physical properties, the selection of appropriate analytical methodologies to accurately quantify a wide range of concentrations in a broad array of environmental and biological matrices becomes critical. An emerging analytical technology, liquid chromatography/mass spectrometry (LC/MS) was found to overcome the limitations of more commonly used approaches. LC/MS allows for separation of the PFOS anion from co-extracted matrix components in the liquid phase followed by mass detection that selectively measures the mass of the characteristic ion in solution. More sophisticated techniques employ tandem MS/MS detection, allowing for the fragmentation of the PFOS anion into unique daughter ions. Since a number of ions can exist with the same mass as PFOS, measurement of the unique daughter ion fragments allows for confirmation of the parent ion.

Analytical techniques employing LC/MS were developed and validated for a number of analytical matrices including biological tissues, soil, and water. In each case, the validation activity included the determination of matrix spike recoveries. Matrix spike recoveries were considered acceptable if PFOS was recovered and determined to be within acceptance criteria of +/- 30%.

In some cases, limited sample size significantly influences the limits of quantitation. Water samples (usually collected as large volumes suitable for analyte concentration techniques) typically have lower limits of quantitation (LLOQ) of 20 parts per trillion while serum samples

(very limited volumes not amenable to analyte concentration techniques) typically have LLOQ's of 1-10 parts per billion.

Analytical methods based on LC/MS/MS technology were developed to encompass a wide array of detection levels and matrices. Environmental studies generally require trace level detection (low part per trillion to part per billion). Toxicology feeding studies often target increased analyte concentrations (mid part per billion to parts per million).

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the actual concentration of the analyte. The precision of the analytical method describes the closeness of individual measurements of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of sample extract. Accuracy and precision are addressed on an individual basis for each validated method and for all performance-based methods.

Data quality objectives have been defined for all GLP and non-GLP studies conducted in the 3M Environmental Laboratory. Adherence to the prescribed data quality objectives assures that the type, quantity, and quality of the analytical data generated will be appropriate for the intended application and that all data are technically and scientifically sound. Specific data quality objectives include linearity, limits of detection, limits of quantitation, duplicate frequency, acceptable accuracy and precision, spike frequency, acceptable spike recoveries, use of surrogates, use of confirmatory methods, and demonstration of analytical method specificity. These same standards are also applied to all studies conducted for 3M by contract laboratory facilities.

Overview

The perfluorooctanyl chemicals 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 using this technology was perfluorooctane sulfonyl fluoride (POSF). Over eight million pounds of this compound were 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 a variety of 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 releases, as manufacturing residuals in products or as products themselves. Possible mechanisms by which PFOS or its precursors can be transported through the environment include: 1) transport in surface water; 2) dispersion in air (for certain volatile compounds); 3) adsorption onto particles present in surface water, sediments, and air; and, 4) uptake by aquatic, avian or terrestrial organisms.

Numerous studies on the environmental fate mechanisms of PFOS have been completed. These include studies of the biodegradability, hydrolysis, and photolysis of PFOS and certain other POSF-based precursors. The studies indicate that PFOS does not degrade through any of these mechanisms, and thus would be considered to be an environmentally stable compound. Because of the low energy inherent in the carbon-sulfur bond in the PFOS molecule, the molecule is degraded through high temperature incineration.

Because PFOS was not the major perfluorochemical compound or product produced, and it was not an ingredient in most POSF-based products, it is also important to understand the degradation products of some of the POSF-based precursors. Based on the studies completed, PFOS is not formed through the hydrolysis or aqueous photolysis of these precursors with the possible exception of POSF. In fact, in experiments involving various precursor molecules, PFOS was only generated through the biotic degradation of N-ethyl-perfluorooctanesulfonamido ethanol (N-EtFOSE). Thus, at this time it is difficult to assess the pathway(s) by which PFOS is found in the environment.

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 exposure to 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 fluorochemical production workers have the highest known blood levels of PFOS. Epidemiological and medical surveillance studies of these workers have not shown health effects attributable to this exposure. An extensive toxicological database on PFOS and specific precursor molecules has been developed. The available information indicates that current levels of PFOS are not expected to result in 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 June 30, 2003. To ensure the most accurate analytical results possible, work was conducted to completely characterize samples used in testing and analysis over the last few years. The results of this purity analysis showed only a slight variation from the initial analysis.

1.0 IDENTITY

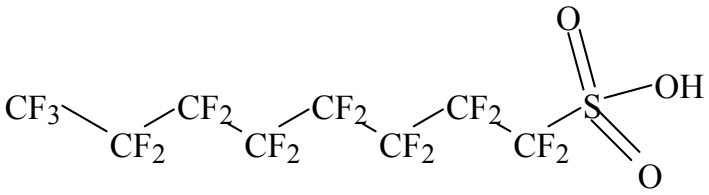
Chemical Name: Perfluorooctane Sulfonic Acid

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: ..... $C_8F_{17}SO_2OH$

Structural formula:



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

2.0 GENERAL INFORMATION ON EXPOSURE

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 perfluorochemical products. PFOS is itself a commercialized product produced from the hydrolysis of POSF. 3M sold approximately 50,000 pounds per year of PFOS in various salt forms. It has been used for a variety of surfactant applications, mainly fire-fighting foams and coating additives.

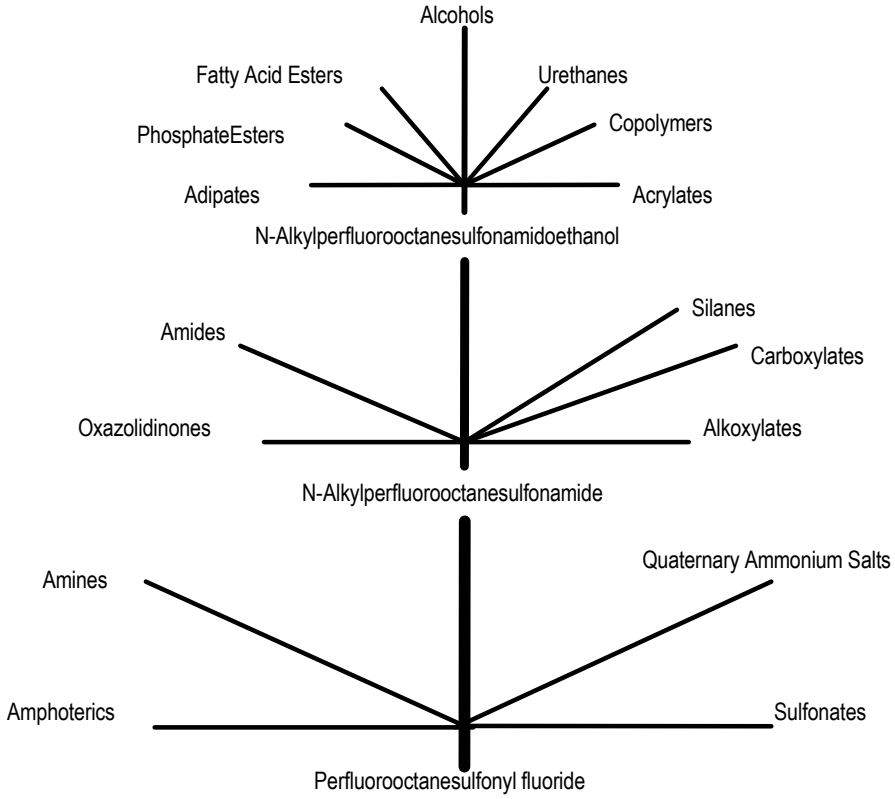
Unique chemistries were created by further derivatization of POSF through the sulfonyl fluoride moiety using conventional hydrocarbon reactions. POSF is reacted with methyl or ethylamine to produce either N-methyl or N-ethyl perfluorooctane sulfonamide. These intermediates were used to make amides, oxazolidinones, silanes, carboxylates and alkoxylates as commercial products. These intermediates were subsequently reacted with ethylene carbonate to form either N-methyl or N-ethylperfluorooctanesulfonamidoethanol (N-EtFOSE or N-MeFOSE). These intermediates were used to make adipates, phosphate esters, fatty acid esters, urethanes, copolymers, and acrylates as commercialized products. See Figure 2-1. It is important to note that most of the POSF-based production was in the form of high molecular weight polymers and surfactants that would contain PFOS precursors and only trace levels of PFOS, if any. As mentioned above, only a relatively small amount of PFOS was manufactured by 3M.

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. These residuals were typically present at a concentration of 1% or less in the final commercialized products.

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 has been shown to be PFOS and other compounds having a C_8 perfluorinated chain. However, it is not expected that all moieties break down to 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 was 7.8 million lbs and in 2001 the production was 385,000 lbs. POSF-derived fluorochemicals (polymers and monomers) are formulated with water or organic solvents, 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-based molecules solely. 3M-produced fluorochemical solids represent the majority of the total global production of POSF-based fluorochemicals. The breakdown of 3M fluorochemical production into different product categories includes:

Figure 2-1. POSF Fluorochemical Reaction Tree



<u>Surface Treatments</u> (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

<u>Paper and Packaging Protectors</u> (Phosphate esters or high MW polymers)

Food Packaging
Paper Products

<u>Performance Chemicals</u> (Lower 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
Insecticide Raw Materials

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

3M substantially discontinued the production of POSF-based products by the end of 2000. Manufacture and distribution of a few, well-defined products having essential uses continued in accordance with EPA's determinations regarding critical uses.

3.0 ENVIRONMENT

This section discusses (1) environmental exposure, including results of environmental monitoring studies and information on fate and transport of PFOS (Section 3.1); (2) toxicity data for aquatic and terrestrial organisms (Section 3.2); and (3) an assessment of potential environmental risk given the exposure and hazard information (Section 3.3).

The environmental data set on PFOS is more extensive than data sets available on most other chemicals. The Appendices to this assessment include Robust Summaries of the ecotoxicity studies, physical and chemical properties, and environmental fate studies.

3.1 Environmental Exposure

This section discusses available information on the presence of PFOS in the environment.

Section 3.1.1 provides an overview of exposure potential.

Physical and chemical tests addressing environmental fate and transport are summarized in Section 3.1.2.

Section 3.1.3 discusses exposure data. Monitoring data have been collected for surface water column, drinking water, sediment, publicly-owned treatment works (POTW) sludge and effluent and landfill leachate samples in six cities. In addition, a market basket survey resulted in the analysis of chicken, pork, hot dogs, fish, eggs, green beans, milk, apples, bread, and ground beef samples from local grocery stores in each of the same cities. Section 3.1.3 also discusses a global biosphere monitoring program including the analysis of more than 1,200 archived serum, liver or other specimens from a wide range of aquatic and terrestrial species. These data are summarized in Tables 3-5, 3-6, 3-7, and 3-8.

3.1.1 General Discussion of 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 does not have complete information on waste streams from other producers and users of these materials.

Generally, the wastes generated from the manufacture and use of POSF-derived substances are not in the form of PFOS itself, but rather in the form of POSF, precursor intermediate molecules, or 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 there is not a complete understanding of the mechanism and the extent to which they may degrade or metabolize to PFOS.

Based on an analysis of waste generated at the 3M Decatur manufacturing site prior to the phase-out, approximately 90% of the waste generated was in the form of solid waste, which was either

incinerated or disposed of in landfills. The remaining Decatur plant wastes were discharged as wastewater (~9%) or as air emissions (~1%).

A Multi-City Study was designed to obtain information about the dispersion of perfluorooctanyl chemicals in the environment, foods, and surface water. Where possible, samples were taken from the surface water column, sediment, drinking water, effluent and sludge from publicly owned treatment works, and landfill leachate. Additionally, a "market basket" of food products was sampled.

Two other studies were completed to characterize the exposure potential of PFOS. The first study involved 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.

The second study was conducted by sampling in the vicinity of a manufacturing facility (Decatur, AL). Samples of groundwater, surface water, sediments, and fish species have been collected and analyzed. This study was repeated in 2002 to obtain more current information that would reflect the changes in production at this manufacturing site. The 2002 study indicates that the levels of PFOS in the various media have declined. For purposes of the risk assessment presented in this document, the older (higher) values were used.

3.1.1.1 Exposure and Release Management

3M announced in May, 2000 that it would voluntarily cease production of POSF chemistry. Even prior to that announcement, activities to reduce releases from 3M manufacturing operations and to improve product quality had been implemented. Product and process understanding were enhanced, the level of residuals and wastes from the manufacturing operations reduced through improved process controls and installation of abatement equipment, and most fluorochemical solid waste materials from 3M manufacturing received thermal treatment. All of these steps have had and will continue to have a significant impact on reducing the exposure potential from 3M manufactured perfluorooctanyl chemicals. 3M, the primary producer, substantially phased out production of POSF-based products by the end of 2000.

3.1.2 Environmental Fate and Transport

A number of tests have been undertaken to evaluate the physical and chemical properties and potential fate and transport mechanisms of PFOS in the environment. Robust summaries of these studies appear in Appendix I, designated as RS-I-1 through RS-I-20. The results are presented below. All studies were conducted utilizing the potassium salt of PFOS.

Table 3-1. Physical and Chemical Properties

Parameter	Report Date	Results
Boiling Point (RS-I-1)		Unable to determine
Melting Point (RS-I-2)	2/24/99	> 400 ºC
Vapor Pressure (RS-I-3)	5/05/99	3.31×10^{-4} Pa @ 20 ºC[a]
Log n-Octanol/Water Partition Coefficient (RS-I-4, RS-I-5, RS-I-6)	5/09/01	–1.08 (calculated from solubility in octanol and water)
Air-Water Partition Coefficient (RS-I-7)	3/19/00	$< 2 \times 10^{-6}$
Solubility in Pure Water (RS-I-8, RS-I-9)	5/03/99 3/30/01	519 mg/L 680 mg/L

[a] The impurities in the PFOS sample most likely were the source of the measured vapor pressure.

3.1.2.1 Water Solubility

Pure water mean: 600 mg/L (Range: 519 – 680 mg/L) (RS-I-8, RS-I-9)
3.5% NaCl solution: 20.0 mg/L (RS-I-10)
Natural sea water: 12.4 mg/L (RS-I-10)

PFOS potassium salt is a moderately water soluble (in pure water) and as the levels of dissolved solids in water increase, the solubility drops dramatically. Results from laboratory studies indicate that the PFOS anion forms strong ion pairs with many cations.

3.1.2.2 Sorption/Desorption

Table 3-2. Description of Soil Types Used in and Results of Sorption/Desorption Studies

Soil Types Utilized in Study

Soil Type	Source	% Organic Carbon	CEC mgq/100 g	% Sand	% Silt	% Clay	pH in $CaCl_2$
Clay	Kittson Co., MN	2.6	54.5	16	22	62	7.2
Clay Loam	Grand Forks Co., ND	2.6	24.7	21	46	33	6.0
Sandy Loam	Grand Forks Co., ND	2.8	23.3	58	22	20	7.8[a]
River Sediment	Grand Forks Co., ND	1.3	17.5	39	42	19	7.7
POTW Sludge	Denver, CO (NIST)	NA[b]	NA	NA	NA	NA	NA

[a] Value is for pH in water, not pH in 0.1 M $CaCl_2$
[b] Not Analyzed

Results

Soil Type	K_d, L/kg	K^{ads}_F[a], L/kg	K^{des}_F[a], L/kg
Clay	18.3	28.2	94.0
Clay Loam	9.72	14.0	60.2
Sandy Loam	35.3	25.1	105
River Sediment	7.42	8.70	44.6
POTW Sludge	<120	338	3130

[a] Freundlich coefficient

In the studies summarized at RS-I-11, PFOS appeared to moderately adsorb to all of the soil/sediment/sludge matrices tested. In both adsorption and desorption studies, an equilibrium was achieved in less than 24 hours, with substantial adsorption (>50%) occurring in some of the time zero samples after approximately one minute of contact. The elevated K_d values for PFOS compared to other ionic chemicals are probably due to the surface-active properties of PFOS, resulting in some adsorption to the soil surfaces. Although PFOS has the ability to sorb to soils, sediments, or sludge, it is expected to be mobile in the aqueous phase at equilibrium.

The use of K_{oc} is probably not the best descriptor for the soil partitioning behavior of PFOS. PFOS is both ionic and surface active, thus other processes, such as adsorption to surfaces and binding to mineral matter or clay, may influence its soil partitioning. The calculation and use of K_{oc} values assume that absorption into organic matter is the only process controlling partitioning,

and therefore all soil partitioning is attributed to organic carbon. This is probably not the case for PFOS. K_d makes no assumptions about the mechanism of partitioning and therefore should be used to describe the soil partitioning rather than Koc.

3.1.2.3 Octanol/Water Partition Coefficient (K_{ow})

PFOS does not partition to octanol and therefore the K_{ow} is not meaningful in modeling. Nevertheless, the log K_{ow} was calculated using laboratory-derived PFOS solubility in water and octanol. See RS-I-4, RS-I-5, RS-I-6, RS-I-8, RS-I-9, RS-I-10. These values are:

Log K_{ow} (Pure water):	-1.08
Log K_{ow} (natural seawater):	0.65
Log K_{ow} (3.5% NaCl):	0.45

The results indicate that PFOS does not partition preferentially to lipids.

3.1.2.4 Bioconcentration

Bluegill sunfish were exposed to 0.086 and 0.87 mg/L PFOS (mean measured concentrations) for 62 and 35 days, respectively (RS-I-12). The fish exposed to 0.87 mg/L PFOS had all died or been sampled by 35 days and were therefore not available for a depuration evaluation. Fish tissues were divided into edible and non-edible portions. Although the results from the last three sample periods during the uptake phase were not statistically different, the tissue concentrations were still apparently increasing and the concentration in the fish tissues was deemed to not be at steady state. Therefore, kinetic bioconcentration factors (BCFKs) were calculated for the edible tissue, non-edible tissue and whole fish rather than bioconcentration factors (BCFs). Analytical results from fish tissues sampled throughout the study are presented in Figure 3-1 for the 0.086 mg/L exposure. As shown there, uptake was greater in the non-edible portions of the fish.

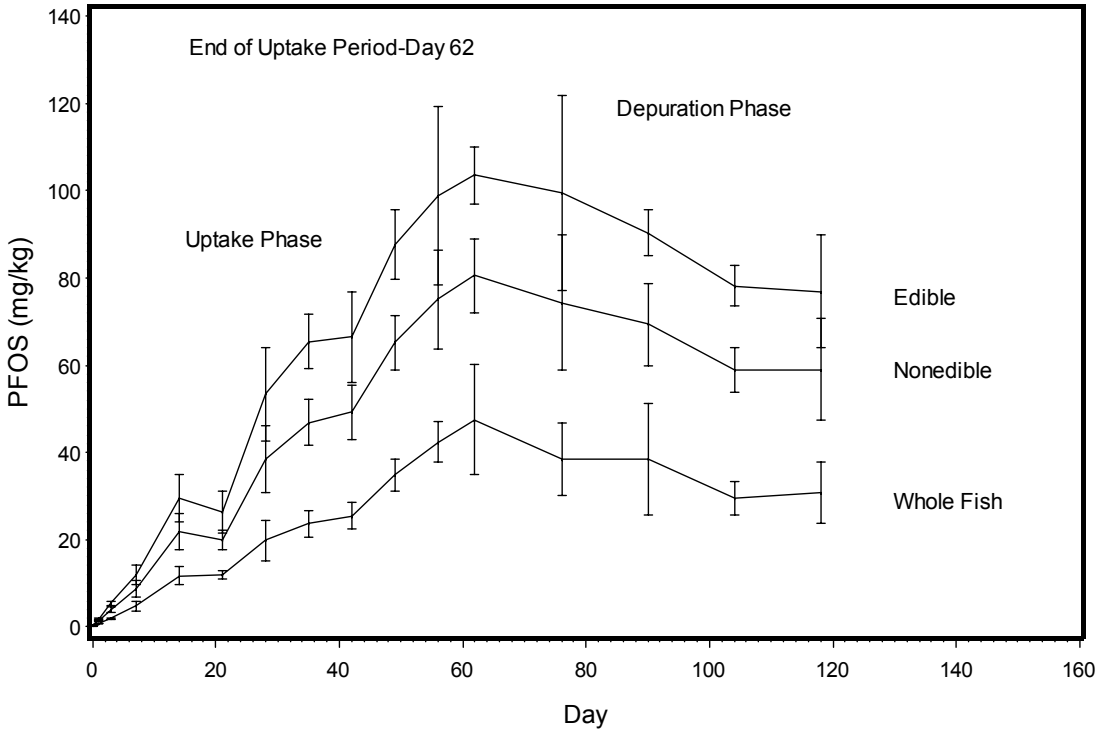
The following BCFK values were calculated for the 0.086 mg/L exposure:

Edible tissue:	1124
Non-edible tissue:	4013
Whole fish:	2796

The depuration phase lasted for 56 days. Estimated time to reach 50% clearance was calculated to be:

Edible tissue:	86 days
Non-edible tissue:	116 days
Whole fish:	112 days

Figure 3-1. Bioconcentration of PFOS in Bluegill Sunfish (*Lepomis macrochirus*) Tissue Concentrations After Exposure to 0.086 mg/L PFOS in Water.



3.1.2.5 Thermal Stability

Under the high temperature conditions of an incinerator, chemical bonds with lower bond energies break first. Calculations based on an electronic structure theory predict that the Carbon-Sulfur bond is the weakest bond in PFOS. Its predicted bond energy is 63 – 64 kcal/mol. For comparison, the bond energies of simple unsubstituted hydrocarbon alkanes are 85 – 90 kcal/mol. Thus, PFOS should undergo primary thermal degradation more readily than alkanes. In addition, during incineration of precursor molecules, any organic transformation products should be predominately non-sulfur-containing compounds. This means that PFOS is likely to be destroyed during incineration of other compounds.

A bench-scale study at the University of Dayton Research Institute (RS-I-13) has borne out these predictions. This laboratory study used a system designed to simulate the thermal degradation conditions likely to occur during municipal or hazardous waste incineration. When perfluorooctane sulfonamide containing products were exposed to these conditions, SO_2 recoveries were 100±25% and LC/MS analysis showed that no quantifiable amounts of PFOS (<0.07% of that stoichiometrically possible) were released from the system. The SO_2 results suggest that the perfluorosulfonamides were substantially destroyed and the LC/MS results strongly suggest that incineration of perfluorooctane sulfonamides would not release PFOS to the

environment. Furthermore, mass spectral extracted ion analysis of the emissions, showed an absence of 67-SOF ions. These ions are indicative of perfluoroalkylsulfonyl compounds, which could potentially transform to PFOS in the environment. The absence of the 67-SOF ions suggests that the carbon-sulfur bond was completely destroyed (and did not reform) in the combustions tests. This result suggests that environmental transformation of combustion products to form PFOS is also unlikely.

When PFOS was exposed to this combustion system, sulfur recoveries varied from 50 to 60% and LC/MS analysis showed that only a small fraction of the PFOS that was volatilized made it through the system. The authors speculate that low sulfur recoveries were likely due to condensation within the apparatus. The fraction of PFOS passing through the system to the PUFs decreased as the temperature of the combustion chamber increased. At 600 °C, about 0.5% of the gasified PFOS made it completely through the system and was collected on the PUFs. When the combustion chamber temperature was increased to 900°C, the amount of PFOS collected on the PUFs was decreased to about 0.07%.

C_1 or C_2 fluoroalkanes (likely products are either CHF_3, CF_4, or C_2F_6), 1,1-difluoroethene (PFOS only) and 1,2-difluoroethene (FC-1395 only) were the only highly fluorinated compounds observed in the system effluent. Fluorobenzene was also detected (FC-1395 and FC-807A only). No higher molecular weight fluorinated polycyclic aromatic hydrocarbons, and no perfluorooctane sulfonyl precursors of PFOS were present in the system effluent at detectable levels.

The report states that the data from this laboratory-scale incineration study indicates that properly operating full-scale incineration systems can adequately dispose of PFOS and the C_8 perfluorosulfonamides. Incineration of these fluorinated compounds is not likely to be a significant source of PFOS into the environment. With the exception of stable C_1 and C_2 fluorocarbons, fluorinated organic intermediates are unlikely to be emitted during the incineration of PFOS or perfluorooctane sulfonamides.

3.1.2.6 Hydrolysis

No loss of PFOS was noted during hydrolytic exposure at 50°C, for 49 days at pH values of 1.5, 5, 7, 9, and 11. Half-life was estimated as $\geq$ 41 years at 25° C. This estimate is based on the analytical limit of quantification; no loss of PFOS was detected in the study (RS-I-14).

3.1.2.7 Photolysis

No evidence of direct photolysis was observed. Aqueous PFOS solutions alone and in the presence of either hydrogen peroxide (H_2O_2), iron oxide (Fe_2O_3) or humic material at environmental concentrations were monitored for up to 167 hours after exposure to a light chamber with an output intensity of 680 w/m^2 in the evaluation of indirect photolysis. The indirect photolytic half-life was estimated as $\geq$ 3.7 years at 25°C using an iron oxide photoinitiator matrix model. This model was chosen because the experimental error was lowest in this matrix. This estimate is based on the analytical limit of quantitation; no loss of PFOS was detected in the study (RS-I-15).

3.1.2.8 Biodegradation

Studies in which PFOS biodegradation was monitored analytically were conducted using a variety of microbial sources and exposure regimes. No biotransformation of PFOS was observed after up to 20 weeks of incubation under aerobic conditions, or after 8 weeks of incubation under anaerobic conditions (RS-I-16, RS-I-17, RS-I-18, RS-I-19, RS-I-20).

3.1.2.9 Summary

PFOS appears to be stable in the environment. This compound is not biodegradable and does not undergo hydrolysis or photolysis; however, PFOS is destroyed by combustion at high temperatures. The solubility of PFOS in pure water has been determined to be 519 and 680 mg/L at two separate testing laboratories. Solubility decreases significantly in seawater to about 12 mg/L. Relative to volatility, obtaining an air/water partition coefficient reading for PFOS has not been possible because the partitioning has been too small to measure. Therefore, due to low volatility, atmospheric dispersion of PFOS in a gaseous state 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 using traditional shake flask methods. Based on the solubility in pure water (680 mg/L) and the solubility in octanol (56 mg/L), the log K_{ow} was calculated to be –1.08. This number is not meaningful and is indicative of the unique characteristics of PFOS.

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

The results from environmental fate and transport testing indicate that PFOS is extremely stable under environmental conditions. The only known degradation mechanism is incineration at high temperatures.

The BCF study demonstrated preferential accumulation in non-edible tissues. Clearance from bluegills occurs at a rate approximately three times slower than it is taken up, with estimated half-lives of 86 – 116 days. Although PFOS has the ability to sorb to soils, sediments, or sludge, it is expected to be mobile in the aqueous phase at equilibrium.

3.1.3 Predicted Environmental Concentrations (PECs)

Monitoring data for PFOS are available from the Multi-City Study (3M Environmental Laboratory, 2001), the Global Biosphere Monitoring program (Giesy and Kannan, 2001, 2002; Kannan *et al*., 2001a,b; Kannan *et al.*, 2002a-d), and a study of the Tennessee River near Decatur, AL, where 3M has a manufacturing plant (Hansen et al., 2002; Entrix, Inc., 2003). Similar data from a study in Japan was recently reported (Taniyasu *et al.*, 2003), and these data are not shown. With the data from the Multi-City Study, Global Biosphere Monitoring program, and Tennessee River study 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 compared.

The sections below provide environmental concentration data for surface water, sediments, biota, and food from these various studies.

As the SIDS Manual indicates, predicted environmental concentration (PEC) should be derived based on monitoring data and/or outputs from exposure models. Extensive monitoring data are available for PFOS so that modeling is not necessary. Moreover, as explained in the previous section, modeling is problematic because of the physical/chemical properties of PFOS. In addition, the nature of exposure routes adds considerable complexity to the development of these models. Consequently, the analyses presented in this document are based on actual monitoring data, which are more reliable than modeling estimates. Thus, what the SIDS Manual refers to as predicted environmental concentrations are in this case actual concentrations.

3.1.3.1 Surface Waters

PFOS concentrations in surface waters were analyzed in samples from six cities in the Multi-City Study and in the Tennessee River study near Decatur, AL.

The Multi-City Study was designed to compare cities having manufacturing or commercial use of POSF-based products (test cities) with cities that did not (control cities). The test cities were Columbus, GA, Pensacola, FL, Mobile, AL, and Decatur AL. The control cities were Cleveland, TN and Port St. Lucie, FL. Descriptions of the sites and test data are presented in Table 3-3. For each sampling site in the initial study, two water samples were analyzed for PFOS concentration. Additional samples were taken at the Port St. Lucie site, a control city, in 2001 to evaluate unusually high PFOS concentrations determined in the quiet water site in the initial study.

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

Location	Sampling Site	No. of Sample Locations	PEC (ng PFOS/L) ppt[a]		
			Site Average	Site Maximum	Location Maximum
Columbus, GA Sampling locations near the Columbus Water Works Influent	Site 1	1	62	64	83
	Site 2	1	80	83	
	Site 3	1	55	55	
	Quiet Surface Water	1	<25	<25	
Pensacola, FL Sampling locations in Texas Bayou	Site 1	1	<25	<25	29
	Site 2	1	26	29	
	Site 3	1	<25	<25	
	Quiet Surface Water	1	<25	<25	
Mobile, AL Sampling locations near a customer's industrial wastewater treatment plant	Site 1	1	<25	<25	43
	Site 2	1	41	43	
	Site 3	1	36	36	
	Quiet Surface Water	1	32	33	
Decatur, AL Sampling locations upstream of the Decatur POTW effluent, downstream of water plant (Multi-City study)	Site 1	1	<25	<25	114
	Site 2	1	<25	<25	
	Site 3	1	<25	<25	
	Quiet Surface Water	1	111	114	
Cleveland, TN Sampling locations upstream of the Cleveland Municipal POTW	Site 1	1	<25	<25	<25
	Site 2	1	<25	<25	
	Site 3	1	<25	<25	
Port St. Lucie, FL Sampling locations in the vicinity of the Northport Wastewater Treatment Plant and Port St. Lucie landfill	Site 1	1	<25	<25	2930[b]
	Site 2	1	<25	<25	
	Site 3	1	<25	<25	
	Quiet Surface Water Site 1, 2000	1	2890	2930	
	Quiet Surface Water Site 1, 2001	5	1940	2340	
	Quiet Surface Water Site 2, 2001	1	<25	25	
	Quiet Surface Water, Site 3, 2001	1	<25	26	
Tennessee River near Decatur, AL Sampling locations: (Entrix 2001)	3M Outfall Area, Including Bakers Cr.	7	60970	156000	156000
	Fox Cr. Area	7	399	592	
	Guntersville Lake	6	<25	<25	
	Decatur WWTP Area	6	53	68	

[a] Limit of quantification is 25 ng/L.

[b] PFOS was initially detected at higher concentrations in the quiet water site in Port St. Lucie relative to concentrations at other sites (1999 study). Further investigation was undertaken and the results were determined to be inconsistent with later values. Therefore the 1999 results were not considered in this analysis.

The Tennessee River near Decatur, AL was sampled in June 2001, as part of a study to characterize PFOS concentrations in surface water, sediment, fish, and Asiatic clams near the 3M manufacturing facility (Hansen *et al*., 2002). Surface water and sediment samples were taken in the 3M Outfall Area, which includes Bakers Creek; in an area several river miles downstream

near Fox Creek; in Guntersville Lake, which is the Tennessee River reservoir immediately upstream of Decatur; and near the City of Decatur wastewater treatment plant. The Guntersville Lake site is considered a reference site. Values of the 2002 repeat study of the Tennessee River were lower than the 2001 values (Entrix, Inc., 2003).

Table 3-3 presents the results of the laboratory analyses. Site average and maximum values are presented for each site. The reporting limits (limits of quantification) are indicated in the table. The average and maximum values are used in the risk assessment analyses presented in Section 3.3. The highest concentrations of PFOS were found in the 3M Outfall Area (which includes Bakers Creek) at Decatur [156,000 ng/L or parts per trillion (ppt)]; and the quiet surface water at Port St. Lucie (2,930 ppt). The concentrations of PFOS at Cleveland, TN, were all less than the limit of quantification (25 ppt). Of those cities with average PFOS concentrations greater than the limit of quantification, Columbus, GA, had the second largest average PFOS concentration (83 ppt) while Pensacola, FL, had the smallest average PFOS concentration (29 ppt).

3.1.3.2 Sediments

PFOS concentrations in sediments were analyzed in samples from the Multi-City Study and in the Tennessee River study. Sediment sampling sites were co-located with the surface water sampling sites. At each Multi-City sampling site, two sediment samples were analyzed for PFOS concentration. For the Tennessee River study (Hansen *et al*., 2002), up to seven sediment samples were analyzed for PFOS in each sampling area.

Table 3-4 presents the results of the laboratory analyses. Site average and maximum values are presented for the Decatur, AL study sites, while only maximum values are presented for the Multi-City sites that were limited to two samples each. In the Tennessee River near Decatur, the largest concentration of PFOS was found in sediments from the 3M Outfall Area (13,300 ppb, dry weight, 2001 value). In the Multi-City Study, concentrations were generally below 1 ppb.

Sediment pore water concentrations of PFOS at each site were estimated by dividing the sediment partition coefficient, 7.42 L/kg (Table 3.2), into the bulk sediment concentration (Table 3-4). Except for the 3M Outfall Area, Fox Creek and Port St. Lucie sampling locations, the estimated pore water concentrations were less than 1 ppb. Because of the high degree of uncertainty in predicting pore water concentrations of PFOS from the sediment partition coefficient, these estimated pore water concentrations should be considered screening-level estimates.

Table 3-4. Environmental Concentrations (PECs) in Sediment and Estimated Maximum Sediment Pore Water Concentration (ppb) for PFOS in Sediments of the Tennessee River near Decatur, AL and From the Multi-City Study

Study	Sampling Site	No. of Sample Locations	PEC (µg PFOS/kg) ppb[a] in Sediment		Est. Max. Water Concentration (ppb)
			Site Average[b]	Site Maximum	
Multi-city	Columbus, GA	3	0.4	0.5	0.1
	Pensacola, FL	3	0.3	0.4	0.1
	Mobile, AL	3	0.5	0.7	0.1
	Decatur, AL	3	0.4	0.8	0.1
	Cleveland, TN	3	<0.2	<0.2	<0.03
	Port St. Lucie 1999	3	0.7	1.1	0.2
	Port St. Lucie 2000	3	<0.3	<0.3	<0.04
	Port St. Lucie Quiet Surface Water Site 1, 2001	4	10.7	14.3	1.9
	Port St. Lucie Quiet Surface Water Site 2, 2001	1	<0.3	<0.3	<0.04
	Port St. Lucie Quiet Surface Water Site 3, 2001	1	<0.3	<0.3	<0.04
Tennessee River near Decatur, AL (Hansen *et a*l., 2002)	3M Outfall Area, Including Bakers Cr. Area	7	2740	13300	1792
	Fox Cr. Area	7	3.5	9.1	1.2
	Guntersville Lake	6	<0.5	0.82	0.1
	Decatur WWTP Area	6	1.7	2.8	0.4

[a] Dry weight.
[b] The dry weight LOQ varies as a function of the percent moisture in the samples.

3.1.3.3 Biota

Concentrations of PFOS in biota are available from the Global Biosphere Monitoring program (Giesy and Kannan, 2001, 2002; Kannan *et al*., 2001a,b; Kannan *et al.*, 2002a-d) and the Tennessee River study (Entrix, Inc., 2003). The Global Biosphere Monitoring program was designed to assess the global distribution of PFOS. Over 1,200 samples of blood, liver, and other tissues were collected from archived specimens of a variety of species from several locations and analyzed for PFOS. Analyzed biota included fish, birds, freshwater mammals, marine mammals, and oysters. Areas of focus included North America (the Great Lakes and marine coast), the Arctic, Asia, and Europe. Analyses of these samples indicated that PFOS is present in the livers, blood, and other tissues of animals, especially in piscivorous (fish-eating) animals.

Tables 3-5 through 3-7 summarize the results of these analyses in liver, blood and other tissues, respectively. The tables present minimum, maximum, and mean values. The means were calculated using all data points, including those that were less than the limit of quantitation. The highest concentrations of PFOS in biota were found in bald eagle plasma and mink livers. Concentrations in other tissues and other species were lower, with a number of results below the limit of quantification.

Table 3-5. Environmental Concentrations (PECs) of PFOS in Liver Samples from Wildlife Species in the Biosphere Monitoring Program

Species	No. of Samples	Location of Maximum Concentration	PEC (µg PFOS/g) ppm (wet weight)		
			Minimum	Maximum	Mean[a]
Baikal Seal (*Pusa siberica*)	24	Lake Baikal, Russia	0.01	<0.03	0.02
Bald Eagle (*Haliaeetus leucocephalus*)	10	Michigan, USA	0.03	1.74	0.33
Black Crowned Night Heron (*Nycticorax nycticorax*)	5	San Diego, CA	<0.08[b]	0.75	0.46
Black Footed Albatross (*Diomedea nigripes*)	5	Sand Is., Midway Atoll	<0.03[b]	<0.03[b]	<0.03[b]
Black Tailed Gull (*Larus crassirostris*)	15	Korea	0.07	0.50	0.17
Black-headed Gull (*Larus ridibundus*)	1	Japan	<0.02[b]	<0.02[b]	<0.02[b]
Bottlenose Dolphin (*Tursiops truncatus*)	26	Sarasota Bay, FL	0.01	1.52	0.42
Brown Pelican (*Pelecanus occidentalis*)	3	East Pascagoula R., MS	0.14	0.62	0.35
Brown Trout (*Salmo trutta*)	10	Great Lakes/Inland Lakes, MI	<0.02[b]	0.03	0.02
California Sealion (*Zalophus californianus*)	6	California Coast	0.01	0.05	0.02
Carp (*Cyprinus carpio*)	4	Saginaw Bay, MI	<0.02[b]	0.03	0.02
Chicken (*Gallus domesticus*)	3	MI, USA	<0.008[b]	<0.008[b]	<0.008[b]
Chinook Salmon (*Oncorhynchus tshawytscha*)	6	Great Lakes, MI	0.03	0.17	0.11

Species	No. of Samples	Location of Maximum Concentration	PEC (μg PFOS/g) ppm (wet weight)		
			Minimum	Maximum	Mean[a]
Clymene Dolphin (*Stenella clymene*)	3	Gulf of Mexico	0.08	0.17	0.12
Common Dolphin (*Delphinus delphis*)	1	Italy	0.94	0.94	0.94
Common Loon (*Gavia immer*)	19	Carteret County, NC	<0.03[b]	0.69	0.17
Cormorant (*Phalacrocorax carbo*)	12	Mediterranean Sea	0.03	0.47	0.10
Elephant Seal (*Mirounga augustirostris*)	5	California Coast	<0.004[b]	0.03	0.02
Fish (Perciformes)	40	Michigan Inland Lakes	<0.004[b]	0.12	0.04
Franklins Gull (*Larus pipixcan*)	4	Red Rock Lakes, MT	<0.08[b]	<0.08[b]	<0.08[b]
Ganges Dolphin (*Platanista gangetica*)	2	River Ganges, India	<0.03[b]	0.08	0.06
Great Black Backed Gull (*Larus marinus*)	2	Carteret County, NC	0.22	0.97	0.60
Great Egret (*Ardea alba*)	7	Naples, FL	<0.08[b]	1.19	0.47
Harbor Seal (*Phoca vitulina*)	3	California Coast	<0.004[b]	0.06	0.03
Herring Gull (*Larus argentatus*)	5	Carteret County, NC	<0.08[b]	0.41	0.23
Lake Whitefish (*Coregonus clupeaformis*)	5	Great Lakes/Inland Lakes, MI	0.03	0.08	0.07
Laysan Albatross (*Diomedea immutabilis*)	3	Sand Is., Midway Atoll	<0.03[b]	<0.03[b]	<0.03[b]
Mink (*Mustela vison*)	77	Illinois	0.04	4.87	1.22
Northern Fur Seal (*Callorhinus ursinus*)	14	Coastal Alaska	<0.03[b]	0.13	0.05
Northern Gannett (*Sula bassanus*)	1	Sacramento Valley, CA	0.10	0.10	0.10
Osprey *(Pandion haliaetus*)	4	Tavernier, FL	<0.08[b]	1.11	0.44
Pilot Whale (*Globicephala melaena*)	1	Italy	0.27	0.27	0.27
Poland Burbot (*Lota lota*)	1	Poland	<0.02[b]	<0.02[b]	<0.02[b]
Polar Bear (*Ursus maritimus*)	31	Alaska	0.08	0.68	0.33
Pygmy Sperm Whale (*Kogia breviceps*)	2	Atlantic Ocean	<0.08[b]	<0.08[b]	<0.08[b]
Red Throated Loon (*Gavia stellata*)	3	Carteret County, NC	<0.08[b]	1.29	0.69
Ringed Seal (*Phoca hispida*)	81	Baltic Sea	0.03	1.10	0.24
River Otter (*Lutra canadensis*)	5	WA/OR Rivers	0.03	0.99	0.33
Rough-toothed Dolphin	2	Tampa Bay	<0.08[b]	<0.08[b]	<0.08[b]

Species	No. of Samples	Location of Maximum Concentration	PEC (μg PFOS/g) ppm (wet weight)		
			Minimum	Maximum	Mean[a]
(*Steno bredanensis*)					
Salmon - Baltic/Finnish (*Salmo salar*)	22	Baltic Sea, Finnish Coast	<0.008[b]	<0.008[b]	<0.008[b]
Sea Eagle (*Haliaeetus albicilla*)	44	Baltic Sea/Germany	<0.003[b]	0.13	0.04
Sea Gull (*Larus sp.*)	28	Japan	<0.02[b]	0.23	0.05
Sea Otter (*Enhydra lutris nereis*)	8	California Coast	<0.004[b]	0.01	<0.01
Skua (*Catharacta sp.*)	1	Antarctica	<0.02[b]	<0.02[b]	<0.02[b]
Snowy Egret (*Egretta thula*)	3	Naples, FL	<0.08[b]	0.48	0.22
Striped Dolphin (*Stenella coeruleoalba*)	6	Venice Inlet	0.07	0.39	0.15
Swordfish (*Xiphias gladius*)	5	Mediterranean Sea	<0.007[b]	0.01	0.01
Tunafish (Thunnus sp.)	8	Mediterranean Sea	0.02	0.09	0.05
Weddell Seal (*Leptonychotes weddellii*)	1	Antarctica	<0.03[b]	<0.03[b]	<0.03[b]
White Faced Ibis (*Plegadus chihi*)	1	Sacramento Valley, CA	<0.08[b]	<0.08[b]	<0.08[b]
White Pelican (P*elecanus erythrorhynchos*)	4	Delevan National Wildlife Refuge, CA	0.08	0.23	0.14
Wood Stork (*Mycteria americana*)	1	Charleston County, SC	0.18	0.18	0.18
Yellowfin Tuna (*Thunnus albacares*)	12	North Pacific Ocean	<0.07[b]	<0.07[b]	<0.07[b]

[a] Mean concentrations were calculated using all data points. The limit of quantitation was used for those values that were less than the LOQ.
[b] Limit of quantitation for the specific analysis.

Table 3-6. Environmental Concentrations (PECs) of PFOS in Blood, Plasma, and Serum Samples from Wildlife Species in the Biosphere Monitoring Program

Species	No. of Samples	Location of Maximum Concentration	PEC ppm		
			Minimum	Maximum	Mean[b]
Bald Eagle (*Haliaeetus leucocephalus*)	42	Pine River, Wisconsin	<0.012[a]	2.57	0.52
Black Footed Albatross (*Diomedea nigripes*)	7	Sand Is., Midway Atoll	0.003	0.02	0.01
Black Tailed Gull (*Larus crassirostris*)	24	Hokkaido, Japan	<0.002[a]	0.01	0.01
Bluefin Tuna (*Thunnus thynnus*)	6	Italian Coast	0.03	0.05	0.04
Bottlenose Dolphin (*Tursiops truncatus*)	4	Italian Coast	0.04	0.21	0.14
Caspian Seal (*Pusa caspica*)	8	Caspian Sea, Russia	<0.012[a]	<0.012[a]	<0.012[a]
Cormorant (*Phalacrocorax carbo*)	22	India	<0.003[a]	<0.003[a]	<0.003[a]
Double Crested Cormorant (*Phalacrocorax auritus*)	12	Little Charity Is., Lake Huron	0.04	0.43	0.17
Grey Seal (*Halichoerus grypus*)	38	Baltic Sea	0.01	0.08	0.04
Herring Gull (*Larus argentatus*)	4	Little Charity Is., Lake Huron	0.07	0.45	0.22
Laysan Albatross (*Diomedea immutabilis*)	6	Sulphur Is., Great Lakes	0.01	0.04	0.02
Northern Fur Seal (*Callorhinus ursinus*)	44	Coastal Alaska	<0.006[a]	<0.006[a]	<0.006[a]
Polar Bear (*Ursus maritimus*)	14	Alaska	<0.003[a]	0.05	0.03
Ringed Seal (*Phoca hispida*)	70	Baltic Sea	<0.003[a]	0.48	0.07
Sea Eagle (*Haliaeetus albicilla*)	58	Japan	<0.003[a]	0.02	0.003
Stellar Sea Lion (*Eumetopias jubatas*)	12	Coastal Alaska	<0.006[a]	<0.006[a]	<0.006[a]
Swordfish (*Xiphias gladius*)	7	Italian Coast	0.004	0.02	0.01

[a] Limit of quantitation for the specific analysis.

[b] Mean concentrations calculated using all data points. The limit of quantitation was used for those values that were less than the LOQ.

Table 3-7. Environmental Concentrations (PECs) of PFOS in Other Tissue Samples from Wildlife Species in the Biosphere Monitoring Program

Species	Tissue	No. of Samples	Location of Maximum Concentration	PEC ppm (wet weight)		
				Minimum	Maximum	Mean[a]
Adelie Penguin (*Pygoscelis adeliae*)	Whole Egg[b]	10	Antarctica	<0.008[c]	<0.008[c]	<0.008[c]
American Oyster (*Crassostrea virginica*)	Whole Body	77	Chesapeake Bay	0.01	0.10	0.06
Bald Eagle (*Haliaeetus leucocephalus*)	Muscle	6	Michigan, USA	0.01	0.10	0.04
Black Footed Albatross (*Diomedea nigripes*)	Kidney	4	Midway Atoll	0.03	0.03	0.03
Brown Trout (*Salmo trutta*)	Eggs	3	Great Lakes, MI	0.05	0.08	0.06
	Muscle	10	Great Lakes, MI	0.01	0.05	0.01
Carp (*Cyprinus carpio*)	Muscle	10	Saginaw Bay, MI	0.06	0.30	0.12
Caspian Tern (*Sterna caspia*)	Whole Egg[b]	4	Michigan, USA	1.91	3.35	2.61
Chicken (*Gallus domesticus*)	Whole Egg[b]	3	Michigan, USA	<0.008[c]	<0.008[c]	<0.008[c]
Chinook Salmon (*Oncorhynchus tshawytscha*)	Muscle	6	Great Lakes, MI	<0.007[c]	0.19	0.09
Common Dolphin (*Delphinus delphis*)	Muscle	1	Italy	0.08	0.08	0.08
Double Crested Cormorant (*Phalacrocorax auritus*)	Whole Egg[b]	6	Michigan, USA	0.57	1.81	1.26
	Yolk	4	Lake Winnipeg, MB	0.03	0.32	0.18
Fin Whales (*Balaenoptera physalus*)	Muscle	1	Italy	<0.02[c]	<0.02[c]	<0.02[c]
Fish (*Perciformes*)	Eggs	19	Michigan Inland Lakes	<0.004[c]	0.22	0.05
	Muscle	172	Belgium	<0.004[c]	0.92	0.04
	Testes	5	Michigan Inland Lakes	<0.004[c]	<0.008[c]	<0.008[c]
Herring Gull (*Larus argentatus*)	Whole Egg[b]	1	Michigan, USA	0.36	0.36	0.36
Krill (*Euphausia superba*)	Whole body	1	Antarctica	<0.02[c]	<0.02[c]	<0.02[c]
Lake Whitefish (*Coregonus clupeaformis*)	Eggs	2	Great Lakes, MI	0.15	0.38	0.26
	Muscle	5	Great Lakes, MI	0.10	0.17	0.13
Laysan Albatross (*Diomedea immutabilis*)	Kidney	3	Midway Atoll	<0.03[c]	<0.03[c]	<0.03[c]
Pilot Whale (*Globicephala melaena*)	Muscle	1	Italy	0.05	0.05	0.05
Ring Billed Gull (*Larus delawarensis*)	Yolk	3	Sulphur Is., Great Lakes	<0.03[c]	0.15	0.08
Sea Otter (*Enhydra lutris nereis*)	Brain	2	California coast	<0.004[c]	<0.004[c]	<0.004[c]
	Kidney	3	California coast	<0.004[c]	<0.004[c]	<0.004[c]
Sharp Spined Notothen (*Trematomus pennellii*)	Whole body	2	Antarctica	<0.02[c]	<0.02[c]	<0.02[c]
Silver Fish (*Pleuragramma antarcticum*)	Whole body	3	Antarctica	<0.02[c]	<0.02[c]	<0.02[c]
Skua (*Catharacta sp.*)	Heart	1	Antarctica	<0.02[c]	<0.02[c]	<0.02[c]
	Kidney	1	Antarctica	<0.02[c]	<0.02[c]	<0.02[c]
	Whole Egg[b]	1	Antarctica	<0.008[c]	<0.008[c]	<0.008[c]
Tree Swallow (*Iridoprocne bicolor*)	Whole Egg[b]	5	Michigan, USA	0.05	0.10	0.07

[a] Mean concentrations calculated using all data points. The limit of quantitation was used for those values that were less than the LOQ.
[b] Homogenate.
[c] Limit of quantitation for the specific analysis.

The Decatur study evaluated the concentration of PFOS in fish and Asiatic clams from the 3M Outfall Area and Guntersville Lake, which is considered a reference site (Table 3-8). The study was designed to maximize the chance of identifying PFOS in a variety of organisms. The study was not designed to obtain a large amount of information for any individual species. PFOS concentrations were considerably higher in fish, but not in clams, collected from the 3M Outfall Area compared with the reference site. The maximum PFOS concentrations in fish and clams were 3.05 ppm and 0.02 ppm, respectively.

Table 3-8. Environmental Concentrations (PECs) of PFOS in Whole Body Fish and Clams From the Tennessee River Near Decatur, AL

Species	No. of Samples	PEC (µg PFOS/g) ppm (wet wt)		
		Minimum	Maximum	Mean
Asiatic Clams (*Corbicula fluminea*)	2	0.01	0.02	0.02
Channel Catfish (*Ictalurus punctatus*)	4	0.01	1.31	0.60
Gar (*Lepisosteus sp.*)	6	0.01	3.05	0.63
Largemouth Bass (*Micropterus salmoides*)	1	0.23	0.23	0.23
Striped Bass (*Morone saxatilis*)	14	0.03	0.11	0.07

3.1.3.4 Market Basket Survey

The market basket survey was conducted to provide an estimate of the potential exposure levels of PFOS to humans from the food supply in each of the six multi-city-study cities. The samples collected consisted of chicken, pork, hot dogs, fish, eggs, green beans, whole milk, apples, bread, and ground beef obtained from three local grocery stores in each of the six cities. Samples were extracted in duplicate and analyzed for PFOS. Of the 180 samples analyzed in duplicate (360 analyses) in this study, PFOS levels were either below the limit of detection or the limit of quantification (0.5 ng/g or ppb) in 354 analyses. Four of the six analyses with quantifiable PFOS concentrations were very close to the limit of quantification (range 0.573 – 0.852 ng/g or ppb). The other two quantifiable PFOS concentrations were from one ground beef sample (0.570 and 0.587 ng/g). Based on these data, diet would not appear to be a significant source of PFOS exposure to humans.

3.2 Ecotoxicological Data

A large body of ecotoxicological data, generated over a period of more than 20 years, exists for various salts of PFOS. Until recently, however, definitive information on the purity of the PFOS tested was not available and validated analytical methodology did not exist to measure exposure concentrations. Data generated before 1998, therefore, are somewhat unreliable because the actual substance(s) and concentrations to which the test organisms were exposed is not known.

The potassium salt of PFOS was chosen for laboratory study because potassium is the most ecotoxicologically understood cation of all the PFOS salts produced by 3M. Additionally, the commercially prepared potassium salt product was available as the neat salt. The lithium,

ammonium, diethanolamine, and didecyldimethylammonium salts used for testing in the past were mixtures containing only 25 – 35% fluorochemical solids. Finally, PFOS potassium salt was produced in higher quantities than the other salts. For example, in 1997, PFOS potassium salt accounted for > 45% of all PFOS salts produced.

Ecotoxicology studies utilizing a well-characterized sample of PFOS potassium salt were conducted between 1998 and 2001. These studies were all conducted in accordance with USEPA and/or OECD Good Laboratory Practices. Where feasible, test concentrations or feed were analyzed for PFOS anion, and results were expressed based on the mean measured concentration during exposure. This assessment focuses on these recent studies except for a few cases where more recently generated data were not available for certain species. The results of the studies are found in Table 3-9 and then discussed below. The studies are summarized in RS-II-1 – RS-II-35 in Appendix II.

Table 3-9. Results from Ecotoxicology Testing on PFOS

Parameter	Result[a]	Units
Wastewater bacteria (OECD 209)		
3-hr EC_{50}	>905[b]	mg/L
Inhibition at highest concentration tested (905 mg/L) = 39%		
***Selenastrum capricornutum* (freshwater green algae)**		
96-hr NOEC (growth rate)	44	mg/L
96-hr ErC_{10} (95% confidence interval)	59 (54 – 63)	mg/L
96-hr ErC_{50} (95% confidence interval)	126 (115 – 138)	mg/L
***Anabaena flos-aquae* (freshwater blue-green algae)**		
96-hr NOEC (growth rate)	94	mg/L
96-hr ErC_{10} (95% confidence interval)	109 (84 – 125)	mg/L
96-hr ErC_{50} (95% confidence interval)	176 (169 – 181)	mg/L
***Naviculla pelliculosa* (freshwater diatom)**		
96-hr NOEC (growth rate)	206	mg/L
96-hr ErC_{10} (95% confidence interval)	243 (209 – 295)	mg/L
96-hr ErC_{50} (95% confidence interval)	305 (295 – 316)	mg/L
***Skeletonema costatum* (marine diatom)**		
96-hr NOEC (growth rate)	>3.20	mg/L
96-hr ErC_{10} (95% confidence interval)	>3.20	mg/L
96-hr ErC_{50} (95% confidence interval)	>3.20	mg/L
Solubility limits precluded obtaining EC_{50} values. No inhib. at 3.2 mg/L		
***Lemna gibba* (duckweed)**		
7-day NOEC (number of fronds)	15	mg/L
7-day IC_{10} (95% confidence interval)	22 (13 – 26)	mg/L
7-day IC_{50} (95% confidence interval)	108 (46 – 144)	mg/L
***Apis mellifera L.* (honey bee)**		
Acute 96-hr Contact NOEL	1.93[b]	µg/bee
Acute 96-hr Contact LD_{50} (95% confidence interval)	4.78 (3.8 – 5.8)	µg/bee
Acute 72-hr Oral NOEL	0.21[b]	µg/bee
Acute 72-hr Oral LD_{50} (95% confidence interval)	0.40 (0.33 – 0.48)[b]	µg/bee
***Daphnia magna* (freshwater water flea)**		
Acute 48-hr NOEC	33	mg/L
Acute 48-hr EC_{10} (95% confidence interval)	53 (<11 – >91)	mg/L
Acute 48-hr EC_{50} (95% confidence interval)	61 (33 – 91)	mg/L

Parameter	Result[a]	Units
Acute 48-hr EC_{90} (95% confidence interval)	63 (<11 – >91)	mg/L
21-day Semi-static Life-cycle Test NOEC	12	mg/L
21-day Semi-static Life-cycle Test LOEC	24	mg/L
Mysidopsis bahia **(marine shrimp)**		
Acute 96-hr NOEC	1.1	mg/L
Acute 96-hr EC_{50} (95% confidence interval)	3.6 (3.0 – 4.6)	mg/L
35-day Flow-through Life-cycle Test NOEC	0.25	mg/L
35-day Flow-through Life-cycle Test LOEC	0.55	mg/L
Artemia salina **(brine shrimp)**		
Acute 48-hr LC_{50} (95% confidence interval)	8.93[c]	mg/L
Unio complamatus **(freshwater mussel)**		
Acute 96-hr NOEC	20	mg/L
Acute 96-hr LC_{50} (95% confidence interval)	59 (51 – 68)	mg/L
Crassostrea virginica **Shell Deposition (eastern oyster)**		
Acute 96-hr NOEC	1.9	mg/L
Acute 96-hr EC_{50}	>3.0	mg/L
Solubility limits precluded obtaining EC_{50} value.		
Inhibition at highest concentration tested (3.0 mg/L) = 28%		
Eisenia fetida **(Earthworm) in Artificial Soil Substrate**		
Acute 7-day NOEC	289	mg/kg
Acute 7-day LC_{50}	398	mg/kg
Acute 14-day NOEC	77	mg/kg
Acute 14-day LC_{50}	373	mg/kg
Xenopus laevis (**frog) Embryo Teratogenesis Assay (FETAX)**		
Acute 96-hr LC_{50} (range for 3 trials)	13.8 – 17.6	mg/L
Acute 96-hr EC_{50} (range for 3 trials)	12.1 – 17.6	mg/L
Minimum Concentration to Inhibit Growth (MCIG) (range for 3 trials)	7.97 – 8.26	mg/L
Teratogenic Index (TI) (range for 3 trials)	0.9 – 1.1	mg/L
Pimephales promelas **(fathead minnow)**		
Acute 96-hr NOEC	3.3	mg/L
Acute 96-hr LC_{50} (95% confidence interval)	9.5 (8.0 – 11)	mg/L
5-day Early Life Stage Flow-through Toxicity Test Hatchability NOEC	4.6	mg/L
47-day Early Life Stage Flow-through Toxicity Test Survival & Growth NOEC	0.30	mg/L
47-day Early Life Stage Flow-through Toxicity Test Survival & Growth LOEC	0.60	mg/L
Lepomis macrochirus **(bluegill sunfish)**		
Acute 96-hr LC_{50}	68[c]	mg/L
Oncorhynchus mykiss **(rainbow trout)**		
Acute 96-hr NOEC, freshwater exposure	6.3	mg/L
Acute 96-hr LC_{50}, freshwater exposure (95% confidence interval)	22 (18 – 27)	mg/L
Acute 96-hr LC_{50}, saltwater exposure (95% confidence interval)	13.7 (10.7 – 17.8)[c]	mg/L
Cyprinodon variegatus **(sheepshead minnow)**		
Acute 96-hr NOEC (1/30 fish discolored at 96-hours)	< 15	mg/L
Acute 96-hr LC_{50}	> 15	mg/L
Anas platyrhynchos (**mallard duck) Dietary Exposure**		
Acute 5-day NOEC (weight loss)	37[b]	mg/kg
Acute 5-day no mortality concentration	146[b]	mg/kg
Acute 5-day LC_{50} (95% confidence interval)	628 (430 – 919) [a]	mg/kg

Parameter	Result[a]	Units
***Colinus virginianus* (bobwhite quail) Dietary Exposure**		
Acute 5-day NOEC	73[b]	mg/kg
Acute 5-day no mortality concentration	73[b]	mg/kg
Acute 5-day LC_{50} (95% confidence interval)	220 (158 – 278)[b]	mg/kg

[a]All values calculated as mean measured concentrations, except where noted.
[b]Results based on nominal concentrations; sample well-characterized.
[c]Results based on nominal concentrations using sample of uncharacterized purity. (Pre 1998 studies)

3.2.1.1 Effects on Microbial Systems

Microbes from a municipal wastewater treatment plant were evaluated for toxic effects to PFOS.

After 3 hours of exposure, the highest concentration tested, 905 mg/L, induced 39% inhibition in respiration rate. It should be noted that this test concentration is greater than the water solubility limit (RS-II-1).

3.2.1.2 Algae and Diatoms

Four species were tested, including freshwater algae (*Selenastrum capricornutum* and *Anabaena flos-aquae*), a freshwater diatom (*Navicula pelliculosa*) and a marine diatom (*Skeletonema costatum*) (RS-II-2, RS-II-3, RS-II-4, RS-II-5). The NOEC and EC_{50} values were calculated using three methods to determine inhibition: cell density, area under the growth curve and average specific growth rate. Three of the species, *S. capricornutum, A. flos-aquae,* and *N. pelliculosa*, demonstrated a concentration-response relationship for growth. The marine diatom, *S. costatum*, was not affected by PFOS at the highest concentration attainable in the media (3.2 mg/L). This study was conducted at the apparent solubility limit in a marine algal medium.

Calculations utilizing cell density and area under the curve resulted in lower effective concentrations than those using average specific growth rate. The effects on all three species were determined to be algistatic (growth resumed when aliquots of the algae in the maximally inhibited concentrations were placed in fresh growth media). Observations of algae and diatom cells during the studies found that there were no signs of aggregation or adherence of the cells to the flasks and there were no noticeable changes in cell morphology after exposure. Since the rate of growth, and not cell mortality appeared to be affected in these studies, evaluation of ecological effects on algae will be confined to the NOEC and EC_{50} values calculated using the average specific growth rate. *S. capricornutum* was the most sensitive algal species studied, with a 96-hour EC_{50} value of 121 mg/L and an NOEC of 42 mg/L.

3.2.1.3 Freshwater Higher Plants

Testing with duckweed (*Lemna gibba*) resulted in a 7-day IC_{50} value of 108 mg/L and an NOEC, based on the number of fronds produced and evidence of sublethal effects, of 15 mg/L (RS-II-6). The sublethal effects noted included root destruction and/or a cupping of the plant downward on the water surface. A recovery period was not evaluated in the duckweed study.

3.2.1.4 Acute Toxicity to Aquatic Invertebrates

Five aquatic invertebrates were evaluated for acute toxicity: two freshwater and three marine species. The freshwater species studied were the water flea (*Daphnia magna*) (RS-II-7, RS-II-8, RS-II-9, RS-II-10) and the mussel (*Unio complamatus*) (RS-II-11, RS-II-12). Marine organisms evaluated were the brine shrimp (*Artemia salina*) (RS-II-13), the mysid shrimp (*Mysidopsis bahia*) (RS-II-14) and the oyster (*Crassostrea virginica*) (RS-II-15). The marine organisms appeared to be more sensitive than the freshwater. Daphnid and mussel EC_{50} values were 61 and 59 mg/L, respectively, while mysid and brine shrimp values were 3.6 and 8.9 mg/L. The brine shrimp value was determined using an uncharacterized sample of unknown purity and the exposure concentrations were not measured. Although there appeared to be a concentration-response relationship in the brine shrimp study, it may have been conducted in excess of PFOS saltwater solubility. The oyster shell deposition EC_{50} could not be determined due to solubility limitations in the unfiltered seawater used in the test. There was an apparent concentration-response relationship with shell growth. The highest concentration tested in oysters, 3.0 mg/L, inhibited shell growth by 28%. NOEC values for aquatic invertebrates, where determined, were 33 mg/L (daphnid), 20 mg/L (mussel), 1.9 mg/L (oyster), and 1.1 mg/L (mysid). Of the five species evaluated, the mysid shrimp was found to be the most sensitive.

The tissues from the freshwater mussels utilized in the acute toxicity study were analyzed for PFOS content. A subset of individuals from each exposure group was removed from their shells, and the entire body was homogenized. A sample of each homogenate was then extracted for analysis. The results obtained are shown in Table 3-10. These data show that PFOS was present in the tissues of *U. complamatus* following 96 hours of exposure. No mortality was associated with tissue concentrations of ≤ 7.3 ppm.

Table 3-10. Analytical Results of PFOS Concentrations Measured in Freshwater Mussel Tissues After 96-Hours Exposure

Mean Measured Exposure Conc., mg/L	Percent Mortality After 96-hours	Mean Measured Tissue Conc., ug/g (ppm) (wet weight)
Negative Control	0	< Limit of quantitation (0.0174)
5.3	0	3.69
12	0	5.22
20	0	7.33
41	5	11.85
79	90	88.8

3.2.1.5 Chronic Toxicity to Aquatic Invertebrates

Survival, growth and reproduction toxicity studies were conducted on one freshwater invertebrate (*Daphnia magna)* (RS-II-16) and one marine invertebrate (*Mysidopsis bahia*) (RS-II-17). There were no adverse effects on survival, reproduction or growth of *D. magna* at

concentrations ≤ 12 mg/L for 21 days (NOEC = 12 mg/L). No sublethal effects, including immobilization of adults, were noted in the study. The only significant endpoint was mortality. The 35-day mysid shrimp reproduction and growth study did demonstrate sublethal effects in addition to mortality. Mysid shrimp exposed to 1.3 and 2.6 mg/L had significantly reduced survival. Those exposed to 0.55 mg/L had significantly reduced reproduction, length and dry weight in comparison to the negative control. Consequently, the NOEC was determined to be 0.25 mg/L. The mysid shrimp were more sensitive than the daphnids.

In the course of these two studies, the young produced (second generation) were removed from the test chambers and briefly exposed to the same concentrations to which the respective first-generation adults were exposed. Survival was monitored for 48 hours (*D. magna*) or 96 hours (*M. bahia*). Second generation daphnids were obtained on Day 14. After 48 hours of exposure, survival in the negative control, 1.4, 2.9, 5.7, 11, and 23 mg/L treatment groups was 95, 100, 100, 100, 90, and 0%, respectively. The results of the daphnid second generation acute exposure indicated an NOEC of 11 mg/L.

After each observation period in which young were present, second-generation mysid shrimp were removed from the negative control, 0.057, 0.12, 0.25 and 0.55 mg/L solutions, and exposed to these test concentrations. Survival after 96 hours was ≥ 95% for all second-generation mysids exposed to these test concentrations. The mysid second-generation acute exposure NOEC was 0.55 mg/L.

3.2.1.6 Acute Toxicity to Fish

Four species of fish were evaluated: fathead minnow (*Pimephales promelas*), sheepshead minnow (*Cyprinodon variegatus*), bluegill sunfish (*Lepomis macrochirus*), and freshwater and marine rainbow trout (*Oncorhynchus mykiss*) (RS-II-18, RS-II-19, RS-II-20, RS-II-21, RS-II-22, RS-II-23, RS-II-24, RS-II-25, RS-II-26). Of the freshwater exposures, fathead minnow was the most sensitive with an LC_{50} of 9.5 mg/L and an NOEC of 3.3 mg/L. The rainbow trout freshwater LC_{50} and NOEC values were 22 and 6.3 mg/L, respectively. The sheepshead minnow study was conducted at only one concentration, 15 mg/L. This was the highest concentration attainable in salt water and required the addition of methanol (0.5 mL/L). There was no mortality observed at this concentration after 96 hours, however, one fish out of 30 was noted as discolored at the end of the exposure period. The 96-hour LC_{50} was reported as >15 mg/L, and the NOEC for sublethal effects as <15 mg/L. The bluegill study was not conducted utilizing a well-characterized sample or with measured concentrations. An LC_{50} of 68 mg/L was reported in the bluegill study. A sublethal effect, erratic swimming, was noted in the fathead minnow study at all concentrations ≥ 5.6 mg/L after 96 hours of exposure. No sublethal effects were observed in the freshwater rainbow trout study or the bluegill study.

The rainbow trout study, conducted with salt-water acclimated fish in 1986, demonstrated a similar sensitivity to PFOS potassium salt as the recent (2002) freshwater study, with an LC_{50} of 13.7 mg/L reported for the saltwater study (versus 22 mg/L for fresh water). No sublethal effects were observed. The 1986 study was not conducted utilizing a well-characterized sample or with measured concentrations. Freshwater rainbow trout were acclimated over 5 days to a final salinity of 30 parts per thousand. It should be noted that although there was an apparent concentration-response relationship in this study, the study appears to have been conducted in

excess of PFOS potassium salt saltwater solubility and thus, the effect values calculated using nominal concentrations may not be reliable.

3.2.1.7 Chronic Toxicity to Fish

Chronic data are available on two species of fish: the fathead minnow (*Pimephales promelas*) and the bluegill sunfish (*Lepomis macrochirus*) (RS-II-27, RS-II-28, RS-II-29). The fathead minnow study exposed fish from an early life stage (eggs through larvae) to PFOS potassium salt for 47 days. Survival data were obtained for bluegill exposed in a bioconcentration study during the 62 days of the uptake phase.

The NOEC derived from the 47-day fathead minnow early-life stage study was 0.30 mg/L. Fish exposed to PFOS at concentrations $\leq$ 0.30 mg/L in this study showed no significant reduction in time to hatch, hatching success, survival or growth. The most sensitive endpoint in this study was survival. There was no statistically significant difference in hatching success at any PFOS concentration tested. There was a statistically significant reduction in post hatch survival, but there were no apparent differences in growth of the surviving larvae.

Juvenile bluegill sunfish, approximately 7 months old, were exposed to PFOS at a concentration of 0.87 mg/L for 35 days and 0.086 mg/L for 62 days during the uptake phase of a bioconcentration study (Figure 3-1). The fish at 0.87 mg/L had all either been sampled or died by 35 days. Only two of the 90 fish (2.2%) exposed to 0.086 mg/L died during the uptake phase. All other fish at this concentration appeared to be normal and healthy throughout the study. This demonstrated a PFOS 62-day NOEC for bluegill survival of 0.086 mg/L. The mean concentrations of PFOS in edible tissues, nonedible tissues and whole fish after 62 days of uptake at 0.086 mg PFOS/L were 48, 104, and 81 mg/kg, respectively. The mean concentrations of PFOS in edible tissues, nonedible tissues and whole fish after 28 days of uptake at 0.87 mg PFOS/L were 120, 340, and 240 mg/L, respectively.

3.2.1.8 Amphibians

A study was conducted to determine the possible developmental effects of PFOS potassium salt on the African clawed frog *(Xenopus laevis*) (RS-II-30). The study indicated low potential for development effects. This Frog Embryo Teratogenesis Assay-Xenopus (FETAX) study evaluated survival, growth and malformations of frog embryos in three replicate assays. Exposure to PFOS caused significant embryo mortality at concentrations $\geq$ 14.4 mg/L. Mortality appeared to be caused by the gut coiling through the body wall. The 96-hour LC_{50} values ranged from 13.8 – 17.6 mg/L.

There was a correlation between PFOS exposure and malformations in each of the three assays. The most common malformations observed were improper gut coiling, edema, notochord abnormalities and facial abnormalities. The 96-hour malformation EC_{50} values ranged from 12.1 – 17.6 mg/L.

Growth of the embryos was not affected by exposure in the first assay. In the second and third assays, the minimum concentration inhibiting growth (MCIG) values were 7.97 and 8.64 mg/L. The reduction in growth may be attributed to the increased incidence of malformed embryos rather than to an overall decrease in growth.

The teratogenic index (TI), defined as the 96-hour LC_{50} divided by the 96-hour EC_{50} for malformations, provides an estimate of the teratogenic risk associated with PFOS. The TIs for the three studies ranged from 0.9 – 1.1. A TI in this range would indicate that exposure to PFOS presents a low risk for developmental effects.

3.2.1.9 Terrestrial Invertebrates

Acute toxicity evaluations were conducted using the honeybee (*Apis mellifera*) and the earthworm (*Eisenia fetida*) (RS-II-31, RS-II-32, RS-II-33).

Both an acute oral and acute contact exposure study were conducted on honeybees (*Apis mellifera*). In the oral study, there was no mortality at the mean intake of 0.21 µg/bee but a steep dose-response was noted between mean intakes of 0.45 and 2.2 µg/bee (NOEC = 0.21 µg/bee). Sublethal effects were observed as knockdown at the highest dose level (4.78 µg/bee) at four hours, with those individuals dying after 24 hours. The resulting 72-hour LD_{50} of 0.40 µg/bee would be classified as highly toxic by the International Commission for Bee Botany (ICBB). If the LD_{50} is converted to dose per kg food, the result is 2.0 mg PFOS/kg sugar solution.

The contact study found no mortality at 1.93 µg/bee but a steep dose-response between 4.24 and 9.30 µg/bee (NOEL = 1.93 µg/bee). No dose-related sublethal effects were observed at any dose level. The 96-hour LD_{50} was 4.78 µg/bee, which is classified as moderately toxic by contact according to the ICBB.

PFOS was incorporated into an artificial soil substrate at five different concentrations and adult earthworms (*Eisenia fetida*) were then exposed to the soil for 14 days. The 14-day LC_{50} and associated 95% confidence interval were determined to be 373 (316 – 440) mg PFOS/kg soil. The worms were also evaluated for burrowing behavior (Days 0 and 7) and body weight (Day 14). At the two highest concentrations tested, 488 and 1042 mg PFOS/kg soil, the worms had significant mortality, aversion to burrowing into the soil, and significant losses of body weight. The 14-Day NOEC, based on survival, was determined to be 77 mg/kg soil. On Day 14, following observations and body weight determination, all surviving worms from each treatment group were placed on moistened filter paper and allowed to purge their gut contents for approximately 24 hours. The entire mass of worms from each treatment group was prepared and then sampled for analysis of PFOS. Table 3-11 below presents the results from the analysis of earthworm tissues. These data show that PFOS was present in the tissues of the earthworm following 14 days of exposure. Mortality or burrowing behavior effects or weight loss did not occur at tissue concentrations of $\leq$ 195 mg/kg (wet tissue weight).

Table 3-11. Analytical Results of PFOS Concentrations Measured in Earthworm Tissues After 14 Days of Exposure

Day 0 Mean Soil Conc., mg/kg (dry weight)	Percent Mortality after 14 Days	Mean Tissue Concentration, mg/kg (wet weight)
Negative Control	0	< limit of quantitation (50)
77	0	195
141	7.5	203
289	2.5	252
488	95	1105[a]
1042	100	No tissue available for analysis

[a] Limited sample size available for this analysis; results may be suspect.

3.2.1.10 Acute Avian Feeding Studies

Acute feeding studies were conducted using 10-day old mallard duck (*Anas platyrhynchos*) (RS-II-34) and northern bobwhite quail (*Colinus virginianus*) (RS-II-35). Each species was offered the dosed feed for 5 days, followed by untreated feed until Day 22. Doses were reported on a nominal concentration basis for eight different doses plus the control group. There were 10 animals per treated dose group and 30 for the negative controls. Studies demonstrating homogeneity of test substance concentrations in diet were conducted and the results served as verification of test substance concentrations. On Day 8, one half of the treatment and control birds were sacrificed, liver weights determined and liver tissue and blood samples were collected. On Day 22 the remaining birds were sacrificed and also sampled for liver weights and tissues and blood. Not all samples collected were analyzed. Livers from birds that died during the period between test initiation and Day 7 were also analyzed for PFOS.

3.2.1.11 Mallard Duck (*Anas platyrhynchos*)

The dietary LC_{50} value for mallard ducks was 628 mg/kg feed. Based on reductions in body weight gain at the 73.2 mg/kg feed concentration, the no observed effect concentration (NOEC) was 36.6 mg/kg feed. When compared to the control group, there was a marked reduction in feed consumption in treatment groups $\geq$ 293 mg/kg feed during the exposure period. This reduction in consumption continued through Day 15. Birds that died during the study were subjected to a gross necropsy. Common observations included thin condition, loss of muscle mass, altered spleen color, empty crops and empty gastrointestinal tracts. Findings were found to be treatment related. Necropsies of birds that survived to Day 8 and Day 22 found similar treatment-related effects in some birds. However, not all birds that survived to Day 22 had observations that were considered to be remarkable, including the four that survived in the 586 mg/kg feed level until terminated at Day 22.

No mortality was observed in the study at any concentration after Day 8. Liver samples from all mallards that died during the time period of Day 0 through Day 7 were analyzed for PFOS content. The results are tabulated below in Table 3-12. Liver concentrations in the range of 116 – 216 ppm were associated with mortality. Below 116 ppm, there was no mortality.

Table 3-12. Summary of Individual Analytical Results for Liver Tissue (µg/g wet) From All Mallards that Died During the Acute Study

Nominal PFOS Conc., ppm	Day 4 PFOS Conc., ppm	Day 5 PFOS Conc., ppm	Day 6 PFOS Conc., ppm	Day 7 PFOS Conc., ppm
293	No mortality	No mortality	No mortality	148
586	No mortality	216	197	180
1171	116, 122	172, 137, 147, 185	131, 121	178

Results from liver and serum analyses of samples from surviving mallards taken on Day 8 and Day 22 are tabulated in Table 3-13. These data show that PFOS was present in the liver and serum of mallard ducks after the exposure period. The liver and serum concentrations decreased as the length of time from the last exposure increased. This could have been a dilution effect due to growth of the mallards during the study. No mortality was associated with liver concentrations of $\leq$ 38.8 ppm or serum concentrations of < 53.9 ppm.

Table 3-13. Summary of Mean Analytical Results for Mallard Liver Tissue (µg/g wet) and Serum (µg/mL) at Day 8 and Day 22

Nominal PFOS Conc., ppm	Percent Mortality, Day 8 and 22[a]	Day 8 Liver Conc., ppm	Day 8 Serum Conc., ppm	Day 22 Liver Conc., ppm	Day 22 Serum Conc., ppm
Negative Control	0	<LOQ (~0.030)	<LOQ (0.005)	<LOQ (~0.030)	<LOQ (0.010)
9.1	0	4.67	7.38	1.03	1.49
18.3	0	6.65	13.8	2.04	4.32
36.6	0	15.3	30.5	3.36	6.82
73.2	0	29.7	48.1	9.21	11.3
146	0	38.8	53.9	12.7	18.9
293	20	55.6	65.8	9.2	13.4
586	30	59.5	107	20.2	34.0

[a] No additional mortality occurred in any treatment level between Day 8 and Day 22. Samples taken at the 1171 ppm treatment level were not analyzed.

3.2.1.12 Northern Bobwhite Quail (*Colinus virginianus*)

The dietary LC_{50} value for quail was 220 mg/kg feed. Based on treatment-related mortality, signs of toxicity and reductions in body weight gain at the 146 mg/kg feed concentration, the no observed effect concentration (NOEC) was 73.2 mg/kg feed. When compared to the control group, there was a marked reduction in feed consumption in treatment groups $\geq$ 293 mg/kg feed during the exposure period. There was no treatment-related effect on feed consumption in any of the surviving treatment groups during the recovery period (Days 6 – 22). Birds that died during the study were subjected to a gross necropsy. Common observations included thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. Findings were found to be treatment related. Necropsies of birds that survived to Day 8 and Day 22 found only one individual with necropsy results (lack of muscle mass and general thinness) that were considered treatment related. All other birds examined on Day 8 and Day 22 had necropsy findings that were unremarkable.

No mortality was observed in the study at any concentration after Day 8. Liver samples from selected quails that died during the time period of Day 0 through Day 7 were analyzed for PFOS content. The results are found in Table 3-14. Liver concentrations associated with mortality ranged from 111 – 249 ppm.

Table 3-14. Summary of Individual Analytical Results for Liver Tissue (µg/g wet) From Selected Quail that Died During the Acute Study

Nominal PFOS Conc., ppm	Day 3 PFOS Conc., ppm	Day 4 PFOS Conc., ppm	Day 5 PFOS Conc., ppm	Day 6 PFOS Conc., ppm	Day 7 PFOS Conc., ppm
146	No mortality	No mortality	No mortality	No mortality	111
293	No mortality	No mortality	249	202	153, 124
586	Not analyzed	Not analyzed	126	235	111
1171	134	132, 123, 124	No live organisms	No live organisms	No live organisms

Results from liver and serum analyses of samples taken from surviving quail on Day 8 and Day 22 are tabulated in Table 3-15.

Table 3-15. Summary of Mean Analytical Results for Quail Liver Tissue (µg/g wet) and Serum (µg/mL) at Day 8 and Day 22

Nominal PFOS Conc., ppm	Percent Mortality, Day 8 and 22[a]	Day 8 Liver Conc., ppm	Day 8 Serum Conc., ppm	Day 22 Liver Conc., ppm	Day 22 Serum Conc., ppm
Negative Control	0	<LOQ (~0.070)	<LOQ (0.010)	0.215	0.115
18.3	0	18.5	<LOQ	3.25	5.89
36.6	0	25.8	3.04	2.92	7.27
73.2	0	44.0	41.2	10.0	26.2
146	10	70.3	41.6	25.8	40.5

[a] No additional mortality occurred in any treatment level between Day 8 and Day 22. Samples taken at the 293, 586, and 1171 ppm treatment level were not analyzed.

These data show that PFOS was present in the liver and serum of bobwhite quail after the exposure period. The liver and serum concentrations decreased as the length of time from the last exposure increased. This could have been a dilution effect due to growth of the quail during the study. No mortality was associated with liver concentrations $\leq$ 44.0 ppm or serum concentrations $\leq$ 41.2 ppm.

The lowest avian dietary LC_{50} value was obtained with the quail (220 mg/kg feed in quail, 628 mg/kg feed in the mallard). However, the mallard NOEC of 36.6 mg/kg feed (based on body weight) was lower than that seen in the quail study (73.2 mg/kg feed, based on signs of toxicity and reductions in body weight gain). Therefore, the mallard duck (*Anas platyrhynchos*) was apparently more sensitive to PFOS exposure than the bobwhite quail (*Colinus virginianus*) in these studies.

3.2.1.13 Pending Studies

A study of uptake in terrestrial plants has recently been received and has not been addressed in this document. A bird reproduction study is still in progress.

3.2.1.14 Predicted No Effect Concentrations (PNECs)

This section describes the selection of PNECs from the ecological toxicity data discussed in the preceding sections. For each group of ecological receptors, PNECs were estimated from the lowest no observed effect concentration (NOEC) for that group (Table 3-16). For acute data, the lowest NOEC was divided by an uncertainty factor of 100 to derive the PNEC. For chronic data, the lowest NOEC was divided by an uncertainty factor of 10 to derive the PNEC. Thus, the PNEC values provide for a margin of safety beyond the levels that cause no effects in the most sensitive species.

Table 3-16. Predicted No Effect Concentrations PNECs for PFOS

Ecological Receptor Group	Toxicity Test	Endpoint	NOEC	Uncertainty Factors[a]	PNEC
Aquatic Life	35-d *M. bahia*	Reproduction and growth	0.25 mg/L	10	0.025 mg/L
Fish	62-d *L. macrochirus*	Survival	80.6 µg/g (wet wt.)	10	8 µg/g (whole body wet wt.)
Mollusks	96-h *U. complamatus*	Survival	7.3 µg/g (wet wt.)	100	0.073 µg/g (tissue wet wt.)
Mammals	2-generation rat study	Reproduction	107 µg/g (liver)	10	10.7 µg/g (liver) (tissue wet wt.)
			47.1 ppm (serum)	10	4.71 ppm (serum)
Birds	19-wk bobwhite quail	Reproduction	Not determined	N/A	Not determined
	19-wk mallard duck	Reproduction	Not determined	N/A	Not determined

[a] 10 for chronic NOECs and 100 for acute NOECs.

3.2.1.15 Aquatic Life

The lowest chronic NOEC for aquatic life exposed to aqueous PFOS was 0.25 mg/L for *M. bahia*. This value is essentially the same as the chronic NOEC for fathead minnows (*P. promelas)*, which is 0.3 mg/L. An uncertainty factor of 10 was applied to the chronic NOEC, which resulted in an aquatic life PNEC of 0.025 mg/L.

3.2.1.16 Fish

In addition to the aquatic life PNEC, a PNEC was derived for whole body concentrations of PFOS in fish from the 62-day *L. macrochirus* study (Section 3.2, Chronic Toxicity To Fish). Although not a life cycle assessment, the 62-day exposure duration represents a lengthy exposure in the lifetime of the test organism. Standard acute toxicity studies have exposure periods of about 4 days using standard protocols. Therefore, for the purpose of the risk assessment, the 62-

day exposure duration is considered to be representative of the poential chronic effects of PFOS. In this study, no effects on survival were observed at 0.086 mg/L. At that test concentration, the whole body concentration of PFOS was 80.6 µg/g wet weight. An uncertainty factor of 10 was applied to this NOEC, which resulted in a fish PNEC of 8 µg/g (whole body concentration).

3.2.1.17 Mollusks

A whole body PNEC for freshwater mollusks also was derived. In the acute 96-hour test with the freshwater mussel, *U. complamatus*, the NOEC for survival was 20 mg/L. At this exposure concentration, the mean measured tissue concentration was 7.33 µg/g wet weight. An uncertainty factor of 100 was applied to this NOEC, which resulted in a mollusk tissue PNEC of 0.073 µg/g (whole body concentration).

3.2.1.18 Mammals

Chapter 4 (Human Health Risk Assessment) presents the results of a growth and reproduction study conducted with rats. Effects on growth and reproduction are typical endpoints used in ecological risk assessments and have a direct correlation to effects at the individual and population level. For the ecological risk characterization for piscivorous wildlife, the NOEC from the dam pre-mating group (47.1 ppm serum PFOS, Table 4-10) was chosen as the PNEC for several reasons. First, this value is taken from a study on reproduction, which has direct relevance at the population level. Second, the mammal serum values from 3M's Biosphere Program are generally derived from adult, non-pregnant animals. Third, end-of-gestation values are affected by the physiologic changes occurring during pregnancy, introducing greater intra- and interspecies variability. Fetal values also tend to be more uncertain than adult values, because neonatal serum is difficult to collect and the risk for contamination or dilution during collection is higher. An uncertainty factor of 10 was applied to the 47.1 ppm maternal rat serum PFOS concentration NOEC, which results in a PNEC of 4.71 µg /g serum PFOS.

For liver values, data are available from dams sacrificed at gestation day 21. The liver concentration associated with the 0.40 mg/kg/day NOEL is 107 ppm (Table 4-10). The 107 ppm liver PFOS concentration was used to derive the PNEC. An uncertainty factor of 10 was applied to this NOEC, which results in a PNEC of 10.7 µg/g liver PFOS.

3.2.1.19 Birds

The only PFOS toxicity data available for birds were from acute feeding studies with mallard ducks and northern bobwhite quail. The chronic reproduction studies were in progress at the writing of this document and therefore, an avian PNEC was not determined.

3.3 Environmental Risk Assessment

The environmental risk of PFOS is estimated by comparing concentrations measured in wildlife in the environment to concentrations of PFOS causing effects on organisms exposed in laboratory testing. In the following risk characterization, direct measures of toxicity and actual measures of PFOS exposure are used in the assessment. The use of direct measurements reduces the uncertainty inherent in modeled exposure concentrations or toxic impacts. Potential risks to ecological receptors were estimated using the ratio of the PEC to the PNEC for aquatic and

mammalian assessments. As indicated in the SIDS manual, a ratio of greater than one (>1) indicates that a hazard may be posed. A ratio of less than one (<1) indicates that 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 risks. The PNECs include uncertainty factors of 10 for chronic toxicity assessments and 100 for acute toxicity assessments that were applied to the lowest NOEC available as a conservative measure.

The methods used to estimate the environmental risk of PFOS are purposefully designed to provide estimates of the greatest possible risk from PFOS exposure, or in other words, to overestimate the risk that can be inferred from the existing data. These conservative risk estimates are achieved by focusing both on the highest PFOS exposure concentrations and the most sensitive indicators of PFOS toxicity. Safety factors are then employed in the risk calculations to provide an additional margin of safety on the risk estimates. This approach to risk estimation is considered a valid and defensible method for assessing potential risk, but is not intended as an accurate measure of actual risk. The U.S. Environmental Protection Agency (U.S. EPA 1992, 1998) describes this approach to risk estimation as a Tier I screening-level risk characterization. The screening analysis is designed to eliminate chemicals from further consideration and to focus the analysis on exposure pathways and mechanisms of toxicity.

The PFOS risk characterization is based on an unusually large database of exposure and effects information. A large number of species, representing over 1,200 tissue samples, were used to establish PFOS exposure to mammals and fish. Direct measures of PFOS levels in surface waters have been collected from multiple sites across the United States. Ecological effects information from over twenty plant and animal species has been compiled for risk estimation. This database is one of the largest informational sources compiled for a single chemical, and represents a global examination of the potential risk of PFOS to the environment. The size of the database reduces the uncertainty in the risk estimates and increases the level of confidence in this overall assessment.

In addition to calculating the PEC/PNEC ratios, the effects and exposure data are presented in graphical form in the sections below. The graphics provide a method for visually comparing the effects and exposure data. From the graphs, the range of effects concentrations and the range of exposure concentrations represented in the current data set can be assessed. Cumulative frequency distributions of effects and exposure are created for the aquatic, fish, and mammalian 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.

3.2.2 Potential Risks to Aquatic Biota from Exposure to PFOS in Surface Waters

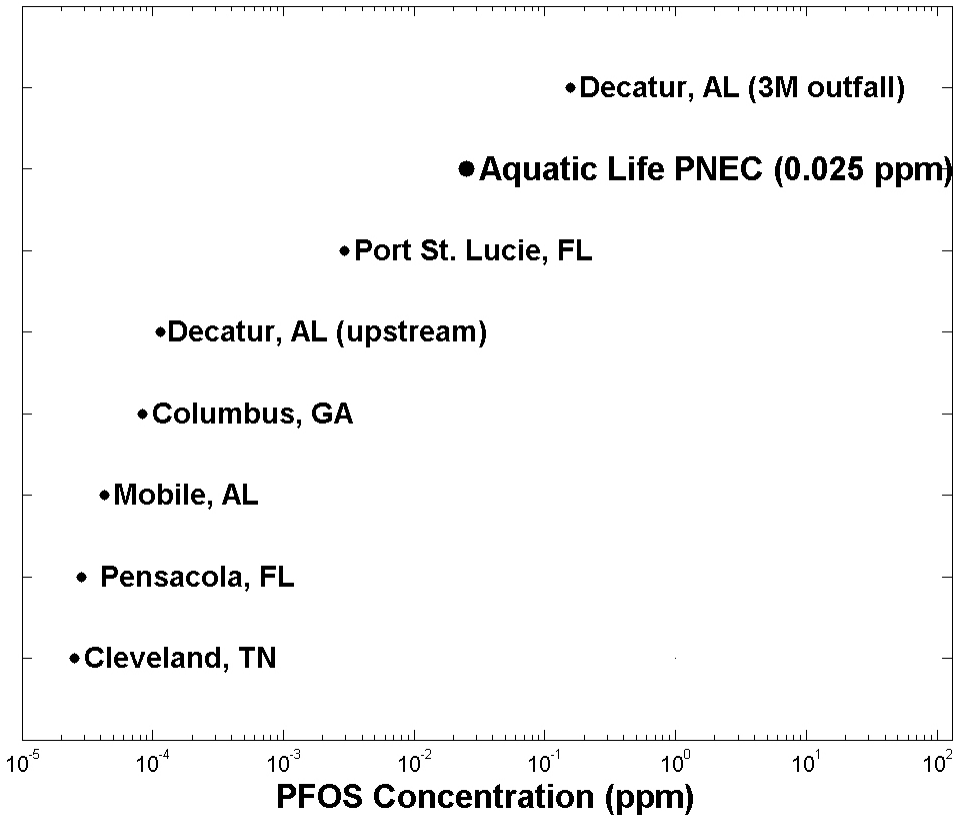
Table 3-17 presents the ratios of average and maximum PECs to PNECs for each city in which aquatic exposure concentrations are available and for the Tennessee River near Decatur, AL. Figure 3-2 provides a visual representation of the maximum site-specific PFOS concentrations at each location and the aquatic life PNEC.

Table 3-17. Ratios of Site Average and Site Maximum PECs to PNEC in Surface Water

Location	Sampling Site	Site Average PEC/PNEC[a]	Site Maximum PEC/PNEC
Columbus, GA Sampling locations near the Columbus Water Works Influent	Site 1	0.003	0.003
	Site 2	0.003	0.003
	Site 3	0.002	0.002
	Quiet Surface Water	0.001	0.001
Pensacola, FL Sampling locations in Texas Bayou (selected for ease of access)	Site 1	0.001	0.001
	Site 2	0.001	0.001
	Site 3	0.001	0.001
	Quiet Surface Water	0.001	0.001
Mobile, AL Sampling locations near a customer's industrial wastewater treatment plant	Site 1	0.001	0.001
	Site 2	0.002	0.002
	Site 3	0.001	0.001
	Quiet Surface Water	0.001	0.001
Decatur, AL Sampling locations upstream of the Decatur POTW effluent, downstream of water plant (Multi-City Study)	Site 1	0.001	0.001
	Site 2	0.001	0.001
	Site 3	0.001	0.001
	Quiet Surface Water	0.004	0.005
Cleveland, TN Sampling locations upstream of the Cleveland Municipal POTW	Site 1	0.001	0.001
	Site 2	0.001	0.001
	Site 3	0.001	0.001
Port St. Lucie, FL Sampling locations in the vicinity of the Northport Wastewater Treatment Plant and Port St. Lucie landfill	Site 1	0.001	0.001
	Site 2	0.001	0.001
	Site 3	0.001	0.001
	Quiet Surface Water Site 1, 2000	0.116	0.117
	Quiet Surface Water Site 1, 2001	0.078	0.094
	Quiet Surface Water Site 2, 2001	0.001	0.001
	Quiet Surface Water, Site 3, 2001	0.001	0.001
Tennessee River near Decatur, AL Sampling locations: (Entrix 2001)	3M Outfall Area, Including Bakers Cr.	2.439	6.240
	Fox Cr. Area	0.016	0.024
	Guntersville Lake	0.001	0.001
	Decatur WWTP Area	0.002	0.003

[a] PNEC = 0.025 mg/L

Figure 3-2. Comparison of the Maximum Site-Specific PFOS Surface Water Concentrations and the Aquatic Life PNEC.



The maximum PEC/PNEC ratios range from < 0.01 at most sites to 6.2 in the immediate area of the 3M Outfall in the Tennessee River near Decatur (using 2001 data). The next largest maximum ratio (3.1) was determined for the Fox Creek area of the Tennessee River near the Decatur manufacturing site. Most of the risk ratios shown in Table 3-17 represent a margin of safety of greater than 1000. The area immediately adjacent to the outfall of the Decatur manufacturing facility is an exception. This maximum PEC/PNEC ratio (6.2) found at the Outfall area reflects the relatively high concentration of PFOS in the effluent and very little dilution with river water. No mixing zone dilution factors have been applied to the PFOS concentrations found at this sampling point. Within a short distance from the discharge point in Bakers Creek, concentrations of PFOS decrease rapidly, which also means that the PEC/PNEC ratio falls to less than one within a very short distance.

3.2.3 Potential Risks to Aquatic Biota from Exposure to PFOS in Sediments

Potential risks to aquatic biota from exposure to PFOS in sediments were assessed by comparing the PNEC for mysid shrimp (0.025 mg/L PFOS in water) with the estimated pore water concentrations in sediments listed in Table 3-4 in Section 3.1.3. These risk ratios are shown in Table 3-18. All sediment pore water PEC/PNEC ratios were less than 1, except for the sediments in the 3M Outfall Area in the Tennessee River near Decatur, where the ratio was 72. This

highest PEC/PNEC ratio indicates that a potential risk to benthic organisms may exist in a small area adjacent to the outfall. However, the majority of the risk quotients indicate a margin of safety of at least 1000, with the Fox Creek Area indicating a safety factor of at least 330. The estimation of pore water PFOS concentrations is highly uncertain, and the risk in sediments based on these values can be considered only as a screening analysis.

Table 3-18. Estimated Maximum Pore Water Concentration of PFOS in Sediments and Ratio of Site Maximum PECs to PNEC from the Tennessee River Near Decatur, AL and the Multi-City Study

Study	Sampling Site	Est. Max. Pore Water Concentration (ppb)	Maximum PEC/PNEC Ratio[a]
Multi-City	Columbus, GA	0.1	0.002
	Pensacola, FL	0.1	0.002
	Mobile, AL	0.1	0.004
	Decatur, AL	0.1	0.004
	Cleveland, TN	<0.03	0.001
	Port St. Lucie 1999	0.2	0.006
	Port St. Lucie 2000	<0.04	0.002
	Port St. Lucie Quiet Surface Water Site 1, 2001	1.9	0.076
	Port St. Lucie Quiet Surface Water Site 2, 2001	<0.04	0.002
	Port St. Lucie Quiet Surface Water Site 3, 2001	<0.04	0.002
Tennessee River near Decatur, AL (Hansen *et al*., 2002)	3M Outfall Area, Including Bakers Cr. Area	1792	71.680
	Fox Cr. Area	1.2	0.049
	Guntersville Lake	0.1	0.004
	Decatur WWTP Area	0.4	0.015

[a] PNEC = 0.025 mg/L

3.2.4 Potential Risks to Fish and Mollusks

Potential risks to fish and mollusks in the Tennessee River near Decatur were assessed by comparing maximum whole body concentrations of PFOS to the PNECs for PFOS in fish and mollusks (Table 3-19). All maximum PEC/PNEC ratios were less than one. Figure 3-3 compares the PNEC for whole body fish PFOS concentration with the cumulative distribution of whole body PFOS concentrations for all species sampled. Each value in the cumulative distribution is the mean of all samples for the particular species.

Labels on Figure 3-3 indicate the species used in the analysis. These data indicate that the whole body PFOS concentrations in fish are below the PNEC. Figure 3-4 compares the minimum, mean, and maximum whole body PFOS concentrations with the fish PNEC for all species. All PFOS concentrations were less than the PNEC.

Table 3-19. Maximum and Mean PEC/PNEC Ratios for Whole Body Fish and Clam Concentrations in the Tennessee River Near Decatur, AL

Species	PEC/PNEC Ratio			
	Maximum		Mean	
	Clam[a]	Fish[b]	Clam	Fish
Asiatic Clams (*Corbicula fluminea*)	0.274		0.274	
Channel Catfish (*Ictaluras punctatus*)		0.164		0.075
Gar (*Lepisosteus sp.*)		0.381		0.079
Largemouth Bass (*Micropterus*		0.029		0.023
Striped Bass (*Morone saxatilis*)		0.014		0.009

[a] Clam tissue PNEC = 0.073 µg/g wet weight
[b] Fish tissue PNEC = 8.0 µg/g whole-body weight

Figure 3-3. Cumulative Distribution of Mean Whole Body Fish PFOS Concentrations in All Species Sampled.

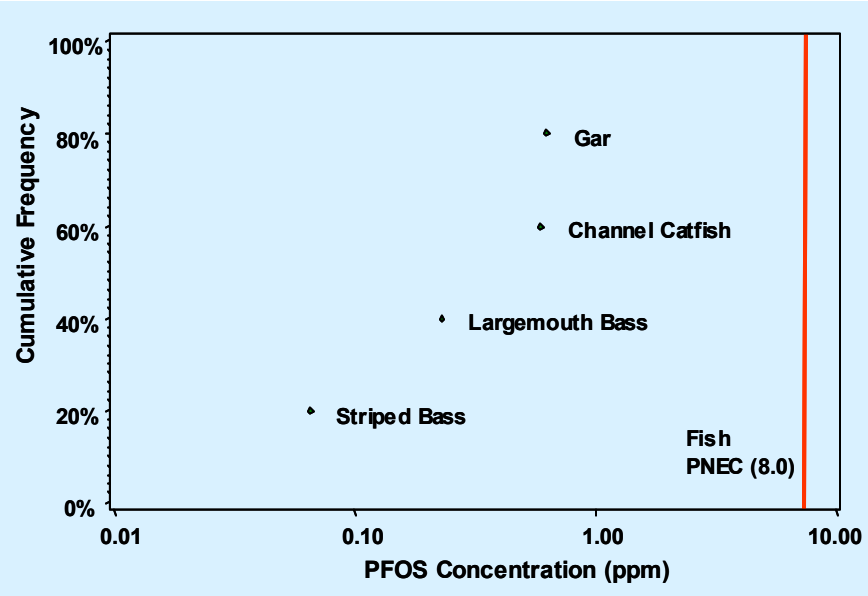
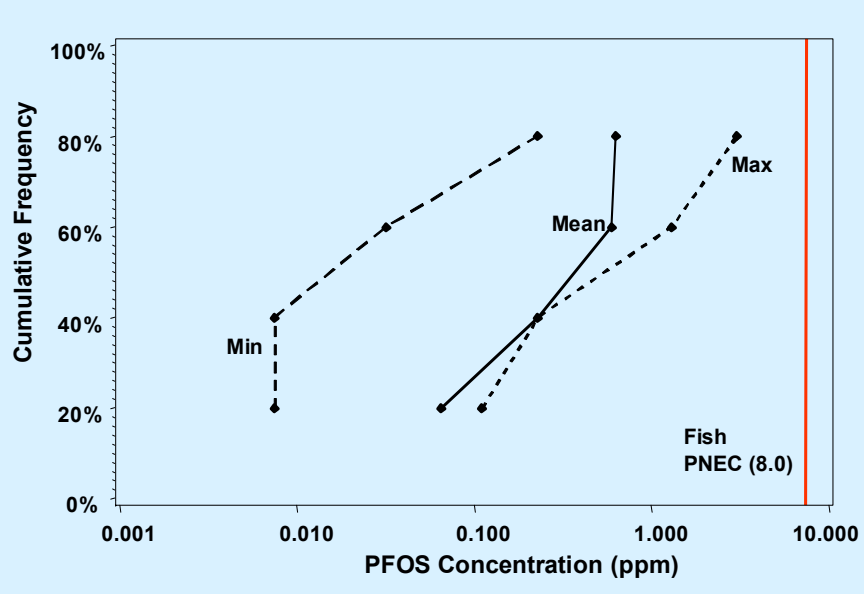


Figure 3-4. Minimum, Mean, and Maximum Whole Body Fish PFOS Concentrations in All Species Sampled.



3.2.5 Potential Risks to Aquatic and Piscivorous Mammals

Risks to aquatic mammals were assessed by comparing serum and liver concentrations of PFOS with serum and liver PNECs. All maximum PEC/PNEC ratios for blood PFOS concentrations in mammals were less than 1 (Table 3-20), with the margins of safety ranging from at least 100 to

1000. For PFOS in mammal livers, all maximum PEC/PNEC ratios were also less than 1 (Table 3-21), with the margins of safety ranging from at least 2.2 to 1000.

Figure 3-5 compares the PNEC for serum PFOS concentration with the cumulative distribution of blood PFOS concentrations for all mammalian species sampled. Each value in the cumulative distribution is the mean of all samples for a particular species.

Figure 3-6 compares minimum, mean, and maximum PFOS concentrations in blood, plasma, and serum with the mammal PNEC for all species. Again, all PFOS concentrations were less than the PNEC.

Figure 3-7 compares the PNEC for mammal liver PFOS concentration with the cumulative distribution of liver PFOS concentrations for all species sampled. Species mean values are used in the cumulative distribution. Figure 3-8 indicates that for the currently available environmental data, the liver concentrations of PFOS in mammal samples are well below the PNEC.

Figure 3-8 compares minimum, mean, and maximum PFOS concentrations in liver from all mammalian species with the mammal PNEC. All PFOS concentrations were less than the PNEC.

Table 3-20. Maximum and Mean PEC/PNEC Ratios for Mammal Serum PFOS Concentrations

Species	PEC/PNEC[a] Ratio	
	Maximum	**Mean**
Bottlenose Dolphin (*Tursiops truncatus*)	0.044	0.030
Caspian Seal (*Pusa caspica*)	0.003	0.003
Grey Seal (*Halichoerus grypus*)	0.017	0.008
Northern Fur Seal (*Callorhinas ursinus*)	0.001	0.001
Polar Bear (*Ursus maritimus*)	0.011	0.006
Ringed Seal (*Phoca hispida*)	0.102	0.015
Stellar Sea Lion (*Eumetopias jubatas*)	0.001	0.001

[a] Mammalian serum PNEC = 4.71 µg/mL

Table 3-21. Maximum and Mean PEC/PNEC[a] Ratios for Mammal Liver PFOS Concentrations

Species	PEC/PNEC Ratio	
	Maximum	Mean
Baikal Seal (*Pusa siberica*)	0.003	0.002
Bottlenose Dolphin (*Tursiops truncata*)	0.142	0.039
California Sea Lion (*Zalophus californianus*)	0.005	0.002
Clymene Dolphin (*Stenella clymene*)	0.016	0.011
Common Dolphin (*Delphinus delphis*)	0.088	0.088
Elephant Seal (*Mirounga augustirostris*)	0.003	0.002
Ganges Dolphin (*Platanista gangetica*)	0.007	0.006
Harbor Seal (*Phoca vitulina*)	0.006	0.003
Mink (*Mustela vison*)	0.455	0.114
Northern Fur Seal (*Callorhinus ursinus*)	0.012	0.005
Pilot Whale (*Globicephala melaena*)	0.025	0.025
Polar Bear (*Ursus maritimus*)	0.063	0.031
Pygmy Sperm Whale (*Kogia broviceps*)	0.007	0.007
Ringed Seal (*Phoca hispida*)	0.103	0.022
River Otter (*Lutra canadensis*)	0.093	0.031
Rough-toothed Dolphin (*Steno bredanensis*)	0.007	0.007
Sea Otter (*Enhydra lutris nereis*)	0.001	0.001
Striped Dolphin (*Stenella coeruleoalba*)	0.036	0.014
Weddell Seal (*Leptonychotes weddellii*)	0.003	0.003

[a] Mammalian liver PNEC = 10.7 µg/g liver tissue wet weight

Figure 3-5. Cumulative Distribution of Mean Blood, Serum, and Plasma PFOS Concentrations in All Mammal Species Sampled.

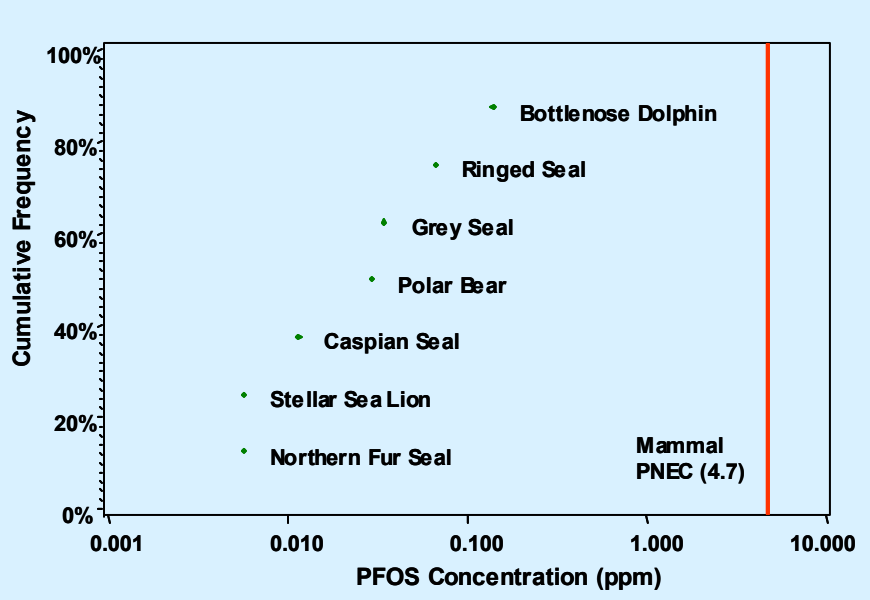


Figure 3-6. Minimum, Mean, and Maximum Blood, Serum, and Plasma PFOS Concentrations in All Mammal Species Sampled.

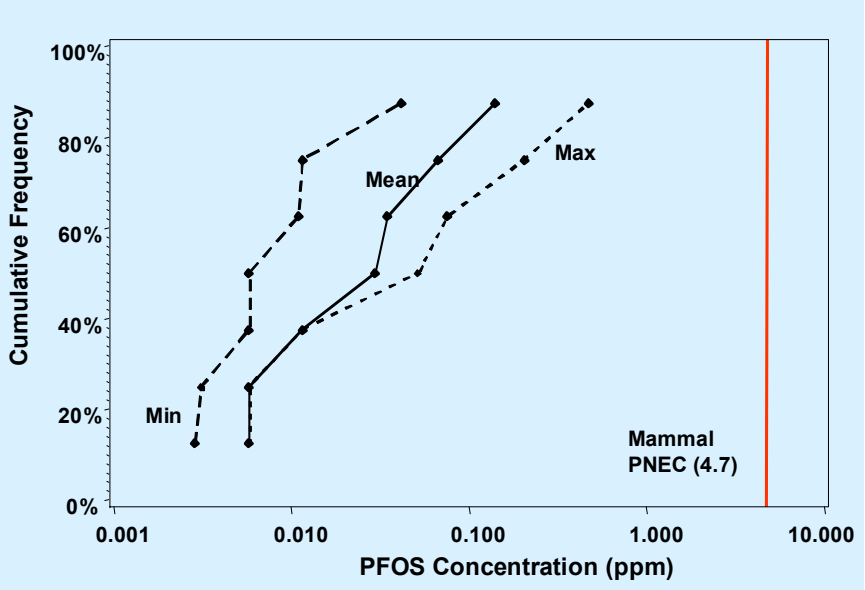


Figure 3-7. Cumulative Distribution of Mean Liver Tissue PFOS Concentrations in All Mammal Species Sampled.

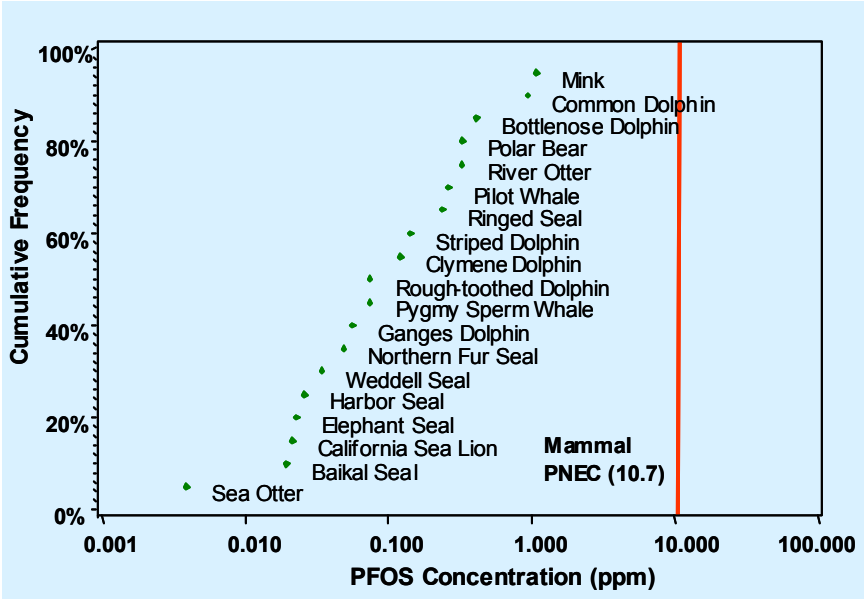
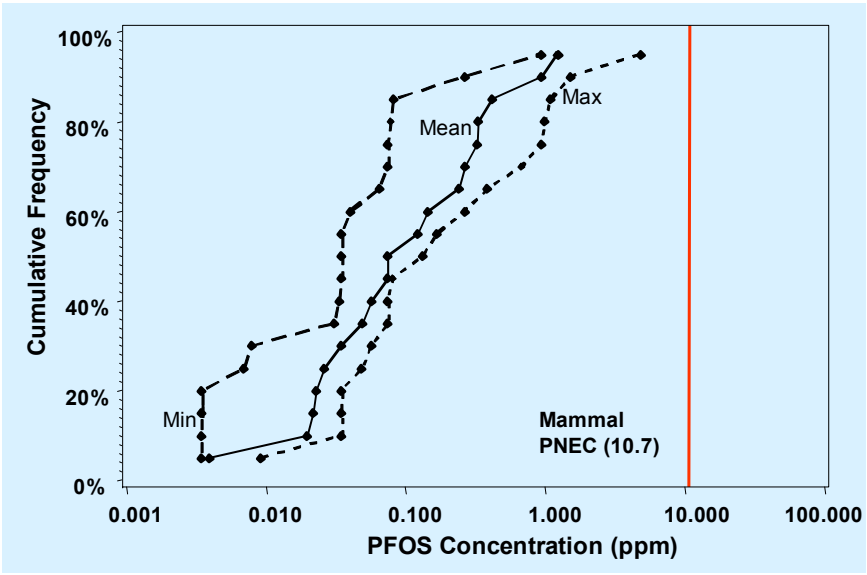


Figure 3-8. Minimum, Mean, and Maximum Liver Tissue PFOS Concentrations in All Mammal Species Sampled.



3.2.6 Potential Risks to Aquatic Birds

An ecological assessment for avian wildlife was not conducted at this time because avian studies are still underway.

3.2.7 Sources of Uncertainty in Assessment of Effects to the Environment

Sources of uncertainty in these risk characterizations include the following:

1. The relative sensitivities of rats to PFOS, compared with mammal wildlife species are unknown; therefore, using laboratory PFOS toxicity data to predict effects to mammalian wildlife may overestimate 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 among species.

2. Uncertainty factors of 10 and 100 were used to estimate the PNEC from the lowest available chronic and acute NOECs, respectively. These uncertainty factors may result in an overly conservative estimate of the actual PNECs for the ecological effects of PFOS. Although these uncertainty factors account for some of the uncertainty present in this initial assessment, the total amount of uncertainty present in the PNECs and PECs is unknown. However, the analysis has focused on the use of maximum exposure concentrations and the most sensitive toxicity endpoints. Therefore, the risk results presented in this report are expected to provide an overestimation of the potential risk due to PFOS exposure.

3. Uncertainty in the risk estimates is also reduced due to the large database containing direct measures of PFOS toxicity and exposure concentrations.

4. The amount of chronic toxicity data for PFOS available at this time for predicting risks to mammalian wildlife is limited, and chronic data for birds are not yet available.

5. Because the biosphere monitoring was done using available archived samples, the existing data set may not be representative of all species or geographic locations. The degree to which the data in the biosphere sampling program are representative of PFOS concentrations in piscivorous wildlife in general is unknown.

3.2.8 Ecological Risk Characterization

The available data indicate that the observed levels of PFOS from a wide variety of samples have not been associated with adverse effects on wildlife or the environment. Most PEC/PNEC ratios are substantially less than one. The only exceptions were the PEC/PNEC ratios for aquatic life in the immediate Outfall Area at 3M's Decatur Plant. It should be recognized, as indicated above, that uncertainty exists in both the PECs and PNECs used in this analysis. An ecological assessment of avian wildlife could not be evaluated as avian chronic studies are underway at this time.

4.0 HUMAN HEALTH

4.1 Introduction

The potential of PFOS to produce adverse effects on human health has been investigated in numerous studies, primarily sponsored by 3M. The data presented and discussed in this section are from laboratory investigations as well as studies of human populations. Robust summaries of all the studies discussed in this section are included in Appendix III. A number of these studies have been or are in the process of being published in the scientific literature.

This human health section is organized in four parts:

- Section 4.2 presents the investigations that have been conducted to understand the concentrations of PFOS in human serum samples from both occupationally exposed and non-occupationally exposed populations.
- Section 4.3 discusses health studies in human populations, including both epidemiologic and medical surveillance studies.
- Section 4.4 presents the adsorption, distribution, metabolism and excretion of PFOS, the findings of numerous toxicological studies, and investigations into the possible mechanism(s) of toxicity.
- Section 4.5 develops the human health risk characterization for PFOS.

Many of the studies discussed in this section include a determination of PFOS concentrations in serum and liver tissues in order to relate these concentrations to the potential effects being monitored in the studies. These PFOS concentration measurements are important to the risk-characterization process. The manner in which humans have been exposed to PFOS is still not completely understood; however, it is likely that there have been multiple pathways of exposure that include both product and environmental sources. Because PFOS is retained for long periods in the body and is not further metabolized, concentrations of PFOS in human fluids and tissues, especially serum, represent an integration of PFOS exposure from multiple sources and can be compared to PFOS concentrations associated with effect and no-effect levels in scientific studies. As will be shown in the following discussion, external doses in toxicology studies are not as correlative as serum PFOS concentrations, which represent an integration of exposures/doses over time. Use of serum PFOS concentrations obviates the need for estimations of external exposure in assessing health risk and in comparing dose-response profiles between species. The overall result is an increase in certainty for human health risk assessment.

4.2 Human Exposure

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

4.2.1 Background

Since the early 1990s, the advancement of chromatographic/mass spectroscopic technology has enabled routine analyses of specific perfluorochemicals from small volumes of serum and large numbers of samples. 3M first used liquid-chromatography/mass-spectrometry (LC/MS) methods in medical surveillance of fluorochemical production workers in 1993. Prior to the 1990s, the analytical technique specific for PFOS was time-consuming and required a large volume of serum, making it unsuitable for routine medical surveillance. Therefore, most measurements prior to the 1990s did not provide concentration information on specific organofluorine compounds but instead measured total organic fluorine. The development of a rapid analytic technique for organic fluorine in the late 1970s allowed large-scale medical surveillance of production employees at detection limits of approximately 0.5 ppm. Using LC/MS techniques, detection limits for PFOS in serum were lowered to 0.05 ppm by 1997. Current detection limits for PFOS in serum approximate 0.001 ppm, with analytical variability in serum measurements that can be as high as ± 30%.

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). However, they observed some variation in the observed spectra from an authentic sample of PFOA, which led them to suggest that branching or the presence of a sulfonate was a possible interpretation of their findings. Subsequent efforts by 3M researchers identified both PFOS and PFOA in blood.

A number of studies over the past quarter century reported levels of organic fluorine in human blood (serum). Table 4-1 presents the study authors, concentrations of organic fluorine determined, populations studied, and methods of analysis. The variety of methods used for determination of fluorine suggests that some caution should be used in comparing results. All reported means were in the hundredths-of-parts per million (ppm). The average of reported values from United States sources was 0.04 ppm total organic fluorine. To compare more recent measurements of PFOS in serum to derive a value for organic fluorine, the reader may multiply the PFOS concentration by the factor 0.65, the fraction of weight of the PFOS molecule contributed by fluorine.

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 *et al.*	0.025	106	Ash	US
1978	Belisle & Hagen	0.020	9	O bomb	US
1979	Singer & Ophaug	0.045	264	Ash	US
1980	Paez *et al.*	0.085	Pooled	Ash	Argentina
1980	Ubel *et al.*	0.045	4	Mod O bomb	US
1981	Belisle	0.011	8	O bomb	China
1989	Yamamoto *et al.*	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

Estimation of external PFOS exposure for workers has been difficult. 3M manufactured perfluorooctane sulfonyl fluoride (POSF), a starting material used to produce PFOS and other fluorochemicals. POSF and some POSF-based chemistries have the potential to degrade or metabolize to PFOS. Employees may have been exposed to POSF and/or other perfluorochemicals in the manufacturing environment by one or more routes (i.e., inhalation, skin contact/absorption, or ingestion). The primary route of exposure may have varied among employees and depended on several factors, including process conditions, job tasks, work location, personal hygiene, personal habits, and general work practices. Because multiple sources and routes of exposure were probable, estimating external worker PFOS exposures is problematic.

Because they allow for an integrated measurement from all sources of exposure, biological monitoring data (e.g., serum PFOS concentrations) have been collected. Serum monitoring data are especially relevant for PFOS due to the fact that PFOS has a very long serum elimination half-life in humans, and serum concentrations can be related to concentrations in the target organ (liver).

Voluntary biennial medical surveillance of production employees began in the late 1970s using total organic fluorine measurements, and routine specific measurement of serum PFOS levels commenced in the mid-1990s. Table 4-2 presents PFOS concentrations in serum obtained from plant manufacturing and laboratory employees in Decatur, Alabama (USA), Antwerp (Belgium), St. Paul, Minnesota (USA), and Sagamihara (Japan) (RS-III-1, RS-III-2, RS-III-3, RS-III-4, RS-III-5, RS-III-6). The Decatur and Antwerp plants have been involved in fluorochemical production; whereas, the Sagamihara facility handled, but did not produce, POSF-based chemistries.

Table 4-2. PFOS Serum Concentrations (ppm): Occupational Populations

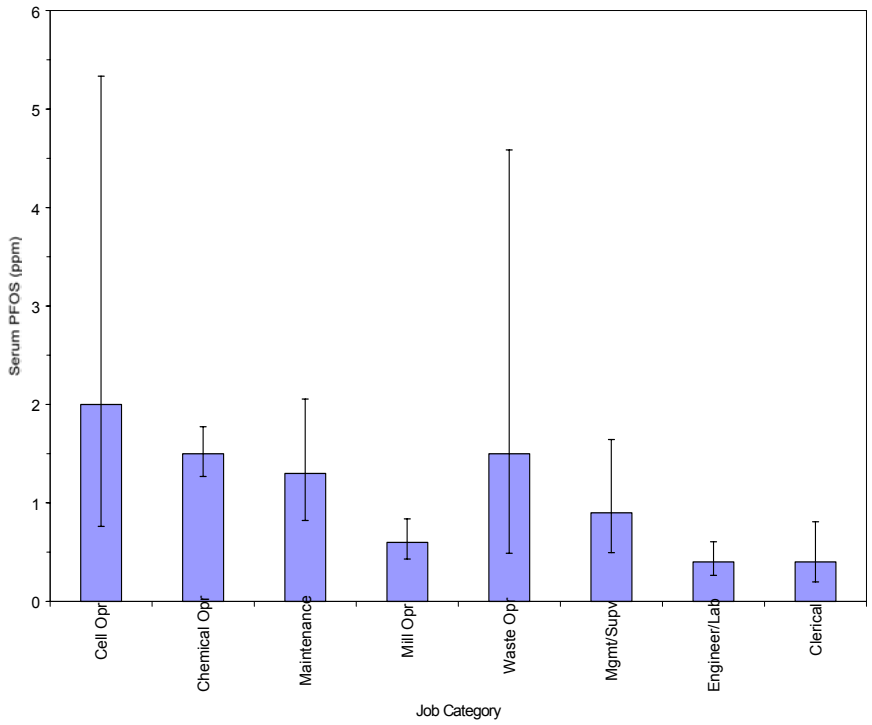
Location & Year	Arithmetic Mean	Range
Decatur (Alabama): Plant		
1994 (n = 100) [a]	2.44	0.25 – 12.83
1997 (n = 84) [a]	1.96	0.10 – 9.93
1998 Chemical (n = 126) [b]	1.51	0.09 – 10.60
1998 Film (n = 60) [b]	0.17	0.02 – 0.95
2000 (n = 263)[a]	1.32	0.06 - 10.06
Antwerp (Belgium): Plant		
1995 (n = 88) [a]	1.93	0.00 – 9.90
1997 (n = 65) [a]	1.48	0.10 – 4.83
2000 (n = 258) [a]	0.80	0.40 - 6.24
St. Paul (Minnesota): Research & Development Laboratory		
2000 (n = 45) [a]	0.18	$\leq$ 0.04 - 1.04
Sagamihara (Japan): Plant		
1999 Production (n = 32)[c]	0.14	$\leq$0.03 – 0. 63
1999 Management (n = 32) [c]	0.04	$\leq$0.03 – 0.06

[a] Voluntary medical surveillance 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.

3M's medical surveillance programs have been voluntary. Because the extent of potential selection 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 (RS-III-6). 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 selected and invited to participate, and 186 (80%) chose to participate. Respondents (n = 186) and non-respondents (n = 46) were comparable with respect to age, gender and employment duration. Of the randomly selected participating employees, 126 were from the chemical plant, and 60 were from the film plant, where fluorochemicals were not manufactured (although, in one production run, a fluorochemical was 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. Individual serum PFOS concentrations ranged from 0.09 to 10.60 ppm. Geometric means and 95% confidence intervals (CI) of the geometric means for chemical plant jobs are presented in Figure 4-1 and included the following geometric means: 1.97 ppm (cell operator); 1.48 ppm (chemical operator); 1.30 ppm (maintenance); 0.59 ppm (mill operator); 1.50 ppm (waste operator); 0.39 ppm (engineer/lab); 0.89 ppm (crew supervisor/mgmt); and, 0.40 ppm (clerical). 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.

Figure 4-1. Geometric Mean and 95% CI For Serum PFOS Concentrations by Job Category, Decatur 1998.



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 is unlikely that employee serum PFOS levels higher than those observed in the medical surveillance programs existed in these fluorochemical-manufacturing populations.

Results from this random-sample assessment were used in the construction of an exposure matrix for the updated retrospective cohort mortality study of the Decatur, Alabama employee population discussed in a subsequent section of this assessment. These biological monitoring data were also used in an episodes-of-care comparison analysis between chemical- and film-plant employees from 1993-1998, which will be discussed in a subsequent section.

In 2000, another voluntary medical surveillance program was offered to both Decatur (RS-III-5) and Antwerp (RS-III-4) chemical-production employees (Olsen *et al*., 2003a). Absolute numbers of participants as well as participation rates were increased compared to previous years. There were 263 participants (53%) from approximately 500 eligible Decatur chemical-plant and site employees and 258 participants (75%) from approximately 340 eligible Antwerp chemical-plant and site employees. (Medical surveillance was also offered to Decatur employees in 2002,

but participation was reduced following 3M's phase out of production of POSF-baswed materials.)

The overall arithmetic mean serum PFOS level for Decatur was 1.32 ppm (range 0.06-10.06) with a geometric mean of 0.91 ppm (95% CI 0.82-1.02). By job, serum PFOS geometric mean levels were comparable to those observed in the random sample study (described above): cell operator (1.57 ppm, 95% CI 1.16-2.13); chemical operator (1.39 ppm, 95% CI 1.23-1.58); maintenance (1.12 ppm, 95% CI 0.83-1.50); mill operator (0.68 ppm , 95% CI 0.58-0.79); waste operator (0.89 ppm , 95% CI 0.42-1.91); manager (0.45 ppm, 95% CI 0.27-0.75); engineer/lab (0.43 ppm, 95% CI 0.34-0.56); and clerical (0.49 ppm, 95% CI 0.30-0.78).

The overall arithmetic mean serum PFOS level for Antwerp was 0.80 ppm (range 0.04-6.24) with a geometric mean of 0.44 ppm (95% CI 0.38-0.51). Serum PFOS geometric mean levels by Antwerp job were comparable to those reported for Decatur: chemical operator (1.66 ppm, 95% CI 1.40-1.97); fluorel (mill) operator (0.51 ppm, 95% CI 0.41-0.64), mechanic (1.19 ppm, 95% CI 0.77-1.84); process engineer (0.26 ppm, 95% CI 0.17-0.40); laboratory QC/QA workers (0.38 ppm, 95% CI 0.28-0.50); and management (0.24 ppm, 95% CI 0.15-0.39).

Both the Decatur and Antwerp employee serum levels were related to work areas as well as job titles. As expected, serum PFOS levels were observed to be highest in those areas involved with actual production (i.e., cell operators and alcohol/amide charging areas).

In 2000, fluorochemical research and development employees located in Building 236 at 3M Center in St. Paul, Minnesota were also offered an opportunity to have their serum analyzed for PFOS (RS-III-6). A total of 45 employees (30%) of approximately 150 eligible voluntarily decided to be tested. Their arithmetic mean was 0.18 ppm (range <LLOQ of 0.04 ppm to 1.04 ppm). The geometric mean was 0.12 ppm (95% CI 0.01-0.15). The values of these employees were comparable to laboratory employees in Antwerp and lower than the engineer/laboratory group of employees in Decatur.

There have been many industrial uses of perfluorochemical-containing compounds, and it is likely that PFOS could be found in serum samples from workers in various downstream-user industries. 3M production employees were exposed to concentrated PFOS precursors; whereas, downstream workers were generally exposed to PFOS precursors in concentrations typically less than 1% as residual materials in perfluorochemical-based products. This suggests downstream workers should have lower serum levels of PFOS than those observed in 3M production employees. The Sagamihara sampling data (RS-III-3) are consistent with this expectation (Table 4-2 above).

4.2.3 Non-Occupational Exposures

Beginning in 1998, three questions were addressed to determine 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-office-based 3M employee population known not to have worked in 3M facilities that manufacture or process

fluorochemicals?

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 or in areas where consumer use of such chemicals is considered to be minimal?

These studies are summarized in Table 4-3. To begin to address the first two questions, several small investigations were conducted using either pooled serum or a small number of individual samples. PFOS could be detected in the serum of adults in the United States (RS-III-7, RS-III-8, RS-III-9) and could be detected in children using a very small amount of serum (0.1 ml) (RS-III-10). PFOS could also be detected in the serum of adults in Belgium, the Netherlands, Germany, Sweden and Japan (RS-III-3, RS-III-11, RS-III-12). No inferences could be developed regarding associations between PFOS and demographics (e.g., age and gender), time trends, or source(s).

To address the third exploratory question, historical samples were obtained to learn whether PFOS could be detected in samples obtained prior to the introduction of POSF-based commercial products (late 1940s to early 1950s) or in areas of limited commercial use of these products. PFOS was not detected in ten pooled samples (ten donors per sample) from U.S. military recruits of the Korean War era (1948-1951). Analysis of ten Swedish samples that were originally collected in 1958 resulted in a range of values from non-detect to 0.002 ppm. A limited number of samples taken during the conduct of several post-1969 epidemiological studies conducted in the United States were analyzed, and, in these, serum PFOS levels ranged from non-detect to 0.059 ppm. Analysis of serum samples from two Chinese rural provinces (Linxian and Shandong) that were collected in 1984 and 1994 showed no detectable PFOS.

Table 4-3. PFOS Serum Concentrations: Non-Occupational Populations

Population	Mean (ppm)	Range (ppm)
3M Corporate Center (n = 31)	0.047	0.028 – 0.096
Tokyo (Japan): 3M Head Office (n = 30)	0.052	≤0.03 – 0.097
Commercial Laboratories [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 (RS-III-8).
[c] pooled samples, 10 donors per sample in Belgium and the Netherlands; 30 donors per sample in Germany (RS-III-12).
[d] RS-III-11.
[e] Pilot analysis. Limited serum (0.1 ml) from children aged 6 or 12 who were enrolled in a group A streptococcal study. RS-III-10. Full study described below.

It appears from this limited sampling that PFOS presence in human serum coincided with the commercial introduction of PFOS-precursor molecules; however, this conclusion is highly uncertain because of the sparseness of the database. Definitive statements regarding time trends cannot be made. A study addressing temporal trends is in progress.

Following on these initial studies, three large research initiatives were implemented whose purpose was to characterize the distribution of individual serum concentrations of PFOS and some of its precursors in human populations. Three study populations were identified:

1. 645 American Red Cross blood donors (ages 20-69) from six blood banks serving the Los Angeles (CA), Portland (OR), Minneapolis-St. Paul (MN), Charlotte (NC), Hagerstown (MD) and Boston (MA) regions (RS-III-13);

2. 238 dementia-free elderly participants in a longitudinal study designed to assess cognitive function in the Seattle region (RS-III-14);

3. 598 pediatric participants (ages 2-12) from 23 states and the District of Columbia involved in a clinical trial of confirmed group A streptococcal infections (RS-III-15).

Blood was collected in the adult and elderly studies during 2001; the pediatric samples were collected between 1994-1995.

PFOS was analyzed using techniques similar to those described by Hansen *et al.* (2001). The serum determinations may have varied, on average, up to 26 percent using human plasma calibration curves. Split samples were analyzed in all three studies (pediatric, adult and elderly), and strong correlations ($r \sim 0.9$) were observed between the split samples for PFOS.

In addition to PFOS, six other analytes were detected and quantified in these studies. These included PFOSAA, M570, M556, PFOSA, PFOA, and PFHS. While of interest, the results for the analytes other than PFOS are not discussed in this document.

Figures 4-2 through 4-4 present the histogram distributions of PFOS for the children (N=598, ages 2-12), adult (N=645, ages 20-69) and elderly (N=238, ages 65-86) populations. All three distributions were similar and log normal.

Figure 4-2. Serum PFOS Distribution (ppm) for the Pediatric Study

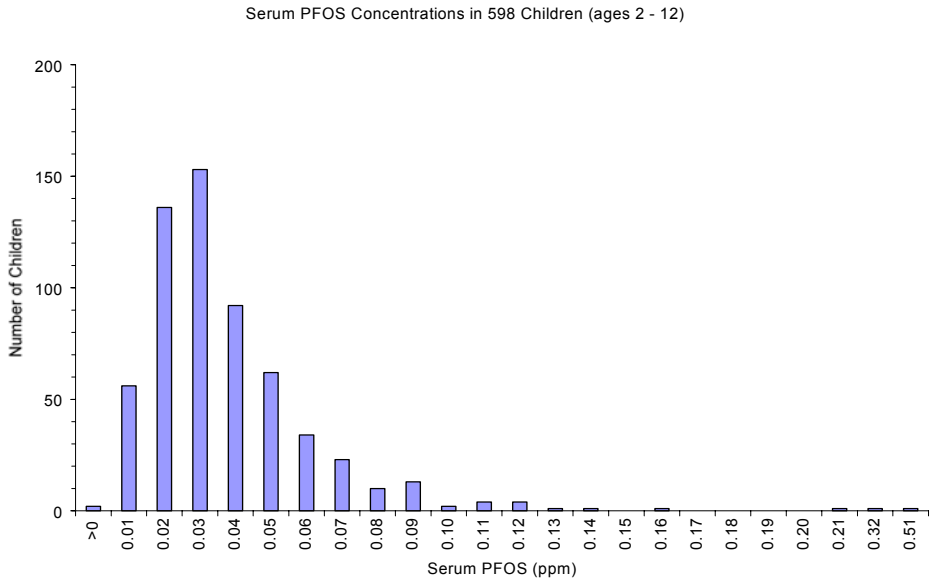


Figure 4-3. Serum PFOS Distribution (ppm) for the Adult Study

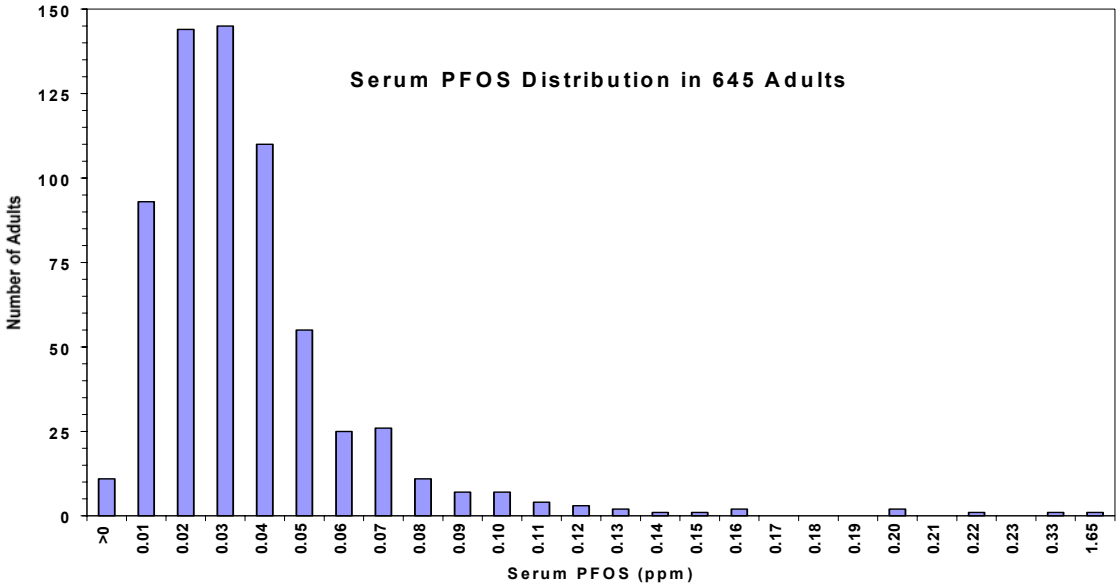


Figure 4-4. Serum PFOS Distribution for the Elderly Study

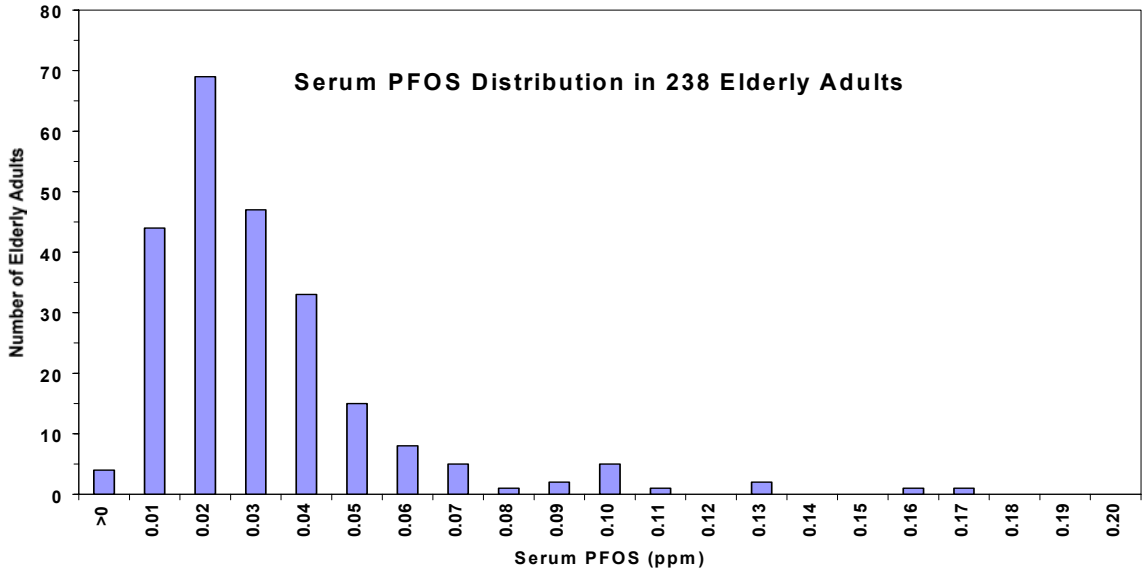


Table 4-4 shows the geometric means, 95% CIs and ranges for the measured concentrations of PFOS for the three study populations. Evaluation of quality control samples injected during the analytical runs indicated that the reported quantitative results may have varied +/- 10% for

precision and accuracy. Thus, the geometric means between the three study populations are nearly identical.

Table 4-4. Geometric Mean Serum PFOS Concentration (ppm), Range and Upper Bound Estimate of 95% Tolerance Limit for the Children, Adult and Elderly Studies

Population	Geo. Mean (95% CI)	Range	Upper Bound of 95% Tolerance Limit Estimate
Pediatric (N = 598)	0.038 (0.036-0.039)	0.007-0.515	0.097
Adults (N = 645)	0.035 (0.033-0.037)	<0.005-1.645	0.100
Elderly (N = 238)	0.031 (0.029-0.033)	<0.003-0.175	0.104

Pediatric serum collected in 1994-95. Adult and elderly serum collected in 2001.

In both the pediatric and adult population studies, males had higher geometric mean PFOS levels than females, although the differences were not substantial. Male adults had a serum PFOS geometric mean of 0.038 ppm (95% CI 0.035-0.040) compared to 0.031 ppm (95% CI 0.030-0.034) in female adults. The serum PFOS geometric mean in male children was 0.040 ppm (95% CI 0.038-0.043) compared to 0.035 ppm (95% CI 0.033-0.037) in female children. Among the elderly, the serum PFOS geometric mean was comparable between sexes (males 0.030 ppm (95% CI 0.027-0.033); females 0.031 ppm (95% CI 0.029-0.036). These differences are not substantial given the inherent variability in the analysis.

For each study, age was not a major predictor of serum PFOS levels, as seen graphically below (natural log scale), when stratified by sex (Figures 4-5 through 4-7). The box covers the 25th to 75th percentile (interquartile range) of the natural log distribution of PFOS. The circle within the box is the mean (natural log). The whiskers extend to the last observation within 1.5 times the interquartile range. The dots with lines through them represent observations outside the 1.5 times interquartile range.

Figure 4-5. Box and Whisker Plots and Natural Log Mean PFOS Concentrations (ppb) in the Pediatric Study Stratified by Age and Gender

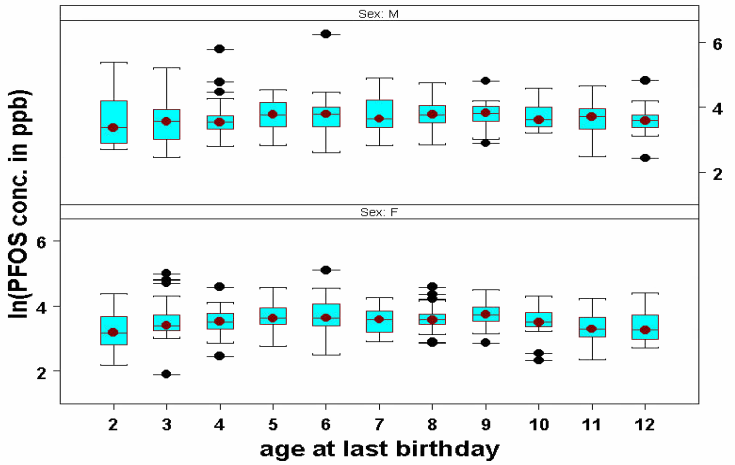


Figure 4-6. Box and Whisker Plots and Natural Log Mean PFOS Concentrations (ppb) in the Adult Study Stratified by Age and Gender

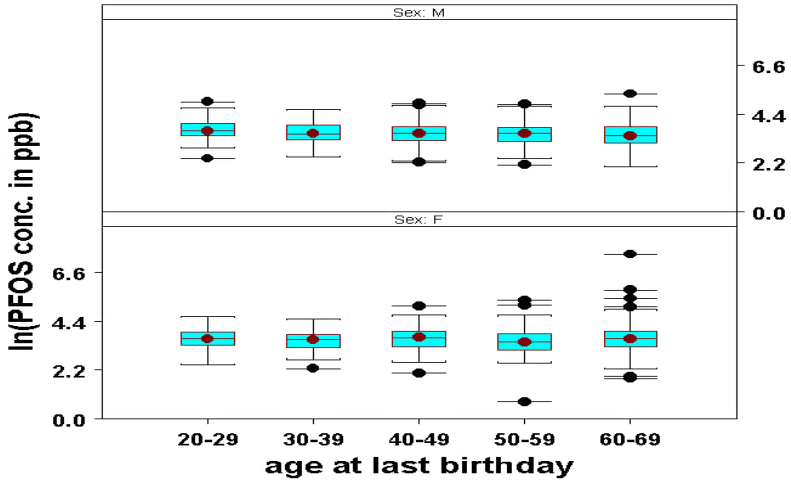
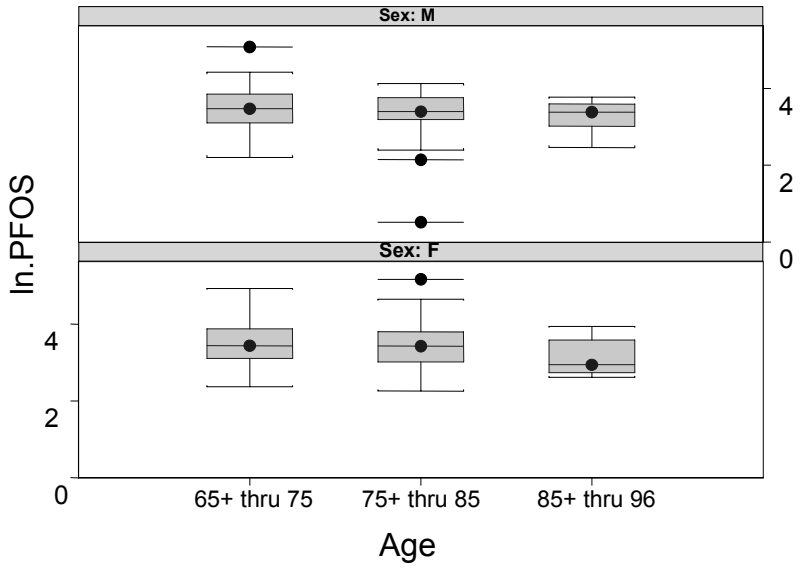


Figure 4-7. Box and Whisker Plots and Natural Log Mean PFOS Concentrations (ppb) in the Elderly Study Stratified by Age and Gender



As seen in Figure 4-8, the natural log mean measured serum PFOS concentrations among children were comparable for the various states, especially when the limited sample size for any state and gender within that state is considered. Similarly, major differences among cities or between genders are not apparent in the adult study (Figure 4-9).

Figure 4-8. Box and Whisker Plots and Natural Log Mean PFOS Concentrations (ppb) in the Pediatric Study Stratified by State and Gender

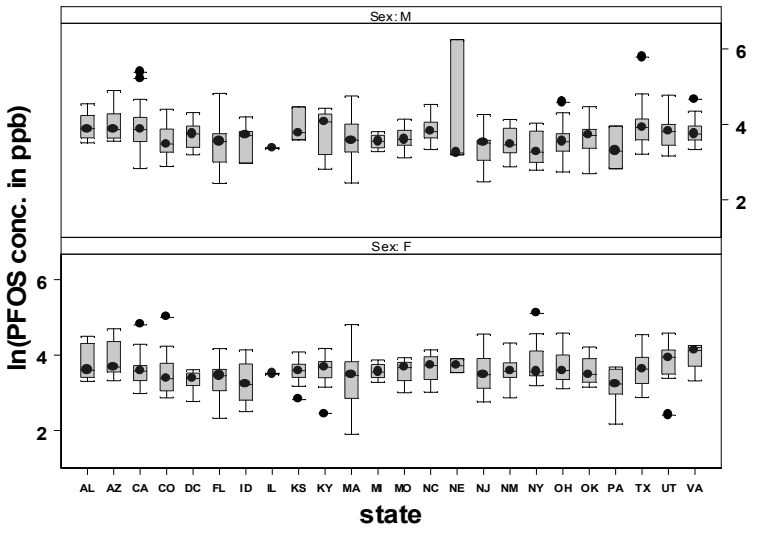
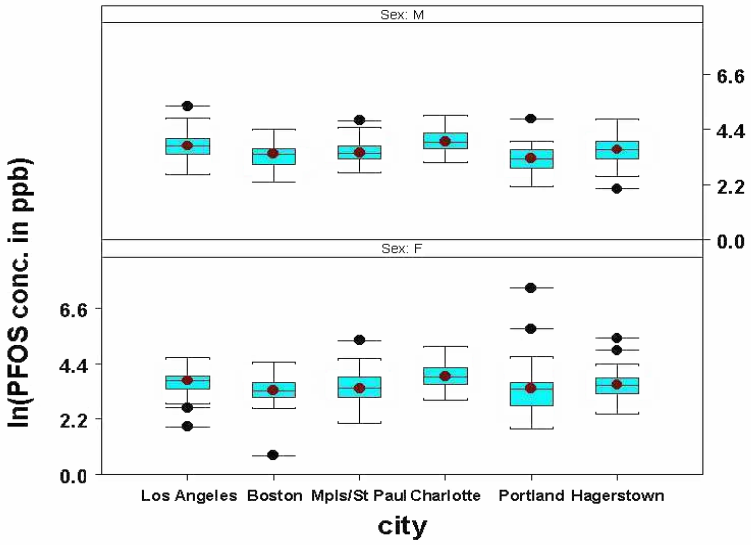


Figure 4-9. Box and Whisker Plots and Natural Log Mean [PFOS] (ppb) in the Adult Study Stratified by Location and Gender



To ensure rigorous determination of the 95th percentile of serum PFOS concentration, the bootstrap method of Efron (1993) was used to generate confidence intervals around the empirical percentiles for serum PFOS concentrations. In this method, a large number of replicated estimates of the percentile are generated from full size samples of the original observations drawn with replacement. Based on this analysis, the upper bound of the 95% confidence interval of the 95th percentile of the serum PFOS levels in these three populations are below 0.100 ppm. Using the same bootstrap technique, it can be said with 95% confidence that 99% of the serum concentrations of PFOS are below values that are approximately 0.200 ppm.

In summary, the findings from this analysis of measured serum PFOS concentrations in 598 children, 645 adults and 238 elderly are consistent with previously reported human data (cf. Table 4-1 with Tables 4-3 and 4-4). The three studies provided consistent results: the geometric means generally ranged between 0.030 and 0.040 ppm. The large individual sample size of these studies facilitated the characterization of the serum fluorochemical distributions, including the calculation of distribution-free tolerance limits and their upper bounds. The 95% tolerance limit for PFOS serum concentrations for these three populations approximated 0.087 ppm with an upper 95% confidence limit of 0.100 ppm. Although slight differences in age and gender were present, serum concentrations of measured PFOS were not demonstrably different by age or gender.

The highest serum PFOS measurement of the 1481 individual serum samples from these three studies was 1.7 ppm. Because donor samples were anonymous, it was not possible to determine anything about this single individual besides gender (male), age (67 years) and location (Portland, OR). This PFOS sample approximated the average serum PFOS levels observed for POSF-related production workers. The next two highest donor serum concentrations for PFOS were considerably lower at 0.515 ppm (child) and 0.329 ppm (adult). The highest serum PFOS concentration in an elderly adult was lower yet, at 0.175 ppm.

As with any interpretation of data obtained from a study population, questions arise regarding the representiveness and generalizability of the data collected. Clearly, American Red Cross blood donors used for the adult samples are a self-selected group from the United States population. The elderly adults were dementia-free voluntary participants in a longitudinal study of cognitive function. The pediatric participants were confirmed group A streptococcal infections. In all three studies, donors were anonymous. No information was obtained about past exposure histories to POSF-related chemistries and materials. However, it is unlikely the selection process of donors used in these three studies resulted in a lack of representiveness of the overall respective study populations from which these donors were sampled. 3M is unaware of any database that can be considered generalizable to the diverse United States general adult population without measures of random and systematic bias incorporated in the data analysis.

4.2.3.1 Organ Donor Study

Besides measurements of serum in non-occupational populations, there has been a limited assessment of human liver tissue concentrations of PFOS in relation to serum levels. Through arrangements with the International Institute for the Advancement of Medicine (IIAM), serum and/or liver samples were obtained from 31 donors over an 18 month time period (RS-III-16) (Olsen *et al*., 2003b). Both serum and liver tissue were harvested from 23 donors; 7 donors contributed liver tissue only and one donor contributed serum only. Arithmetic mean age was 50 years (SD 15.6, range 5-69) for male donors (n = 16) and 45 years (SD 18.5, range 13-74) for female donors (n = 15). Causes of death were intracranial hemorrhage (n = 16, 52%), motor vehicle accidents (n = 7, 23%), head trauma (n = 4, 13%), brain tumor (n = 2, 6%), drug overdose (n = 1, 3%) and respiratory arrest (n = 1, 3%). Serum and liver PFOS values determined to be less than the lower limit of quantitation (LLOQ) were assigned the midpoint value between zero and the LLOQ.

Fifteen (50%) of the 30 liver donors had liver PFOS analyses <LLOQ. The arithmetic mean liver PFOS concentration was calculated at 0.019 ppm (range <LLOQ (0.004) - 0.057 ppm). The geometric mean for liver PFOS was 0.153 ppm (95% CI 0.012-0.020). The arithmetic mean serum PFOS level was 0.018 ppm (range < LLOQ (0.006) - 0.058 ppm) for the 24 serum donors' samples analyzed. The geometric mean for serum PFOS was 0.015 ppm (95% CI 0.011-0.019). Mean PFOS concentrations were comparable between male and female donors for liver (male = 0.019 ppm; female = 0.018 ppm) and serum (male = 0.018 ppm; female = 0.017 ppm). No associations were observed between measured PFOS concentrations and age. The mean liver to serum ratio was 1.3:1 (95% CI 0.9:1 - 1.7:1 for the 23 donors who contributed both serum and liver. Restricted to only those donors (n = 10) who had serum and liver concentrations > LLOQ, the mean liver to serum ratio was 1.4:1 (95% CI 0.8:1 - 1.9:1) These data, although limited in numbers, were nevertheless consistent with serum PFOS concentrations reported above for the general population. Furthermore, the mean liver to serum ratio of 1.3 among the 23 donors who contributed both serum and liver tissue was comparable to the ratios reported in a month PFOS feeding study of cynomolgus monkeys (Seacat *et al.*, 2002a).

4.2.4 Pending Studies

A time-trend analysis of concentration of fluorochemicals, including PFOS, in serum collected in Washington County, Maryland, is underway. Serum samples were collected in 1974 and 1989

from the same 59 individuals, as well as 120 different individuals in the two time periods (ages 20-60+).

4.3 Human Studies

Studies in humans evaluating potential health effects include: medical surveillance of fluorochemical production workers at 3M plants in Decatur, Alabama and Antwerp, Belgium; a mortality study of the Decatur plant workers; and a hypothesis-generating study of episodes of medical care based on medical insurance claims from Decatur plant employees. There have been no epidemiological studies of the general (non-occupational) population.

4.3.1 Background and Early Medical Surveillance

Following reports of the finding of organic fluorine in serum samples, a fluorochemical medical surveillance program began at 3M's Decatur manufacturing facility in the late 1970s. The voluntary program has generally consisted of biennial tests of clinical chemistries, pulmonary function, and blood counts, accompanied by routine biomonitoring of fluorochemical exposure. Until the mid-1990s, 3M medical surveillance included a periodic measurement of total organofluorine in workers. Since the mid-1990s, these medical surveillance programs have incorporated serum measurements of PFOS and other analytes by high performance liquid chromatography-mass spectrometry methods rather than total organic fluorine. During the course of the medical surveillance since the late 1970s, company physicians have reviewed each employee's results. These physicians did not, and have not, found abnormalities in individuals that they believed were related to fluorochemical exposure. Pre-existing medical conditions, medications and lifestyle factors have adequately explained any observed clinical abnormalities.

4.3.2 Medical Surveillance Studies in 1994, 1995 and 1997

An aggregate analysis of measured serum PFOS concentrations was conducted of the Decatur and Antwerp male employees participating in the 1994/1995 (n = 178) and 1997 (n = 149) medical surveillance examinations (RS-III-2). (There were too few female employees to allow group 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 Decatur and Antwerp 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 the 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 serum PFOS concentration (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, the most sensitive clinical chemistry endpoint in rats and monkeys with increasing exposure to PFOS 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 a 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.

4.3.3 Medical Surveillance Study in 2000

In 2000, voluntary medical surveillance was again offered to Antwerp and Decatur employees involved in fluorochemical production (RS-III-4, RS-III-5, RS-III-17). There were substantially more employees who voluntarily participated in the medical surveillance program in 2000 than participated in 1995 and 1997. Altogether, there were 255 Antwerp employees (206 male and 49 female) and 263 Decatur employees (215 male and 48 female). This represented approximately 75 percent and 53 percent of the eligible employees at these two locations, respectively. Seventy three percent of the participating Antwerp employees and 75 percent of the Decatur employees worked in production activities; the remaining participants were administrative, site and laboratory employees. Only 12 percent of the Antwerp female employees worked in production activities compared to 63 percent of the Decatur female employees.

The study measured PFOS, PFOA and five additional fluorochemical analytes and provided a calculated value, total organic fluorine (TOF), which included the total organofluorine contribution of the seven measured analytes. PFOS and PFOA were the predominant contributors to TOF. Male production workers from the Decatur plant had higher serum PFOS concentrations than their Antwerp counterparts. Because the majority of Decatur female employees worked in production jobs, their serum PFOS levels were also higher than those reported for Antwerp female employees who were primarily nonproduction workers.

Table 4-5 presents the PFOS measurements from both plants for males and females combined.

Table 4-5. Year 2000 Medical Surveillance: Male and Female Serum PFOS Concentrations (ppm).

Production Facility	Arithmetic Mean	Geometric Mean	Arithmetic Mean of Fourth Quartile
Antwerp	0.80 (range: 0.04-6.24)	0.44 (95% CI: 0.38-0.51)	2.61 (range: 1.67-6.24)
Decatur	1.32 (range: 0.06-10.1)	0.91 (95% CI: 0.82-1.02)	3.22 (range: 2.31-10.1)

A standard set of hematological and clinical chemistry tests were analyzed. Six thyroid hormones/indices were also assayed: thyroid stimulating hormone (TSH; μIU/ml); serum thyroxine (T4; μg/dL); free thyroxine (free T4; ng/dL); serum triidothyronine (T3; pg/mL); thyroid hormone binding ratio (THBR, %, previously referred to as T3 Uptake) and free thyroxine index (FTI).

As observed previously (and discussed above with the 1995/1997 medical surveillance data), Antwerp employees, compared to Decatur employees, were significantly younger, had lower mean BMIs, worked fewer years, drank more alcoholic beverages per day, had higher mean HDL and total bilirubin values, and lower mean triglyceride, alkaline phosphatase, GGT, AST and ALT values.

Among all Antwerp and Decatur male employees, a statistically significant positive association between PFOS and serum cholesterol and triglycerides was reported. This positive association is inconsistent with the results of numerous toxicological studies which have shown PFOS to decrease serum cholesterol and triglyceride levels in research animals and then only at serum levels approximately two orders of magnitude higher than the average serum PFOS levels observed in these production employees.

Among the Decatur production employees, a significantly greater mean ALT value for those workers in the highest serum PFOS quartile distribution was reported (compared to the other three quartiles). This highest quartile of Decatur employees also had the largest percentage of employees with ALT (28%) and GGT (15%) above the reference range. Because of the potential confounding positive association with serum triglycerides, this variable, along with other important confounding factors (including age, BMI, number of alcoholic drinks per day, cigarettes smoked per day and location), were added to the hepatic clinical chemistry multivariable models as independent variables. In these models of male production and nonproduction employees, there was no significant association between PFOS and the liver enzymes after adjusting for these potentially confounding factors.

Among the combined multivariable analysis of Antwerp and Decatur male production and nonproduction employees, serum PFOS was not associated with TSH, T4, free T4, THBR or FTI. There was a weak positive significant association with T3. This T3 model with several independent variables, including PFOS, explained minimal variation ($r^2 = 0.12$). Although there was an association with T3, it is unlikely that this statistical finding was related to a hyperthyroid or hypothyroid condition based on the entirety of these thyroid hormone data.

Urinalyses were conducted only of the Decatur employee population. There was no association between the prevalence of abnormal urinalyses, as measured by a microstick (albumin, blood and glucose), and employee serum PFOS concentrations.

The 2000 medical surveillance data also offered the initial opportunity to examine, in aggregate, serum PFOS concentrations among Antwerp and Decatur female employees. The mean PFOS concentration for the Antwerp female employees was 0.13 ppm. These employees were primarily engaged in nonproduction activities. The mean PFOS level for Decatur female production and nonproduction employees was 0.93 ppm and more than 90% were engaged in production-related activities. A univariate analysis demonstrated the highest quartile of female employees (primarily Decatur production employees) were significantly different than the lowest quartile females (primarily Antwerp nonproduction employees) in many factors. Highest quartile females were older, had higher BMIs, smoked more, drank less, had higher triglyceride levels, alkaline phophatase levels and GGT levels, and had lower total bilirubin vales when compared to the lowest quartile females. There were no differences in the thyroid hormones measured or in the urinalyses.

In summary, the findings from three voluntary medical surveillance programs conducted at Antwerp and Decatur in 1994/95, 1997 and 2000 have continued to suggest that these POSF-related production employees do not have substantial changes in their serum cholesterol, lipoproteins or hepatic enzymes that are consistent with toxicological findings of PFOS observed in laboratory animals. The two employee populations, Antwerp and Decatur, have substantial demographic differences which can be associated with clinical chemistry tests (e.g., Decatur employees have significantly higher BMIs which influence hepatic enzyme test results); thus, it is important to take into account these potentially confounding factors when interpreting the data.

4.3.4 Longitudinal Analysis of Medical Surveillance Data

When a larger number of employees participated in the 2000 cross-sectional analysis, it became possible to undertake longitudinal analysis. (See RS-III-18 summarizing longitudinal study).

The longitudinal assessment was conducted on males only since there was an insufficient number of females (14) to produce a meaningful longitudinal assessment. One hundred and seventy-five (175) male employees voluntarily participated in the 2000 program and at least one of the two previous programs (1994/95, 1997). The breakdown was as follows:

- 41 (24 percent) participated in all three years (Antwerp = 21, Decatur = 20);
- 65 (37 percent) participated in 1994/95 and 2000 (Antwerp = 45, Decatur = 20); and
- 69 (39 percent) participated in 1997 and 2000 (Antwerp = 34, Decatur = 35).

PFOS was assayed in each surveillance program year, although the method of analysis (high performance liquid chromatography mass spectrometry) differed slightly between years. A different research laboratory was used to assay PFOS in each year.

The same hospital laboratory analyzed the clinical chemistries for all three surveillance years (1994/95, 1997, and 2000). Chemistries analyzed in this longitudinal assessment included cholesterol, HDL, triglycerides, alkaline phosphatase, GGT, AST, ALT, total and direct bilirubin. Most reference ranges remained relatively constant over the time except for ALT. In

each surveillance year, age, BMI, number of alcoholic drinks per day and cigarettes smoked per day were quantified and considered covariates in the analysis.

The continuous outcomes of lipid and hepatic clinical chemistry tests were evaluated as repeated measures incorporating the random subject effect fitted to a mixed model by the MIXED procedure in the SAS statistical package. Restricted maximum likelihood estimates of variance parameters were computed. Adjusted regression models were built by introducing all covariates and testing the covariance structure.

Adjusting for potential confounders in the statistical analyses, there were no temporal changes associated with hepatic or lipid clinical chemistry tests and PFOS.

4.3.5 Mortality Studies

There have been two mortality studies of Decatur production workers. In the first, University of Minnesota researchers conducted a retrospective cohort mortality study of employees who worked at least one year (1961-1990) at the 3M Decatur manufacturing site to determine whether the mortality experience of the production workforce was significantly different from that which would be expected (RS-III-19). 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 or 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.

The retrospective cohort study was subsequently updated for eligibility and vital status through 1998 (RS-III-20). In addition, an exposure matrix was developed for the updated assessment through computerization of the work history records of the study cohort (the original study had only extracted start and termination dates). With the knowledge accrued about the serum PFOS concentrations of the major job groups at Decatur (obtained through the voluntary medical surveillance program and a random sample study described previously) each unique job and department combination in the work history records was assigned to one of three POSF-related exposure categories: 1) no workplace exposure to POSF-based fluorochemicals (encompassed the film plant jobs at the Decatur facility); 2) low potential workplace exposure to POSF-based fluorochemicals (included such jobs as engineers, quality control technicians, environmental, health and safety workers, administrative assistants and managers); and 3) high potential workplace exposure to POSF-based fluorochemicals (included cell operators, chemical operators, maintenance workers, mill operators, waste operators and crew supervisors).

In the updated mortality study, there was a total of 50,970 person-years of follow-up among the 2083 eligible cohort members. The non-exposed (n = 812), low-exposed (n = 289) and high-exposed (n = 982) cohorts accumulated 20,304, 6823 and 21,876 person-years, respectively.

Altogether, there were 145 deaths identified in the entire cohort, and 139 death certificates (96%) were obtained. The overall SMR for all causes was 0.63 (95% CI 0.53-0.74). There were 39 deaths from all malignant neoplasms for the entire cohort (SMR = 0.72, 95% CI 0.51-0.98). Table 4-6 provides the causes of death for those male cohort members who worked for at least one year in a high exposure job (N = 782).

Table 4-6. Mortality Analysis for Male Employees Who Worked at Least One Year in a High Exposure Job at the Decatur Chemical Plant (n = 782)

Cause of Death	Observed	SMR	95% Confidence Interval
All Causes	53	0.70	0.54-0.95
All Malignant Neoplasms	14	0.80	0.46-1.41
Digestive Organs & Peritoneum	2	0.66	0.08-2.37
Esophagus	1	2.73	0.07-15.16
Biliary Passages & Liver Primary	1	2.57	0.06-14.26
Cancer of the Bronchus, Trachea, or Lung	6	0.96	0.35-2.09
Urinary Organs	3	5.11	1.05-14.93
Bladder	3	16.12	3.32-47.14
Malignant Melanoma of Skin	1	1.67	0.04-9.25
All Lymphatic & Hematopoietic Tissue	1	0.56	0.01-3.08
Cerebrovascular Disease	2	0.93	0.11-3.37
All Heart Disease	12	0.61	0.32-1.07
Cirrhosis of Liver	2	1.03	0.13-3.73
Accidents (motor vehicle & other)	13	1.04	0.56-1.78
Violence (suicides & homicides)	2	0.74	0.09-2.67

All three cases of bladder cancer deaths that occurred were in male employees who had worked in high exposure jobs for at least five years. The job classifications of the three workers, although classified as high exposure, were not exclusively related to chemical production jobs. The majority of their work history entailed work in maintenance, in the plant incinerator, or in the wastewater treatment plant. No other bladder cancer deaths were observed in the cohort. The investigators offered several possible explanations for the bladder cancer excess which included: 1) exposure to PFOS and other fluorochemicals; 2) another, undetermined occupational exposure(s); 3) non-occupational factors such as smoking or other personal habits; and 4) chance. Given the magnitude of the risk estimate, it would take many years of additional follow-up with no further deaths from bladder cancer for this excess to fully attenuate to null. Current toxicological evidence does not indicate that the bladder is a target of PFOS. The two-year dietary bioassay in rats with PFOS did not show an increased risk of bladder tumors or increases in urothelial hyperplasia. Most known bladder carcinogens are genotoxic or precipitate in the urine (Cohen, 1998). PFOS did not produce genotoxicity in multiple test systems and urinalyses from medical surveillance at the Decatur plant have not shown abnormalities attributable to PFOS exposure. Since the solubility of PFOS in human urine is 305 µg/mL at 23-24 deg. C (RS-III-21), it is unlikely that PFOS is insoluble in the urine of these workers. Other occupational exposures were investigated at this plant by developing a list of known or potential bladder carcinogens based on a review of the medical literature. Compounds on this list were compared,

by CAS numbers, with the historic raw material inventory records kept by the company. Five compounds were recognized as being used at the Decatur plant site. Four materials were found to have been part of former inactive processes, including 4,4 methylene-dianiline, orthotoluidine, benzidine salts, and butyl benzyl phthalate. The use of these materials ended in the 1960's and 1970's. These chemicals were not widely used in the plant, however, the available exposure monitoring and use information was limited. A fifth chemical, melamine, has been used in the last decade. Qualitative exposure assessments have indicated exposures to melamine were low based on short exposure task duration and exposure control practices.

Altogether, there were two deaths from liver cancer among workers with at least one year of work experience in either high or low exposure jobs (expected = 0.65, SMR = 3.08, 95% CI 0.37-11.10). Of these two liver cancer deaths, one individual had worked in a high exposure job (expected = 0.39, SMR = 2.57, 95% CI 0.06-14.26). As seen by the wide confidence intervals, it is not possible to draw any inference as these associations are based on very few observed and expected deaths. The only other liver disease category of death reported in the Decatur mortality study was for cirrhosis of the liver. Among the employees who worked at least one year in high exposure jobs there were two deaths from cirrhosis of the liver (expected 1.94, SMR = 1.03, 95% CI 0.13-3.73).

There are several limitations to this mortality study. A death certificate was not obtained from six known decedents. The extent to which this would affect the results is unknown, but would have the largest effect on the analysis if the non-coded causes of death were attributed to relatively rare causes of death including liver and bladder cancer. Although the exposure matrices were guided with biomonitoring data from a previous assessment, which likely reduced misclassification, this may not have completely eliminated misclassification. Potential confounders (e.g., smoking) were not available for this cohort. The size of the cohort currently precludes a detailed analysis by exposure, particularly for less common diseases.

At the request of 3M, researchers from the University of Minnesota Division of Environmental and Occupational Health are in the midst of a case ascertainment study to determine the bladder cancer incidence experience of all past and present Decatur employees who have worked at the manufacturing site (chemical or film) since the plant began production in the early 1960s. The study design includes a self-reported survey with medical validation for those individuals who report a bladder cancer diagnosis. Other survey questions may provide further perspective regarding the findings reported in the mortality and episodes of care studies.

Except for medical surveillance activities, epidemiologic research studies (e.g., mortality research) have not been conducted at the Antwerp, Belgium plant. A retrospective cohort mortality study was not feasible at the Antwerp plant due to the confidential nature of death certificate registration in Belgium.

4.3.6 Episodes of Care

A major strength of a retrospective cohort mortality study, as described above, is the fact that, if the complete study population is identified, a comprehensive analysis of the occupational cohort's death experience can be ascertained. However, if the occupational exposure is likely to lead to non-fatal outcomes, then the utility of a mortality study may be limited. Because the

average Decatur employee's serum PFOS levels were approximately two orders of magnitude below the earliest clinical effect observed (lowered serum cholesterol levels) in the six-month PFOS oral dosing study with cynomolgus monkeys, it is not anticipated that mortality outcomes would be found to be associated with exposure to POSF-based fluorochemicals. A morbidity study (i.e., episodes of care) was therefore designed to examine the health experience of Decatur chemical and film plant employees (RS-III-22).

A health care episode is defined as a series of events all of which are related to a particular health problem that exists continuously for a delineated period of time. For a given individual, medical claims which may seem unrelated can be grouped, via proprietary software, for a given disease or condition, and these grouped claims are referred to as episodes. The episode of care concept does not translate into a well-defined epidemiologic endpoint, as it may include incident cases, prevalent cases, tentatively diagnosed cases and misclassified cases that are the routine consequence of the differential diagnoses that individuals may undergo in the course of disease diagnosis, treatment and management. Nevertheless, the episode of care concept may be a useful screening method for the potential risk of diseases and/or conditions that do not lead to traditional occupational epidemiologic study endpoints such as mortality, or for morbidity outcomes that would be difficult to assess without formal investigations involving comprehensive medical-record reviews to validate the diagnoses.

The purpose of this study was to compare the ratio of observed to expected episodes of care experience from January 1, 1993 through December 31, 1998 of 652 Decatur chemical plant employees to the ratio of observed to expected episode of care experience of 659 film plant employees at the 3M Decatur manufacturing site. Health claims data were not available for analysis purposes prior to 1993. Clinical Care Group™ (CCG) software, developed by Ingenix Employer Group, was 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. Based on the toxicology and epidemiology research conducted to date, episodes of care that were considered *a priori* interests for this study included liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, gastrointestinal disorders of the liver and biliary tract, and reproductive, pregnancy, congenital and perinatal disorders.

Altogether for the six-year study period, there were 34,053 outpatient and 204 inpatient claims among the 652 chemical plant employee subjects compared to 40,174 outpatient and 237 inpatient claims from the 659 film plant employees. Once categorized by the CCG software, these individual medical claims resulted in 10,608 episodes of care for the 652 chemical plant employees compared to 11,957 episodes of care among the 659 film plant employees.

The overall episodes of care experience was comparable between chemical and film plant employees for most diseases and conditions and between the four comparison groups described in the robust summary (RS-III-22). Table 4-7 presents an abbreviated sample of the more than 400 RRE_pCs for the overall comparison. Where increased RRE_pCs (observed:expected ratio in the chemical plant divided by observed:expected ratio in the film plant) were observed, they were often attributed to a deficit of observed episodes of care in the film plant as much as any observed excess of episodes of care in the chemical plant. These high RRE_pC values often had

wide 95% confidence intervals which did not exclude, or barely excluded, the null value (e.g., see malignant neoplasm of prostate in Table 4-7).

Table 4-7. Episodes of Care Study Results

	Disease or Condition	*Chemical Plant* Obs	Exp	Unique Number of Individuals (%)	*Film Plant* Obs	Exp	Unique Number of Individuals (%)	RRE_pC[a]	95% CI
1.	Infectious Diseases	582	599.6	402 (69)	680	612.7	432 (64)	0.9	0.8 – 1.0
2.	Cancers and Benign Growths	303	270.7	192 (63)	273	320.4	174 (64)	1.3	1.1 – 1.6
	Malignant neoplasm of colon	4	1.8	4 (100)	1	2.4	1 (100)	5.4	0.5 – 265
	Malignant neoplasm of liver	0	0.5	0 (-)	1	0.6	1 (100)	-	-
	Malignant neoplasm of pancreas	1	0.4	1 (100)	0	0.6	0 (-)	-	-
	Malignant neoplasm of rectum and anus	4	1.3	4 (100)	3	1.8	3 (100)	1.8	0.3 – 12.4
	Benign colonic polyps	48	24.6	37 (77)	47	33.7	38 (81)	1.4	0.9 – 2.1
	Neoplasms of the respiratory tract	2	2.5	2 (100)	1	3.3	1 (100)	2.6	0.1 – 155
	Benign neoplasm of skin	94	71.9	93 (99)	82	79.8	79 (96)	1.3	0.9 – 1.7
	Malignant neoplasm of female breast	2	3.5	2 (100)	0	4.0	0 (-)	-	-
	Benign neoplasm of breast	10	16.8	9 (90)	10	17.6	10 (100)	1.1	0.4 – 2.8
	Malignant neoplasm of bladder	0	1.0	0 (-)	1	1.5	1 (100)	-	-
	Malignant neoplasm of kidney	0	0.7	0 (-)	1	0.9	1 (100)	-	-
	Malignant neoplasm of prostate	5	3.1	5 (100)	1	4.7	1 (100)	7.7	0.9 – 364
	Malignant neoplasm of testicle	2	0.6	2 (100)	0	0.6	0 (-)	-	-
	Neoplasms of the nervous system	1	1.6	1 (100)	1	1.9	1 (100)	-	-
	Malignant neoplasm of thyroid	1	1.0	1 (100)	0	1.1	0 (-)	-	-
	Other benign or unspecified neoplasm	66	71.7	64 (97)	63	85.4	63 (100)	1.3	0.9 – 1.8
	Leukemia, chronic	0	0.8	0 (-)	3	1.1	3 (100)	0.0	0.0 – 3.1
	Lymphoma	3	2.4	3 (100)	3	2.8	3 (100)	1.2	0.2 – 8.7
	Multiple myeloma	1	0.4	1 (100)	1	0.5	1 (100)	-	-
3.	Endocrine Disorders	370	367	224 (61)	453	438.8	266 (59)	1.0	0.9 – 1.1
	Disorders of the thyroid	31	51.2	22 (71)	33	57.1	28 (85)	1.1	0.6 – 1.8
	Diabetes (Type I and II)	69	66.7	41 (59)	91	84.4	62 (68)	1.0	0.7 – 1.3
	Disorders of lipid metabolism (hyperlipidemia)	157	139.2	141 (90)	194	172.7	173 (89)	1.0	0.8 – 1.3
4.	Hematologic Disorders	44	46.4	36 (82)	43	53.0	39 (91)	1.2	0.8 – 1.8
5.	Neurologic Disorders	186	211.4	129 (69)	236	236.9	160 (68)	0.9	0.7 – 1.1

Table 4-7. Episodes of Care Study Results (continued)

Disease or Condition		*Chemical Plant* Obs	Exp	Unique Number of Individuals (%)	*Film Plant* Obs	Exp	Unique Number of Individuals (%)	RRE_pC[a]	95% CI
6.	Cardiovascular Disorders	413	423	234 (57)	571	533	282 (49)	0.9	0.8 – 1.0
	Hypertension	157	149.4	145 (92)	203	185.4	186 (92)	1.0	0.8 – 1.2
	Atherosclerotic coronary vascular disease and cardiac arrest	82	76.1	51 (62)	134	102.6	65 (49)	0.8	0.6 – 1.1
7.	Pulmonary Disorders	849	691.8	381 (45)	897	757.6	394 (44)	1.0	0.9 – 1.1
	Lower respiratory infections	315	305.0	181 (57)	327	337.9	181 (55)	1.1	0.9 – 1.3
	Asthma	23	36.9	22 (96)	28	39.5	27 (96)	0.9	0.5 – 1.6
	Chronic obstructive pulmonary disease	30	23.3	30 (100)	32	29.2	31 (97)	1.2	0.7 – 2.0
8.	Disorders of Gastrointestinal System	631	520.5	264 (42)	755	604.3	306 (41)	1.0	0.9 – 1.1
	Disorders of the liver	3	6.0	3 (100)	3	6.9	3 (100)	1.2	0.2 – 8.6
	Disorders of the biliary tract	26	25.7	13 (50)	20	30.6	12 (60)	1.6	0.8 – 2.9
	Disorders of the pancreas	7	4.2	1 (14)	3	5.4	2 (67)	3.0	0.7 – 18
9.	Urologic Disorders	411	269.0	163 (40)	481	318.4	205 (43)	1.0	0.9 – 1.2
	Cystitis	56	23.3	19 (34)	40	24.3	31 (78)	1.5	1.0 – 2.2
	Urinary tract infections, not specified	71	71.2	47 (66)	67	75.1	45 (67)	1.1	0.8 – 1.6
	Calculus of urinary tract	73	32.2	34 (47)	85	38.9	44 (52)	1.0	0.8 – 1.4
	Prostatic hyperplasia	59	40.6	53 (95)	83	58.1	79 (95)	1.0	0.7 – 1.4
	Prostatitis, acute	73	39.2	55 (58)	100	48.7	60 (60)	0.9	0.7 – 1.2
10.	Gynecologic and Repro. Disorders	354	378.4	182 (51)	323	374.1	193 (60)	1.1	0.9 – 1.3
11.	Pregnancy	40	44.7	13 (33)	23	26.3	8 (35)	1.0	0.6 – 1.8
	Complicated pregnancy or delivery	29	30.2	12 (41)	16	18.6	8 (50)	1.1	0.6 – 2.2
	Preterm labor	7	3.1	3 (43)	1	1.7	1 (100)	3.9	0.5 – 172
	Normal or unspecified preg. and del.	11	14.5	9 (82)	7	7.7	5 (71)	0.8	0.3 – 2.6
	Spontaneous abortion	2	1.2	2 (100)	0	0.7	0 (-)	-	-
	Pregnancy	9	13.3	9 (100)	7	7.0	5 (71)	0.7	0.2 – 2.2
12.	Congenital Anomalies	25	28.4	25 (100)	27	31.2	26 (96)	1.0	0.6 – 1.8
13.	Perinatal Disorders	1	2.7	1 (100)	4	2.6	4 (100)	0.2	0.0 – 2.4

[a] RRE_pC = observed:expected ratio in the chemical plant compared to observed:expected ratio in the film plant.

For *a priori* interests, increased episodes of care among chemical plant workers as opposed to film plant workers were not observed for liver tumors or liver disease, bladder cancer, thyroid and lipid metabolism disorders or reproductive, pregnancy and perinatal disorders.

Because of a previously reported increased mortality risk for bladder cancer among the chemical plant employees, particular attention was given to those episodes of care which involved the urogenital tract. One episode of care for bladder cancer was reported for a film plant employee who had never worked in the chemical plant. There was an increased risk ratio in episodes of care among chemical plant employees for lower urinary tract infections (RRE_pC = 1.3, 95% CI 1.0-1.6). This increased risk ratio in episodes of care was greater among the long-term high exposure chemical plant workers (RRE_pC = 2.2, 95% CI 1.4-3.3). There was also a greater percentage of these long-term high exposure chemical plant workers who had recurring lower urinary tract infections (59%) than film plant workers (31%). It is not known whether this reoccurrence is a result of occupational exposure(s) or nonoccupational-related factors. It was noted that the prevalence of episodes of care for lower and unspecified urinary tract infections (number of unique individuals divided by population at risk) was comparable between the chemical (9.5%) and film (9.9%) plant employees.

Other associations from Table 4-7 include malignant neoplasm of colon (RRE_pC = 5.4, 95% CI 0.5 - 265) and rectum (RRE_pC = 1.8, 95% CI 0.3-12.4). There was a total of 5 unique chemical plant employees who constituted the 8 episodes of care for the cancer of the colon and rectum conditions. The frequency of adenomatous polyps often parallels colorectal cancer incidence. Benign colonic polyps was nonsignificantly increased (RRE_pC = 1.4, 95% CI 0.9-2.1). It should be noted that results from a two-year PFOS bioassay study in rats did not report an increased incidence of colon tumors. Malignant neoplasm of the prostate had an RRE_pC = 7.7 (95% CI 0.9 - 364). The magnitude of this association was partially attributable to the 1 observed malignant neoplasm of prostate in the film plant compared to 4.7 expected. Prostatic hypertrophy was not increased among chemical plant employees (RRE_pC = 1.0, 95% CI 0.7 - 1.4). The RRE_pC for disorders of the pancreas was elevated among chemical plant employees but this was based on only one unique chemical plant worker.

Among gynecologic and reproductive disorders, pregnancy, congenital anomalies and perinatal disorders, only pre-term labor had a RRE_pC greater than 2, with a very wide 95% confidence interval (RRE_pC = 3.9, 95% CI 0.5 - 172). Perinatal disorders were lower among chemical plant employees (RRE_pC = 0.2, 95% CI 0.0 - 2.4).

An important methodologic strength of this study was the comparison of two local study groups: one likely to have been exposed to POSF-based chemicals (chemical plant) and a reference group of employees (film plant) who were unlikely to have been exposed. This study design limited the influence of regional differences or variations in diagnostic and therapeutic patterns. Another strength of the study was the use of the metric, episodes of care, which was likely comprehensive for all health outcomes experienced among these study subjects from 1993 through 1998 as long as they remained employed at the Decatur site. Study limitations included: 1) the episode of care concept does not readily translate into a well-defined epidemiologic metric (incidence or prevalence data); 2) the study time period was only six years; 3) not all employees were covered (approximately 40 employees had elected to join a Health Maintenance Organization and were not part of the study); and 4) former employees were not included as they

would not be covered by the company's health insurance plan, although retirees would be covered.

Several of the associations reported in this episode of care investigation will be investigated further in the University of Minnesota case ascertainment study described above in the mortality section.

4.4 Toxicology Studies

The sections below describe data from toxicology studies of PFOS:

- Section 4.1 - Toxicokinetics and Metabolism;
- Section 4.2 - Acute Toxicity Studies;
- Section 4.3 - Subchronic Repeated Dose Studies;
- Section 4.4 - Reproductive and Developmental Toxicity;
- Section 4.5 - Genotoxicity;
- Section 4.6 - Chronic Studies and Oncogenicity;
- Section 4.7 - Mechanisms of Toxicity; and
- Section 4.8 - Other Information Relevant to Toxicity.

4.4.1 Toxicokinetics, Metabolism

The absorption, tissue distribution, potential metabolism and excretion of PFOS have been studied most extensively in rats by both radiolabel (^{14}C) and direct quantitation. Data relating oral dose to serum and liver concentrations of PFOS in the cynomolgus monkey during dosing and recovery were determined from direct quantitation. Using direct quantitation, serum PFOS concentrations in retired 3M chemical workers have been followed in an attempt to estimate an elimination rate constant.

4.4.1.1 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 [^{14}C]PFOS demonstrated that > 95% of the dose was absorbed in the first 24 hours (RS-III-23).

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 (RS-III-24). 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, the data are 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, although an LC_{50} of 5.2 mg/L has been estimated from an inhalation study (RS-III-31). Due to the exceptionally low vapor pressure of PFOS, inhalation exposure to vapor would be unlikely. If inhalation exposure does occur, it would be most likely associated with aerosols or particulates containing PFOS. In the production of PFOS and related chemistries, exposure to the volatile PFOS-precursor, POSF, was possible. In addition to POSF, certain other precursor molecules, such as N-EtFOSE and N-MeFOSE, are known to sublime. These precursors represent a more likely inhalation exposure pathway than exposure to PFOS itself.

4.4.1.2 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 [^{14}C]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 [^{14}C]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 ($\leq$0.5) were measured in adrenals, skin, testes, muscle, fat and eye. No radioactivity (<0.05) was detected in brain. (RS-III-25).

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 (RS-III-26; Johnson *et al.*, 1984).

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) (Seacat, *et al.*, 2002a). 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. 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, a fact that allows accurate

estimation of expected serum concentration from delivered dose as well as accurate estimation of delivered dose from serum concentration.

In a 26-week capsule-dosing study in cynomolgus monkeys, a similar pattern of increasing serum concentration with cumulative dose was observed (Seacat *et al.*, 2002a). At the lower experimental doses, 0.03 and 0.15 mg/kg/day, serum levels increased in fairly linear fashion (Figure 4-10). Mean serum and liver concentrations for males and females appeared similar in all dose groups and are presented in Table 4-8.

Three male and three female cynomolgus monkeys given a single intravenous dose of 2.0 mg/kg PFOS were found to have.volumes of distribution ranging from 178 - 220 mL/kg in males and 231 - 327 mL/kg in females (Noker and Gorman, 2003). These values are in general agreement with observations from the oral toxicity studies in cynomolgus monkeys, as described above.

PFOS was found to be 99% to 100% bound to rat, monkey, and human plasma over a concentration range of 1 to 500 μg/mL (approximately 2 μM to 1 mM) *in vitro* (Kerstner-Wood *et al.*, 2003). This suggests that major differences in plasma binding capacity for PFOS do not exist between species.

In order to identify primary binding proteins for PFOS in human plasma, isolated human plasma protein fractions were made up in buffer at physiological concentration, and PFOS was introduced at a final nominal concentration of 10 μg/mL (Kerstner-Wood *et al*., 2003). The percent of PFOS found bound to protein was 99.8% for albumin, 95.6% for beta-lipoprotein, 59.4% for alpha-globulin, 24.1% for gamma-globulin, and < 0.1% for each of fibrinogen, alpha-2-macroglobulin, and transferrin.

Figure 4-10. Serum PFOS concentrations in monkeys given PFOS for 183 days.

Male (closed symbol, - - -) and female (open symbol, —) Cynomolgus monkeys treated with 0.03 (■,□); 0.15 (▲, Δ) or 0.75(●, o) mg PFOS/kg/day.

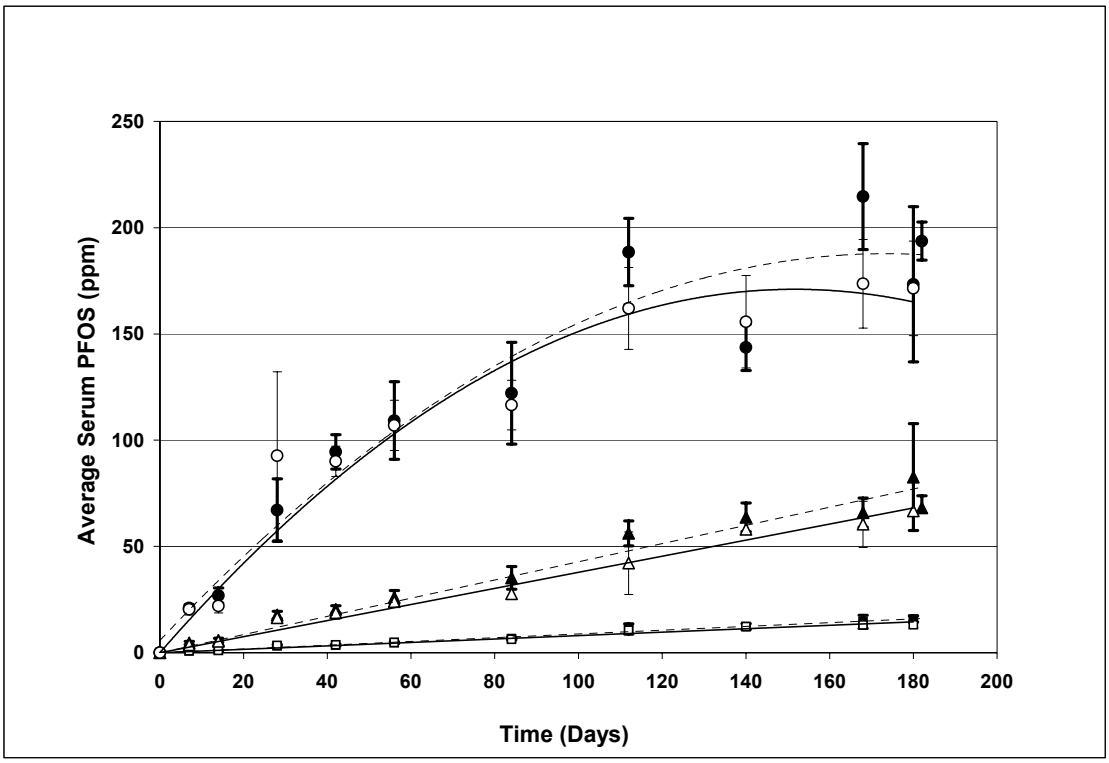


Table 4-8. Mean Serum and Liver PFOS Concentrations (ppm) in Male (M) and Female (F) Monkeys Dosed Orally for Six Months with PFOS.

Dose Group	$[PFOS]_{serum}$, M	$[PFOS]_{serum}$, F	$[PFOS]_{liver}$, M	$[PFOS]_{liver}$, F
Control	0.05 ± 0.01	0.05 ± 0.02	0.12 ± 0.03	0.11 ± 0.02
0.03 mg/kg/day	15.8 ± 1.4	13.2 ± 1.4	17.3 ± 4.7	22.8 ± 2.1
0.15 mg/kg/day	82.6 ± 25.2	66.8 ± 10.8	58.8 ± 19.5	69.5 ± 14.9
0.75 mg/kg/day	173 ± 37	171 ± 22	395 ± 24	273 ± 14

Male and female CR:CD rats continuously exposed to PFOS in the diet for 14 weeks (RS-III-27) also showed a linear relationship between dose and serum concentration, as shown by the data in Table 4-9.

Table 4-9. Serum Concentrations in Male and Female Rats After 14 Weeks of Exposure to PFOS in the Diet.

	Dose Group			
	Males			
Dietary Dose [a] (ppm)	0.5 ppm	2.0 ppm	5.0 ppm	20 ppm
Delivered Dose [b] (mg/kg)	0.04	0.10	0.35	1.37
Cumulative Dose [c] (mg/kg)	3.2	12.6	31.1	123
Serum PFOS concentration (ppm)	4.22	17.9	45.6	134
	Females			
Dietary Dose [a] (ppm)	0.5 ppm	2.0 ppm	5.0 ppm	20 ppm
Delivered Dose [b] (mg/kg)	0.04	0.16	0.41	1.6
Cumulative Dose [c] (mg/kg)	3.6	14.6	37.0	144
Serum PFOS concentration (ppm)	6.7	26.9	62.9	216

[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
[c] Delivered dose times days of dosing

Distribution across the placenta and exposure of the fetus *in utero* has been demonstrated. Serum and liver PFOS concentration measurements were obtained from a pharmacokinetic study conducted during pregnancy (RS-III-28). 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. Pooled fetal serum was obtained by litter on gestation day 21. The pre-mating (GD0), gestation day 21 (GD21), and lactation day 22 values are represented in Table 4-10.

Table 4-10. Maternal and Fetal PFOS Serum and Liver PFOS Concentrations Associated with Gestation and Lactation (Rats).

Dose Group	Biological Matrix	PFOS Concentration, ppm			
		Pre-mating Dams[a]	GD21[b] Dams	GD21 Fetal Litter[c]	LD22[d] Dams
0.1	Serum	10.3 ± 1.3	4.91 ± 1.23	10.5 ± 1.0	0.04 ± 0.02
	Liver	---	23.4 ± 3.8	9.17 ± 1.08	
0.4	Serum	47.1 ± 5.0	30.3 ± 17.0	39.7 ± 5.9	5.28 ± 0.36
	Liver	---	107 ± 25	42.5 ± 19.4	
1.6	Serum	185 ± 14	158 ± 87	117 ± 15	18.9 ± 1.3
	Liver	---	440 ± 316	100 ± 31	
3.2	Serum	367 ± 24	180 ± 42	191 ± 26	82 ± 18
	Liver	---	598 ± 84	265 ± 70	

[a] Dams were treated daily by oral gavage for 42 days prior to mating, during mating and through gestation. Pre-mating samples were taken after 42 days of dosing.
[b] Gestation day 21
[c] Pooled sample by litter
[d] Lactation day 22

The potential for exposure during lactation has been demonstrated in a cross-foster study with PFOS. Dams were exposed to either 0 or 1.6 mg/kg/day PFOS for 42 days prior to mating with breeder males and through cohabitaiton and gestation. Each dam was allowed to deliver naturally, and each resulting litter was immediately placed in the care of another dam such that no dam raised her natural litter. The design was such that four foster groups resulted: 1) pups from control dams raised by control dams; 2) pups from control dams raised by treated dams; 3) pups from treated dams raised by control dams; and, 4) pups from treated dams raised by treated dams. Table 4-11 presents the serum PFOS concentrations in dams and pups (pooled by litter) at the end of the 21-to-22-day lactation period. Pup serum PFOS concentrations were greatest in pups that were from treated dams and raised by treated foster dams. Pups from treated dams that were raised by control dams had the second highest serum PFOS concentrations. Lactational transport is suggested by the fact that pups from untreated dams that were raised by treated dams had the third highest serum PFOS concentrations. Transfer via milk has recently been supported by the analysis (by CDC validated analytical method for milk) of two milk samples taken at lactation day 14 from treated dams in which PFOS was found; there was no PFOS above the lower limit of quantitation in eight control milk samples taken at the same time (Dr. Antonia Calafat, CDC, personal communication).

Table 4-11. PFOS Serum Values in Foster Dams and Pups from a Cross-Foster Study with Potassium PFOS at Time of Necropsy (LD 21/22) in ug/mL (ppm)

Fetal/Pup Exposure		Lactation Day 22 $[PFOS]_{serum}$ in ppm (mean)	
Natural Mother Tresatment during Gestation [a] (Pup Exposure *in utero)*	Foster Mother Treatment during Gestation [b] (Pup Exposure during Lactation)	Foster Nursing Dams	Pups (pooled by litter)
0	0	0.05[c] (12) [d]	0.05 (6)
0	1.6	83.0 (13)	22.4 (6)
1.6	0	2.02 (13)	53.9 (6)
1.6	1.6	89.0 (12)	89.7 (6)

[a] Gestational period exposure is from dams treated with 1.6 mg PFOS/kg/day for 42 days prior to mating, during a variable mating period and through gestation.
[b] Lactational period exposure is for pups and presumed to be from milk of dams exposed through gestation; therefore, "0" represents foster dam was not dosed with PFOS.
[c] 0.05 µg/mL (ppm) is Lower Limit of Quantitation. All PFOS concentrations are from samples taken at the end of the lactational period (22 days after birth).
[d] Number in parentheses is number of samples.

Figure 4-11 plots maternal dose in a two-generation reproduction study with PFOS in rats against mean serum concentration of PFOS in parental-generation females at two points in time, just prior to mating (42 days of dosing) and on gestation day 21 (~65 days of dosing). The plot demonstrates the linearity of mean serum PFOS levels prior to mating when plotted against maternal dose; however, at the end of gestation, mean serum levels at the higher dose tended to plateau. Up to approximately 1 mg/kg/day maternal dose, the differences between serum concentrations of PFOS measured prior to mating and at the end of gestation are less prominent than those differences measured in the range where perinatal toxicity has been observed (above 1 mg/kg/day). Figure 4-12 plots mean maternal liver concentrations from gestation day 21 against maternal dose. The plots in Figures 4-11 and 4-12 can be used to interpolate expected serum or liver concentrations at other PFOS doses.

Serum concentrations of PFOS from the 14-week dietary study and the reproduction study show relatively good correspondence when daily dose is compared to serum concentration in the females. Differences can be explained by the difference in the length of the dosing periods and methods of dosing.

PFOS has the ability to compete with endogenous substrate for binding sites on albumin and liver fatty acid binding protein (L-FABP) (Luebker *et al.*, 2002).

Figure 4-11. Plot of Maternal Dose in a Rat Reproduction Study with PFOS Against Mean Serum PFOS Concentration (µg/mL) at either Just Prior to Mating (42 Days Dosing) or at the End of Gestation (~70 Days of Dosing).

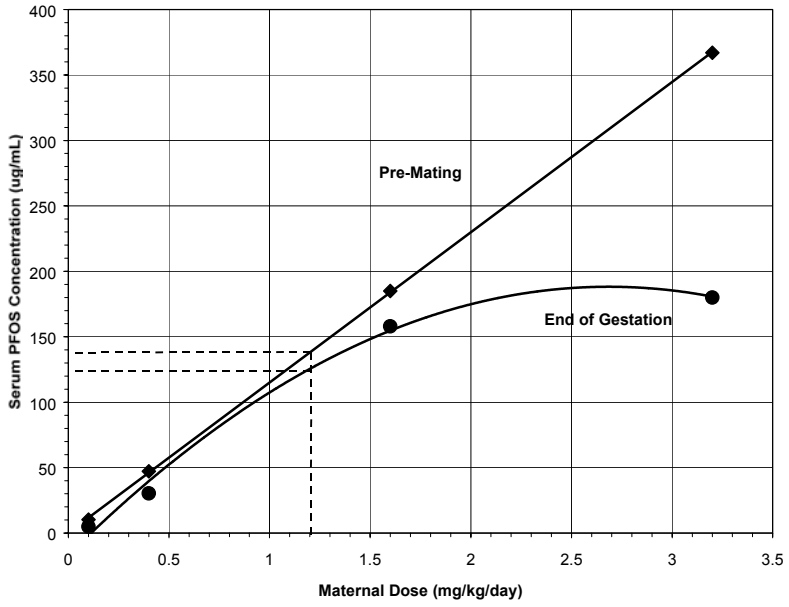
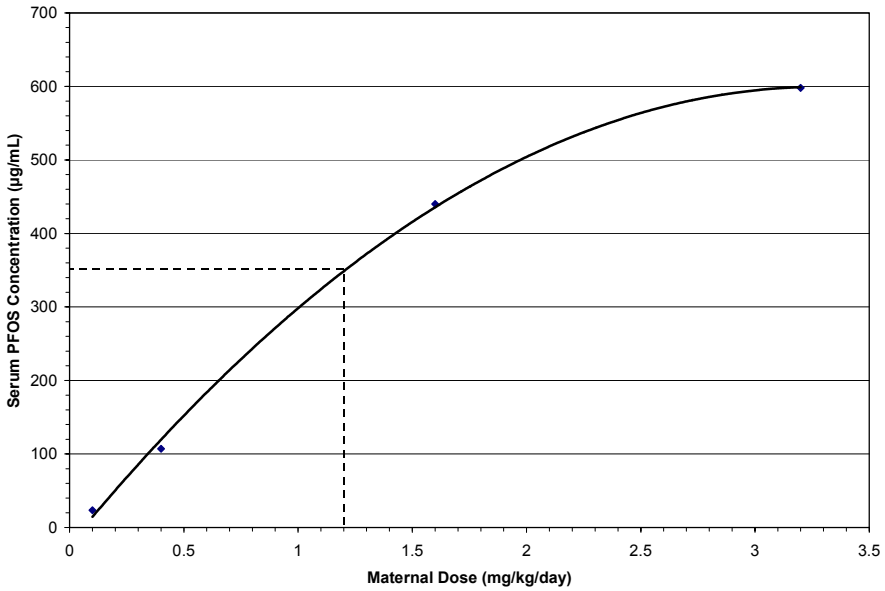


Figure 4-12. Plot of Maternal Dose in a Rat Reproduction Study with PFOS against Mean Liver PFOS Concentration (µg/mL) at the End of Gestation (~ 70 Days of Dosing).



4.4.1.3 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 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 (RS-III-29). This represented 4.4% of the administered dose.

4.4.1.4 Excretion

Single intravenous doses (mean 4.2 mg/kg) of [^{14}C]PFOS in 0.9% NaCl were administered to male rats (RS-III-25). 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. 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. In an oral study, 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 (RS-III-23).

Fecal and total excretion of ^{14}C were increased in male rats administered cholestyramine (~ 2.7 g/kg/d) in their diet following single intravenous doses of [^{14}C]PFOS (Johnson *et al.*, 1984; RS-III-26). 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 (9.4µg/g), plasma (0.9µg/ml), and red blood cells (0.3 µg/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; however, due to the enhanced fecal elimination, the extent of total elimination of ^{14}C (urine plus feces) was higher in the cholestyramine-treated rats.

Cynomolgus monkeys (two per sex per dose group) were followed for one year in recovery after six months of daily oral dosing by capsule at 0.15 or 0.75 mg/kg/day (Seacat *et al.*, 2002a). While the numbers of animals are limited, the values suggest mean serum elimination half-lives of ~ 200 days were calculated for the mid- and high-dose recovery groups, suggesting that 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.

After a single intravenous dose of 2.0 mg/kg potassium PFOS was given to three male and three female cynomolgus monkeys, terminal elimination half-lives ranged from 122-146 days (mean = 132 days) in males and 88 - 138 days (mean = 110 days) in females (Noker and Gorman, 2003). Excretion of PFOS in urine was ≤ 0.1% of total administered dose in any given 24-hour collection period. Total body clearance ranged from 1.0 - 1.2 mL/kg/day in males and 1.6 - 2.1 mL/kg/day in females.

Of the species examined, humans have been determined to have the longest apparent elimination half-life. Twenty-four (21 male, 3 female) retirees from the Decatur (AL) manufacturing plant and three male retirees from the Cottage Grove (MN) manufacturing plant have voluntarily agreed to participate in a research effort to determine the serum half-life of PFOS. The retirees were invited to participate based on having prior work assignments in the chemical division. All participants were long-term 3M employees. To date, there have been six collection periods for the Decatur retirees: November 1998, June 1999, November 1999, May 2000, February 2001 and January 2002. These correspond to t_0 through t_5 time periods, respectively.

A second interim report regarding serum PFOS half-life analysis has been done of the t_0 through t_3 time periods for nine of the 27 subjects, all of whom were Decatur retirees (7 males, 2 females) (RS-III-30). In an effort to minimize experimental error including systematic and random error in the analytical method (high performance liquid chromatography electrospray tandem mass spectrometry), the nine subjects had their serum measured in triplicate for the four time periods (t_0 through t_3). This approach allowed for statistical evaluation of the precision of the measurement and assured that all experimental biases equally affected each sample used for half-life determination. Serum half-lives were calculated using a one compartmental model. The average age of these nine retirees at the study initiation (t_0) was 61 years (SD = 4.8). Their number of months retired at study initiation (t_0) from Decatur averaged 19 months (SD = 3.6). Their mean measured PFOS concentration at study initiation (t_0) was 0.89 ppm (range 0.11-3.53 ppm; SD = 1.1). Their mean serum half-life for PFOS was 8.7 years (range 2.3-21.3 years, SD = 6.1). Multivariable regression analyses examined the influence of age, body mass index (BMI), number of years worked or years since retired on the serum-half life. None of these variables was a significant predictor of the serum half-life for PFOS.

Blood collection is scheduled for completion by January 2004 with a final report of all study subjects presented within six months. No further interim reports are scheduled until the study is completed. Data from the interim report should be regarded as preliminary.

As noted in the discussion of human exposure data, there has been a limited assessment of human liver tissue concentrations of PFOS in relation to serum levels. The mean liver to serum ratio was 1.3 for the 23 donors who contributed both serum and liver. This liver to serum ratio is comparable to the 1:1 ratio reported in the low- and mid-dose groups in the six month PFOS feeding study of cynomolgus monkeys, whose serum levels averaged 20 and 75 ppm, respectively.

4.4.1.5 Summary

The data provide no evidence for PFOS metabolism in any species. PFOS is readily absorbed after oral exposure, but the rate of absorption by the dermal route is low. There is evidence that absorbed PFOS is distributed primarily in blood and liver. PFOS undergoes entero-hepatic recirculation. There is slow whole body elimination in both sexes. At lower and moderate experimental doses, body burden is proportional to cumulative dose. Liver-to-serum PFOS concentration ratios from non-occupationally exposed humans appear to be similar to the ratios observed in cynomolgus monkeys after six months of dosing.

In rat studies it is clear that PFOS can traverse the placenta and expose the fetus *in utero.* PFOS is also distributed into the milk of lactating females.

4.4.2 Acute Toxicity Studies

Several acute toxicity studies have been conducted on PFOS. In a study to determine the median lethal concentration (LC_{50}), the potassium salt of PFOS as dust in air was administered to Sprague-Dawley rats at levels of 1.89 to 45.97 mg/l PFOS (RS-III-31). An LC_{50} of 5.2 mg/l was estimated from this study. Two oral toxicity studies on PFOS have been performed. In one acute oral toxicity study, the acute oral LD_{50} and 95% confidence limits were determined to be 251 (199-318) mg/kg (RS-III-32). Another study tested only two doses and determined the acute oral LD_{50} to be greater than 50 mg/kg and less than 1500 mg/kg. 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 (RS-III-24). Thus, PFOS is acutely toxic in laboratory animals by inhalation and ingestion.

4.4.3 Subchronic Repeated Dose Toxicity

PFOS has been studied in a 90-day subchronic dietary study in rats (RS-III-33), at subchronic time points in a chronic study in rats (RS-III-27), in a 90-day gavage study in rhesus monkeys (RS-III-34, RS-III-35), and in a 26-week oral (capsule) study in cynomolgus monkeys (Seacat *et al.*, 2002a). Results from repeat-dose studies in rats and monkeys are summarized in Tables 4-12 and 4-13 below, respectively. Doses in the older studies ranged higher than the more recent studies. An improvement in the recent studies is the measurement of serum and liver PFOS concentrations, allowing for these concentrations to be associated with doses producing effects and no effects. The rat and monkey studies are described in detail in the following discussion.

4.4.3.1 Rats

In a 90-day dietary study reported in 1978, rats were fed 0, 30, 100, 300, 1,000 and 3,000 ppm PFOS (RS-III-33). At 30 ppm, mean body weights were slightly lower when compared to the controls, and slight elevation of blood glucose, serum alkaline phosphatase, blood urea nitrogen and gamma-glutamyl transpeptidase activities were reported. PFOS at doses of 100 ppm and

greater caused liver enzyme elevations, hepatic vacuolization, hepatocellular hypertrophy, weight loss, convulsions and death.

As part of a chronic (104-week) dietary study of PFOS in rats, observations for subchronic toxicity were made at 4, 14, and 53 weeks (Seacat *et al.*, 2003; RS-III-27). Male and female rats (Crl:CD® (SD) IGS BR) were fed diets containing the potassium salt of PFOS at 0, 0.5, 2.0, 5.0, and 20.0 ppm for 4, 14, or 52 weeks. The potential role of early hepatocellular peroxisomal and cellular proliferation as factors in liver response, and liver and serum PFOS concentrations were also measured. Blood and liver samples were collected at weeks 4 and 14 (all dose groups), and at week 53 (high dose and controls).

Subchronic results at 4, 14 and 53 weeks during the 104-week study demonstrated the liver as the target organ (Seacat *et al.*, 2003). At 4 weeks, effects included decreased serum glucose in the 20-ppm dose-group males. At 14 weeks, the 20 ppm dose group males had increased liver weight, decreased serum cholesterol, increased non-segmented neutrophils, and increased alanine amino transferase. Relative liver weights and urea nitrogen were increased in both sexes. Hepatocytic hypertrophy and cytoplasmic vacuolation were observed in the 5 and 20 ppm male and the 20 ppm female dose groups. Although there was a weak (< two-fold) increase in hepatic palmitoyl CoA oxidase activity in 20 ppm dose-group males at four weeks, this was not observed at 14 weeks. The average hepatocyte proliferation index was not increased, although individual animals had mild increases. Serum PFOS concentrations were generally higher in females than in males (see Table 4-21). The liver-to-serum PFOS ratios ranged from approximately 3:1 to 12:1 and were generally greater in males than in females. After 14 weeks, the no-observed-effect levels (NOEL) were 2 ppm and 5 ppm in males and females, respectively. These no-effect dose levels corresponded to average serum PFOS concentrations of 17 ± 1 ppm (males, N=5) and 64 ± 6 ppm (females, N=5) and average liver PFOS concentrations of 74 ± 6 ppm (males, N=5) and 370 ± 22 ppm (females, N=5).

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) were 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 hepatocellular vacuolation were increased in incidence in males at 20 ppm. High-dose females showed an increased incidence of centrilobular hepatocellular hypertrophy and pigment. There were no effects on hepatic palmitoyl-CoA oxidase activity or cell proliferation (as measured by BrDU) at 53 weeks. In the high-dose group for which tissue concentrations were measured, serum and liver PFOS concentrations were essentially the same at week 53 as at week 14 (see Table 4-21).

Results for the study did not provide strong evidence for hepatocellular peroxisomal or cellular proliferation at the doses tested**.** Liver toxicity was present at lower dietary dose levels than had been observed in the 1978 study. Histologic evidence of liver effects (vacuolation and

hypertrophy) seen at the 5 and 20 ppm dose levels in the recent study were not observed at 30 ppm in the 1978 study but were present at 100 ppm in the latter study.

Table 4-12. Summary of Sub-Chronic Toxicity Studies for PFOS in Rats

Diet Dose Level	Observations Related to Toxicity
90-Day Dietary Study in CD Rats (1978)	
30 ppm	↓ body weight; ↑ ALT, AST; liver discoloration
100 ppm	3 pre-term deaths; ↓ food consumption, hemoglobin, hematocrit, erythrocyte count, reticulocyte count (females), leukocyte count; ↑ CK, AP, Glucose, BUN, sensitivity to external stimuli; liver enlargement, hepatocellular hypertrophy & necrosis; stomach discoloration & hemorrhage
300 ppm	Fatal pre-term; symptoms of emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy, & dermal acanthosis
1000 ppm	Fatal early pre-term; hunched posture
3000 ppm	Fatal early pre-term; hypoactivity
NOAEL	None - the lowest dose tested (30 ppm) was an effect dose
14-Week Dietary Study in Crl:CD® (SD) IGS BR Rats (2002)	
0.5 ppm	None
2 ppm	None
5 ppm	Hepatocellular hypertrophy & vacuolation (males)
20 ppm	↓ body weight; ↓ total serum cholesterol (males); ↑ liver weight; hepatocellular hypertrophy & vacuolation
NOAEL	5 ppm (males); 5 ppm (females) $[PFOS]_{serum}$ = 44 ± 1 ppm (males); 64 ± 6 (females) $[PFOS]_{liver}$ = 74 ± 6 (males); 370 ± 22 (females)

4.4.3.2 Monkeys

In a six-month oral-dosing study in cynomolgus monkeys (Seacat *et al.*, 2002a), groups of six male and six female monkeys (four per sex at the low dose) received 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS by oral capsule for 182 days. Two monkeys per sex from each group, except the 0.03 mg/kg/day dose group, were assigned to recovery groups and were monitored for one year after the last treatment. Significant adverse effects only occurred in the 0.75 mg/kg/day dose group. These included:

1) compound-related mortality in two of six male monkeys;
2) decreased body weights;
3) increased liver weights;
4) lowered serum total cholesterol;
5) lowered triiodothyronine concentrations (without evidence of hypothyroidism);
6) lowered estradiol levels.

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, fewer feces (mucoid, liquid and black-colored), 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 uncertain, although pulmonary necrosis with severe acute inflammation was a potential factor. On day 179, a second male from the 0.75 mg/kg/day dose group was sacrificed in a moribund condition. Clinical signs noted included low food consumption, excessive salivation, labored respiration, hypoactivity and ataxia. Despite thorough pathological review, the cause of death could not be determined and did not appear to involve the liver. However, both cases are believed to be the result of PFOS-treatment.

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 enlarged liver. Three of four 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.

The decrease in serum total cholesterol occurred at the high dose of 0.75 mg/kg/day at serum PFOS concentrations greater than 100 ppm. High-density lipoprotein (HDL), a component of serum total cholesterol, also appeared to decrease at the high dose.

The study data suggested a statistically significant reduction in HDL in females in the 0.15 mg/kg/day dose group when analyzed against controls using Dunnett's test. Two of the six values were slightly below the reported reference range of 30-150 mg/dL. Serum HDL is usually

proportional to serum total cholesterol, which was not reduced at this dose level. Because of the small number of females (six), and the facts that pre-study baseline values for HDL were not obtained, and HDL was only measured at the end of the six-month dosing period, it is difficult to interpret if this apparent marginal reduction was a result of PFOS treatment. The statistically significant reductions in HDL and total cholesterol observed in the 0.75 mg/kg/day dose group females are consistent with the expected reduction in HDL during total cholesterol lowering. If HDL was actually reduced as a result of treatment in the 0.15 mg/kg/day dose group females, lowering of HDL may be a more sensitive clinical chemistry response than lowering of serum total cholesterol (see discussion in Seacat *et al*., 2002a). Even HDL was reduced in females in the 0.15 mg/kg/day dose group as a result of PFOS treatment, the individual values suggest that the change is not toxicologically significant.

The only histomorphologic findings were hepatocellular hypertrophy and lipid vacuolation, both present at term in the 0.75 mg/kg/day dose group. No peroxisomal or cell proliferation was detected in liver tissue.

During the recovery period, complete reversal of clinical and hepatic effects and significant decreases in serum and liver PFOS occurred within 211 days post-treatment.

The no-observed-adverse-effect level (NOAEL) was established as 0.15 mg/kg/day. Serum concentrations associated with no adverse effects (0.15 mg/kg/day) were 82.6 ± 25.2 ppm for males and 66.8 ± 10.8 ppm for females, and the mean of males and females was 75 ppm. Mean liver-to-serum PFOS ratios were comparable in all PFOS-treatment groups, with a range of 1:1 to 2:1.

Data from the cynomolgus monkey studies and earlier studies in rhesus monkeys (RS-III-34 and RS-III-35) are summarized in Table 4-13.

Table 4-13. Summary of Sub-Chronic Toxicity Studies for PFOS in Monkeys

Dose Level	Observations Related to Toxicity
Aborted 90-Day Oral (Gavage) Study in Rhesus Monkeys (1978)	
0 mg/kg (2M/2F)	None
10 mg/kg (2M/2F)	Pre-term fatality within 11-20 days; ↓ body weight; marked weakness; anorexia; ↓ activity; emesis; diarrhea; tremors; prostration; hemorrhage; lipid depletion of the adrenal cortex
30 mg/kg (2M/2F)	Pre-term fatality within 7-10 days; same symptoms as 10 mg/kg
100 mg/kg (2M/2F)	Pre-term fatality within 3-5 days; same symptoms as 10 mg/kg
300 mg/kg (2M/2F)	Pre-term fatality within 2-4 days; same symptoms as 10 mg/kg
NOAEL	None (<10 mg/kg)
90-Day Oral (Gavage) Study in Rhesus Monkeys (1979)	
0 mg/kg (2M/2F)	None
0.5 mg/kg (2M/2F)	No adverse effect; slight, intermittent ↓ activity in 3 of 4 monkeys
1.5 mg/kg (2M/2F)	Blood & mucous in stools; diarrhea; dehydration; tremors; ↓ body weight; ↓ cholesterol; marked ↓ activity
4.5 mg/kg (2M/2F)	Fatal pre-term within 7 weeks; marked ↓ cholesterol; ↓ AP; ↑ AST; atrophy of pancreatic exocrine cells & submandibular salivary gland serous alveolar cells; diffuse lipid depletion of adrenals; ↓ body weight; black/bloody stools; dehydration; rigidity; convulsions; prostration
NOAEL	0.5 mg/kg
182-Day Oral (Gavage) Study in Cynomolgus Monkeys (2002)	
0 mg/kg (6M/6F)	None
0.03 mg/kg (4M/4F)	None
0.15 mg/kg (6M/6F)	None
0.75 mg/kg (6M/6F)	1 pre-term death & 1 pre-term moribund sacrifice (males); ↓ body weight; ↑ liver weight; ↓ total serum cholesterol, HDL, T3, E2; mottled liver (2 males/1 female); centrilobular or diffuse hepatocellular hypertrophy and vacuolation
NOAEL	0.15 mg/kg
NOAEL ($[PFOS]_{serum}$)	83 ± 25 ppm (males); 67 ± 11 ppm (females)
NOAEL ($[PFOS]_{liver}$)	59 ± 20 (males); 70 ± 15 (females)

4.4.3.3 Summary

For PFOS, similar toxic effects are produced in subchronic studies in rats and monkeys. These effects are generally characterized as body-weight or body-weight gain decreases, liver-weight or relative liver-weight increases, liver cell hypertrophy, in some cases with vacuolation, and a reduction in serum cholesterol levels.

4.4.4 Reproductive and Developmental Toxicity

Developmental toxicity studies in rats and rabbits and 2-generation studies in rats for PFOS are available. In addition, the results of cross-fostering and mechanistic studies are presented and discussed. All studies employed the oral (gavage) route for administering PFOS.

4.4.4.1 Developmental Studies

The effects of oral exposure of pregnant rats and rabbits on prenatal development of their embryos and fetuses were assessed. PFOS caused maternal and fetal toxicity. Initial studies with PFOS reported a lesion in the lens of the eye in all treated groups (RS-III-36). The study director subsequently retracted the causal association between this effect and chemical exposure 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 a repeat study in the same laboratory that conducted the original study.

Since those original studies were conducted, additional prenatal developmental toxicity studies have been performed in the rat and rabbit (Case *et al.*, 2001a). Key aspects of all developmental studies with PFOS are summarized in Table 4-14.

Results in the three PFOS studies were similar. Maternal toxicity and developmental toxicity were 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 were the same, 1 mg/kg body weight. The maternal toxicity NOAEL was 0.1 and 1.0 mg/kg for rabbit and rat, respectively. Fetal toxicity, rather than anatomical malformations, characterized the principal effect of PFOS. Developmental effects were seen only in conjunction with maternal toxicity, indicating that PFOS is not a selective developmental toxicant.

In a recent study, findings of cleft palate, anasarca, ventricular septal defect, and enlargement of the right atrium were found in rat and mouse fetuses, primarily at maternal doses of 10 and 20 mg/kg/day, respectively (Thibodeaux *et al.*, 2003). In these studies, pregnant rats were given doses of 1, 2, 3, 5, or 10 mg/kg/day from gestational day (GD) 2 through GD 20, and pregnant CD-1 mice were given 1, 5, 10, 15, and 20 mg/kg/day from GD 1 through GD 17. Maternal weight gains and serum triglycerides were reduced in a dose-dependent manner in both species, and pregnant mice experienced marked liver enlargement at 10, 15, and 20 mg/kg/day dose levels. The numbers of implantation sites and live fetuses at birth were not altered as compared to controls; however, fetal weight in the rats was slightly reduced. The birth defects that occurred were at doses that produced significant maternal toxicity.

Table 4-14. Summary of PFOS Developmental Toxicity Studies (by Oral Gavage)

Design	NOAEL[a]	LOAEL[a]	Effects	Reference
Rat SD Group size: 22 Dose[a]: 0, 1, 5, 10	Mat.[b] 5 Dev.[c] 10	Mat. 10, Dev. None	Mat. Body weight.	RS-III-36
Rat SD Group size: 25 Dose[a]: 0, 1, 5, 10	Mat. 1 Dev. 1	Mat. 5 Dev. 5	Mat. Body wt. Clinical signs, g.i. lesions. Dev. Body wt, visc. anom., skel. var.	RS-III-37
Rabbit NZW Group size: 22 Dose[a]: 0, 0.1, 1, 2.5, 3.75	Mat. 0.1 Dev. 1	Mat. 1 Dev. 2.5	Mat. Body wt. Abortions Dev. Body wt. Delayed ossification	RS-III-38

[a] (mg/kg). Rats dosed on GD 6-15. Rabbits dosed on GD 7-20.
[b] Mat. = maternal
[c] Dev. = developmental

4.4.4.2 Two-Generation Study

A two-generation study in the rat was conducted with potassium PFOS (RS-III-39). No effects on mating, fertility, or natural delivery were noted, with the exception of a decrease in implantations and litter size in F_0 females at 3.2 mg/kg, the highest dose tested. The early adverse response in adults and in pups was reduced body weight gain in both sexes. Most significant was the death of all F_1 pups in the perinatal period at the maternal dose of 3.2 mg/kg/day. Mortality was also seen in F_1 pups from dams that received 1.6 mg/kg. The dose-response for this effect was 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 groups, respectively. Severity of effect on F_1 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 three dose groups proceeded through a mating, pregnancy and postnatal evaluation phase until F_2 pups were 21 days of age. The F_1 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 F_2 pups were modest and transient. The NOAELs from the study and the effect(s) seen at the next higher dose are represented in Table 4-15 below.

Maternal body weight changes are observed in the pre-natal teratology studies and the two-generation studies. Results of the two-generation study indicate that fertility and reproductive

performance are not impaired at doses that cause adverse body weight effects on males and females. 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 two-generation studies, pup development until the time of birth was fairly normal. 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 contributed to this effect.

Table 4-15. NOAELs and LOAELs (mg/kg/day) from a Two-Generation Reproduction/Developmental Study with PFOS.

Generation	Effect of Interest	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)
	PFOS		
F_0	↓ Body Weight Gain	0.1	0.4
F_0	↓ Implantation & Litter Size	1.6	3.2
F_1	↑ Neonatal Mortality & ↓ Body Weight	0.4	1.6
F_1	Reproductive Parameters	0.4	Not Established (no effects at highest dose tested)
F_2	All Measured Parameters	0.4	Not Established (no effects at highest dose tested)

4.4.4.3 Cross Foster Study

A PFOS cross-foster study was performed to ascertain the role of pre-natal, post-natal, or combined exposure on pup mortality and health (RS-III-40) (Case *et al.*, 2001b). 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-day premating period, mating, pregnancy, and a lactation period of 21 days. At birth, 25 litters from control or treated dams were cross-fostered with either control or PFOS treated dams. Thus, four groups were established. The results of the study that ended on post-natal day 21 are summarized in Table 4-16 below.

Mortality was increased (9.6% versus 1.6% in control) in pup litters whose exposure was solely *in utero.* Mortality was greatest (19.2%) in pup litters exposed *in utero* who also were nursed by treated females. There was no increase in mortality in pup litters not exposed *in utero* that were nursed by treated females; body weight gain was reduced. The greatest reduction in weight gain was in pup litters that had *in utero* and lactation exposure. PFOS serum levels were determined in litters and dams from this study. These data were summarized in Table 4-11 above.

The data indicate that treatment of a pregnant dam can result in *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 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 above (RS-III-28), 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 premating serum concentration of dams in this dose group averaged 185 ppm.

In summary, *in utero* exposure to 1.6 mg/kg PFOS via the dam led to perinatal mortality and reduced growth. In a separate study, maternal exposure to 1.6 mg/kg led to serum levels of 117 ppm in fetuses just prior to birth. *In utero* and peri-natal exposure to 1.6 mg/kg appeared 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 perinatal toxicity is directly associated with PFOS serum concentration.

Table 4-16. Cross-Foster PFOS Study Post-Natal Pup Effects During 21 Day Lactation Period

PFOS Exposure[a]		No. Pups Dead	Total Pups	Mortality, %	No. Litters Affected	Pup Weight[b]
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] refers to daily female dose of 0 or 1.6 mg/kg PFOS.
[b] mean weight in Grams on LD 14

4.4.4.4 Further Study of the Perinatal Mortality Effect

A one-generation reproductive/developmental study of PFOS was performed to better define the dose-response for perinatal mortality and to explore the role of hypolipidemia and other factors as potential mechanisms responsible for the perinatal mortality observed in the prior multi-generation reproduction/developmental study in rats (RS-III-41). This study will be discussed in two parts. The first is related to dose-response of perinatal mortality, and the second is related to the results of the effect on pup viability after supplementation of maternal rats with high doses of cholesterol and its metabolic precursor, mevalonate, along with doses of PFOS known to cause perinatal death in pups.

4.4.4.5 Dose-Response of Perinatal Mortality

To better define the dose-response for perinatal mortality, groups of female rats were administered potassium PFOS by daily oral intubation at dose levels of 0, 0.4, 0.8, 1.0, 1.2, 1.6 or 2.0 mg/kg/day (Luebker *et al.*, 2002; Butenhoff *et al.*, 2002). The female rats were dosed for 42 days prior to mating with untreated males. After mating, dosing continued in the females throughout gestation and five days of lactation. At day 21 of gestation, fetuses from eight litters in the 0, 1.6, and 2 mg/kg dose groups were taken by cesarean section for serum chemistry and liver PFOS determination. All remaining dams were allowed to give birth and surviving pups and dams were sacrificed at day 5 of lactation. A viability index was calculated for pups through day 5 of lactation. Total serum cholesterol, HDL, LDL, triglycerides, glucose, free and total T3, free and total T4, TSH, and serum PFOS concentrations were determined in fetuses/pups and dams at gestation day 21 and lactation day 5. Liver PFOS concentrations were determined in the 0.0, 0.4, 1.6, and 2.0 mg/kg dose groups (dams and pups) on lactation day 5.

Table 4-17 represents the results with respect to viability through day 5 of lactation. Based on significant increased mortality at maternal doses of 1.6 and 2.0 mg/kg/day, the no-observed effect level for perinatal mortality and pup growth under the study conditions was 1.2 mg/kg/day.

Results of thyroid hormone determinations require a special discussion due to the fact that the initial finding of significant reductions as compared to controls in thyroxin (T4) and triiodothyronine (T3) in dams and pups without a corresponding increase in thyroid stimulating hormone (TSH) may be largely influenced by method issues. As initially measured, changes in the levels of thyroid hormones were present in all treated groups. On lactation day 5, free and total T4 were significantly reduced in pups from all dosed groups. Total T3 was reduced in pups in the 1.6 and 2.0 mg/kg/day dose groups (analysis of free T3 in pups was complicated by most control and dosed group values being below detection). TSH values in pups were elevated only in the 1.6 mg/kg/day group and decreased in the 0.4 mg/kg/day group. Dams in all groups receiving PFOS had lower free and total T4 and T3 at gestation day 21 when compared to controls with no significant changes in TSH. At lactation day 5, dams in all dosed groups had lower total and free T4, all dose groups above 0.4 mg/kg/day had lower total T3, all dose groups above 1.0 mg/kg/day had lower free T3, and all groups had comparable TSH. The reductions in T4 without corresponding increases in TSH do not represent clinical hypothyroidism and could result from method interferences. Direct spiking of the RIA assays with up to one mM PFOS did

not result in method interference; however, equilibrium dialysis followed by RIA assay of free T4 from dams in all dose groups did not indicate that free T4 was decreased, indicating the presence of an interfering substance (Chopra, 1997 and 1998). Thus, it is likely the reductions are artifactual, based on the presence of normal TSH values and the results of equilibrium dialysis. In addition, histological evaluation of pup thyroids did not indicate abnormalities.

Light microscopy was performed on hearts and thyroids from lactation day 5 pups in the 0.0, 0.4, 1.6, and 2.0 mg/kg/day dose groups. No pathology findings were observed in any of the tissues.

Mean serum PFOS levels were generally similar in dams and pups at lactation day 5 and increased in a dose response manner (Table 4-17). Liver PFOS levels in dams at lactation day 5 were generally similar to the lactation day 5 serum levels. Liver PFOS levels in the pups at lactation day 5, however, were approximately twice as high as the pup serum levels and the dam liver levels (Table 4-17).

4.4.4.6 Other Studies

The perinatal mortality that originally observed in the two-generation study has also been observed in rats and mice after dosing of pregnant dams during gestation alone (Lau *et al.*, 2003; Grasty *et al.*, 2002). In the study reported by Lau *et al.* (2003), pregnant rats were given doses of 1, 2, 3, 5, and 10 mg/kg/day from GD 2 through GD 21, and pregnant CD-1 mice were given doses of 1, 5, 10, 15, and 20 mg/kg/day from GD1 through GD 18. Neonate mortality within 24 hours of birth was 95% at doses of 5 mg/kg/day and 15 mg/kg/day for rats and mice, respectively. Mouse pups from treated dams had increased liver weights. As in the 3M cross-foster study, cross-fostering the pups from PFOS-treated dams to control dams failed to improve survival. Growth lags occurred in surviving pups from treated dams. Slight delays in eye-opening were noted. Learning and memory acquisition were not affected. It is noteworthy that hypothyroxemia (reduced serum thyroxin) was noted without a feedback increase in thyroid stimulating hormone or evidence of thyroid toxicity. Serum PFOS concentrations in neonates were similar to maternal serum PFOS concentrations and were dose dependent.

Grasty *et al.* (2002) have reported that PFOS-induced perinatal mortality occurs from dosing pregnant Sprague Dawley rats with PFOS (25 mg/kg/day) for four-day intervals covering GD 2 through GD 20, with neonatal mortality increasing as the dosing period approaches the end of gestation. Maternal serum PFOS concentrations were directly related to neonatal mortality. This suggests that the body burden in pregnant dams toward the end of gesatation is most influential in producing mortality of the neonate.

Table 4-17. Dose-Response Study: Percent Viability of F1 Rat Pups through Day 5 of Lactation

Maternal Dose (mg/kg/day)	0.0	0.4	0.8	1.0	1.2	1.6	2.0
Viability Index (%)	97.3	97.6	93.1	88.8	81.7	49.2**	17.1**
$[PFOS]_{serum}$ (ppm), Dams	0.02 ± 0.01(6)	27 ± 19 (6)	43 ± 7 (6)	52 ± 26 (6)	86 ± 10 (6)	169 ± 32 (6)	134 ± 27 (6)
$[PFOS]_{serum}$ (ppm), Pups	0.03 ± 0.02(6)	36 ± 4 (6)	53 ± 28 (6)	84 ± 18 (6)	147 ± 162 (6)	NA[a]	138 (1)
$[PFOS]_{liver}$ (ppm), Dams	1.6 ± 0.5 (6)	48 ± 5 (4)	NA	NA	NA	110 ± 13 (6)	146 ± 20 (6)
$[PFOS]_{liver}$ (ppm), Pups	< LOQ[b] (6)	73 ± 31 (6)	NA	NA	NA	248 ± 131 (6)	245 ± 93 (4)

** Statistically significant difference from control, $p < 0.01$ (Student's t-test)
[a] Not analyzed
[b] Below limit of quantitation (< 12.5 ng/g)

4.4.4.6 Dietary Supplementation with Cholesterol and Mevalonic Acid Lactone

To test the hypothesis that hypolipidemia may be a factor in (PFOS)-induced neonatal mortality, groups of female rats were supplemented with either 500 mg/kg of mevalonate (as mevalonic acid lactone) twice daily or 500 mg/kg cholesterol once daily and compared to non-supplemented controls (Luebker *et al.*, 2002; Butenhoff *et al.*, 2002; RS-III-53). Dose groups consisted of vehicle control, cholesterol/vehicle control, mevalonate/vehicle control, 1.6 mg/kg/day PFOS, 1.6 mg/kg/day PFOS + mevalonate, 1.6 mg/kg/day PFOS + cholesterol, 2.0 mg/kg/day PFOS, 2.0 mg/kg/day PFOS + cholesterol, and 2.0 mg/kg/day PFOS + mevalonic acid. The female rats were dosed for 42 days prior to mating to untreated males. After mating, dosing continued in the females throughout gestation and five days of lactation. At day 21 of gestation, fetuses from eight litters per dose group were taken by cesarean section. All remaining rats were allowed to give birth and pups and dams were sacrificed on day 5 of lactation. Total serum cholesterol, HDL, LDL, TG, glucose, and PFOS concentration were determined for both fetuses/pups and dams on day 21 of gestation and day 5 of lactation. Plasma mevalonic acid levels were determined in the mevalonate supplemented and non-supplemented dose groups at both time points.

Table 4-18 summarizes key serum chemistry measurements for supplemented and non-supplemented groups for day 21 of gestation, and results for pup viability through post-natal day 5. Plasma mevalonic acid levels were significantly higher in the mevalonic acid supplemented animals than in the non-supplemented animals. Serum cholesterol levels, however, did not differ between the cholesterol supplemented dose groups and the non-supplemented dose groups. Maternal supplementation with mevalonate or cholesterol had no positive effect on perinatal survival through lactation day 5, and neonatal mortality was not associated with reduced serum lipid in the study.

Table 4-18.Supplementation Study: Lactation Day 5 Viability Index and Gestation Day 21 Serum Parameters

Maternal Dose (mg/kg)	Tween 80 Control	1.6 mg/kg PFOS	2 mg/kg PFOS	MAL Control	1.6 mg/kg PFOS + MAL	2 mg/kg PFOS + MAL	CHOL Control	1.6 mg/kg PFOS + CHOL	2 mg/kg PFOS + CHOL
Neonate Viability through Day 5 of Lactation (Percent)									
Day 5 Viability Index (%)	97.3%	49.2%**	17.1%**	98.7%	41.4%*	1.1%**	98.0%	42.0%*	14.3%**
Serum Chemistry Parameters on Day 21 of Gestation (Group Mean Values)									
Maternal cholesterol (mg/dl)	84.3	78.1	72.3	92.5	91.0	88.9	89.6	79.3	77.9
Maternal Mevalonate (mg/dl)	47.7	20.6	86.2	1663.8	1369.8	1482.5	NA	NA	NA
Maternal PFOS (µg/ml)	0.007	142	125	NA	NA	NA	NA	NA	NA
Fetal Cholesterol (mg/dl)	50.8	61.3	61.5	53.1	68.3**	63.1	45.1	59.1**	64.0**
Fetal Mevalonate (mg/dl)	54.5	67.0	104.8	18971	16563	20175	NA	NA	NA
Fetal PFOS (µg/ml)	< LOQ	142	170	NA	NA	NA	NA	NA	NA

* Statistically significant difference from control, $p < 0.05$ (Student's t-test)
** Statistically significant difference from control, $p < 0.01$ (Student's t-test)
LOQ, limit of quantitation, 0.005 µg/ml
NA, not analyzed

4.4.5 Genetic Toxicity

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* (RS-III-42, RS-III-43, RS-III-44, RS-III-45, RS-III-46), a test for gene conversion in the D4 strain of *Saccharomyces cerevisiae* (RS-III-46), an *in vitro* assay for chromosomal aberrations in human whole blood lymphocytes (RS-III-47), the mouse micronucleus assay (RS-III-48), and an assay for unscheduled DNA synthesis (UDS) in primary rat liver cell cultures (RS-III-49). PFOS was negative in all assays in which it was tested.

PFOS potassium salt 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* WP2*uvr*A, 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 (RS-III-43, RS-III-46).

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% (RS-III-42).

PFOS potassium salt 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 (RS-III-47). Nor did it induce UDS in primary cultures of rat hepatocytes when tested at up to cytotoxic levels (RS-III-49).

In the *in vivo* mouse micronucleus assay, PFOS 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 (RS-III-48).

4.4.6 Chronic Study and Oncogenicity

One dietary carcinogenicity study has recently been completed on PFOS in Sprague Dawley rats (RS-III-27; Seacat *et al.*, 2002b).

4.4.6.1 Animals and Dosing

Male and female Crl:CD®(SD)IGS BR rats were given potassium PFOS (86.7 %) in the diet at 0, 0.5, 2, 5, and 20 ppm for up to 104 weeks. A recovery group received PFOS for 52 weeks, after which they were dosed with the same diet as control animals. The group designations and details of dosing duration are presented in Table 4-19. Average doses in each group calculated from feed consumption and body-weight data are represented in Table 4-19. Results from interim sacrifices at weeks 4, 14 and 53 were previously described in the discussion of subchronic results. This section describes observations from 53 weeks through term.

Table 4-19. PFOS Cancer Bioassay: Dose Groups and Estimated Amount of PFOS Consumed by Rats

Dietary Dose Group (ppm PFOS in Diet)	Group Size (Male & Female)	Mean Achieved Dose Levels - Range (mg/kg body weight/day)	
		Males	Females
Control[a, b, c]	70	-	-
0.5 ppm[b]	60	0.015 - 0.057	0.015 - 0.052
2 ppm[b]	60	0.064 - 0.226	0.073 - 0.213
5 ppm[b]	60	0.153 - 0.570	0.186 - 0.559
20 ppm[b, c]	70	0.643 - 2.205	0.838 - 2.149
20 ppm (Recovery)[d]	40	0.732 - 2.336	1.047 - 2.160

[a] The control animals received the control diet (basal diet with acetone).

[b] Five animals/sex in Groups 1 through 5 were sacrificed at Weeks 4 and 14 for hepatocellular proliferation rate measurements, biochemical analyses (palmitoyl-CoA oxidation), and histopathology (Week 14 only).

[c] Ten animals/sex in Groups 1 and 5 were designated as interim sacrifice animals. These animals were sacrificed after at least 52 weeks of treatment.

[d] Animals in Group 6 were treated for at least 52 weeks, then treatment was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 52 weeks post-treatment. During recovery, the animals received basal diet only.

4.4.6.2 Survival and body weight, and feed consumption

Survival through term was increased significantly for males in the 5 and 20 ppm dose groups, 50% and 45%, respectively, as compared to 22% in controls. Females in the 20 ppm dose group experienced survival similar to control females; however, survival in the 0.5, 2, and 5 ppm dose groups was lower, significantly so in the 2 ppm dose group, which was humanely sacrificed during study week 102 due to low numbers of survivors. Mean body weight gain was lower with statistical significance lower for males and females in the 20 ppm dose group during certain time periods during the study. Animals at lower dose levels occasionally had statistically significantly decreases in body weight gain; however, these occurrences were inconsistent over time between sexes and were not clearly dose-related. Similarly, food consumption was sometimes reduced in the 20 ppm dose group and was unaffected at lower dose levels.

4.4.6.3 Clinical Pathology

The reduced serum total cholesterol, seen at earlier time points, was no longer apparent after 104 weeks of treatment.

4.4.6.4 Non-Neoplastic Lesions

Absolute and relative liver weights in males and relative liver weights in females were increased at 20 ppm. At the terminal sacrifice, the livers of animals given 5 or 20 ppm exhibited a slight

increase in macroscopic findings, including enlarged, mottled, diffuse darkened, or focally lightened. Adaptive changes in liver cells were present at doses of 0.5 ppm and higher in males (hepatocellular cystic degeneration) and 5 ppm and higher in females (hepatocellular hypertrophy). Adverse hepatocellular findings in males were observed in the 5 ppm dose group and higher (hepatocellular hypertrophy with vacuolation). Females presented with hepatocellular hypertrophy in the 5 ppm and higher dose groups. Necrosis was present in males and females in the 20 ppm dose groups, along with centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, centrilobular hepatocytic vacuolation. Females in the 20 ppm dose group also had increases in periportal hepatocellular hypertrophy, lymphohistiocytic and pigmented macrophage infiltrates of the liver. Females also had decreased zymogen granules in the pancreas at all treatment levels and in the 20-ppm recovery group. Hepatotoxicity was not present in 20-ppm recovery-group animals. The data suggest that the hepatotoxicity did not persist in the recovery-sacrifice animals.

In the unscheduled sacrifices between Weeks 54 and 105, increased hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment were noted among the 20-ppm dose-group rats. Increased hepatocellular centrilobular hypertrophy was seen in rats given 5 ppm.

Liver samples were obtained from the 20 ppm dose-group rats during necropsy at the scheduled terminal sacrifice (104 weeks) for examination by transmission electron microscopy (TEM). Liver samples were assessed for ultrastructural changes. Electron microscopic evaluation identified mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy as the prominent differences between the 20 ppm dose-group and the control rats. Other changes included a slight increase in the amount of glycogen in treated animals compared with controls.

Mechanistic endpoints possibly related to tumor formation were assessed and are discussed in Section 4.4.7 below. Briefly, there were no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) immunohistochemistry at weeks 4 and 14, or by bromodeoxyuridine (BrdU) immunohistochemistry at week 53. A mild increase in peroxisome proliferation as measured by palmitoyl CoA oxidase was observed only at four weeks and only in high-dose males.

4.4.6.5 Neoplastic lesions

There was no effect of the test material on the incidence of palpable masses. Survival-adjusted, statistically significant increases in neoplastic findings are presented in Table 4-20 males and females, respectively.

For males, there was a statistically significant, albeit marginal ($p = 0.05$), increase in benign liver tumors (hepatocellular adenoma) compared to controls at 20 ppm, as well as a statistically significant ($p = 0.03$) positive trend for this tumor type. No malignant liver tumors occurred in males. Hepatocellular adenoma was not observed in the 20-ppm recovery group. Thyroid follicular cell adenoma was increased pair-wise for the 20-ppm recovery group ($p = 0.028$), which was outside the range of historical control values. However, there was not a similar

response in 20-ppm dose-group males or in females, and no other indications of thyroid abnormality were found.

For 20-ppm dose-group females, benign liver tumors (hepatocellular adenoma) were increased compared to controls at 20 ppm ($p = 0.04$) and for trend ($p = 0.02$). Combined benign and malignant liver tumors (hepatocellular adenoma/carcinoma) were also increased relative to controls ($p = 0.02$) and for trend ($p = 0.01$), although there was only one malignant liver tumor observed among 20-ppm females, and malignant liver tumors were not elevated. While mammary tumors were increased in females at the 0.5 and 2 ppm dose levels, the lack of response at higher dose levels is notable. In addition, mammary fibroadenoma was decreased for trend ($p = 0.015$) and pair-wise at 20 ppm ($p = 0.024$).

The hepatocellular tumors are believed to be a result of treatment with PFOS. Due to the demonstrated lack of genotoxicity of PFOS, the hepatocellular tumors are likely the result of a non-genotoxic mechanism.

The increase in thyroid follicular cell adenoma in the high-dose recovery males is interpreted as being spurious and unrelated to treatment since this finding was not observed in the high-dose group. No increase was present in males and females at the high dose or in recovery group females. Also, no other evidence of thyroid involvement was seen in the study.

Table 4-20. Results of Statistical Analyses of Neoplastic Lesions in SD Rats after Chronic PFOS Exposure.

Dose Group	Control	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm Recovery	20 ppm vs. 20 ppm Recovery [a]
				Males			
Liver - Hepatocellular, Adenoma	0/60	3/50	3/50	1/50	7/60*E	0/40	*
Thyroid - Follicular Cell, Adenoma	3/60	5/49	4/50	4/49	4/59	9/39*	*
Thyroid - Follicular Cell, Carcinoma	3/60	1/49	1/50	2/49	1/59	1/39	
Thyroid - Follicular Cell, Combined Adenoma/Carcinoma	6/60	6/49	5/50	5/49	5/59	10/39	*
				Females			
Liver - Hepatocellular, Adenoma	0/60*[b]	1/50	1/49	1/50	5/60*	2/40	
Liver - Hepatocellular, Carcinoma	0/60	0/50	0/49	0/50	1/60	0/40	
Liver - Hepatocellular, Adenoma/Carcinoma	0/60**	1/50	1/49	1/50	6/60*	2/40	

[a] The 20 ppm dose group was compared to the 20 ppm recovery dose group, and the significance for those comparisons are indicated in the far right column

[b] Significance in control column refers to significance of trend

* = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$;

E = Exact permutation test

4.4.6.6 Serum and liver PFOS measurements

Blood samples were collected for serum analyses from five animals/sex/group in all dose groups from the PFOS cancer study. Samples were collected at weeks 4 and 14 (all dose groups), at week 53 (high dose and controls), and at terminal and recovery sacrifices.

Liver samples were obtained for analysis of PFOS at scheduled sacrifices on weeks 4, 14 and 53, as well as at scheduled or unscheduled termination between weeks 53 and 105.

Results of serum and liver PFOS concentration measurements through week 53 are considered reliable for understanding the comparable body burdens of PFOS generated during dosing in the PFOS cancer study. The data through week 53 are presented in Table 4-21. Beyond week 53, aging of animals resulted in much higher variability in serum and liver concentrations of PFOS.

4.4.6.7 Summary

Chronic dietary exposure of male and female Sprague Dawley rats to PFOS caused a low-level increase in hepatocellular adenoma at the highest dose tested (20 ppm in diet), consistent with a generalized non-neoplastic response seen in the liver in this study. Peroxisome proliferation and cell proliferation were not strongly indicated as possible mechanisms for the low incidence of hepatocellular adenoma. Given the rather weak response in terms of benign hepatocellular adenoma, taken together with the demonstrated lack of genotoxicity of PFOS, PFOS should not present a risk of cancer to humans at the levels of exposure that have been determined. Between weeks 14 and 52 of the cancer study in rats, male and female rat serum PFOS concentrations had reached steady state and were approximately 140 ppm and 220 ppm in males and females in the high-dose group (20 ppm potassium PFOS in diet), respectively (Table 4-21).

Table 4-21. Serum and Liver PFOS Concentrations in Males and Females after 4, 14, 52 and 102/104 Weeks of Dosing

			Nominal Dietary Dose of Potassium Perfluorooctanesulfonate (ppm)				
Weeks	Sex	Parameter	0 (Control)	0.5	2	5	20
4	Male	$[PFOS]_{serum}$ (µg/mL)	< LOQ [a]	0.91 ± 0.06	4.33 ± 1.16	7.57 ± 2.17	41.8 ± 7.9
		$[PFOS]_{liver}$ (µg/g)	0.10 ± 0.07	11.0 ± 2.3	31.3 ± 5.8	47.6 ± 12.5	282 ± 45
		$[PFOS]_{Liver:Serum}$ [b]	NA [c]	12.2 ± 3.1	7.6 ± 2.0	6.3 ± 0.2	7.2 ± 1.0
4	Female	$[PFOS]_{serum}$ (µg/mL)	0.03 ± 0.01	1.61 ± 0.21	6.62 ± 0.50	12.6 ± 1.7	54.0 ± 7.3
		$[PFOS]_{liver}$ (µg/g)	0.11 ± 0.05	8.71 ± 0.55	25.0 ± 6.1	83.0 ± 14.1	373 ± 44
		$[PFOS]_{Liver:Serum}$	4.1 ± 1.5	5.6 ± 0.6	3.7 ± 0.7	6.6 ± 0.3	6.9 ± 0.4
14	Male	$[PFOS]_{serum}$ (µg/mL)	< LOQ	4.04 ± 0.80	17.1 ± 1.22	43.9 ± 4.9	148 ± 14
		$[PFOS]_{liver}$ (µg/g)	0.46 ± 0.06	23.8 ± 3.5	74.0 ± 6.2	358 ± 29	568 ± 107
		$[PFOS]_{Liver:Serum}$	NA	6.2 ± 2.2	4.4 ± 0.5	8.2 ± 1.2	3.9 ± 0.9
14	Female	$[PFOS]_{serum}$ (µg/mL)	2.67 ± 4.58	6.96 ± 0.99	27.3 ± 2.3	64.4 ± 5.5	223 ± 22
		$[PFOS]_{liver}$ (µg/g)	12.0 ± 22.4	19.2 ± 3.8	69.2 ± 3.5	370 ± 22	635 ± 49
		$[PFOS]_{Liver:Serum}$	3.5 ± 0.8	2.8 ± 0.7	2.5 ± 0.1	5.8 ± 0.5	2.9 ± 0.3
52	Male	$[PFOS]_{serum}$ (µg/mL)	0.0249 ± 0.0182	ND [d]	ND	ND	146 ± 33 (4)
		$[PFOS]_{liver}$ (µg/g)	0.635 ± 1.04	ND	ND	ND	435 ± 97 (9)
		$[PFOS]_{Liver:Serum}$	28 ± 26	ND	ND	ND	2.9
52	Female	$[PFOS]_{serum}$ (µg/mL)	0.395 ± 0.777	ND	ND	ND	220 ± 44 (5)
		$[PFOS]_{liver}$ (µg/g)	0.923 ± 1.77	ND	ND	ND	560 ± 180 (10)
		$[PFOS]_{Liver:Serum}$	2.6 ± 1.4	ND	ND	ND	2.6
104	Male	$[PFOS]_{serum}$ (µg/mL)	0.0118 ± 0.0104	1.31 ± 1.30	7.60 ± 8.60	22.5 ± 23.5	69.3 ± 57.9
		$[PFOS]_{liver}$ (µg/g)	0.114 ± 0.148	7.83 ± 7.34	26.4 ± 20.4	70.5 ± 63.1	189 ± 141
102 [e]/104	Female	$[PFOS]_{serum}$ (µg/mL)	0.0836 ± 0.134	4.35 ± 2.78	20.2 ± 13.3[e]	75.0 ± 45.7	233 ± 124
		$[PFOS]_{liver}$ (µg/g)	0.185 ± 0.184	12.9 ± 6.81	55.1 ± 31.5[e]	131 ± 61.4	381 ± 176

[a] All values were less than the lower limit of quantitation (0.0091 µg/mL at 4 weeks; 0.0457 µg/mL at 14 weeks)
[b] Ratio of the concentration of PFOS in liver to the concentration in serum
[c] Not applicable due to serum concentrations being below the limit of quantitation
[d] Not determined
[e] Sacrifice of 2 ppm dose-group females occurred on study day 719 after 102 weeks of dosing.

4.4.7 Mechanisms of Toxicity

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 (Luebker *et al.*, 2002), cholesterol synthesis (Haughom and Spydevold, 1992) and bioenergetics (Starkov and Wallace, 2002) have been studied. In addition, PFOS has been reported to induce peroxisome proliferation (Sohlenius *et al.*, 1993, Ikeda *et al.*, 1987, Berthiaume and Wallace, 2002).

The cause of perinatal mortality is still under investigation. Luebker *et al.* (2002) have reported that the perinatal mortality associated with PFOS treatment before and during pregnancy is not a result of reduced serum lipids. Administration of supplemental mevalonic acid lactone or cholesterol to dams before and during gestation did not reduce perinatal mortality. Reductions in thyroid hormones (T3 and T4) have been noted in rat dams and pups (Butenhoff *et al.*, 2002; Lau *et al.*, 2003; Thibodeaux *et al.*, 2003) without increases in TSH or indications of thyroid pathology. In rat dams, free T4 was unchanged when measured after equlibrium dialysis, suggesting the presence of an interfering substance (Butenhoff *et al.*, 2002). Pregnant mice also showed a similar reduction in T4 (Thibodeaux *et al.*, 2003). Thyroid hormone reductions were not attributed as the primary cause of perinatal mortality. The critical period in gestation for PFOS- induced mortality appears to be near birth (Grasty *et al.*, 2002). Underdeveloped lungs as a cause of death in the perinatal period have been suggested based on gross dissection observations (Grasty *et al.*, 2002); however, electron microscopy of gestation day 21 rat fetal lungs did not reveal significant abnormalities in Type II alveolar cells or lamellar bodies (RS-III-40).

PFOS did not increase the biogenesis of mitochondria after a single intraperitoneal injection of 100 mg/kg in adult male rats (Berthiaume and Wallace, 2002). Although mitochondrial biogenesis has not been determined for PFOS-treated monkey livers, these data suggest that the liver weight increase seen in monkeys may be due to other mechanisms.

The mitochondrial effects of PFOS are likely due to non-specific detergent action on mitochondrial membranes as opposed to proteinophoric uncoupling or induction of the mitochondrial permeability transition (Berthiaume and Wallace, 2002; O'Brien and Wallace, 2002). Hu *et al.* (2002a) have reported a species and tissue non-specific inhibition of gap junctional intercellular communication. The *in vitro* EC_{50} was 30μM and the *in vivo* LOEC was 54 ppm in liver tissue. Hu *et al*. (2003a) have reported an increase in the membrane fluidity of fish leukocytes and alterations of mitochondrial membrane potential, with concentration thresholds between 5 and 15 mg PFOS/L.

Jones *et al.* (2002) reported an investigation of binding displacement of steroid hormones in fish and birds as well as *in vitro* binding assays with bovine serum albumin. They concluded that, at current environmental concentrations of PFOS, it is extremely unlikely that PFOS would cause significant displacement of hormones from their serum binding proteins in wildlife.

Hu *et al.* (2002b) have used differential display and gene chips to identify genes responsive to PFOS-treatment of rats (3 days or 3 weeks) or H4IIE rat liver hepatoma cells (96 hours). Genes which were expressed were generally clustered as those coding for fatty-acid metabolizing

enzymes, nuclear binding factors, and signal transduction pathway proteins. While replication gave consistent results, *in vitro* and *in vivo* studies showed only limited similarity.

Seacat *et al.* (2002a) have speculated that the morbidity associated with PFOS in a six-month oral dosing study in cynomolgus monkeys may have been due to metabolic disturbances leading to skeletal muscle injury and lactic acidosis. At present, this appears to be supported by elevations in creatine kinase in the two male monkeys which became moribund toward the end of the dosing period. Also supportive are the findings of increased urea nitrogen and potassium in the monkey for which clinical chemistry is available from the day of sacrifice.

The increase in benign liver tumors observed on chronic dosing of rats with PFOS may be a rodent-specific phenomenon, based on the interspecies differences in the biochemical response to cholesterol lowering through inhibition of hydroxymethylglutamate-CoA reductase (HMG-CoA reductase). PFOS has been reported to reduce the activity of HMG-CoA reductase, and this is a possible explanation for the lowering of cholesterol (Haughom and Spydevold, 1992). The production of hepatic tumors has been observed in rodents after chronic exposure to high doses of statin drugs, which are also HMG-CoA reductase inhibitors (Bernini *et al.*, 2001; Newman and Hulley, 1996). Rodents respond to statins by increasing HMG-CoA reductase production rather than by increasing LDL-C receptors, the latter being the human response to statin therapy. Consequently, HMG-CoA reductase accumulates in proliferated smooth endoplasmic reticulum in rodents, which results in a microscopic hepatocellular atypia. Dogs, which also respond to statins by increasing LDL-C receptors, have not responded with an increase in hepatocellular tumors. Therefore, some researchers believe that statin-induced hepatocellular tumors may be specific to rodents due to the difference between rodents and higher species in physiological and biochemical response to statins (MacDonald *et al.*, 1988; Gerson *et al.*, 1989). The lack of overt hepatocellular peroxisome proliferation or cell proliferation that was observed in the cancer bioassay discussed above suggests that those mechanisms may not explain the increase in hepatocellular tumors. Perhaps the response to HMG-CoA reductase reduction (not measured in the studies discussed above) may be an operative mechanism that would distinguish the rodent response from a primate response.

Continuing a possible analogy to statin drugs, thyroid follicular cell tumors have been observed in rodents exposed chronically to high doses of simvastatin or fluvastatin, and this increase is thought to be due to elevations in thyroid stimulating hormone (TSH) (Newman and Hulley, 1996; Smith *et al.*, 1991). While mild to substantial reductions in thyroxin (T4) and triiodothyronine (T3) have been observed in repeated-dose studies with rats and monkeys reported herein, corresponding increases in TSH were either not present or weak. This may be explained by the presence of an interfering substance (Chopra, 1998), as shown in one case (Butenhoff *et al.*, 2002), or by the occurrence of non-thyroidal-illness syndrome (Chopra, 1997; De Groot, 1999). The increase in thyroid follicular cell adenoma in high-dose recovery male rats in the PFOS study may be spurious or may relate to alterations in thyroid homeostasis; however, thyroid hormones were not measured in the cancer bioassays.

The possible interactions of PFOS with critical quaternary amines (e.g., choline, phosphocholine, and phosphatidylcholine) are currently being explored by 3M.

No conclusions have been reached at this time on the relative importance of any of these possible mechanisms of toxicity. To date, exposure of humans to both statins and PFOS and related compounds has not been associated with an increased risk of liver or thyroid cancer (Bernini *et al.*, 2001; current document).

4.4.8 Other Information Relevant to Human Health

4.4.8.1 Ocular Irritation

PFOS was found to be mildly irritating to the eyes of albino rabbits when placed in the conjunctival sac 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 (RS-III-50).

4.4.8.2 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 (RS-III-51).

4.4.8.3 Sensitization

No reports on the sensitization potential of PFOS are available.

4.4.8.4 Human Data

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

4.4.8.5 Conclusions

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

4.5 Assessment for Human Health

4.5.1 Health Effects of PFOS

4.5.1.1 Human Studies

Beginning in the late 1970s, biennial hematological and clinical chemistry tests, along with tests of pulmonary function, were performed on workers at 3M's Decatur manufacturing facility. In the years of testing, occupational physicians have reported no clinical abnormalities that they believe to be related to any given individual employee's serum organic fluorine concentrations.

Until the mid-1990s, measurement of fluorochemical exposure was based on the level of serum organic fluorine. In 1994/1995, 1997 and 2000, with improvements in analytical techniques, serum levels of PFOS were measured and were used in evaluating the health of employees at 3M's Decatur and Antwerp production facilities. Measured serum PFOS levels averaged 1-2 ppm, and ranged up to 10-13 ppm. These levels were comparable to those found in a randomized study at Decatur in 1998.

The medical surveillance testing has included standard sets of clinical chemistry and hematological tests. In addition, assays for different hormones were performed on a subset of samples of the workers' blood. Adjusting for potential confounding factors such as age and body-mass index, no significant hematological, clinical chemistry, or hormonal abnormalities were associated with serum PFOS. In particular, serum total cholesterol was not decreased. This finding is significant because of the experimental observation that PFOS above 100 ppm in monkeys decreased serum total cholesterol.

Longitudinal analysis evaluating workers who participated in multiple rounds of medical surveillance also showed no PFOS-associated abnormalities with lipid or hepatic clinical chemistry tests.

A retrospective cohort mortality study at the Decatur manufacturing site found no increase in mortality believed to be associated with PFOS. The study included 50,970 person-years of follow-up among 2,083 eligible cohort members. Follow-up was excellent, although, the mortality study has limited statistical power. While an elevation in the SMR for bladder cancer was observed, PFOS does not appear to have the properties of known bladder carcinogens and has not shown any bladder effects in toxicology studies. It is neither genotoxic nor insoluble in urine at room temperature. Further investation of the bladder cancer incidence is underway.

The Episodes of Care Study (based on insurance claims experience) provides evidence that the majority of health endpoints were not associated with POSF-related job groups in the Decatur chemical facility. These endpoints include neonatal diagnoses and other endpoints related to reproductive outcomes. Those associations that were observed are being further investigated among current and past employees. Results of this investigation are expected in 2003.

Collectively, these studies of production workers provide important information for risk assessment. They involve direct attempts to identify possible adverse health effects in the most

highly exposed human populations. They also include a wide range of outcomes, including mortality, morbidity; and clinical evaluations. The multiple medical surveillance exams provide convincing evidence that serum PFOS concentrations, as measured in these employees, are not associated with aberrations in serum lipids or other clinical chemistry tests.

4.5.1.2 Toxicology Studies

The extensive database on the effects of PFOS from toxicological studies demonstrates a consistency of effects in monkeys, rats, and rabbits. In recent repeat-dose studies in rats, PFOS at high doses reduced serum cholesterol levels, caused reductions in body weight gain, and caused liver enlargement and vacuolization. The same pattern of effects was observed in PFOS exposure studies involving monkeys. At the highest experimental doses tested, PFOS has caused unexplained deaths in monkeys and rats.

When PFOS was administered to rats in their diet for up to two years, an increase in hepatocellular adenoma (males and females) and combined hepatocellular adenoma/carcinoma (but not carcinoma alone) (females) occurred only at the high dose of 20 ppm in the diet. While this increase had marginal statistical significance ($p = 0.05$), these liver tumors are considered to be related to treatment and to arise from non-genotoxic mechanisms.

PFOS has been evaluated for teratogenic effects in rats and rabbits and for adverse reproductive and developmental effects in a two-generation reproduction/developmental study in rats and several follow-up studies. PFOS caused reduced neonatal survival and body weight gains in pups at the high doses in studies that assessed post-natal effects. No effects were observed at the low doses in the studies.

At this time, the mechanisms underlying the toxic effects exhibited by PFOS in experimental studies are not fully understood, and several types of exploratory investigations may begin to shed light on how PFOS produces toxicity.

4.5.2 Approach to Risk Assessment

A margin-of-exposure (MOE) approach will be used in this assessment. For the MOE comparison, measured human serum or estimated human liver PFOS concentrations are compared to serum or liver PFOS concentrations derived from toxicology studies that correspond to either a NOAEL or a calculated benchmark dose (BMD).

Use of a BMD has the advantage of using a value that is calculated based on the dose-response from all study data, and represents a specified level of response or risk (U.S. EPA, 1999; Gaylor and Kodell, 2002). The calculation uses the results across the entire dose-response curve and therefore has more sensitivity to detect and estimate biological effects than comparison of the results from individual, somewhat arbitrarily determined dose groups. The benchmark response level that is used to calculate the benchmark dose may vary depending on the endpoint that is being benchmarked. For most categorical data from toxicology studies, a 10% response level is fairly representative of the limits in which a change can be accurately determined. Certain continuous responses, such as body-weight change, may be accurately measured at a 5% response level. Therefore, for some values, a 10% response level is used, and for others a 5% reposnse level is used in this assessment. To be conservative, the lower 95% CI of the BMD (LBMD) is used.

Both NOAELs and the LBMD values are taken from the external (administered) doses in the toxicology studies. However, these levels can then be equated with the serum or liver concentrations that occurred in the animals at those doses. These serum or liver concentrations are then compared with human serum or liver concentrations to evaluate the MOE. In this risk characterization, serum and liver PFOS concentration values associated with external dose and the dose-response curve have been used to derive a benchmark internal concentration (BMIC) for the hazard endpoints of interest. The lower 95% CL of the BMIC (LBMIC) for a given endpoint at a specified reponse level (5% or 10%) has been used. Appendix IV presents derivations of the LBMD and LBMIC for a number of endpoints from the PFOS toxicology studies by Dr. David W. Gaylor.

It should be pointed out that, while many endpoints in toxicology studies may be benchmarked, the toxicological relevance of each endpoint as well as the appropriate response level should be carefully considered when deciding which endpoints to review for selecting a point of departure for risk assessment. As an example, changes in clinical chemistry values or organ weights may not be meaningful toxicologically in the absence of a clear pattern of clinical, biochemical, and histologic changes.

As noted previously, rats have a relatively higher liver-to-serum PFOS concentration ratio compared to humans and monkeys. Therefore, assuming that the liver is the primary target organ, the use of rat serum PFOS concentration data is believed to be conservative.

The LBMD/LBMIC doses from toxicology studies that are considered for potential use in assessing potential health risk are shown in Table 4-22. Because benchmark doses could not be calculated for clear toxic responses of the liver, the NOAELs for liver toxicity, expressed as both external dose and serum or liver PFOS concentration are presented in Table 4-23.

It is noteworthy that these values are distributed over a narrow range of external doses and PFOS concentrations. The discussion that follows develops the approach to selecting points of departure to derive MOE values to characterize potential risk for human populations based on serum and liver PFOS concentrations. These points of departure are presented in Table 4-24.

4.5.2.1 Use of Serum Concentrations for Risk Assessment

PFOS has been identified in serum samples from both occupationally and non-occupationally exposed human populations. Mean serum PFOS concentrations in non-occupationally-exposed populations are in the range of 0.030-0.040 ppm.

Based on an estimated mean elimination half-life of nine years and an estimated volume of distribution at steady state of 0.2 L/kg body weight, the daily external PFOS exposure that may result in a steady state serum concentration of 0.040 ppm can be estimated and is approximately 2×10^{-6} mg/kg. This level of exposure is on the order of 10^{-4} to 10^{-6} of the external doses administered in toxicological studies. Because external exposures are so small and can occur from a variety of environmental and commercial-use pathways involving not only PFOS but a diverse group of PFOS-generating compounds, the sources and routes of exposure that result in PFOS being present in the human body are not easily characterized in a quantitative manner. Therefore, most of the toxicology and human monitoring studies have included quantification of serum PFOS concentration as a measure of integrated exposure from the various potential sources and routes of exposure. In addition to quantifying serum PFOS concentrations, most of the toxicology studies have also included quantification of liver PFOS concentrations.

The repeat-dose toxicology studies have shown that the occurrence of toxic effects is related strongly to the accumulation of PFOS beyond a critical body burden threshold for effects. Therefore, the occurrence of toxicity depends more directly upon cumulative body burden than on the magnitude of the external dose. The toxicology and toxicokinetic studies have demonstrated that serum concentration of PFOS is highly correlated and linearly proportional to cumulative dose. This correlation is present in the serum PFOS concentration range observed in occupational and non-occupational human population monitoring studies. As a result, serum PFOS concentration can be considered to be a direct reflection of body burden (or internal dose) as well as of integrated external exposure.

Accordingly, PFOS serum values can be used to compare the levels to which humans are exposed to the levels from laboratory toxicology studies, in order to evaluate potential risk to humans. This comparison shows the "margins of exposure" (MOEs), which indicates the ratio between the levels producing no effects in experimental studies and human exposures.

4.5.2.2 Use of Liver Concentration for Risk Assessment

The liver is a primary target organ for PFOS, and liver PFOS concentrations are likely to be more directly related to the toxicodynamic action of PFOS than serum concentrations. Liver

data are available from the animal studies, but human liver data are limited to the organ donor study.

Liver PFOS concentrations in humans are only available from the limited number of human liver samples obtained in the organ donor study. Because these donor liver samples were matched to serum samples from the same individuals ($N = 23$), they provide valuable information on the human liver-to-serum PFOS concentration ratio. The average liver-to-serum PFOS concentration ratio from the donor study is 1.3:1 (95% CI = 0.9-1.7). This is consistent with observations from the six-month cynomolgus monkey study, in which the mean liver-to-serum PFOS concentration ratios ranged from 0.9:1 to 2.7:1.

The liver-to-serum PFOS concentration ratios from the donor study make it possible to estimate human liver PFOS concentrations from measured serum data, and then to derive MOE comparisons between estimated human liver levels and the measured liver concentrations from animal studies. In developing the liver PFOS-concentration based risk characterization based, human serum PFOS concentration values derived from the larger studies involving Red Cross adult blood donors, children from a *Streptococcal* A clinical trial, and dementia-free elderly enrolled in a prospective thought-change study were multiplied by a factor of 1.7 (the upper 95% CI value for measured liver-to-serum PFOS concentration ratios in human donor samples) to derive estimated human liver values.

Although using estimated values of liver PFOS concentration to derive an MOE based on liver concentration is less exact than using measured serum PFOS concentration data in the MOE based on serum PFOS, the liver MOE comparison is likely to be more directly relevant to the toxicodynamics of PFOS. For that reason, it is important to consider and present the MOE values derived from these estimates of general population liver concentrations.

4.5.2.3 Potential Points of Departure for Serum Comparison

As can be seen by inspecting Tables 4-22 and 4-23, the most conservative value for use as a point of departure for the serum-based assessment is the LBMIC for a 5% response (decrease) in body-weight gain through lactation in rat pups, 26 µg/mL (ppm). This value is derived from data from the two-generation reproduction/developmental study (pup weight gain through lactation) and the serum PFOS concentration measurements on gestation day 21 made in a separate toxicokinetic study during pregnancy at the same dose levels. It can be argued that this value is conservative in that rat dam serum concentrations fall during the 21 days of gestation, partially due to volume expansion and transplacental transfer. Few, if any of the human serum measurements are expected to have come from women in late pregnancy. While perinatal mortality in rat pups is influenced by body burden in late gestation (Grasty *et al*., 2002), the relationship of dam PFOS body burden in late gestation to the benchmarked endpoint, pup weight gain in lactation, is not well understood at this time. The same effect has an associated LBMIC (5% response level) of 36 µg/mL (ppm) when using corresponding dam serum PFOS concentrations measured at the time of mating (pre-gestational). Because the pre-mating and end-gestational values are not greatly different, the average of the two values, 31 ppm, will be used.

Litter size on lactation day 4, prior to culling, reflects a more serious effect. The LBMIC (5% response level) for this effect is either 30 ppm or 39 ppm , based on gestation day 21 or pre-gestational serum PFOS measurements, respectively. These values average 35 ppm.

Perinatal mortality in pups is the most notable effect seen in rat reproduction studies. From the two-generation reproduction/developmental study data for perinatal mortality, the LBMIC (5% response level) for this effect is either 71 ppm or 84 ppm, based on gestation day 21 or pre-gestational serum PFOS measurements, respectively. A one-generation study undertaken to better define the dose-response for perinatal mortality in pups gave a LBMIC (5% response) value of 83 ppm based on pre-gestational serum PFOS measurements. These values average 78 ppm.

Liver weight increase, absent other clinical, biochemical, or histological evidence of toxicity would normally be considered an adaptive response. With PFOS, liver weight increase is a response to treatment, and the benchmarks fall in the range of the effects from the reproduction studies. For example, the LBMICs are 44 and 49 ppm for male rats and female monkeys, respectively, at the 10% response level.

The NOAELs associated with liver toxicity (Table 4-23) may be of more relevance to risk assessment than LBMICs derived from liver weight increase. The lowest value in Table 4-23 is 44 ppm for male rats, a value equivalent to the LBMIC for liver weight increase at a 10% response level (Table 4-22). The other serum PFOS concentration values associated with NOAELS for liver toxicity in female rats and male and female monkeys are all within a factor of two higher.

The rat cancer bioassay showed a weak tumorigenic response. A 10% response level (BMD_{10}) is likely most appropriate for these categorical data. The "Guidelines for Carcinogen Risk Assessment" proposed by the U.S. EPA (U.S. EPA, 1999) recommend the use of the benchmark dose as a point of departure for low-dose cancer risk assessment. The $LBMD_{10}$ provides a conservative value that takes into account the potential experimental variation of the two-year cancer bioassay. The same guidelines suggest that a margin-of-exposure analysis is applicable if a non-linear dose-response function containing a significant change in slope can be presumed from the data. This is the case for the PFOS dataset. The LBMIC, expressed as serum PFOS concentration for liver tumorigenesis, 10% response level, is 62 and 92 ppm for male and female rats, respectively. Although corresponding benchmark dose calculations for liver PFOS concentration could not be calculated, study data and the high liver-to-serum PFOS concentration ratio in the rat suggest that they are several times higher.

The serum PFOS measurements associated with the BMD for liver tumors in rats were those from week 14 of the two-year bioassay. This is because the 104-week serum measurements were highly variable and deemed unreliable. The variability in serum measurements at 104 weeks may be the result of nephritic syndrome in aging rats, which could produce a loss of PFOS bound to serum proteins. The effect on cholesterol was no longer apparent at 104 weeks; this may also be due to nephritic syndrome. Serum and liver PFOS concentrations did not differ greatly at the high dose between weeks 14 and 53.

Table 4-22. Serum and Liver PFOS Concentrations Associated Benchmark Dose Values

Species/Sex	Study	Benchmarked Endpoint	Benchmark Response Level (%)	LBMD[a] (mg/kg)	LBMIC[a] (µg/mL) Serum	LBMIC (µg/mL) Liver
Rat	2-Gen Repro/Dev	F_1 Pup Weight Gain (LD21)[b, c]	5	0.34	26[c]	74[c]
Rat	2-Gen Repro/Dev	F_1 Pup Weight Gain (LD21)[d]	5	0.34	36[d]	NS[d]
Rat - female	2-Gen Repro/Dev:	F_1 Litter Size (LD4)[c]	5	0.39	30[c]	85[c]
Rat - female	2-Gen Repro/Dev	F_1 Litter Size (LD4)[d]	5	0.39	39[d]	NS
Rat	1-Gen Repro/Dev	F_1 Litter Size (LD5)[c]	5	0.83	71[c]	200[c]
Rat	2-Gen Repro/Dev	F_1 Pup Mortality (LD4)[c]	5	0.84	71[c]	199[c]
Rat	1-Gen Repro/Dev	F_1 Pup Mortality (LD5)[d]	5	0.83	83[d]	NS
Rat	2-Gen Repro/Dev	F_1 Pup Mortality (LD4)[d]	5	0.84	84[d]	NS
Rat - male	14-Week Dietary	Liver Weight	10	0.40	44	154
Monkey-female	6-Month Oral	Liver Weight	10	0.22	49	77
Rat - male	104-Week Bioassay	Liver Tumors	10	~0.5[g]	62	NC
Rat - female	104-Week Bioassay	Liver Tumors	10	~0.5[g]	92	NC

[a] LBMD and LBMIC = lower 95th CI of the benchmark dose (BMD) or benchmark internal concentration, respectively, for given level of response response.
[b] LD = lactation day, i.e., day after birth
[c] Based on samples taken on presumed gestation day 21 after ~65 days of dosing
[d] Based on samples taken on presumed gestation day 0 following 42 days of dosing; NS = no sample
[e] Estimated based on 3.9 ppm in diet
[f] A benchmark dose could not be calculated from available data.
[g] Estimated based on 7.9 ppm in diet.

Table 4-23. Serum and Liver PFOS Concentrations Associated with No Observed-Effect Levels for Liver Toxicity from Toxicology Studies.

Species/Sex	Study	NOAEL		
		External Dose (mg/kg/day)	$[PFOS]_{serum}$ (ppm)	$[PFOS]_{liver}$ (ppm)
Rat - male	14-Week Dietary	~0.4[a]	44	358
Rat - female	14-Week Dietary	~0.4[a]	64	223
Monkey -female	6-Month Oral	0.15	67	70
Monkey - male	6-Month Oral	0.15	83	59

[a] 5 ppm in diet

Table 4-24 Selected Points of Departure for Risk Assessment.

Media	Endpoint	Point of Departure [PFOS] ppm	Basis
Serum	Pup weight gain through lactation, rat pups	**31 ppm**	LBMIC at 5% response level, using mean of gestation day 21 and pre-gestational serum levels in dams
Liver	Liver toxicity, male monkeys	**59 ppm**	NOAEL for liver toxicity [LBMIC could not be calculated for males and is higher for females]

The following factors support the use of a margin-of-exposure approach to assessing the potential increased risk for liver tumors based on the rat two-year bioassay:

- The tumors are likely secondary to liver toxicity. Statistically significant elevations in hepatocellular adenomas occurred at the dose at which overt liver toxicity was present at 14 weeks and 53 weeks into the study. Tumor incidence was reduced (significantly in males) when dosing was suspended at one year. PFOS has a demonstrated lack of genotoxicity.

- The tumor-incidence dose-response curve suggests a non-linear relationship between dose and increased lifetime risk of excess liver tumors.

- The striking similarity of the serum PFOS values associated with the NOAEL or $LBMD_{10}$ in toxicology studies -- whether for liver effects, reproductive outcomes or tumorigenicity -- suggests a threshold body burden for effect.

Therefore, the margin-of-exposure approach is considered more appropriate than a linear low-dose extrapolation.

The narrow distribution of values for external dose and PFOS serum concentration associated with the LBMIC or NOAEL presented in Table 4-22 and 4-23 is noteworthy and lends support to the concept that effects of PFOS are related to a critical body burden. Taking all of the LBMIC and NOAEL serum PFOS concentration values into consideration, potential points of departure are 26 ppm or 36 ppm for pup body-weight gain (gestational day 21 and pre-gestational values, respectively), 44 ppm for liver toxicity in male rats, and 62 ppm for tumor response. Using the mean of gestational day 21 and pre-gestational values for the LBMIC at the 5% response level for pup body-weight gain through lactation, 31 ppm, is appropriate and conservative. Use of this level is protective for liver toxicity and liver tumors.

4.5.2.4 Selection of Points of Departure for Liver Comparisons

The endpoint most sensitive to liver concentration is liver toxicity in the male monkey. A liver concentration of 59 ppm is associated with the NOAEL of 0.15 mg/kg/day for the six-month oral toxicity study in male cynomolgus monkeys (Table 4-23). A BMD for liver weight in male monkeys could not be calculated given the small sample size and poor fit of the models. The NOAEL for liver toxicity in female monkeys is 70 ppm in the liver, and the LBMIC for liver weight increase (10% response) in females is 77 ppm liver concentration. The LBMIC (5% response) based on liver PFOS concentration in rat dams at gestation day 21 that is associated with decreased pup weight gain is 74 ppm. Thus, the liver PFOS concentration associated with the NOAEL for liver effects in male monkeys (59 ppm) represents the most sensitive value and will be used as a point of departure for MOE calculations based on liver concentration.

4.5.2.5 Comments on Uncertainty

The use of an MOE approach to risk characterization does not provide a quantitative measure of uncertainty for PFOS. The adequacy of an MOE must be considered in the context of an analysis of uncertainty related to the potential for adverse effects to the population of concern. Residual areas of uncertainty as well as factors that tend to reduce uncertainty in this risk characterization are discussed below. These factors should be considered when assessing the adequacy of MOE values.

The approach used in this risk characterization has the advantage of deriving MOE values using multiple biological responses in rodents and monkeys by comparison of internal dose (concentration of PFOS in serum). In addition, the use of LBMD and LBMIC values as points of departure has an advantage over the use of a NOAEL or LOAEL in that the benchmarked values

represent a defined level of excess response (risk), providing a better view of species differences in response by normalizing response at a specified level. Uncertainty related to variations in response within and between species is further reduced by the fact that PFOS is not metabolized, and the pharmacokinetics of PFOS have been investigated in rodents and primates. The use of internal dose as a measure of exposure also has advantages in that internal dose is more directly related to biological response (toxicodynamics), and the influence of rates of absorption and elimination (toxicokinetics) on response when using external (administered) dose are minimized. When all of the above advantages are combined, the result is reduction in uncertainty.

One major source of uncertainty is the extrapolation of results in animals to humans. A second major source of uncertainty is the variability in sensitivity among individuals. Further, human exposures are typically much lower than those used in experimental studies, which are conducted at doses many times higher than human exposure levels in order to elicit adverse effects in a relatively small number of animals. Low-dose studies for direct observation of risks below 1 in a 1,000 would require tens of thousands of animals, which would be prohibitive. Hence, a third major source of uncertainty is the extrapolation from high-dose laboratory studies to low-dose human exposure levels.

In utilizing serum or liver PFOS concentrations as a measure of exposure or internal dose for health risk assessment, the potential for interspecies variation requires attention. Two major differences have been noted when comparing the toxicokinetics of PFOS in rats, monkeys and humans. The first of these relates to serum PFOS elimination half-life. The current estimates of serum PFOS elimination half-lives in rats, monkeys, and humans are ~ 100 days, ~ 200 days, and several years, respectively. However, in all three species, the serum elimination half-lives are relatively long, with the effect that frequent exposures to PFOS or PFOS-generating compounds would be expected to result in increasing body burdens.

The second difference noted is in the distribution of PFOS to the liver in rats as compared to monkeys and humans. Rats have been shown to have liver PFOS concentrations ranging from 3-12 times that of their serum PFOS concentrations (Table 4-21; Seacat *et al.*, 2003). In contrast, the human organ donor study showed that the mean liver-to-serum PFOS concentration ratio among the matched sets of liver and serum samples was 1.3:1 (95% CI 0.9-1.7). Data from the six-month toxicity study in monkeys have shown mean liver-to-serum PFOS concentration ratios in controls and treated groups ranging from 0.9:1 to 2.7:1. Assuming that PFOS concentration in the liver, the primary target organ, is related more directly to toxicity than serum concentration, this would suggest that the rat liver would respond at lower serum concentrations than monkeys or humans, because the liver-to-serum concentration ratio is higher in the rat. Indeed, NOAEL and LBMIC values based on liver response and expressed as serum PFOS concentration suggest that the male rat appears to be more sensitive. However, the NOAEL and LBMIC values based on liver toxicity and expressed as liver PFOS concentration are several times lower in monkeys than in rats, suggesting that monkey liver tissue may be more sensitive on a PFOS-concentration basis (Table 4-23).

Current methods for qualitatively assigning uncertainty factors in a deterministic approach to assessing risk have been recently reviewed by Kalberlah *et al*. (2003). Most methods currently assign uncertainty factors for intraspecies and interspecies extrapolation. In the past several

years, subdividing these two uncertainty factors into sub-factors for toxicokinetics and toxicodynamics has gained acceptance. Renwick (1991) first suggested that uncertainty could be regarded by consideration of two factors: 1) toxicokinetic (the relationship between external dose and internal dose in time); and, 2) toxicodynamic (the relationship between internal dose and effect). The same author later proposed that the usual default uncertainty factors of 10 could be divided into two subfactors, one each for toxicokinetics and toxicodynamics, to allow for available compound-specific scientific data to be used in modifying uncertainty factors (Renwick, 1993). The World Health Organization has adopted this concept (WHO, 1994).

For PFOS, information is available to significantly reduce both the toxicokinetic and the toxicodynamic aspects of uncertainty. The use of internal dose in this assessment is possible because the relevant toxicology studies have included measures of internal dose (serum and liver PFOS concentration) associated with external dose and effect, and human bio-monitoring studies have been conducted that provide an understanding of internal dose. In addition, measurements of specific biochemical parameters in workers have have been related to internal dose and provide valuable information in support of the risk assessment. The continuing investigation of the PFOS elimination rate in retired workers is also helpful to the risk assessment. Thus, use of internal dose has great advantage in that it is directly related to biological response (toxicodynamics), and the influence of rates of absorption and elimination (toxicokinetics) on response when using external (administered) dose are minimized. The result is reduction in uncertainty.

Several factors can be represented as reducing uncertainty as a result of intraspecies variability. Perhaps the most important of these is the fact that PFOS is not known to be metabolized or otherwise degraded, a fact that eliminates uncertainty related to individual differences in metabolic capacity as a result of genetics, health status, diet, and age. The human population monitoring data available for PFOS also decreases uncertainty related to the variability in exposure, since serum PFOS concentrations have been characterized in a group of children, in adult Red Cross blood donors, and in an elderly population. The fact that these serum measurements are distributed over a relatively narrow range and do not show major differences between age or gender groups reduces the chance that large portions of the population may not be adequately represented in a risk characterization. With respect to PFOS pharmacokinetics, PFOS has been shown to distribute mainly to extracellular space in rats and monkeys, and there is no reason to believe that the distribution would be different in humans. In addition, PFOS has been shown to have similar plasma binding characteristics in rats, monkeys, and humans. The elimination rate of PFOS in humans continues to be investigated, and current information provides a reasonable understanding of the potential variability in elimination of PFOS. In addition to the above factors, medical monitoring of human workers exposed to PFOS has not shown evidence of liver function abnormalities (liver enzymes in serum) or other measured endpoints at serum PFOS concentrations within an order of magnitude of the benchmark internal concentrations for effects, and there is no evidence of altered health status attributable to PFOS exposure. Taken together, these factors argue for consideration of reducing the overall intraspecies uncertainty, especially uncertainty associated with toxicokinetics.

In the case of uncertainty in the extrapolation between species, several factors can be considered to reduce uncertainty. The fact that PFOS is not metabolized or otherwise degraded eliminates

potential variation between species and, as noted above, within species. In both rats and monkeys, similar responses to treatment have been observed have been observed, and there do not appear to be major qualitative differences in response. The volume of distribution in rats and cynomolgus monkeys is quite similar, suggesting that PFOS is distributed primarily in extracellular spaces. Serum binding capacity also appears similar in rats, monkeys, and humans. Major differences in elimination rate between sexes in rats and monkeys do not appear to exist. The use of serum concentrations as a measure of internal dose can compensate for differences in elimination rate between species, and this, combined with other factors, has the effect of reducing uncertainty with regard to toxicokinetics.

Observations from experimental studies suggest that body burden thresholds exist for the expression of effects due to PFOS exposure. The latter point is exemplified by cholesterol reduction in male and female cynomolgus monkeys, which occurs concurrently with an apparent plateauing of serum PFOS concentration and by the steep dose-response in perinatal mortality that appears to be dependent on body burden in late gestation or at the time of birth. Clinical chemistry markers of response have been followed in toxicological studies in rats and monkeys, as well in worker monitoring studies, and these have been related to internal dose. Remarkably, most of the LBMD, LBMIC, and NOAEL values for rat or monkey liver effects in Tables 4-22 and 4-23 (whether expressed as external dose or PFOS concentrations) are relatively similar in magnitude. LBMD and LBMIC values for perinatal effects and tumorigenic effects shown in Table 4-22 fall in the range of those for liver toxicity (Table 4-23). This narrow range of serum and liver concentrations and external doses reinforces the concept of a critical threshold body burden for expression of toxicity. Therefore, while interspecies differences may exist, these differences do not appear to be pronounced with respect to differences in sensitivity of the liver. These facts, together with the fact there is no evidence that PFOS is metabolized in rats, rabbits, monkeys and humans, suggest that major uncertainties in interspecies comparisons based on serum PFOS concentration do not exist. The above factors argue for reductions in uncertainty when assessing the adequacy of MOE values.

4.5.3 Assessment of Risk

The use of serum and liver concentrations of PFOS as a measure of exposure and a factor in dose-response for risk characterization is supported by the clinical and experimental data. As noted previously, a measure of human health risk can be obtained by calculating the ratio of the serum or liver PFOS concentration associated with NOAEL or LBMIC values from toxicology studies to human exposure levels. The resulting value is the MOE and can be used in risk characterization (U.S.E.P.A., 1999).

4.5.3.1 Points of Departure

Based on the foregoing discussion, a serum PFOS value of 31 ppm and a liver PFOS value of 59 ppm are appropriate and protective as points of departure for calculating MOEs. Comparisons can also be made with serum values of 44 for liver toixicity (male rats), and 62 ppm for liver tumors. These points of departure are shown in Table 4-24 above.

As noted above, the advantage to using serum and liver PFOS concentrations associated with either the NOAEL or the LBMIC from toxicology studies is in reducing uncertainty in cross-species extrapolation and in providing an integrated measure of exposure.

4.5.3.2 Exposure Data

Worker biomonitoring data are available from the medical surveillance of exposed workers. For the general population, individual serum PFOS concentrations have been measured in three human populations: 1) children; 2) adult blood donors; and, 3) the elderly. These serum data can be used for risk assessment.

For exposure based on liver concentration, human liver concentration can be estimated based on the observed liver-to-serum PFOS concentration ratios in a limited number of organ donors and in cynomolgus monkeys treated with PFOS for six months. The mean human liver-to-serum PFOS concentration ratio from the organ donor study was 1.3:1, and the monkey values were generally in the range of roughly 1:1 to 2:1. To estimate a conservative value for representative human liver concentrations for this risk assessment, measured individual serum PFOS concentrations will be multiplied by 1.7. The factor of 1.7 represents the upper 95% CI of mean liver-to-serum PFOS concentration ratios from the donor study.

4.5.3.3 Occupational Exposures and Risks

Serum levels of PFOS in 3M production workers have averaged less than 2 ppm. The great majority of employee serum PFOS concentrations have been measured at less than 6 ppm with a few workers levels up to 10-13 ppm. No evidence of effects has been observed by standard clinical chemistry tests and by assays of different hormones. In particular, serum cholesterol levels are not affected in this range of serum PFOS concentrations measured in production workers. In a retrospective cohort mortality study, no increases in mortality other than bladder cancer, a finding believed to be unrelated specifically to PFOS, were observed. Based on the years of medical surveillance and all the available data, this information suggests that workers are not at risk at the serum levels reported.

Few serum PFOS data are available for occupational exposures in various user facilities. 3M production workers were exposed to unreacted, concentrated starting materials. The majority of downstream workers were exposed to fluorochemical products that typically contained less than one percent residual unreacted starting material that could be absorbed and metabolized to PFOS. It is probable that such workers had lower exposure levels than 3M production workers, and therefore would have larger MOE values. The information that 3M is aware of, including the study of non-production workers' serum in 3M's Japanese facility, suggests that downstream workers' serum concentrations approximated 1/10th of the mean 3M fluorochemical production workers' serum PFOS concentrations.

Table 4-25 presents the MOE analysis for workers. As noted above, 3M's workers have been monitored and studied for almost three decades without finding adverse effects attributable to

PFOS exposure. Clinical chemistry, hematological and hormonal measurements have not shown effects, nor did the mortality study find effects attributable to PFOS.

Table 4-25. Human-Health Risk Assessment for PFOS Body Burden: Margin-of-Exposure Analysis based on Worker Serum and Estimated Worker Liver PFOS Concentrations

Mean Concentration in Humans (C), ppm	Point-of-Departure (POD), ppm	Endpoint	Margin of Exposure (Ratio POD:C)
Serum:			
2	31 [a]	Pup weight gain, rats	16
2	44 [b]	Liver effects, rats	22
2	62 [c]	Liver tumors, rats	31
Liver: Human liver concentrations estimated from serum assuming a liver-to-serum ratio of 1.7:1.			
3.4	59 [d]	Liver effects, male monkeys	17

[a] LBMIC at 5% response level, using mean of gestation day 21 and pre-gestational serum levels in dams.
[b] NOAEL for liver toxicity (LBMIC could not be calculated for male rats and is higher than the male NOAEL for female rats). It should be noted that the LBMIC (10% response level) for increased liver weight in male rats is also 44 ppm.
[c] LBMIC at 10% response level.
[d] NOAEL (as liver-tissue [PFOS]) for liver toxicity (LBMIC could not be calculated for male monkeys and is higher than the male NOAEL for female monkeys).

4.5.3.4 General Population (Non-Occupational) Exposures

The available data from sampling of blood from three segments of the general population (children, adults, and elderly) show that the overall mean serum PFOS concentrations are generally between 30 and 40 ppb. A mean serum value of 40 ppb is used for the comparisons. The upper 95% confidence limit for serum PFOS concentration of the 95% tolerance limit (upper bound) for these three populations are all essentially 0.1 ppm.

Using these serum values and multiplying by 1.7 (the upper 95% CL of human liver-to-serum PFOS cponcentration ratios) to conservatively estimate liver concentration, estimated mean liver concentrations are between 0.060 and 0.080 ppm. Deriving an upper bound liver concentration by doubling the upper bound serum concentration leads to an estimate of 0.2 ppm. This is higher than the highest liver value observed in the human organ donor study of 0.057 ppm.

Table 4-26 presents the MOE analysis based on these values and the associated points of departure. Based on this analysis, margins of exposure for children, adult blood donors, and the elderly are two to three orders of magnitude compared to laboratory animal no-effect levels for the various endpoints.

In addition, general population serum concentrations are well below the levels found in 3M fluorochemical production workers, who have been monitored for many years without evidence of adverse effects attributable to PFOS (Table 4-27).

The assessment presented above is based on a substantial body of health effects data, covering a variety of species and endpoints. Because it is based on an interspecies comparison of serum PFOS levels, it is more certain than typical assessments, which are based on inter-species comparisons of external doses. This reduced uncertainty translates to a need for smaller uncertainty factors for cross-species extrapolation.

Table 4-26. Human-Health Risk Assessment for PFOS Body Burden: Margin-of-Exposure Analysis based on Human Serum and Estimated Human Liver PFOS Concentrations

PFOS Concentration in Humans (C), ppm	Point-of-Departure (POD), ppm	Endpoint	Margin of Exposure (Ratio POD:C)
Serum:			
0.040 (mean)	31 [a]	Pup weight gain	775
0.040 (mean)	44 [b]	Liver effects, rats	1100
0.040 (mean)	62 [c]	Liver tumors, rats	1550
0.100 (upper bound) [d]	31	Pup weight gain	310
0.100 (upper bound)	44	Liver effects, rats	440
0.100 (upper bound)	62	Liver tumors, rats	620
Liver: Human liver concentrations estimated from serum assuming a liver-to-serum ratio of 1.7:1.			
0.068 (mean)	59 [e]	Liver effects, monkeys	868
0.17 (upper bound) [f]	59	Liver effects, monkeys	347

[a] LBMIC at 5% response level, using mean of gestation day 21 and pre-gestational serum levels in dams.
[b] NOAEL for liver toxicity (LBMIC could not be calculated for male rats and is higher than the male NOAEL for female rats). It should be noted that the LBMIC (10% response level) for increased liver weight in male rats is also 44 ppm.
[c] LBMIC at 10% response level.
[d] Upper 95% confidence limit at 95% tolerance limit.
[e] NOAEL (as liver-tissue [PFOS]) for liver toxicity (LBMIC could not be calculated for male monkeys and is higher than the male NOAEL for female monkeys).
[f] Upper 95% confidence limit at 95% tolerance limit based on assumed liver-to-serum PFOS concentration ratio of 1.7:1.

Table 4-27. Ratio of Worker Serum to General Population Serum [PFOS]

Worker Serum [PFOS]	General Population Serum [PFOS]	Ratio of Mean Worker Serum [PFOS] to Population Serum [PFOS]
2 ppm (mean)	0.04 ppm (mean)	50
	0.100 ppm (upper bound)[a]	20

[a] Upper 95% confidence limit at 95% tolerance limit.

Based on the discussion above (see section 4.5.2.5), there are justifiable reasons to reduce the default uncertainty factors of 10 for intraspecies and interspecies uncertainty in the traditional approach to uncertainty analysis. The fact that the lowest MOE determined in this risk characterization is approximately 300 suggests a more-than-adequate level of protection for the general population.

4.5.4 Work in Progress

3M continues to sponsor research investigating mechanisms of toxicity of PFOS and its precursors. Worker health will continue to be monitored, and an update of the Decatur mortality study is planned within three years. Completion of the bladder cancer incidence study in this cohort should occur within the next twelve months. All final reports will continue to be shared with the EPA and other agencies.

5.0 CONCLUSION

This report summarizes the information that is available as of June 30, 2003. There is a substantial body of data addressing human and wildlife exposures to PFOS and the potential environmental and health effects of PFOS. These data, when taken in the context of ecological and health risk, demonstrate adequate margins of exposure in wildlife and human populations. Uncertainty is reduced by the use of internal dose to assess risk. Therefore, adverse effects are not expected in either wildlife or humans.

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APPENDIX I

SUMMARY REPORTS FOR PHYSICAL/CHEMICAL PROPERTIES AND ENVIRONMENTAL FATE STUDIES

APPENDIX I

SUMMARY REPORTS FOR PHYSICAL/CHEMICAL PROPERTIES AND ENVIRONMENTAL FATE STUDIES

CONTENTS

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RS-I-1: 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-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

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

RS-I-2: MELTING 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-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, ^{1}H-HMR, ^{19}F-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, 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

RS-I-3: VAPOR PRESSURE

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, ^{1}H-HMR, ^{19}F-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 vapor pressure was done by using the Spinning Rotor Gauge method.

RESULTS

Vapor Pressure: $3.31X10^{-4}$ Pa
Temperature °C: 20°C
Decomposition (yes/no/ambiguous): No

Remarks: The measured vapor pressure was repeatable.

CONCLUSIONS

Remarks: The vapor pressure of the test substance was determined to be $3.31X10^{-4}$ 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

RS-I-4: OCTANOL/WATER PARTITION COEFFICIENT

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, ^{1}H-HMR, ^{19}F-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

RS-I-5: SOLUBILITY IN OCTANOL– SHAKE FLASK METHOD

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 re-crystalized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. Purity determined to be 97.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840.
GLP (Y/N): Yes
Year completed: 2001

Octanol Source: Aldrich
Screen Test: Lot JU0873804, 99+%
Definitive Test: Lot 06238CI, 99.9+% HPLC Grade

Remarks: The definitive test consisted of placing ~0.010 g test substance with ~10 mL octanol in centrifuge tubes. The tubes were shaken at ~150 rpm at ~30°C for 24, 48 or 72-hours followed by 24-hours of equilibration at 22 - 23°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

RESULTS

24-hours: 56.9 mg/L
48-hours: 55.7 mg/L
72-hours: 55.4 mg/L

Mean solubility of PFOS in octanol = 56.0 mg/L

The 24, 48, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentration

CONCLUSIONS

The overall mean solubility concentration of the test substance in pure octanol was 56.0 mg/L.

Typically, the Column Elution Method is recommended for use with substances with solubility screening results of < 10 mg/L. However, the shake flask method was utilized in this study because of the difficulty in obtaining tubing compatible with octanol and the possible explosion hazard posed by possible leaks of a flammable solvent in an incubator.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

Studies conducted at the 3M Company, St Paul, MN, 2001, Environmental Laboratory Project Number EOO-1716.

OTHER

Last changed: 6/22/01

RS-I-6: OCTANOL-WATER PARTITION COEFFICIENT CALCULATION

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 re-crystalized from a production lot of FC-95, and assigned the internal reference number of TCR-00017-046. Purity was determined to be 97.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method/guideline followed: Calculated from n-octanol solubility and water solubility according to OPPTS 830.7550 and OECD 107.
Type: n-Octanol/Water partition coefficient (at saturation)
GLP (Y/N): Yes
Year: 2001
Test Temperature: n-Octanol solubility value at 23 - 24°C
Water solubility value at 24-25°C

Remarks: The physical properties of PFOS do not allow determination of the partition coefficient by the shake flask method per guidance provided in the OPPTS and OECD guidelines. Therefore, this study does not bring the two phases (n-octanol and water) into contact with PFOS at the same time, and this testing reflects the partition coefficient for the subject material at saturation only. The n-Octanol / Water partition coefficient was calculated by dividing the solubility of PFOS in n-octanol by the solubility in water and expressing it as the logarithmic value.

RESULTS

The calculated log K_{ow} for PFOS was determined to be –1.08 at saturation (log (56 mg/L in n-octanol / 680 mg/L in water)).

CONCLUSIONS

No conclusions can be derived from this information. It applies only to a saturated system, which would not likely exist in the environment.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

Remarks: The solubility studies were conducted properly. However, the applicability of this data point is limited. Application of this value in a risk assessment has limited or no value as it only applies to saturated systems.

REFERENCES

Studies conducted at the 3M Company, St. Paul, MN, 2001, Environmental Laboratory Project Number E00-1716.

OTHER

Last changed: 6/21/01

RS-I-7: AIR/WATER PARTITION COEFFICIENT

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. 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/m^3 or 0.044 Pa m^3/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, Minnesota, 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

RS-I-8: WATER SOLUBILITY

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, ^{1}H-HMR, ^{19}F-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

RS-I-9: WATER SOLUBILITY IN PURE WATER- SHAKE FLASK METHOD

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 re-crystalized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. Purity determined to be 97.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840.
GLP (Y/N): Yes
Year completed: 2001

Water Source: ASTM Type I water, Millipore

Remarks: The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 225 rpm at 30°C for 24, 48 and 72-hours followed by 24-hours of equilibration at 24 - 25°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

RESULTS

ASTM Type I Water: 680 mg/L at 24 - 25ºC

Description of solubility: Slightly soluble.

The 24, 48, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations.

CONCLUSIONS

The overall mean solubility concentration of the test substance in pure water was 680 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

Studies conducted at the 3M Company, St Paul, MN, 2001, Environmental Laboratory Project Number EOO-1716.

OTHER

Last changed: 6/26/01

RS-I-10: WATER SOLUBILITY IN NATURAL SEAWATER AND 3.5% SODIUM CHLORIDE SOLUTION – SHAKE FLASK METHOD

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 re-crystalized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. Purity determined to be 97.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840.
GLP (Y/N): Yes
Year completed: 2001

Water Sources:

Natural Seawater = Ocean Scientific, lot # LN 58, salinity = 3.5%
Sodium Chloride = EM Science, 99% pure, mixed with ASTM Type I water to achieve salinity of 3.5%

Remarks: The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 150 rpm at 30°C for 24, 48 and 72-hours followed by 24-hours of equilibration at 22 - 24°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

RESULTS

Natural Seawater: 12.4 mg/L at 22 - 23ºC
3.5% NaCl Solution: 20.0 mg/L at 22 - 24ºC

Description of solubility: Slightly soluble.

The 24, 48, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations for the natural seawater. The 24-hour values were not included in the mean solubility of sodium chloride calculation because when the coefficient of variation was calculated for all of the replicate analyses for that day, it was > 15%.

CONCLUSIONS

The overall mean solubility concentration of the test substance in natural seawater it was 12.4 mg/L and in a 3.5% NaCl solution it was 20.0. PFOS solubility decreases with increasing ionic strength of the medium.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

Studies conducted at the 3M Company, St Paul, MN, 2001, Environmental Laboratory Project Number EOO-1716.

OTHER

Last changed: 6/26/01

RS-I-11: SOIL ADSORPTION

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was re-crystalized from a production lot of FC-95. Purity determined to be 97.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: Based on OECD 106
GLP (Y/N): Yes
Year completed: 2001. Amended report issued 2002
Statistical methods: Statistical analysis and plotting of the data was done according to OECD Method 106 using Microsoft Excel.
Temperature: Room Temperature (19-30°C)
Stock and test solution preparation: The test concentrations and conditions were determined in a Preliminary experiment. For the definitive experiment, test solutions were made by diluting a stock solution of un-radiolabeled Perfluorooctanesulfonate to a final test substance concentration of approximately 0.5 mg/L in aqueous 0.01 M $CaCl_2$.

Soil Characteristics:

Soil Class	Clay	Clay Loam	Sandy Loam	River Sediment	Domestic Sludge
Source	Kittson County, MN	Grand Forks County, ND	Grand Forks County, ND	Grand Forks County, ND	NIST, from Denver, CO POTW
Lot Number	00-2407	00-2405	99-2564	00-2406	2781
Physical Description	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	200 mesh, oven-dried, sterilized
% Organic Carbon	2.6%	2.6%	2.8%	1.3%	Not analyzed
% Sand	16%	21%	58%	39%	Not analyzed
% Silt	22%	46%	22%	42%	Not analyzed

Soil Class	Clay	Clay Loam	Sandy Loam	River Sediment	Domestic Sludge
% Clay	62%	33%	20%	19%	Not analyzed
CEC (meq/100g)	54.5	24.7	23.3	17.5	Not analyzed
pH in 0.01 M $CaCl_2$	7.2	6.0	7.8[1]	7.7	Not analyzed

(1) Value is for pH in water not pH in 0.1M $CaCl_2$

Test Conditions:

Adsorption Kinetics: Replicate study samples containing the soils (or sediments or sludges) were equilibrated by shaking for at least 12 hours at room temperature with 0.01 M $CaCl_2$. Study samples were dosed with the test substance at approximately 0.5 mg/L and placed on an orbital shaker. Replicate sets of these study samples were removed at designated time points throughout a 48 hour time period. Study samples were then prepared and analyzed for the target analyte. The adsorption kinetics were determined using this data. The last set of study samples (48 hour) were saved and used for the desorption kinetics portion of the method.

Desorption Kinetics (One concentration): After the adsorption kinetics experiment, the 48 hour study samples were centrifuged and the aqueous phase removed. The volume of solution removed was replaced by an equal volume of 0.01 M $CaCl_2$ without test substance. The new mixture was agitated until the desorption equilibrium was reached. During a 48-hour period, at defined time intervals, small aliquots of the aqueous phase were removed and analyzed for the target analyte. The desorption kinetics were determined using this data.

RESULTS

Adsorption Kinetics of PFOS, 1:5 Soil:Solution Ratio, 48 Hour Time Point:

Soil Type	Average Distribution coefficient, K_d, L/Kg	Percentage of Organic Carbon in Soil	Average Organic Carbon normalized Adsorption Coefficient, K_{oc}, L/Kg
Clay	18.3	2.6	704
Clay Loam	9.72	2.6	374

Soil Type	Average Distribution coefficient, K_d, L/Kg	Percentage of Organic Carbon in Soil	Average Organic Carbon normalized Adsorption Coefficient, K_{oc}, L/Kg
Sandy Loam	35.3	2.8	1260
River Sediment	7.42	1.3	571
Domestic Sludge	< 0.120	Not available	Not calculable

The data indicate that adsorption occurred within the first few hours of exposure and the test substance concentration did not vary significantly after 16 hours.

Apparent Desorption Kinetics of PFOS, 1:5 Soil:Solution Ratio, 48 hour Time Point:

Soil Type	Desorption Coefficient, K_{des}, L/Kg
Clay	47.1
Clay Loam	15.8
Sandy Loam	34.9
River Sediment	10.0
Domestic Sludge	<237

The river sediment displayed the most desorption at 39% after 48 hours. The sludge samples did not desorb a detectable amount of test substance. Desorption that did occur was accomplished rather quickly; after the 8 hour time point the test substance concentration did not vary significantly.

Adsorption Isotherms:

Soil type	Log K^{ads}_F	K^{ads}_F (1), L/Kg	Regression constant, 1/n	Regression Constant, n
Clay	1.40	25.1	0.884	1.13
Clay Loam	1.15	14.0	0.841	1.19

Sandy Loam	1.45	28.2	0.829	1.21
River Sediment	0.939	8.70	0.989	1.01
Domestic Sludge	2.53	338	1.26	0.795

(1) Freundlich adsorption coefficient. The units for the isotherm assume that the term n=1. More accurately, the units are $(\mu g^{1-1/n}(L)^{1/n}Kg^{-1})$

Desorption Isotherms:

Soil Type	Log K^{des}_F	K^{des}_F(1), L/Kg	Regression Constant, 1/n	Regression Constant, n
Clay	2.02	105	0.860	1.16
Clay Loam	1.78	60.2	0.954	1.05
Sandy Loam	1.97	94.0	0.985	1.02
River Sediment	1.65	44.6	1.02	0.981
Domestic Sludge	3.50	3130	0.977	1.02

(2) (1) Freundlich desorption coefficient. The units for the isotherm assume that the term n=1. More accurately, the units are $(\mu g^{1-1/n}(L)^{1/n}Kg^{-1})$

Freundlich adsorption isotherms relate the amount of test substance adsorbed on the soil to the amount present in the aqueous solution at equilibrium. The values calculated for the regression constant indicate that the data obtained for the test substance over two orders of magnitude is slightly non-linear.

CONCLUSIONS

Perfluorooctanesulfonate (PFOS) appeared to adsorb to all of the soil/sediment/sludge matrices tested. In either case, adsorption or desorption, an equilibrium is achieved in less than 24 hours, with substantial adsorption (>50%) occurring in some of the time 0 samples after approximately 1-minute of contact.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

3M Technical Report "Soil Adsorption/Desorption Study of Potassium Perfluorooctanesulfonate (PFOS)". Mark E. Ellefson, Project Number E00-1311, Final Report Completion Date June 4, 2001. Amended Report Completion Date May 24, 2002.

OTHER

Last Changed: June 12, 2002

RS-I-12: BIOCONCENTRATION

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method/guideline followed: US EPA OPPTS 850.1730 and OECD 305
Type: Flow-through exposure with flow-through depuration phase.
GLP (Y/N): Yes
Year: 2001, Report amended 2002
Species: Bluegill (*Lepomis macrochirus*)
Supplier: Osage Catfisheries, Inc., Osage Beach, Missouri
Length and weight at test termination:
Mean length = 62 mm, range 56-66 mm
Mean weight = 2.70 g, range 2.03 – 3.32 g
Loading: 0.48 g fish/L/day (based on initial loading of 90 fish per tank, using mean fish weight at the end of the study and volume of water that passed through test chamber in 24-hours).
Fish age: Approximately 7 months at test initiation
Analytical monitoring: Concentration of PFOS in water and fish.
Pretreatment: None
Number of concentrations: Two plus a negative control
Test concentrations (mean measured): Negative control, 0.086 and 0.87 mg/L
Uptake period: 62-days (0.086 mg/L exposure)
35-days (0.87 mg/L exposure – this exposure ended after 35-days due to fish mortality)
Depuration period: 56-days (0.086 mg/L exposure)
None (0.087 mg/L exposure)
Test conditions:
Dilution water: Moderately–hard well water
Dilution water chemistry:
Specific conductance: 313 (310 – 315 umhos/cm)
Hardness: 130 (128 – 132 mg/L)
Alkalinity: 178 (176 – 178)
pH: 8.1 (8.0 – 8.2)
Measured during the 4-week period immediately preceding the test.

Test conditions (cont.):

Stock and test solution preparation: Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless.
Diluter flow rate: Approx. 6.3 volume additions per 24-hours
Exposure vessels: 104 L stainless steel aquaria filled with approximately 80 L solution.
Number of replicates: None – one vessel per concentration
Number of fish per vessel: 90
Diet: Flake food, Ziegler Brothers, Inc., Gardners, PA

Water chemistry ranges during the study:

	Neg. Control	0.086 mg/L	0.87 mg/L
Dissolved oxygen, mg/L:	6.8 – 8.6	6.8 – 8.6	6.4 – 8.2
Temperature, °C:	21.8 – 22.0	21.7 – 22.0	21.7 – 21.9
pH:	7.9 – 8.2	7.9 – 8.2	7.9 – 8.2

Photoperiod: 16 hours light and 8 hours dark with a 30 minute transition period.
Light intensity: 278 lux at surface of the negative control vessel at test initiation

Collection of tissue samples: Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed and measured. Fish then rinsed with dilution water, blotted dry again and dissected into edible and nonedible tissue fractions. The fractions were individually weighed. The head, fins and viscera were considered to be nonedible tissue. The remaining tissue, including skin was considered to be edible tissue.
Statistical methods: Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values were originally calculated from the tissue concentrations at apparent steady-state using the BIOFAC model. Upon further investigation, this model was deemed inappropriate for use with this data set. The tissue concentrations had reached a point where there were not statistically significant differences between the last three sample days. However, a plot of the data clearly shows a trend of increasing concentrations in the tissues. In addition, the BIOFAC program is not accepted as appropriate for surfactants. An amended report was issued with BCFK values calculated as outlined in the draft OPPTS 850.1730 Guidance Document. The kinetic bioconcentration factor (BCFK), uptake rate (k_1) and

depuration rate (k_2) were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L. These rate constants were then used to calculate a BCFK (BCFK = K_1/K_2) and half-lives for clearance for each tissue type. The results from this data reanalysis are presented below.

RESULTS

Nominal concentrations: Negative control, 0.1 and 1.0 mg/L
Mean measured concentrations: < 0.05, 0.086 and 0.87 mg/L

Kinetic Bioconcentration factors (BCFK):

0.086 mg/L exposure

	Edible	Nonedible	Whole Fish
BCFK:	1124	4013	2796
Time to reach 50% clearance:	86 days	116 days	112 days

0.87 mg/L exposure

Although BCF values were calculated using the BIOFAC software, the results are not reported here. All of the fish had died or been sampled prior to achieving steady-state. As a result, the BCF values were underestimated and are not relevant.

PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L:

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	0.167, 0.155, 0.144, 0.182	0.415, 0.519, 0.417, 0.497	0.293, 0.351, 0.286, 0.363
1	0.734, 0.726, 0.631, 0.806	1.68, 1.85, 1.72, 2.07	1.26, 1.34, 1.29, 1.53
3	1.73, 2.07, 2.03, 2.11	4.59, 5.50, 5.47, 5.97	3.21, 4.04, 4.18, 4.38
7	3.73, 4.25, 4.73, 6.25	10.2, 10.6, 11.9, 15.2	7.33, 7.66, 8.73, 11.4
14	11.4, 9.07, 13.7, 12.6	27.3, 23.2, 35.3, 32.6	20.2, 16.9, 26.0, 24.6
21	11.7, 12.0, 12.9, 10.6	33.3, 22.7, 24.6, 24.4	23.3, 18.4, 19.8, 18.5
28	18.3, 13.7, 23.9, 23.1	49.4, 40.7, 65.3, 57.9	35.3, 29.2, 45.4, 44.1

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
35	22.6, 27.7, 23.8, 20.6	67.1, 73.3, 62.0, 59.1	46.3, 53.8, 46.6, 40.9
42	27.6, 25.3, 21.2, 27.6	64.0, 68.1, 54.4, 79.6	50.1, 49.4, 40.9, 56.3
49	33.3, 36.2, 39.0, 30.6	85.0, 95.1, 93.1, 77.7	62.8, 69.6, 70.8, 57.4
56	48.3, 38.9, 44.1, 38.3	122, 94.2, 73.2, 106	90.6, 71.6, 63.3, 74.8
62	42.4, 66.2, 42.2, 39.2	101, 112, 105, 96.4	77.0, 92.7, 79.6, 73.1
Depuration Day			
14	48.5, 31.8, 31.6, 42.0	124, 79.4, 81.8, 113	90.3, 60.4, 61.6, 85.3
28	26.0, 33.3, 38.7, 55.8	85.7, 95.1, 85.7, 94.8	58.2, 70.1, 68.1, 81.1
42	24.1, 31.2, 30.0, 33.0	71.7, 80.6, 78.3, 82.1	51.4, 61.4, 61.0, 62.2
56	21.1, 37.6, 32.9, 31.2	57.7, 80.3, 85.4, 84.4	41.6, 66.5, 65.8, 62.1

PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L:

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	1.46, 1.48, 1.19, 1.39	3.52, 4.37, 4.22, 4.06	2.71, 3.08, 2.84, 2.89
1	4.68, 6.59, 5.56, 5.64	11.1, 14.2, 13.3, 12.1	8.00, 10.9, 10.2, 9.47
3	17.3, 15.8, 19.0, 20.8	39.3, 42.0, 43.8, 51.8	30.5, 30.7, 34.5, 39.1
7	42.0, 44.0, 57.7, 46.8	100, 102, 102, 120	74.9, 77.0, 85.3, 89.8
14	87.1, 81.6, 90.7, 73.3	177, 207, 245, 214	141, 157, 180, 158
21	79.4, 117, 104, 102	201, 278, 246, 229	146, 210, 185, 172
28[(1)]	102, 131, 107, 133	289, 372, 320, 361	205, 267, 232, 263

[(1)] Sampling of fish stopped after Uptake Day 28 due to mortality.

Test organism mortality:
Negative control: None during the uptake phase (62 days) or depuration phase (35 days)
0.086 mg/L exposure: One fish died after 49 days and one after 59 days of exposure in the uptake phase, none during the depuration phase (total of 2.2% mortality during the study).
0.87 mg/L exposure: Mortality first noted on Day 9 and continued through Day 35 of the uptake phase at which time all of the fish had either died or had been sampled
Analytical methodology: Analyses of test solutions and fish tissues were performed at Wildlife International, Ltd. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

CONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. The BCFK values calculated for the edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were calculated to be 1124, 4013, and 2796, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were 86, 116 and 112 days, respectively.

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Wildlife International, Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723. The report was amended and reissued June 6, 2002.

OTHER

Last changed: 6/11/02

RS-I-13: Decomposition of PFOS by Combustion Processes

LABORATORY-SCALE THERMAL DEGRADATION OF PERFLUORO-OCTANYL SULFONATE AND RELATED SUBSTANCES

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or $C_8F_{17}SO_3^-K^+$. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3). Sample container was a clear glass vial with black plastic screw on cap. Label read "C8F17SO3$^-$K$^+$, 98-0211-3916-1, Lot 217". The purity of this substance is 86.9%. Storage conditions were not described.

Remarks: Thermal degradation of FC-807A and FC-1395 (C_8 perfluorooctyl sulfonamides) was also evaluated as a potential source of PFOS in the environment. FC-807A is a mixture of mono- and di-phosphate esters of fluorochemical alcohols (HOCH2CH2N(R)SO2C8F17). FC-807A had previously been used in production of oil-resistant papers and packaging material. FC-1395 is a urethane polymer to which the fluorochemical alcohol is bound through urethane linkages. FC-1395 had previously been used as a carpet treatment material prior to the production phase-out in December 2000. The sample container for FC-807A was made of clear glass with a metal screw cap. The Label for this substance read "Material FC-807A/8681/BC AS/Time: 11:10/Lot No. 30177/Drum T1/Step/Date: 12-22-2K/Sampled by C. Senior." The sample container for FC-1395 was an amber glass vial with black plastic screw on cap. The label for this substance read "Name: FC-1395/Lot#: 90086/Date: 11/7/00." Storage conditions were not described for FC-807A and FC-1395. Purity was also not noted for these test substances.

METHOD

Method: Method developed by Takahiro Yamada and Philip H. Taylor, Environmental Sciences and Engineering Group, University of Dayton Research Institute, based on the use of batch-charged continuous flow reactors developed at UDRI to study the thermal stability of organic materials (Rubey and Carnes, 1985, Rubey and Grant, 1988).

Method comments: Laboratory-scale study simulating a full-scale hazardous waste incinerator. The System for Thermal Diagnostic Studies (STDS) was used for the incineration study. The instrument consists of several major components: a thermal reaction compartment; a transfer

line; an analytical gas chromatograph (GC), a mass selective detector and a computer workstation.

Year completed: Phase I testing began in 2001 and the final report was written and completed in 2003.

GLP: No

Test Overview:

Testing was completed in 3 phases. Phase I tested the initial test protocol and project objectives, Phase II consisted of the method development work, and Phase III revised and applied the test protocol. The overall goal of this study was to determine if incineration is a potential source of perfluoroalkyl sulfonates, e.g., perfluoro-octanyl sulfonates (PFOS), which has been found widespread in wildlife tissue samples

Phase I:

Objectives of Phase I were to determine if C_8 perfluorosulfonamides form combustion products in the form of perfluorooctane sulfonate (PFOS) or a perfluoro precursor of perfluorooctane sulfonate, to determine the extent of conversion of PFOS under conditions representative of hazardous or municipal waste incineration, to identify the major fluorinated combustion products, and to determine if the sulfur present in the PFOS is quantitatively converted to sulfur dioxide and/or thionyl fluoride (SOF_2) and sulfuryl fluoride (SO_2F_2) at high temperature, fuel-lean combustion conditions.

The development of the test protocol was based on the use of batch-charged continuous flow reactors developed at UDRI to study the thermal stability of organic materials. Analysis was performed using in-line gas chromatography/mass spectrometry (GC/MS). The analytical focus was identification of stable fluorinated organic intermediates and the quantification of sulfur oxides in an attempt to recover 100% of the initial sulfur in the sample. Sulfur quantification was performed using a mass selective detector (MSD).

Gas-phase thermal stability analysis was performed to determine 1) the temperature needed to gasify sample, 2) if the phase change is the result of evaporation or decomposition, and 3) if a non-volatile residue is deposited by the sample. Then testing was performed to assess whether the gasification products have the ability to be transported under the flow reactor conditions.

The STDS was selected to carry out this incineration study. Methane was chosen as the fuel for samples that were hydrogen deficient. Two temperatures (600 and 900°C) were selected for the study based on preliminary combustion tests.

Phase II:

The purpose of Phase II of the incineration study was to verify gasification of PFOS and transport of PFOS through the UDRI thermal instrumentation system. Recovery efficiencies and detection limits for sulfur compounds (SO_2, SOF_2, and SO_2F_2) and perfluorooctane sulfonyl fluoride (POSF, a precursor of PFOS) were determined through analysis using a GC/MS system. Measurements were performed in duplicates. Hexafluoropropene (HFP) was selected as the surrogate volatile fluorocarbon. Recovery efficiencies and detection limits of volatile C_1-C_4 fluorocarbons were also established. A quantitative method of sampling the reactor effluent was established. ORBO PUF cartridges were used for sampling PFOS and precursors from the reactor effluent.

LINEAR FIT EQUATIONS AND DETECTION LIMITS

Sample Name	Linear Fit (Y: peak area, X: concentration (ppm))	R	Detection Limit (ppm)
SO_2	Y = 5.8813E3* X - 3.8541E5	0.9971	78.5
SOF_2	Y = 8.3335E3* X - 7.0267E4	0.99941	30.3
SO_2F_2	Y = 1.0331E4*X + 1.8273E6	0.99708	20.1
POSF	Y = 1.0423E5*X – 8.4043E5	1.0	14.1
HFP	Y = 1.4975E4*X - 2.8253E6	0.9997	3.9

TRANSPORT EFFICIENCY

System Transport				Direct Injection			Efficiency
Peak Area				Peak Area			(%)
Sample	1st	2nd	AVG (1)	1st	2nd	AVG (2)	(1)/(2)×100
SO_2	9130332	8980717	9055525	11952302	11762267	11857285	76.4
SOF_2	25244352	25203780	25224066	24862639	24773683	24818161	101.6
SO_2F_2	86850304	85572809	86211557	84435720	79738316	82087018	105.0
POSF	1280370	1228718	1254544	1064431	1067947	1066189	117.7
HFP	148679354	145606343	147142849	148372504	142271896	145322200	101.3

Phase III:

Phase III consisted of 8 different tests (SO_2 Transfer Efficiency Tests, Laboratory Spike Analysis for PFOS, Heated Blank Combustion Test,

Combustion Tests for PFOS and two C_8 perfluorosulfonamides, Heated Blank Combustion Test (repeat), Transfer Efficiency Test for PFOS, Sulfur Recovery Analysis as SO_2, and Extracted Ion Analysis). In-line and off-line GC/MS analysis was conducted on the combustion gases. The off-line GC/MS analysis was used in replicate runs in order to capture the more volatile compounds and because there were resolution issues with the in-line sampling approach for the sulfur recovery rate. PUF (polyurethane foam) collection of the reactor effluent and chemical extraction of the reactor and associated transfer lines was completed. PUF cartridges and extracts were delivered to 3M for analysis of PFOS by LC/MS.

SO_2 Transfer Efficiency Tests were performed to confirm results from phase II. The Heated Blank Combustion Tests were used to examine system contamination. Four analyses were conducted for the Heated Blank Combustion test (in-line GC/MS analysis, PUF collected off-gas sample analysis, off-line GC/MS analysis using Tedlar bag, and reactor/transfer line system extraction using methanol).

RESULTS

SO_2 Transfer Efficiency Test:

SO_2 transport efficiency was 83.7% in Phase III, slightly higher than the Phase II results, 76.4%, which gives average value of 80.1%. The transport efficiency for SOF_2 was 100% in Phases II and III.

Laboratory Spike Analyses for PFOS:

Phase III laboratory spikes included fourteen 1 μg and twelve 10 μg spikes of PFOS (as the PFOS anion) into the PUFs (see Appendix 5.), and a single 1 μg PFOS spike (as the PFOS K salt) of the tubing used as the reactor (combustion chamber) and transfer line. For the PUF spikes, the spiking solution was injected just below the surface, and allowed to dry for 30 minutes, and then extracted as in the test procedure. An average of 82% of the PFOS was recovered from the 1 μg PUF spikes with a relative standard deviation of 10%. An average of 92% of the PFOS was recovered from the 10 μg PUF spikes with an RSD of 7%. The single combustion chamber spike involved injecting a 1 μg sample of PFOS into the reactor as a 1 μg/μl solution in HPLC grade methanol, and drying by blowing with high purity nitrogen. After drying, the transfer line was assembled to the reactor and the 1.1 mL volume assembly was extracted by pumping of 5.5 mL of methanol through the assembly in two sequential washings. The total PFOS recovery from this reactor/transfer line spike was 188%. This single spike result suggests that an error likely occurred during preparation, extraction or analysis. Nevertheless, this spike result confirms that PFOS can be extracted from reactor/transfer lines.

PFOS Laboratory Reactor/Transfer Line Spike Analysis:

Sample Extracts	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS 1st Extracts	232	1.6
PFOS 2nd Extracts	40.5	0.28

Heated Blank Combustion Analysis:

The flow profile and carrier flow volume used for the heated blank analyses at 600°C and 900°C are given in the following two tables.

Flow Rate Profile for Heated Blank Analysis at 600°C

Time Period (sec)	Reactor Flow Rate (ml/min)	Pyroprobe Flow Rate (ml/min)	Total Flow Rate (ml/min)	Total Volume (ml)	Sampled Volume[d] (ml)
0 – 120	10.5	0.80	11.30	22.60	20.60
120 – 130	10.5	0.80 → 4.63[a]	11.30 → 14.63	2.16	1.99
130 – 140	10.5	4.63	15.13	2.52	2.35
140 – 160	9.03 (He)[b]	4.53 (He)[c]	13.56	4.52	4.19
			Total Volume (ml)	31.80	29.13

[a] Linear increase (approximate). [b,c] Switched to helium for sweep. [d] Sampled volume for PUF and Tedlar bag collection.

Flow Rate Profile for Heated Blank Analysis at 900°C

Time Period (sec)	Reactor Flow Rate (ml/min)	Pyroprobe Flow Rate (ml/min)	Total Flow Rate (ml/min)	Total Volume (ml)	Sampled Volume[d] (ml)
0 – 150	7.60	0.70	8.30	20.75	18.25
150 – 160	7.60	0.70 → 4.63[a]	8.30 → 12.23	1.71	1.54
160 – 170	7.60	4.63	12.23	2.04	1.87
170 – 190	6.54 (He)[b]	4.53 (He)[c]	11.07	3.69	3.36
			Total Volume (ml)	28.19	25.02

[a] Linear increase (approximate). [b,c] Switched to helium for sweep. [d] Sampled volume for PUF and Tedlar bag collection.

There was no measurable contamination for either temperature (600 and 900°C) for both in-line and off-line GC/MS analysis. A small amount of PFOS (0.08μg) was detected in the reactor/transfer line extract in the first heated blank combustion test. The amount of PFOS extracted in the second heated blank combustion test was below detection limits.

Combustion Tests for PFOS and two C_8 perfluorosulfonamides:

PFOS in Transfer-Line Extracts: Table 5.4.1.1 of the report shows that in the four sequential combustion tests on PFOS, a total of 1.83 mg of PFOS were gasified in the pyroprobe.

0.45+0.38+0.5+0.5 = 1.83 mg

Tables 5.4.2.1 and 5.4.3.1 of the report show that, in the four sequential combustion tests on FC-807A and FC-1395, totals of 2.16 mg and 2.02 mg, respectively, of product dry mass were gasified in the pyroprobe. For example, for FC-807A:

0.59+0.59+0.45+0.53 = 2.16 mg

The gasified samples then passed through heated (260°C) transfer lines to a combustion chamber. In the combustion chamber the volatilized gases mixed with near-stoichiometric amounts of air (FC-807A and FC-1395) or excess air (PFOS) and were exposed to 600 or 900°C for about 2 seconds. From here, the combustion products passed through 260°C transfer lines to ambient temperature polyurethane foam containing cartridges or Tedlar bags.

Following the combustion tests at 900°C, two sequential methanol extracts of the second half of the combustion chamber and the transfer lines down-stream of the combustion chamber were analyzed for PFOS. The following table shows the concentration and amounts of PFOS (as the K salt), detected in the chamber/transfer line. This is the amount that accumulated in the chamber/transfer line from the four sequential tests, two at 600°C and two at 900°C, for each product. The total amount of PFOS detected in the extracts was equivalent to about 0.04% of the PFOS gasified in each of the four tests or, as shown in the following equation, 0.009% of the 1.83 mg of PFOS gasified in the four sequential tests.

100 X (0.00011+0.00005)/1.83 = 0.009%

Following the four sequential combustion tests on each of the two perfluorooctylsulfonamide products, PFOS was below detection limits in the chamber/transfer line extracts. Based on the detection limits, the amount of PFOS that could have been present in each of the two sequential extract was less than 0.035 μg. Based on the stoichiometry given in the Table 1 on page 7 of Appendix 4, the 2.16 mg and 2.02 mg of FC-807A or FC-1395 solids gasified in the four sequential tests could have formed a maximum of 1.87 mg or 1.55 mg of PFOS (as the K salt), respectively. For example, for FC-807A:

2.16 mg X 0.519/0.600 = 1.87 mg

where 0.519 and 0.600 are the fractions of fluorine in FC-807A and PFOS (as the K salt). The following shows how this calculation was done for PFOS using the data in Table 1, page 7 of Appendix 4:

Fraction F in $PFOS^-K^+$ = (19*17)/(12*8+19*17+16*3+32*1+39.1*1)
= 0.600

Where 19, 12, 16, 32 and 39.1 are the molecular weights of fluorine, carbon, oxygen, and potassium, respectively.

Thus, this analysis shows the amount of PFOS in the chamber/transfer lines was less than 0.004% and less than 0.005% of the maximum amount of PFOS that could have been formed from FC-807A or FC-1395 respectively. For example, for FC-807A:

100 X (< 0.000035+ < 0.000035)/1.87 = < 0.004%.

Transfer-Line Extraction Results:

PFOS		
Extraction	**$PFOS^-K^+$ (pg/μl)**	**$PFOS^-K^+$ (μg)**
1	15.4	0.11
2	8.61	0.059
FC-1395		
Extraction	**$PFOS^-K^+$ (pg/μl)**	**$PFOS^-K^+$ (μg)**
1	<5.00	<0.035
2	<5.00	<0.035
FC-807A		
Extraction	**$PFOS^-K^+$ (pg/μl)**	**$PFOS^-K^+$ (μg)**
1	<5.00	<0.035
2	<5.00	<0.035

PFOS in PUF Extracts: The calculation below, using data in the following table, shows that the amount of PFOS making it through the combustion system and extracted from the PUFs was equivalent to less than 0.5% of the 0.45 mg of PFOS gasified at 600 °C.

100 X (0.00062 + 0.0016)/0.45 = 0.49%

The data in this table also show that about 0.07 % of the 0.5 mg of PFOS gasified at 900°C makes it thought the system and was extracted from the PUFs.

100 X (0.00011 + 0.00022)/0.5 = 0.066%

PUF Extraction Results:

Substance	Temp (Degrees C)	Extraction	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	600	1,2	25.1, 64.0	0.62, 1.6
	900	1,2	4.31, 9.01	0.11, 0.22
FC-1395	600	1,2	<5.00, <5.00	<0.12, <0.12
	900	1,2	<5.00, <5.00	<0.12, <0.12
FC-807A	600	1,2	<5.00, <5.00	<0.12, <0.12
	900	1,2	<5.00, <5.00	<0.12, <0.12

The PUF Extraction Results table (above) also shows that, following combustion of perfluoroalkylsulfonamide compounds, no PFOS was detected in PUF extracts at concentrations above the lower limit of quantification (LLOQ). Based on the detection limits, the amount extracted from each of the two sequential PUFs was less than 0.12 μg. Of the 0.45 and 0.55 mg of FC-807A and FC-1395 gasified at 900°C, combustion could have formed a maximum of 0.39 mg or 0.42 mg of PFOS (as the K salt), respectively. For example for FC-807A:

0.45*0.519/0.6 = 0.39 mg

where 0.519 and 0.600 are the fractions of fluorine in FC-807A and PFOS K salt respectively. The following equations thus show that the amount of PFOS in the combined PUF extracts of the 900°C tests was less than 0.07% of the PFOS that could have been formed from both FC-807A:

100 X (<0.12 + <0.12)/390 = <0.062%

and FC-1395:

100 X (<0.12 + <0.12)/420 = <0.057%.

Transport Efficiency Tests for PFOS:

Three types of tests were conducted to examine transport efficiency. These tests were done to validate that PFOS could be detected if it were generated during the combustion of fluorochemicals in the test system. The first test examines the transfer efficiency of samples gasified in the pyroprobe and transported through the reactor to the PUF cartridges. The second test investigates the possibility that the volatilized PFOS condensed on the walls of the pyroprobe/reactor transfer line. The third test examines how much PFOS volatilized in the combustion reactor could be transferred to the PUFs and how much would condense in the reactor/transfer line tubing.

In the 1st Transfer efficiency test 0.48 mg of PFOS was volatilized. The following table shows that no detectable amount of the volatilized PFOS was recovered from the PUF cartridge. This result indicates that the sample was either thermally dissociated in the pyroprobe chamber or the gasified sample was completely condensed in the pyroprobe/reactor transfer line tubing.

PUF Extraction Results for 1st Transfer Efficiency Test

Sample	PUF Extracts	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	1st	<5.00	<0.12
	2nd	<5.00	<0.12

The next two tables show that, in the 2nd transfer efficiency test, measurable amounts of the 0.47 mg of PFOS that was volatilized survive pyrolysis conditions of the pyroprobe, and collected in the heated transfer lines. However, no detectable amount of PFOS survives transit to the PUF sampling cartridge.

Methanol Extraction Results for 2nd Transfer Efficiency Test

Sample	Extracts	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	1st	897	21
	2nd	<10.0	<0.24

PUF Extraction Results for 2nd Transfer Efficiency Test

Sample	PUF Extracts	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	1st	<10.0	<0.25
	2nd	<10.0	<0.25

The following two tables show results of the 3rd transfer efficiency test. In this test, PFOS was volatilized in the combustion chamber (reactor) by heating it to 575°C.The first table shows that measurable amounts of volatilized PFOS passed from the reactor to the PUFs. Calculations using data from the first of these tables show that 12.8% of the 0.46 mg of PFOS volatilized in He reached the PUF, as did 5.2% of the 0.48 mg of PFOS volatilized in air.

100 X (0.058 + 0.0011)/0.46 = 12.8%

100 X 0.025/0.48 = 5.2%

The second table below shows methanol extract data obtained in the 3rd transfer efficiency test. These were from extracts of: 1) the 260°C reactor/transfer line tubing and 2) the room temperature valve and associated transfer line tubing just upstream of the PUF cartridges. Calculations using data from this second table show that larger amounts of PFOS (5.6% air, 39.3% He) accumulated in the reactor/transfer lines upstream of the PUF cartridges. The majority of the PFOS accumulated in the portion of the transfer line heated to 260°C, suggesting that this compound could condense, or was in a particulate form, at this temperature.

100 X (0.024 + 0.00045 + 0.0024 + 0.000079)/0.48 = 5.6%

100 X (0.171 + 0.0019 + 0.0077 + 0.00035)/0.46 = 39.3%

3rd Transfer Efficiency Test PUF Extraction Results

Sample	Carrier Gas	Cartridge	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	He	1st	2330	58
		2nd	44	1.1
	Air	1st	997	25
		2nd	<10.0	<0.12

3rd Transfer Efficiency Test Reactor/Valve Transfer Line Extraction Results

Sample	Gasification	Location	Extracts	$PFOS^-K^+$ (pg/μl)	$PFOS^-K^+$ (μg)
PFOS	Air	Reactor[1]	1st	1908	24
			2nd	35.4	0.45
		Valve[2]	1st	696	2.4
			2nd	22.8	0.079
	He	Reactor[1]	1st	13530	171
			2nd	150	1.9
		Valve[2]	1st	2218	7.7
			2nd	102	0.35

Notes:

1. The location labeled "Reactor" included the second half of the combustion chamber (575°C) and the heated (260°C) transfer line leading from the combustion chamber to the valve.

2. The location "Valve," included the valve and the transfer line leading from the valve to the PUF. The valve and this transfer line were at room temperature during the test.

Sulfur Recovery Rate:
Based on the in- and off-line GS/MS analyses, sulfur was found mainly as SO_2. No SOF_2 and SO_2F_2 were detected. Because the SO_2 peaks using the off-line GC/MS system were much sharper than SO_2 peaks observed using in-line GC/MS, it was decided to use off-line GC/MS analytical results to quantitatively analyze the sulfur recovery as SO_2.

SO_2 Calibration Results Using the off-line PLOT Column

Conc. (ppm)	Mol. #	Area 1	Area 2	Area (Avg)
1000	4.09E-08	7191079	6980771	7085925
700	2.86E-08	4414365	4366705	4390535
400	1.63E-08	2304594	2295497	2300046
100	4.09E-09	425431	416699	421065

In the SO_2 transfer efficiency test conducted using the off-line analysis approach-the average recovery rate was 75.6%. This is very similar to the recovery rates obtained from the in-line analysis, i.e. 83.7 and 76.4%, suggesting that the lack in 100% recovery is due to sample losses in the combustion system and not the sampling and analysis procedures.

Standard SO_2 Transfer Efficiency

Volume (ml)	Area	Calculated Mol. #	# of Mol. Used	Transfer Efficiency (%)
22.13	10591947	1.36E-06	1.63E-06	83.4
22.13	8515987	1.11E-06	1.63E-06	67.8
			Average	75.6

Extracted Ion Analysis:
The following ions (51-CF2H, 69-CF_3, 119-C_2F_5, and 67-SOF) were among those extracted from the total ion chromatograms from tests on PFOS, a lower molecular weight perfluoroalkylsulfonate (designated as PFXS), and the C_8 perfluorosulfonamides. This was done for both the in-line and off-line GC/MS analyses in order to analyze for the presence of perfluorinated and sulfonate-containing intermediates. Importantly, the inability to detect the 67-SOF ion in any of these analyses suggested low levels or an absence of perfluoroalkylsulfonyl compounds in the

combustion products. This result strongly suggests that incineration is not likely to be a significant source of compounds that are environmental precursors of PFOS or other perfluoroalkyl sulfonates. Extracted ion chromatograms for 51, 69, and 119 ions, when evaluated in conjunction with hydrogen flame ionization detector (HFID) chromatograms, suggest that tri- and tetrafluoromethane and hexafluoroethane were likely combustion products.

Due to an apparent loss of sensitivity of the in-line MS detector during the PFOS test, the extracted ion analysis generated from PFOS combustion did not indicate the presence of fluorinated combustion products, unlike the results for all other compounds tested. An evaluation of the PFOS HFID data in conjunction with the HFID and extracted ion analysis data for a lower molecular weight perfluoroalkylsulfonate (PFXS) strongly suggested that PFOS combustion also generated some volatile (low MW) fluorochemical combustion products. The PFXS data showed fluorochemicals were present at the same retention time as HFID peaks. Additionally, the retention times of the HFID response from PFOS and PFXS combustion were nearly identical. Thus, the same fluorochemical combustion products likely formed from these two different compounds.

The following two tables show that the HFID peak area at 900°C is approximately 1% of that at 600°C. This result suggests nearly complete destruction of fluorochemicals combustion products at 900°C.

Integrated HFID Peak Area of PFXS and PFOS at 600ºC

Sample	Peak Area	Net Amount of Gasified Sample (mg)
PFXS	1190193	0.52
PFOS	3547614	0.38

Integrated HFID Peak Area of PFOS at 900ºC

Sample	Peak Area	Net Amount of Gasified Sample (mg)
PFOS	39041	0.50

CONCLUSIONS

This laboratory study used a system designed to simulate the thermal degradation conditions likely to occur during municipal or hazardous waste incineration. When perfluorooctane sulfonamide containing products (FC-1395 and FC-807A) were exposed to these conditions, SO_2 recoveries

were 100±25% and LC/MS analysis showed that no quantifiable amounts of PFOS (<0.07% of that stoichiometrically possible) were released from the system. The SO_2 results suggest that the perfluorosulfonamides were substantially destroyed and the LC/MS results strongly suggest that incineration of perfluorooctane sulfonamides would not release PFOS to the environment. Furthermore, mass spectral extracted ion analysis of the emissions, showed an absence of 67-SOF ions. These ions are indicative of perfluoroalkylsulfonyl compounds, which could potentially transform to PFOS in the environment. The absence of the 67-SOF ions suggests that the carbon-sulfur bond was completely destroyed (and did not reform) in the combustions tests. This result suggests that environmental transformation of combustion products to form PFOS is also unlikely.

When PFOS was exposed to this combustion system, sulfur recoveries varied from 50 to 60% and LC/MS analysis showed that only a small fraction of the PFOS that was volatilized made it through the system. The authors speculate that low sulfur recoveries were likely due to condensation within the apparatus. The fraction of PFOS passing through the system to the PUFs decreased as the temperature of the combustion chamber increased. At 600 °C, about 0.5% of the gasified PFOS made it completely through the system and was collected on the PUFs. When the combustion chamber temperature was increased to 900°C, the amount of PFOS collected on the PUFs was decreased to about 0.07%.

C_1 or C_2 fluoroalkanes (likely products are either CHF_3, CF_4, or C_2F_6), 1,1-difluoroethene (PFOS only) and 1,2-difluoroethene (FC-1395 only) were the only highly fluorinated compounds observed in the system effluent. Fluorobenzene was also detected (FC-1395 and FC-807A only). No higher molecular weight fluorinated polycyclic aromatic hydrocarbons, and no perfluorooctane sulfonyl precursors of PFOS were present in the system effluent at detectable levels.

The report states that the data from this laboratory-scale incineration study indicates that properly operating full-scale incineration systems can adequately dispose of PFOS and the C_8 perfluorosulfonamides. Incineration of these fluorinated compounds is not likely to be a significant source of PFOS into the environment. With the exception of stable C_1 and C_2 fluorocarbons, fluorinated organic intermediates are unlikely to be emitted during the incineration of PFOS or perfluorooctane sulfonamides.

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

DATA QUALITY

Reliability: Klimisch ranking 2. Purity/Composition of FC-807A and FC-1395 were not properly characterized in the study.

REFERENCES

Study conducted at the request of 3M Company by University of Dayton Research Institute.

OTHER

Last changed: 7/03/03

RS-I-14: STABILITY IN WATER (HYDROLYSIS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS-potassium salt, 1-perfluorooctanesulfonic acid-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptdecafluorosulfonate-potassium salt. CAS: #2795 39-3.

Remarks: 3M production lot number was 171. Test substance is a light-colored powder at 25°C.

METHOD

Method: Based on OPPTS: 835.2110
GLP (Y/N): No, but many GLP procedures followed.
Year completed: 2001
Type: Hydrolysis as a function of pH
Test sample preparation: Test solutions consisted of 1.0 mL buffered aqueous solutions at six pH levels (1.5, 3.0, 5.0, 7.0, 9.0, and 11.0). The resulting PFOS concentration in all test samples (sample triplicates and matrix spike samples) was approximately 500 µg/L. Samples were shielded from light during incubation at 50° for periods of 0 to 49 days. Control samples and blanks addressed potential non-hydrolytic degradation routes.
Analytical Procedures: Samples were analyzed by quantitative HPLC/MS.

Remarks: This study was conducted at 50°C in order to facilitate hydrolysis. Rates derived at 50°C were extrapolated to 25°C by dividing by a factor of 10, which is valid for reactions with Arrhenius heats of activation near 18 kcal/mole.

RESULTS

Breakdown products: Not applicable.
Degradation: The analytical results indicate no degradation of PFOS or dependence on pH. The mean and standard deviation of all observed PFOS concentrations, pooled over the six observed pH levels, indicate that the pseudo-first order hydrolytic half-life of PFOS is greater than 41 years.

CONCLUSIONS

The analytical results indicate no degradation of PFOS or dependence on pH. The study indicates that the hydrolytic half-life of PFOS in water is greater than 41 years.

Submitter: 3M Company, Environmental Laboratory
P.O. Box 3331
St Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking: 2

Remarks: Study was well conducted, though not under GLP.

REFERENCES

This study was conducted at the 3M Environmental Laboratory and Pace Analytical Services, Minneapolis, MN under Lab Request Number W1878. See 3M Environmental Laboratory Report No. W1878 (dated March 27, 2001).

OTHER

Last Changed: June 21, 2001

RS-I-15: STABILITY IN WATER (PHOTOLYSIS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS potassium salt, 1-Perfluorooctanesulfonic acid-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluoro-potassium salt. CAS: #2795 39-3.

Remarks: 3M production lot number 171. Test substance is a light-colored powder at 25°C. Purity determined to be 86.4% by LC/MS, ^{1}H-NMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

References: Based on OPPTS: 835.5270 and OECD Draft Document "Phototransformation of
Chemicals in Water - Direct and Indirect Photolysis", August 2000.
GLP (Y/N): No, but many GLP procedures were employed.
Year completed: 2001
Type: Direct and Indirect Photolysis
Light source: Suntest CPS+ or Suntest XLS+ lamp
Light intensity: 680 w/m^2
Wavelength range: 290-800 nm
Duration: 67-167 hours
Test matrices:
Direct: Water
Indirect: H2O2/water (1:1 molar equivalent)
Fe2O3/water (Fe^{3+} at 24X molar excess)
Fe2O3/water with H2O2
Commercial (Aldrich) humic material prepared as in OPPTS 835.5270
All tests included a series of unexposed controls (kept in the dark) for the evaluation of any degradation reactions occurring without the presence of light.
Temperature: 25 ± 3ºC
Test sample preparation: Aliquots of PFOS were added to three separate sets of VOA screw cap vials (exposed, unexposed, and control vials) containing 5 ml of appropriate matrix. Test vials were placed in the photoreactor. Control vials were wrapped in aluminum foil, sealed in a plastic bag, and placed in the photoreacter.
Analytical Procedures: A UV/Vis spectrum of a saturated aqueous solution of PFOS was recorded between 190 and 1100 nm. Samples were analyzed by quantitative LC/MS and GC/MS techniques.

RESULTS

Degradation and breakdown products: No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. Direct photolytic decomposition

of PFOS was not observed based on loss of starting material, nor were any of the predicted degradation products detected above their limits of quantitation.

Data obtained from the Fe2O3 matrix samples (with and without H2O2) were pooled to provide sufficient statistical data to estimate the minimum half-life. The mean and standard deviation of these data indicate that the minimum environmental half-life of PFOS due to indirect photolysis at 25°C is greater than 3.7 years.

CONCLUSIONS

No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. The mean and standard deviation of the observed PFOS concentrations in an aqueous Fe2O3/H2O2 matrix indicate that the indirect photolytic half-life of PFOS at 25°C is greater than 3.7 years.

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

DATA QUALITY

Reliability: Klimisch ranking: 2

Remarks: Study was well conducted, though not under GLP.

REFERENCES

This study was conducted at the 3M Environmental Laboratory under Lab Request Number W2775. See 3M Environmental Laboratory Report No. W2775 (dated April 23, 2001).

OTHER

Last Changed: January 25, 2002

RS-I-16: BIODEGRADATION

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or $C_8F_{17}SO_3^-K^+$. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: PFOS was a white powder. The original 3M production lot number was not noted. The PFOS was HPLC purified and assigned 3M standard identification # TCR-00017-046. Upon receipt at the testing laboratory, the test article was given a test, control and reference (TCR) number CATCR02-014. An Interim Certificate of Analysis, reports the purity to be 97.9%. All results in this study were calculated assuming 100% purity.

METHOD:

Method: Based on EPA Guidelines OPPTS 835.3200
Test Type: Aerobic
GLP: No
Year Initiated: 2000
Year Completed: 2001
Contact time: 18 days

> **Note:** a previous study[(1)], conducted to evaluate the biodegradability of NEtFOSE alcohol and to determine its metabolites, was conducted for 35 days. This robust summary describes the test conditions for an 18-day study of the NEtFOSE alcohol microbial metabolite, PFOS.

Inoculum: Activated sludge collected 7/31/00 from the aeration basin at the Metro Wastewater Treatment Plant, St Paul, MN. The MLSS was determined to be 2,280 mg/L when first collected. The MLSS was stored at 4ºC for approximately 5 weeks prior to being used for this study. The sludge was allowed to settle and the solids used for inoculum. The settled sludge constituted approximately 20% of the volume (~200 mL) of the MLSS used.
Test medium: Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Methanol (1 mL per liter) was added per liter of mineral medium. Fifty mL of settled sludge was added per liter of mineral salts medium. Mineral medium plus sludge was prepared 9/7/00, while fresh mineral medium without sludge abiotic controls) was prepared 8/10/00.
Study design:

> Blank Sludge Controls (mineral medium, inoculum)
> Abiotic Controls (mineral medium, PFOS)
> Test Substance (mineral medium, inoculum, PFOS)

Test vessels were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

Test concentration: 2.455 mg/L.
Incubation conditions:

Temperature: 25°C +/- 3°C
Agitation: ~200 rpm
Test vessels: Sterile 125 mL Nalgene polycarbonate culture flasks containing 25 mL of media

Dosing procedure: Test vessels were spiked with 6 µL of an 10,230 mg/L solution of PFOS in methanol yielding 2.455 mg/L.
Sampling Frequency: Days 0 and 18.
Analytical method: The day zero test vessels were prepared and immediately placed in a freezer that was maintained at –20°C until analyzed. After 18 days, the test vessels were removed from the incubator and frozen until final sample preparation by solid phase extraction (SPE). Following thawing, test vessel contents were adjusted to 1% acetic acid and then passed through a conditioned SEP-VAC C18 6cc SPE cartridge. Methanol was then added to the emptied culture flask, shaken vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative -ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to PFOS, the additional compounds quantified are specified below. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

Compound Name
Acronym
Chemical Formula

2-(N-Ethyl Perfluorooctane sulfonamido) acetic acid
N-EtFOSAA
$C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$

2-(Perfluorooctane sulfonamido)
acetic acid
M556
$C_8F_{17}SO_2NH(CH_2COOH)$

N-Ethyl Perfluorooctane sulfonamide
N-EtFOSA
$C_8F_{17}SO_2NH(C_2H_5)$

Perfluorooctane sulfonamide
FOSA
$C_8F_{17}SO_2NH_2$

Perfluorooctane sulfinate
PFOSulfinate
$C_8F_{17}SO_2^- K^+$

Perfluorooctanoate, ammonium salt
PFOA
$C_7F_{15}COO^- NH_4^+$

2-(N-ethylperfluorooctanesulfonamido) ethyl alcohol
EtFOSE-OH
$C_8F_{17}SO_2N(CH2CH3)(CH_2CH_2OH)$

Reference substance: None. When results from an EtFOSE Alcohol study conducted at the same time are compared to the previous EtFOSE Alcohol 35-day study, the viability of the microbial inoculum is confirmed.

RESULTS

After 18 days, the analytical results[2] demonstrate that when exposed to municipal wastewater treatment sludge, 2.455 mg/L PFOS was not measurably degraded biotically or abiotically. Mass balance for PFOS test vessels was excellent and ranged from 104 - 108%.

CONCLUSIONS

No loss of PFOS was demonstrated. Mass balance was 104 - 108%. The results from this study confirm the results from other aerobic biodegradation studies of PFOS.

DATA QUALITY

Klimisch ranking: 2. The study was conducted as a non-GLP study but with the understanding that good data quality objectives be met.

Determination of analyte recovery from spiked sample matrices was not deemed necessary as they have been determined twice previously at several different concentrations, and in both instances recoveries were near 100% with some exceptions for M556. Methanol and sample (mineral medium) blanks contained no detected target analytes.

Analyses of the blank sludge controls (mineral media plus inoculum) at days 0 and 18 demonstrated that the inoculum source did not contain endogenous concentrations of test substance or its metabolites.

A calculation error was discovered. The PFOS molar conversion calculation should have used 538 ng/nmole rather than 522 ng/nmole. The corrected value for PFOS on page 13 of the report should be 92.9 nM.

REFERENCES

[1]Biodegradation Study Report, "*The Aerobic Biodegradation of N-EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge"*, Contract Analytical Project ID: CA058, October 30, 2000. LIMS E00-2252
[2]Biodegradation Study Report, "*The 18-Day Aerobic Biodegradation Study of Perfluoroctanesulfonyl-Based Chemistries"*, Contract Analytical Project ID: CA097, February 23, 2001.

Both of the above studies were conducted at the request of the 3M Company by Pace Analytical Services, Inc., Minneapolis, MN.

OTHER

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

Last changed: 6/11/01

RS-I-17: BIODEGRADATION (Acclimated Activated Sludge and Sediment Cultures)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. The 3M production lot number was 217 (TN-A-2130). Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques subsequent to analyses cited in the attached report.

METHOD

Method: Designed by Springborn Laboratories, Inc. Includes 3 elements:
Toxicity evaluation,
Acclimation phase with biodegradability evaluation,
Acclimated inoculum, closed vial biodegradation studies.
Test Type: Aerobic
GLP: No
Year completed: 2000

Toxicity Evaluation Study:
Contact time: 48-hours
Inoculum source: Activated sludge from the Wareham Wastewater Treatment Plant aeration basin, Wareham, Massachusetts.
Inoculum loading in test vessels: 30 mg/L (dry weight)
Test medium: Appropriately diluted OECD 301A media plus 5.0 mg/L [^{14}C]sodium benzoate.
Test concentrations: 20.8 mg/L (nominal)
Study design:
Inoculum control: media, inoculum, [^{14}C] sodium benzoate, water
Test: media, inoculum, [^{14}C] sodium benzoate, PFOS stock soln
Incubation conditions
Temperature: 22°C
Agitation: Shaker table at 150 rpm
Test vessels: Single stoppered 250 mL Erlenmeyer flask containing 100 mL OECD mineral salts media and a small headspace trap containing 2.0 mL 1M KOH for each test and control system.
Dosing procedure: Each test flask received all components at test initiation.
Sampling frequency: 6, 24, and 48-hours
Analytical method: Liquid scintillation counting of KOH trap samples

Acclimation phase with biodegradability evaluation:
Contact time: 16 weeks

Inoculum source: Activated sludge from the Wareham Wastewater Treatment Plant aeration basin, Wareham, Massachusetts, secondary return activated sludge from New Bedford, Massachusetts Wastewater Treatment Plant, one disk from a rotating biological contacter plant at Bridgewater, Massachusetts, sediment from below the Wareham activated sludge plant's outfall, and a sandy soil from Wilson County, North Carolina.
Initial inoculum loading in test vessels: sludge at 1000 mg/L (dry weight) on Day 0, soil at 10,000 mg/L and sediment at 1000 mg/L (dry weight) added on Day 7.
Test medium: Primary influent from the Wareham wastewater treatment plant, OECD 301A media, trace minerals, yeast extract, and activated sludge extract. The bioreactors were acclimated to this media for approximately 27 days prior to dosing.
Test concentrations: Initial nominal dose 20.8 mg/L PFOS, gradual additions to system over 112 days resulted in final measured concentration in system of 28.4 mg/L PFOS.
Study design:

Inoculum control: media, inoculum
Abiotic control: media, PFOS
Test: media, PFOS, inoculum

Incubation conditions

Temperature: 22 $\pm$ 2°C
Agitation: Shaker table, in environmental chamber, at 150 rpm
Test vessels: One 300 mL baffled Erlenmeyer flask containing 100 mL media for the test system. More than one (number not specified) 2 liter Erlenmeyer flasks containing 1000 mL each for the inoculum and abiotic controls. The inoculum and abiotic controls set up after 21-days into the study.

Dosing procedure: Weekly removal and replacement of 70% of flask contents. Removal accomplished by first pulling out 10 mL of total flask contents (biomass and media) and then taking 60 mL of supernatant resulting from centrifugation of remaining volume (90 mL) of flask contents. This was replaced with an equal volume (70 mL) of fresh medium, including freshly collected sewage influent, and inoculum, and adding 1.4 mL of the 1060 mg/L PFOS stock solution. The inoculum control flasks did not receive the PFOS stock solution.
Sampling frequency: Weekly; samples analyzed only on days 7, 14, 21, 28, 35, and 112.
Analytical method: LC/MS analyses of biomass and media for PFOS. TOC via carbon analyzer obtained weekly on supernatants after 1.0 μm filtration.

<u>Acclimated inoculum, closed vial biodegradation studies</u>
Contact time: 61 and 63 days
Inoculum source: Acclimated sludge from the acclimation phase above (after 12 weeks of acclimation)
Initial inoculum loading in test vessels: 200 mg/L (dry weight)
Test medium: OECD 301A medium plus yeast extract and resazurin
Test concentrations: 0.212 and 105 mg/L (nominal), set up on two different days
Study design:

Blank control: media only
Inoculum control: media, inoculum

Abiotic control: media, PFOS
Test: media, PFOS, inoculum

Incubation conditions

Temperature: Not noted
Agitation: Not noted
Test vessels:

0.212 mg/L exposure: 22 mL serum vials, containing 15 mL test solution. 12 set for blank controls, 12 for inoculum controls, 20 for abiotic controls, and 20 for the test.
105 mg/L exposure**:** 22 mL serum vials, containing 15 mL test solution. 16 set for inoculum control, 16 for abiotic control, 16 for the test.

Dosing procedure: Each test flask received all components at test initiation.
Sampling frequency:

0.20 mg/L exposure: Days 0, 16, 30, 61
105 mg/L exposure: Days 0, 15, 30, 63

Analytical method: LC/MS for PFOS, both exposure levels. Headspace analyses of CO_2 via carbon analyzer on half of the samples collected for the 105 mg/L exposure.
Remarks: Stock solutions used to dose the toxicity and biodegradation test systems were prepared at concentrations of 1.060 and 1.010 mg/L. These concentrations are approximately twice the water solubility of PFOS

RESULTS

Toxicity Evaluation Study:
Evolution of $^{14}CO_2$ from the control flask (46,700 DPM at 48-hours) was not substantially different from that of the flask with the presence of PFOS (47,000 DPM at 48-hours). Concludes that 20.8 mg/L will not inhibit microbes.

Acclimation phase with biodegradability evaluation
No clear evidence was seen of biodegradation of PFOS in this study. Two possible confounding factors, which might have affected the mass balance, were postulated:

1. PFOS was found to be initially distributed primarily in the medium, and as time went by, was found primarily associated with the biomass (cells).

2. A white precipitate was observed in the flasks after 2 weeks; appears that PFOS concentrations were exceeding water solubility and precipitating out of solution.

Acclimated inoculum, closed vial biodegradation studies
No clear evidence was seen of biodegradation of PFOS in this study. Concentrations of PFOS remained relatively constant during the course of the 0.20 mg/L exposure study. Approximately a 20% decline in PFOS concentration was noted in the 105 mg/L exposure study. However, the loss mechanism could not be clearly differentiated between matrix effects and biodegradation. Analyses of CO_2 in the headspace in the 105 mg/L exposure study found very little difference in CO_2 production between the blank and PFOS-containing vessels.

CONCLUSIONS

The overall conclusion of these studies is that PFOS is very recalcitrant in the activated sludge/sediment system.

DATA QUALITY

Reliability: Klimisch ranking = 3. The stock solution used to dose the systems was prepared at twice the water solubility. There were no initial measured concentrations taken in the acclimation phase study. As a result, it is unclear exactly what the bioavailable and/or soluble concentration of PFOS was in this study. This also affects the concentration of PFOS that was transferred to the test flasks for the acclimated biodegradation study. Without an accurately determined starting concentration, mass balance calculations are not feasible. In addition, the acclimated inoculum closed vial biodegradation study did not include details on sample incubation conditions.

REFERENCES

This study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts at the request of the 3M Company. Report completed 11/3/00. Lab Project number E01-0434.

OTHER

Last changed: 6/12/01

RS-I-18: BIODEGRADATION (Aerobic Soil and Sediment Cultures)

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 3M production lot number was 217 (TN-A-2130). The test substance is a white powder. Sample was stored AT 16-20^{o}C prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques subsequent to analyses cited in the attached report.

METHOD

Method: Designed by Springborn Laboratories, Inc.
Test Type: Aerobic
GLP: No
Year completed: 2000
Contact time: 20-weeks
Inoculum sources: Soils collected from a hardwood forest in Hanson, MA, a pine forest on Onset, MA and a river bank in Bridgewater, MA. Sediments collected from brackish sites below the Wareham, MA wastewater treatment plant outfall and from the Narrows area in Wareham, MA. Biomass was determined on day 83 by both the fumigation/extraction and standard plate count methods. Biomass was reported as 17.4 mg C/100 g soil and 6 x 10^{5} cells/g, respectively.
Test medium: Soil and sediment samples were air dried, 2.00 mm sieved, and mixed together in equal dry weight portions. A nutrient mixture was prepared by combining a sterile potting soil extract, a trace mineral solution, a yeast extract and reagent water. The soil/sediment mixture was adjusted to 75% of the water holding capacity using the above nutrient mixture. Soil moisture was monitored weekly during the study, and adjusted using reagent water as needed.
Test concentration: ~21.2 mg/kg (nominal)
Study design:

Inoculum control: soil/sediment mixture, nutrient mixture, methanol
Test: soil/sediment mixture, nutrient mixture, PFOS stock solution

Incubation conditions

Temperature: 22 $\pm$ 3°C
Lighting: Conducted in the dark
Agitation: Not noted
Test vessels: 40 mL I-Chem glass vials with silicone/Teflon®-lined septum screw caps containing 10 g (dry weight) soil/sediment mixture.

Dosing procedure: Each test flask received all components at test initiation.
Sampling frequency: Days 7,14, 21, 28, 35, 42, 49, 56, 63

Analytical method: Entire sample extracted with methanol via accelerated solvent extraction. Extracts passed through a 0.2 μm nylon filter prior to analysis. Samples diluted as necessary in methanol, and analyzed via LC/MS.

Remarks: Stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L. This concentration is approximately twice the water solubility of PFOS

RESULTS

Results of PFOS analyses indicated that essentially no PFOS metabolism occurred during the study.

CONCLUSIONS

PFOS is recalcitrant in the soil/sediment system.

DATA QUALITY

Reliability: Klimisch ranking = 2. Study not conducted following Good Laboratory Practice Standards. The stock solution used to dose the systems was prepared at twice the water solubility. No Day 0 samples were taken to determine starting concentrations.

REFERENCES

This study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

OTHER

Last changed: 5/26/01

RS-I-19: BIODEGRADATION (Anaerobic Sludge)

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 3M production lot number was 217 (TN-A-2130). The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques subsequent to analyses cited in the attached report.

METHOD

Method: Designed by Springborn Laboratories, Inc.
Test Type: Anaerobic
GLP: No
Year completed: 2000
Contact time: 56-days
Inoculum source: Anaerobic digestor at the Rockland, MD wastewater treatment plant.
Inoculum loading in test vessels: 300 mL anaerobic sludge per liter medium
Test medium: Mixture containing dried sludge extract (from rotating biological contacter wastewater treatment, Bridgewater, MA), unspecified OECD mineral media, and resazurin indicator. Media prepared under nitrogen purge to exclude oxygen.
Test concentration: ~20.8 mg/L (nominal)
Study design:
Inoculum control: inoculum, nutrient media
Test: inoculum, nutrient media, PFOS stock solution
Incubation conditions
Temperature: 35°C
Lighting: Conducted in the dark
Agitation: Not noted
Test vessels: Twenty 160 mL serum bottles, containing 100 mL test solution, (purged with nitrogen after filling), with crimped butyl rubber tops.
Dosing procedure: Each test flask received all components at test initiation.
Sampling frequency: Days 7,14, 21, 28, 35, 42, 49, 56 – test bottle
Days 7, 56 – inoculum control bottles
Analytical method: Aliquot removed from each bottle and centrifuged. Both the supernatant and the solid biomass portion of each aliquot were then analyzed via LC/MS.

Remarks: Stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L. This concentration is approximately twice the water solubility of PFOS

RESULTS

Results of PFOS analyses indicated no apparent PFOS biodegradation over the 56-day period.

CONCLUSIONS

PFOS is recalcitrant in the anaerobic system.

DATA QUALITY

Reliability: Klimisch ranking = 2. Study not conducted following Good Laboratory Practice Standards. The stock solution used to dose the systems was prepared at twice the water solubility. No Day 0 samples were taken to determine starting concentrations.

REFERENCES

This study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

OTHER

Last changed: 6/12/01

RS-I-20: BIODEGRADATION (Pure Microbial Cultures)

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 3M production lot number was 217 (TN-A-2130). The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques subsequent to analyses cited in the attached report.

METHOD

Method: Springborn Laboratories, Inc./Betts *et al*, 1974, two study types:
Pure Culture
Closed Vial Headspace
Test Type: Aerobic
GLP: No
Year completed: 2000

Pure Culture Studies
Contact time: 7-days
Inoculum: 4 separate pure cultures tested:
Cunninghamella echinulata var. echinulata (fungi, ATCC #9244)
Mucor circinelloides f. griseocyanus (fungi, ATCC #1207a)
Phanerochaete chrysosporium (fungi, ATCC #24725)
Streptomyces griseus (actinomycete, ATCC #13273)
Source: American Type Culture Collection (ATCC)
Inoculum loading in test vessels: 6 mL of Stage II cultures into 60 mL media, allowed 24-hr of agitation prior to PFOS addition
Test medium: soybean grits-glucose (SGG)
Test concentrations: ~20.9 mg/L (nominal)
Study design:
Inoculum control: media, inoculum
Test: media, inoculum, PFOS stock solution
Incubation conditions
Temperature: 26°C
Agitation: Shaker table at 250 rpm
Test vessels: Not noted
Dosing procedure: Each test flask received all necessary components at test initiation. All work utilized strict aseptic technique until harvest at Day 7.
Sampling frequency: Day 0 and 7
Analytical method: Separation of broth and cells via centrifugation. Analysis of both by LC/MS

Closed Vial Headspace Study:
Contact time: 3-days
Inoculum: *Phanerochaete chrysosporium* (fungi)
Inoculum source: American Type Culture Collection (ATCC)
Initial inoculum loading in test vessels: not noted
Test medium: soybean grits-glucose (SGG) medium at 1/10 and 1/100 strength plus resazurin
Test concentration: 0.2 mg/L.
Study design:
Inoculum control: media, inoculum
Abiotic control: media, PFOS (not sterile)
Test: media, PFOS, inoculum
Incubation conditions
Temperature: 26°C
Agitation: Shaker table, in environmental chamber, at 250 rpm
Test vessels: 22 mL sterile vials
Dosing procedure: Each test flask received all necessary components at test Initiation. Headspace purged with oxygen, vial immediately crimped.
Sampling frequency: Once – Day 3
Analytical method: Separation of broth and cells via centrifugation. Analysis of both by LC/MS

Remarks: Stock solutions used to dose the biodegradation test systems were prepared at a concentration of 1,060 mg/L (Pure Culture Study) and 1,011 mg/L (Closed Vial Study). These concentrations are approximately twice the water solubility of PFOS,

RESULTS

The studies with *Cunninghamella, Mucor,* and *Streptomyces* did not provide any indication of biotransformation of PFOS.

Preliminary analytical results of the first *Phanaerochaete* study indicated possible biotransformation of PFOS (90% mass balance). Therefore, closed vial studies were set up to confirm. Difficulties were encountered in maintaining aerobicity, and only 3 days of exposure were maintained.

The results from the closed vial study indicated no significant biotransformation of PFOS by *Phanaerochaete* fungi.

CONCLUSIONS

It does not appear that the four species studied are capable of metabolizing PFOS.

DATA QUALITY

Reliability: Klimisch ranking = 2. The stock solution used to dose the systems was prepared at twice the water solubility. There were no initial measured concentrations taken in the closed vial study. These studies were not conducted in accordance with the principles of Good Laboratory Practices.

REFERENCES

This study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts at the request of the 3M Company. Report completed 11/3/00. Lab Project number E01-0434.

OTHER

Last changed: 6/12/01

APPENDIX II

SUMMARY FOR AQUATIC TOXICOLOGY STUDIES (PNECs)

APPENDIX II

SUMMARY FOR AQUATIC TOXICOLOGY STUDIES (PNECs)

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RS-II-1: ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

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, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OECD 209
Test type: Static acute
GLP: Yes
Year Completed: Study completed 1999. Report completed 2000.
Analytical monitoring: Dissolved oxygen concentrations.
Statistical methods: Probit analysis using the computer software of C.E. Stephan.
Test organism source: Activated sludge collected from the Prospect Bay Wastewater Treatment Facility, Grasonville, Maryland.
Test conditions:

Dilution water: NANOpure®
Synthetic Sewage:

1 liter municipal water
16.0 g peptone
11.0 g meat extract
3.0 g urea
0.7 g NaCl
0.4 g $CaCl_2$ $2H_2O$
0.2 g $MgSO_4$ $7H_2O$
2.8 g K_2HPO_4

Reference and test solution preparation: A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL of 1N NaOH, diluted to 30 mL with NANOpure® water, then brought to the point of incipient precipitation with 1N H_2SO_4, and diluted to 1 L with NANOpure® water. The pH of the reference solution was measured to be 7.18.
PFOS was added directly to test vessels rather than volumetric addition of a stock solution. This method was deemed appropriate based on the observed solubility of the test substance in water.
Test vessels: Mixtures were prepared and aerated in 500 mL Erlenmeyer flasks and then transferred into 300 mL Biochemical Oxygen Demand (BOD) bottles
Number of concentrations: 7 plus 3 reference controls and 2 Blank controls
Temperature: 19-21°C

Total Suspended Solids and pH for sludge on day of testing: 4380 mg/L and 7.87 respectively.
Element Basis: Respiration inhibition as determined by dissolved oxygen concentration.

Method Remarks: Stock solutions of PFOS that were prepared at nominal concentration of approximately 500 and 1000 mg/L in NANOpure® water contained test material that was not in solution after 20-minutes of sonication. Therefore, direct weight addition was employed to administer PFOS to the test system.

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

RESULTS

Nominal concentrations: Two blank controls, three reference substance controls, 0.90, 2.7, 9.0, 27, 90, 271, 905 mg/L test material solutions
Statistical Analyses: EC_{50} values were calculated for the reference material by probit analysis using the computer software of C.E. Stephan. An EC_{50} value could not be calculated for the test substance.
Analytical Methodology: Analysis of DO concentrations in all test solutions were performed at Wildlife International Ltd. using a YSI Model 50B Dissolved Oxygen Meter. Dissolved oxygen readings were recorded every 10 seconds for 10 minutes or until the dissolved oxygen dropped below 1.0 mg/L

Respiration Rates and Percent Inhibitions:

Treatment	Respiration Rate mg O_2/L/hour	Percent Inhibition
Control 1	39.6	NA
Control 2	41.1	NA
3,5-dichlorophenol 3 mg/L	31.1	22.9
3,5-dichlorophenol 15 mg/L	14.6	63.8
3,5-dichlorophenol 50 mg/L	5.1	87.4
Test substance 0.90 mg/L	38.9	3.6

Treatment	Respiration Rate mg O_2/L/hour	Percent Inhibition
Test substance 2.7 mg/L	35.1	13.0
Test substance 9.0 mg/L	33.5	17.0
Test substance 27 mg/L	37.9	6.1
Test substance 90 mg/L	32.7	19.0
Test substance 271 mg/L	28.1	30.4
Test substance 905 mg/L	24.7	38.8

Control response: satisfactory

CONCLUSIONS

The test substance exhibited a maximum inhibitory effect of 39% upon respiration at a nominal test substance concentration of 905 mg/L. The EC_{50} (respiration inhibition) is therefore greater than the solubility of the test substance.

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

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

RS-II-2: TOXICITY TO AQUATIC PLANTS (FRESHWATER ALGAE, *SELENASTRUM CAPRICORNUTUM*)

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, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OECD 201, OPPTS 850.5400, ASTM 1218-90E
Test: Static acute
GLP: Yes
Year completed: Study completed 1999. Report completed 2000
Species: *Selenastrum capricornutum*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: PFOS measured at 0, 72, 96-hours
Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate
Exposure period: 96-hours
Start date: 4/12/99
End date: 4/16/99
Analytical monitoring: Test concentrations measured at 0, 72, and 96-hours.
Test organisms laboratory culture: Algae cultures had been actively growing in freshwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Test temperature range: 23.6 – 25.8°C
Growth medium: ASTM Standard Guide 1218-90E, 1990

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_20$	12.16	mg/L
$CaCl_2 \cdot 2H_20$	.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H20$	0.416	mg/L
$ZnCl_2$	3.28	ug/L
$FeCl_2 \cdot 6H_20$	0.1598	mg/L
$CoCl_2 \cdot 6H_20$	1.428	ug/L
$Na_2Mo0_4 \cdot 2H_20$	7.26	ug/L
$CuCl_2 \cdot 2H_20$	0.012	ug/L
$Na_2EDTA \cdot 2H_20$	0.300	mg/L
$NaNO_3$	25.50	mg/L
$MgSO_4 \cdot 7H_20$	14.70	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L

Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 $\pm$ 0.1 using 10% HCl and the medium was sterilized by filtration (0.22 m) prior to use.
Stock and test solution preparation: A primary stock solution was prepared in algal medium at a concentration of 183 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with algal medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.
Exposure vessels: Sterile 250 mL polycarbonate Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.
Agitation: Shaken continuously at 100 rpm
Number of replicates: three
Initial algal cell loading: 1.0×10^4 cells/mL

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

Water chemistry:

pH range (0 – 96 hours)

7.5 – 8.1 (control exposure)

7.4 – 7.5 (179 mg/L exposure)

Test temperature range (0 – 96 hours)

23.6 – 25.8°C

Light levels: (0 – 96 hours)

3870 – 4610 lux from continuous cool-white fluorescent lighting

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

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

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

Element value:

24-hour EC_{50} (cell density) = 163 (74-191) mg/L
24-hour E_bC_{50} (area under curve) = 122 (19-176) mg/L
24-hour E_rC_{50} (growth rate) = 136 (30 - 204) mg/L
48-hour EC_{50} (cell density) = 81 (72 – 90) mg/L
48-hour E_bC_{50} (area under curve) = 84 (67 – 146) mg/L
48-hour E_rC_{50} (growth rate) = 142 (107 - 185) mg/L
72-hour EC_{10} (cell density) = 37 (<0 – 64) mg/L
72-hour E_bC_{10} (area under curve) = 46 (<0 – 56) mg/L
72-hour E_rC_{10} (growth rate) = 53 (23 – 64) mg/L
72-hour EC_{50} (cell density) = 70 (44 – 78) mg/L
72-hour E_bC_{50} (area under curve) = 74 (55 – 82) mg/L
72-hour E_rC_{50} (growth rate) = 120 (103 – 132) mg/L
72-hour EC_{90} (cell density) = 153 (130 – 165) mg/L
72-hour E_bC_{90} (area under curve) = 165 (145 – 176) mg/L
72-hour E_rC_{90} (growth rate) = >179 mg/L (C.I. not calculable)
96-hour EC_{10} (cell density) = 49 (43 – 50) mg/L
96-hour E_bC_{10} (area under curve) = 49 (40 – 50) mg/L
96-hour E_rC_{10} (growth rate) = 59 (54 – 63) mg/L
96-hour EC_{50} (cell density) = 71 (66 – 73) mg/L
96-hour E_bC_{50} (area under curve) = 71 (67 – 74) mg/L
96-hour E_rC_{50} (growth rate) = 126 (115 – 138) mg/L
96-hour EC_{90} (cell density) = 137 (105 – 153) mg/L
96-hour E_bC_{90} (area under curve) = 145 (125 – 155) mg/L
96-hour E_rC_{90} (growth rate) = >179 mg/L (C.I. not calculable)
72-hour NOEC (growth rate, cell density, area under the curve): 44 mg/L
96-hour NOEC (growth rate, cell density, area under the curve): 44 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits at 72 and 96. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilks's test and Bartlett's test, respectively. The treatment groups were then compared to the control using Dunnett's test. Results of the statistical analyses were used to determine the NOEC values.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.1. Samples collected at test initiation had measured values from 82.0 to 98.1% of nominal. Measured values for samples taken at 72-hours ranged from 91.7 to 105% of nominal. Measured values for samples taken at 96-hours ranged from 90.3 to 102% of nominal. For the abiotic controls, measured values for samples taken at 72-hours ranged from 81.9 – 105% of nominal and for samples taken at 96-hours, 90.3 – 103% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
5.7	4.73, 6.04, 5.84	5.5	96
11	10.7, 11.2, 12.1	11	100
23	19.8, 23.1, 20.7	21	91
46	42.7, 41.9, 46.3	44	96
91	83.3, 86.0, 88.3	86	95
183	179, 186, 172	179	98
183 (abiotic)	not analyzed, 150, 188	169	92

Control response: satisfactory

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	2,740,000	-	-	-
5.5	3,040,000	-11	-8.5	-1.9
11	2,880,000	-5.1	-3.3	-0.84
21	3,240,000	-18	-13	-3.0
44	3,080,000	-12	-5.3	-2.0
86	626,667	77	75	27
179	33,667	99	98	79

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

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

CONCLUSIONS

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

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

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

RS-II-3: TOXICITY TO AQUATIC PLANTS (FRESHWATER ALGAE, *ANABAENA FLOS-AQUAE*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400
Test: Acute static
GLP: Yes
Year completed: 2001
Species: *Anabaena flos-aquae*
Source: Originally from UTEX – The Culture Collection of Algae at the University of Texas at Austin, and maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: PFOS measured at 0, 72, 96-hours
Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate
Exposure period: 96-hours
Start date: 1/28/00
End date: 6/5/00

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

Test Conditions:

Freshwater Algal medium:

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_2O$	12.16	mg/L
$CaCl_2 \cdot 2H_2O$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H_2O$	0.416	mg/L
$ZnCl_2$	3.28	µg/L
$FeCl_3 \cdot 6H_20$	0.1598	mg/L
$CoCl_2 \cdot 6H_20$	1.428	µg/L
$Na_2MoO_4 \cdot 2H_20$	7.26	µg/L
$CuCl_2 \cdot 2H_2O$	0.012	µg/L
$Na_2EDTA \cdot 2H_20$	0.300	mg/L
$NaNO_3$	25.5	mg/L
$MgSO_4 \cdot 7H_2O$	14.7	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L

Dilution water source: The pH of the medium was adjusted to 7.5 $\pm$ 0.1 and it was sterilized by filtration (0.22µm) prior to use.
Test solution preparations: Individual test solutions were prepared in algal medium at each of the six nominal concentrations. The solutions were stirred with magnetic stir plates for approximately 18 hours. The final test solutions appeared clear and colorless.
Exposure vessels: Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.
Agitation: Shaken continuously at 100 rpm
Number of replicates: six.
Initial algal cell loading: 1.0×10^4 cells/mL
Cell counts method: hemacytometer and microscope

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

Water chemistry:

pH range (0 – 96 hours)

7.4 – 7.6 (control exposure)

7.4 – 7.4 (329 mg/L exposure)

Test temperature range (0 – 96 hours)

22.8 – 23.8ºC

Light levels: (0 – 96 hours)

1990 – 2310 lux from cool-white fluorescent lighting

Photoperiod: 24-hours light

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 37.9, 58.6, 88.8, 139, 216, 331 mg/L plus 331 abiotic replicate

Measured concentrations: <LOQ, 37.9, 63.9, 93.8, 143, 235, 329 mg/L; abiotic replicate = 349 mg/L

Element values (95% confidence interval):

24-hour EC_{50} (cell density) = 105 mg/L (C.I. not calculable)
24-hour E_bC_{50} (area under curve) = 90 (40 - 150) mg/L
24-hour E_rC_{50} (growth rate) = 94 (33 - 145) mg/L
48-hour EC_{50} (cell density) = 117 mg/L (C.I. not calculable)
48-hour E_bC_{50} (area under curve) = 103 mg/L (C.I. not calculable)
48-hour E_rC_{50} (growth rate) = 128 mg/L (C.I. not calculable)
72-hour EC_{10} (cell density) = 43 (34 – 84) mg/L
72-hour E_bC_{10} (area under curve) = <38 mg/L (C.I. not calculable)
72-hour E_rC_{10} (growth rate) = 82 (49 - 116) mg/L
72-hour EC_{50} (cell density) = 120 (92 - 139) mg/L
72-hour E_bC_{50} (area under curve) = 116 (49 - 142) mg/L
72-hour E_rC_{50} (growth rate) = 174 (146 - 208) mg/L
72-hour EC_{90} (cell density) = 224 (193 - 275) mg/L
72-hour E_bC_{90} (area under curve) = 204 (134 - 226) mg/L
72-hour E_rC_{90} (growth rate) = 275 (162 - 330) mg/L
96-hour EC_{10} (cell density) = 82 (29 - 123) mg/L
96-hour E_bC_{10} (area under curve) = 56 (26 - 107) mg/L
96-hour E_rC_{10} (growth rate) = 109 (84 - 125) mg/L
96-hour EC_{50} (cell density) = 131 (106 - 142) mg/L
96-hour E_bC_{50} (area under curve) = 124 (104 - 138) mg/L
96-hour E_rC_{50} (growth rate) = 176 (169 - 181) mg/L
96-hour EC_{90} (cell density) = 213 (203 - 219) mg/L

96-hour E_bC_{90} (area under curve) = 209 (197 - 218) mg/L
96-hour E_rC_{90} (growth rate) = 225 (220 - 235) mg/L
72-hour NOAEC (cell density, area under curve): 37.9 mg/L
72-hour NOAEC (growth rate): 93.8 mg/L
96-hour NOAEC (cell density, growth rate): 93.8 mg/L
96-hour NOAEC (area under curve): 63.9 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. The EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits were calculated by linear interpolation with treatment response and exposure concentration data using TOXSTAT Version 3.5. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality and homogeneity of variances using the Shapiro-Wilk's test and Levene's test, respectively. Where the data were normally distributed with equal variances, the treatment groups were compared to the control using Dunnett's test. In the one instance where data were not normally distributed, the non-parametric Kruskal-Wallis test was used. Results of the statistical analyses were used to determine the NOAEC values.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.80 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 103%. Samples collected at test initiation had measured values from 100 to 112% of nominal. The measured values for the samples taken at 72-hours were 99.0 - 110% of nominal. The measured values for the samples taken at 96-hours were 99.0 - 109% of nominal. For the abiotic replicate, the measured value for the sample taken at 72-hours was 103% of nominal and for the sample taken at 96-hours, 107% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
37.9	37.9, 38.2, 37.6	37.9	100
58.6	65.6, 62.7, 63.4	63.9	109
88.8	94.3, 97.4, 89.8	93.8	106
139	142, 146, 142	143	103

216	230, 238, 236	235	109
331	331, 328, 329	329	99.4
331 (abiotic)	Not analyzed, 342, 356	349	105

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	569,167	-	-	-
37.9	605,000	-6.3	3.0	-1.3
63.9	585,833	-2.9	13	-0.97
93.8	492,500	13	24*	3.6
143	228,833	60*	66*	22*
235	2,333	100*	99*	100*
329	8,500	99*	100*	96*

*Indicates a significant difference from the negative control using the appropriate statistical test (p <0.05)

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any treatment group. In addition, there were no noticeable changes in cell morphology when compared to the negative control.

Reversibility of Growth Inhibition: Aliquots of the 235 and 329 mg/L test solutions were diluted with algal medium and cultured for nine days after the exposure phase of the study concluded. Based on the increase in growth observed by Day 9 of the recovery phase, the effect on algal growth was algistatic at a concentration of 235 mg/L. However, no algal cells were detected during the recovery phase in the 329 mg/L treatment, indicating that PFOS was algicidal at that concentration.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC50 and 95% confidence interval for *Anabaena flos-aquae* was determined using three calculation methods. By cell density, it was 131 (106 – 142) mg/L, by area under the growth curve it was 124 (104 – 138) mg/L and by growth rate 176 (169 – 181) mg/L. The 96-hour NOAEC values were determined to be 63.9 mg/L using the area under the growth curve, and 93.8 mg/L with the cell density and growth rate calculation method. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. PFOS was determined to be algistatic at a concentration of 235 mg/L and algicidal at 329 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/12/01

RS-II-4: TOXICITY TO AQUATIC PLANTS (FRESHWATER DIATOM, *NAVICULA PELLICULOSA*)

TEST SUBSTANCE

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

Remarks: Sample obtained from 3M production lot number 217. The test substance is a white powder. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400
Test: Acute static
GLP: Yes
Year completed: 2001
Species: *Navicula pelliculosa*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: PFOS measured at 0, 72, 96-hours
Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate
Exposure period: 96-hours
Start date: 2/25/00
End date: 2/29/00
Test organisms laboratory culture: Algae cultures had been actively growing in freshwater algal culture medium with silica and selenium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Growth medium

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_20$	12.16	mg/L
$CaCl_2 \cdot 2H_20$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H_20$	0.416	mg/L
$ZnCl_2$	3.28	ug/L
$FeCl_3 \cdot 6H_20$	0.1598	mg/L
$CoCl_2 \cdot 6H_20$	1.428	ug/L
$Na_2Mo0_4 \cdot 2H_20$	7.26	ug/L
$CuCl_2 \cdot 2H_20$	0.012	ug/L
$Na_2EDTA \cdot 2H_20$	0.300	mg/L
$NaNO_3$	25.50	mg/L
$MgSO_4 \cdot 7H_20$	14.70	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L
$Na_2SiO_3 \cdot 9H_20$	20.0	mg/L
$Na_2SeO_3 \cdot 5H_20$	0.010	mg/L

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

Test solution preparation: A primary stock solution was not prepared for this study. Individual test solutions were prepared in algal medium at each of the seven nominal concentrations. The individual test solutions were stirred with a magnetic stir plate for approximately 24 hours. All final test solutions appeared clear and colorless.

Exposure vessels: Sterile 250 mL plastic Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.
Agitation: Shaken continuously at ~100 rpm
Number of replicates: three.
Initial algal cell loading: 1.0 X 10^4 cells/mL
Number of concentrations: seven plus a negative control plus an abiotic control at the highest concentration tested
Water chemistry:
pH range (0 – 96 hours)
7.5 – 8.6 (control exposure)
7.5 – 7.7 (335 mg/L exposure)
Test temperature range (0 – 96 hours)
23.1 – 24.6ºC
Light levels: (0 – 96 hours)
3910 – 4510 lux from continuous cool-white fluorescent lighting
Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 61.5, 81.3, 110, 147, 198, 264, 347 mg/L plus 347 mg/L abiotic control.

Measured concentrations: <LOQ, 62.3, 83.2, 111, 150, 206, 266, 335 mg/L; abiotic control = 339 mg/L

Element value (95% confidence interval):
24-hour EC_{50} (cell density) = 281 (214 - 312) mg/L
24-hour E_bC_{50} (area under curve) = 262 (205 - 308) mg/L
24-hour E_rC_{50} (growth rate) = 279 (212 - 306) mg/L
48-hour EC_{50} (cell density) = 261 (219 - 306) mg/L
48-hour E_bC_{50} (area under curve) = 259 (227 - 303) mg/L
48-hour E_rC_{50} (growth rate) = 294 (271 - 307) mg/L
72-hour EC_{10} (cell density) = <62.3 (C.I. not calculable) mg/L
72-hour E_bC_{10} (area under curve) = <62.3 (C.I. not calculable) mg/L
72-hour E_rC_{10} (growth rate) = 221 (190 - 252) mg/L
72-hour EC_{50} (cell density) = 242 (200 - 276) mg/L
72-hour E_bC_{50} (area under curve) = 246 (210 - 277) mg/L
72-hour E_rC_{50} (growth rate) = 295 (288 - 305) mg/L
72-hour EC_{90} (cell density) = 317 (306 - 326) mg/L
72-hour E_bC_{90} (area under curve) = 318 (307 - 325) mg/L
72-hour E_rC_{90} (growth rate) = 335 (323 - 335) mg/L
96-hour EC_{10} (cell density) = <62.3 (C.I. not calculable) mg/L
96-hour E_bC_{10} (area under curve) = <62.3 (C.I. not calculable) mg/L
96-hour E_rC_{10} (growth rate) = 243 (209 - 295) mg/L
96-hour EC_{50} (cell density) = 263 (217 - 299) mg/L

96-hour E_bC_{50} (area under curve) = 252 (220 - 285) mg/L
96-hour E_rC_{50} (growth rate) = 305 (295 - 316) mg/L
96-hour EC_{90} (cell density) = 322 (310 - 328) mg/L
96-hour E_bC_{90} (area under curve) = 319 (308 - 326) mg/L
96-hour E_rC_{90} (growth rate) = >335 mg/L (C.I. not calculable)
72-hour NOAEC (cell density, area under the curve): <62.3 mg/L
72-hour NOAEC (growth rate): 206 mg/L
96-hour NOAEC (cell density): 150 mg/L
96-hour NOAEC (area under the curve): <62.3 mg/L
96-hour NOAEC (growth rate): 206 mg/L
All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilkes's test and Levene's test, respectively. The treatment groups were then compared to the control using Dunnett's test. Results of the statistical analyses were used to determine the NOAEC values.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 106% of nominal. Measured values for samples taken at 72-hours ranged from 98.5 to 106% of nominal. Measured values for samples taken at 96-hours ranged from 94.8 to 101% of nominal. For the abiotic controls, the measured value for the sample taken at 72-hours was 98.2% of nominal and for the sample taken at 96-hours, 96.8% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
61.5	62.3, 63.6, 61.1	62.3	101
81.3	83.8, 84.7, 81.0	83.2	102
110	109, 113, 110	111	101
147	147, 154, 149	150	102
198	209, 209, 199	206	104
264	271, 268, 258	266	101
347	334, 342, 329	335	96.5
347 (abiotic)	Not analyzed, 341, 336	339	97.7

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	2,726,667	-	-	-
62.3	2,366,667	13	24*	2.5
83.2	2,366,667	13	22*	2.7
111	2,373,333	13	24*	2.5
150	2,473,333	9.3	16	1.7
206	2,093,333	23*	24*	4.7
266	1,330,000	51*	58*	13*
335	35,333	99*	99*	79*

*Indicates a significant difference from the negative control using Dunnett's test ($p \leq 0.05$)

Control response: satisfactory
Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative control, although at 72 and 96 hours of exposure a few cells in the 335 mg/L treatment group appeared small in comparison to the control.
Reversibility of Growth Inhibition: The 335 mg/L treatment group was maximally inhibited after 96-hours. The treatment group was diluted to a concentration of the test substance that would not inhibit growth and exposed for 7 days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC_{50} and 95% confidence interval for *Navicula pelliculosa* was determined using three calculation methods. By cell density, it was 263 (217 - 299) mg/L, by area under the growth curve it was 252 (220 - 285) mg/L and by growth rate 305 (295 - 316) mg/L. The 96-hour NOAEC was determined by Dunnett's procedure ($p \leq 0.05$) to be 150 mg/L using cell density, <62.3 mg/L when using area under the curve and 206 mg/L by growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/19/01

RS-II-5: TOXICITY TO AQUATIC PLANTS (MARINE DIATOM, *SKELETONEMA COSTATUM*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400
Test: Acute static
GLP: Yes
Year completed: 2001
Species: *Skeletonema costatum*
Source: Originally from The Culture Collection of Algae and Protozoa, Dunstaffnage marine Laboratory at Oban Argyll, Scotland, and maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: PFOS measured at 0, 72, 96-hours
Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate
Exposure period: 96-hours
Start date: 5/19/00
End date: 5/23/00
Analytical monitoring: Test concentrations measured at 0, 72, and 96-hours.
Test organisms laboratory culture: Algae cultures had been actively growing in saltwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water. Solutions were then diluted in artificial saltwater to prepare final growth media.

Test Conditions:

Algal saltwater medium:

Compound	Nominal Concentration	Units
$FeCl_3 \cdot 6H_20$	0.72	mg/L
$MnCl_2 \cdot 4H_2O$	2.16	mg/L
$ZnSO_4 \cdot 7H_2O$	0.675	mg/L
$CuSO_4 \cdot 5H_2O$	2.36	µg/L
$CoCl_2 \cdot 6H_20$	6.06	µg/L
H_3BO_3	17.1	mg/L
$Na_2EDTA \cdot 2H_20$	15.0	mg/L
K_3PO_4	3.0	mg/L
$NaNO_3$	50.0	mg/L
$Na_2SiO_3 \cdot 9H_20$	20.0	mg/L
Thiamine Hydrochloride	0.25	mg/L
Biotin	0.05	µg/L
B_{12}	0.5	µg/L

Dilution water source: The stock nutrient solutions were prepared by adding the appropriate volumes reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The algal medium was prepared by adding appropriate volumes of the stock nutrient solutions to artificial saltwater at 30 ppt salinity. The pH of the medium was 8.1 and it was sterilized by filtration (0.22µm) prior to use.
Test solution preparation: A single test solution (3.46 mg/L) was prepared for this study in algal saltwater medium. The solution was sonicated for approximately 30 minutes and was stirred with a magnetic stir plate for approximately 43 hours. The final test solution appeared clear and colorless.
Exposure vessels: Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.
Agitation: Shaken continuously at 100 rpm

Number of replicates: six.
Initial algal cell loading: 7.7 X 10^4 cells/mL
Number of concentrations: one plus a negative control plus an abiotic control at the highest concentration tested
Water chemistry:
pH range (0 – 96 hours)
8.0 – 8.4 (control exposure)
8.0 – 8.4 (3.20 mg/L exposure)
Test temperature range (0 – 96 hours)
20.2 – 21.4ºC
Light levels: (0 – 96 hours)
3880 – 4710 lux from cool-white fluorescent lighting
Photoperiod: 14-hours light and 10 hours dark

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 3.46 mg/L plus 3.46 mg/L abiotic control. This is apparently the highest concentration of PFOS attainable in this saltwater algal media.

Measured concentrations: <LOQ, 3.20 mg/L; abiotic control = 3.18 mg/L

Element value (95% confidence interval):
72 and 96-hour EC_{10} via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

24, 48, 72, and 96-hour EC_{50} via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72 and 96-hour EC_{90} via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72-hour NOAEC (cell density, area under the curve, growth rate):
3.20 mg/L

96-hour NOAEC (cell density, area under the curve, growth rate):
3.20 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. The EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits could not be calculated using statistical methods. Cell densities, areas under the growth curve and growth rates at 72 and 96

hours were evaluated for normality using the Shapiro-Wilk's test and for equality of variance using an F-test. The treatment group were then compared to the control using ANOVA and a 2-sample t-test. Results of the statistical analyses were used to determine the NOAEC values.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). The 72 and 96-hour samples were centrifuged approximately 10 minutes at approximately 2000 rpm prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.480 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 88.5% of nominal. The measured value for the sample taken at 72-hours was 92.2% of nominal. The measured value for the sample taken at 96-hours was 91.2% of nominal. For the abiotic control, the measured value for the sample taken at 72-hours was 97.1% of nominal and for the sample taken at 96-hours, 86.8% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
3.46	3.26, 3.19, 3.15	3.20	92.5
3.46 (abiotic)	Not analyzed, 3.36, 3.00	3.18	91.9

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	2,481,667	-	-	-
3.20	2,601,667	-4.8	-7.3	-1.3

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or the treatment group. In addition, there were no noticeable changes in cell morphology when compared to the negative control.
Reversibility of Growth Inhibition: After 96-hours of exposure, there was no significant inhibition of growth in the highest concentration tested (3.20 mg/L). Therefore, a recovery phase was not conducted.

CONCLUSIONS

A single concentration of potassium perfluorooctanesulfonate was evaluated for toxicity to *Skeletonema costatum.* This mean measured concentration, 3.20 mg/L, was the highest concentration attainable in this algal media. The 96-hour EC_{50} and 95% confidence interval for *Skeletonema costatum,* as determined by cell density, area under the growth curve, and by growth rate was found to be > 3.20 mg/L. The 96-hour NOAEC was determined by ANOVA and a 2-sample t-test to be 3.20 mg/L calculated using cell density, area under the curve and growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/12/01

RS-II-6: TOXICITY TO AQUATIC PLANTS (DUCKWEED, *LEMNA GIBBA* G3)

TEST SUBSTANCE

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

Remarks: Sample obtained from 3M production lot number 217. The test substance is a white powder. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.4400
Test: Static acute
GLP: Yes
Year completed: 2001
Species: *Lemna gibba G3*
Source: Originally from The United States Department of Agriculture. Maintained in culture medium at Wildlife International Ltd., Easton, MD
Analytical monitoring: Test concentrations measured at 0, 3, 5, and 7-days
Element basis: Number of fronds
Exposure period: 7-days
Start date: 3/3/00
End date: 3/10/00
Test organisms laboratory culture: Duckweed cultures had been actively growing in freshwater medium (20X AAP) for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Test temperature range: 24.2 – 25.2ºC
Light levels: 5000 $\pm$ 750 lux from continuous warm-white fluorescent lighting
Growth medium: USEPA OPPTS 850.4400 20X AAP, 1996

Compound	Nominal Concentration	Units
$MgCl_2 \cdot 6H_20$	243.2	mg/L
$CaCl_2 \cdot 2H_20$	88.0	mg/L
H_3BO_3	3.712	mg/L
$MnCl_2 \cdot 4H20$	8.32	mg/L
$ZnCl_2$	65.6	ug/L
$FeCl_3 \cdot 6H_20$	3.196	mg/L
$CoCl_2 \cdot 6H_20$	28.56	ug/L
$Na_2Mo0_4 \cdot 2H_20$	145.2	ug/L
$CuCl_2 \cdot 2H_20$	0.240	ug/L
$Na_2EDTA \cdot 2H_20$	6.00	mg/L
$NaNO_3$	510	mg/L
$MgSO_4 \cdot 7H_20$	294	mg/L
K_2HPO_4	20.88	mg/L
$NaHCO_3$	300	mg/L

The pH of the medium was adjusted to 7.5 $\pm$ 0.1 using 10% HCl.

Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 $\pm$ 0.1 using 10% HCl and the medium was sterilized by filtration (0.22 μm) prior to use.
Stock and test solution preparation: A primary stock solution was prepared in duckweed medium at a concentration of 351 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with duckweed medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.
Exposure vessels: 250 mL plastic beakers containing 100 mL test solution, each covered with a disposable petri dish lid.
Agitation: None
Number of replicates: three plys 2 additional replicates for

analytical sampling on Days 3 and 5
Initial loading: 5 plants/replicate, 15 fronds/replicate
Number of concentrations: six plus a negative control plus abiotic controls at the highest concentration tested
Water chemistry:
pH range (0 – 96 hours)
7.9 – 8.9 (control exposure)
8.4 – 8.7 (230 mg/L exposure)
Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at Days 0, 3, 5, and 7.

RESULTS

Nominal concentrations: Negative control, 11, 22, 43.9, 87.9, 176, and 351 mg/L plus 351 mg/L abiotic control.
Measured concentrations: <LOQ, 7.74, 15.1, 31.9, 62.5, 147, 230 mg/L; abiotic control = 231 mg/L
Element value and 95% confidence interval (based on frond number):
3-day IC_{10}: 101 mg/L (C.I. not calculable)
3-day IC_{50}: > 230 mg/L (C.I. not calculable)
3-day IC_{90}: > 230 mg/L (C.I. not calculable)
5-day IC_{10}: 30.7 mg/L (13.3 – 142 mg/L)
5-day IC_{50}: 182 mg/L (89.1 – 240 mg/L)
5-day IC_{90}: > 230 mg/L (C.I. not calculable)
7-day IC_{10}: 22.1 mg/L (13.3 – 26.0 mg/L)
7-day IC_{50}: 108 mg/L (45.7 – 144 mg/L)
7-day IC_{90}: > 230 mg/L (C.I. not calculable)
7-day NOAEC (number of fronds): 15.1 mg/L

All element values based on mean measured concentrations

Statistical methods: Mean plant and frond numbers, percent inhibition values and the percentages of necrotic, chlorotic and dead fronds were calculated using "Microsoft Excel Version 5.0", while statistical analyses were conducted using "TOXSTAT Version 3.5". Percent inhibition values were calculated for each treatment group as the percent reduction in mean frond number relative to mean frond number in the control replicates. The IC_{10}, IC_{50}, and IC_{90} values and 95% confidence intervals were determined, when possible, using linear interpolation with frond number and exposure concentration data. The percentages of dead, chlorotic and necrotic fronds also were calculated relative to the total number of fronds in each test chamber. The frond number data was evaluated for normality and homogeneity of variances ($p = 0.05$) using the Shapiro-Wilks' and Levene's tests, respectively. The data were normally distributed and the variances were homogeneous, thus statistically significant differences between the control and treatment groups were identified using ANOVA and Dunnett's test. Results of the statistical analyses, as well as an evaluation of the concentration-response pattern and other observations of effects were used in the determination of the no-observed-adverse-effect-concentration (NOAEC).

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were centrifuged as necessary prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Samples collected at test initiation had measured values from 64.2 to 82.6% of nominal. Measured values for samples taken at Day 3 ranged from 67.3 to 83.3% of nominal. Measured values for samples taken at Day 5 ranged from 65.4 to 85.4% of nominal. Samples collected at test termination (Day 7) ranged from 63.9 to 83.8% of nominal. For the abiotic controls, measured values for samples taken at Day 3, Day 5, and Day 7 ranged from 64.2 – 66.9% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at Days 0, 3, 5, and 7, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
11	7.57, 8.35, 7.47, 7.55	7.74	70
22	15.2, 15.4, 14.6, 15.2	15.1	69
43.9	32.2, 31.9, 31.8, 31.7	31.9	73
87.9	63.5, 63.1, 61.5, 61.8	62.5	71
176	145, 146, 150, 147	147	84
351	226, 237, 232, 224	230	66
351 (abiotic)	not analyzed, 225, 235, 232	231	66

Biological observations after 7-Days:

Counts:

Mean Measured Concentration, mg/L	Mean Number of Plants	Mean Number of Fronds	Percent Inhibition via Frond Number
Negative Control	19	197	-
7.74	18	177	10
15.1	20	219	-11
31.9	14	151*	24
62.5	11	134*	32
147	15	69*	65
230	17	37*	81

*Statistically significant difference ($p < 0.05$) from the negative control using ANOVA and Dunnett's Test.

Effects:

Mean Measured Concentration, mg/L	Mean Dead Fronds, %	Mean Chlorotic Fronds, %	Mean Necrotic Fronds, %
Negative Control	0	0	0
7.74	0	0	0
15.1	0	1.1	0
31.9	0	0	0.23
62.5	0	0.9	0.61
147	1.0	11	4.5
230	3.8	9.4	19

Control response: satisfactory. Plants appeared healthy and exhibited normal growth throughout the test with the exception of one necrotic frond observed on Day 3 and Day 5 of the test.
Observations: Duckweed exposed to 147 and 230 mg PFOS/L exhibited a dose-responsive increase in the incidence of dead, chlorotic or necrotic fronds during the test. By Day 7, all treatment groups $\geq$ 31.9 gm/L showed evidence of sublethal effects, including root destruction and/or a cupping of the plant downward on the water surface.

CONCLUSIONS

The potassium perfluorooctanesulfonate 7-Day IC_{50} and 95% confidence interval for duckweed was determined to be 108 (45.7 – 144) mg/L. The 7-Day NOAEC, based on the inhibition of frond production and evidence of sublethal effects, was 15.1 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

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

OTHER

Last changed: 6/19/01

RS-II-7: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (*DAPHNIA*)

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, ^{1}H-HMR, ^{19}F-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: EC_{50} 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 $CaCO_3$
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 ºC (control exposure)
19.3 – 20.1 ºC (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

RS-II-8: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (*DAPHNIA*)

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-Karber 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 mmhos/cm (control exposure)
120 – 124 mmhos/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-Karber 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

RS-II-9: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (*DAPHNIA*)

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: The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Not noted.
Test type: Static acute
GLP: No
Year completed: 1994
Species: *Daphnia magna*
Analytical monitoring: pH and DO content
Statistical methods: EC_{50} values calculated using Trimmed Spearman-Karber 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 ºC
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 EC50 values and 95% confidence intervals were calculated using the Trimmed Spearman-Karber 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

RS-II-10: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (*DAPHNIA*)

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-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test sample is FM-3820, a mixture of the test substance in diethylene glycol butyl ether and water (approximately 24-28% test substance in diethylene glycol butyl ether and water). Calculations were made to adjust test values using the upper limit concentration of the test substance (28%) in the test sample and no adjustment was made for the presence of the diethylene glycol butyl ether or water when noted below. These calculations assumed that all toxicity was due to the presence of the Perfluorooctanesulfonate substance.

METHOD

Method: OECD 202
Test type: Static acute
GLP: Yes
Year completed: 1991
Species: *Daphnia magna*
Analytical monitoring: DO, pH, Conductivity, and temperature were monitored daily.
Statistical methods: EC_{50} 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/m^2. 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 ºC (control exposure)

20.7 – 20.9 ºC (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.

RS-II-11: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (FRESHWATER MUSSEL)

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, ^{1}H-HMR, ^{19}F-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 complamatus*
Analytical monitoring: Test substance concentrations measured by LCMS at 0, 48, 96-hours

Statistical methods: LC_{50} 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 $CaCO_3$
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 ºC (control exposure)
21.8 – 23.7 ºC (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 LC50 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

RS-II-12: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (FRESHWATER MUSSEL)

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, ^{1}H-HMR, ^{19}F-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 complamatus*
Analytical monitoring: Test substance concentrations measured by LCMS at 0, 48, 96-hours

Statistical methods: LC_{50} 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 µm filtered well water
Dilution water chemistry (during the 4-week period immediately preceding the test):
hardness: 126 (120-132) mg/L as $CaCO_3$
alkalinity: 174 (170-178) mg/L as CaCO3
pH: 8.3 (8.1-8.5)
TOC: < 1.0 mg/L
Conductivity: 321 (310-330) µmhos/cm
Ca/Mg ratio: 35/13.5
Na/K ratio: 21.3/6.62

Lighting: Colortone® 50 fluorescent lights, intensity approximately 369 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.
Stock and test solutions preparation: A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for approximately 24 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.
Exposure vessels: 25 liter polyethylene aquaria containing approximately 20 L of test solution. The approximate depth of test solution was 23.2 cm.
Number of replicates: two
Number of test organisms per replicate: ten
Number of concentrations: five plus a negative control
Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):
5.8 – 8.5 mg/L (control exposure)
5.0 – 8.6 mg/L (79 mg/L exposure)

pH range (0 – 96 hours)
8.0 – 8.4 (control exposure)
7.9 – 8.5 (79 mg/L exposure)

Test temperature range (0 – 96 hours)
21.4 – 21.8ºC (control exposure)
21.8 – 23.7ºC (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 LC50 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 Conc., mg/L	24 Hours	48 Hours	72 Hours	96 Hours
Neg. 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, Minnesota, 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

RS-II-13: TOXICITY TO AQUATIC INVERTEBRATES (*ARTEMIA SP*)

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 of uncharacterized purity.

METHOD

Method: Draft International Standards Organization (Vanhaecke and Persoone, 1981)
Type: Acute static
GLP: No
Year completed: 1985
Species: *Artemia sp.*
Artemia source: Salt lake Brine Shrimp Inc.
Artemia age at test initiation: naupuli < 24 hours old.
Exposure period: 48-hours
Analytical monitoring: Dissolved oxygen, pH, conductivity
Statistical methods: Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC_{50}.

Test conditions:

- **Dilution water:** 30 parts per thousand NaCl solution
- **Dilution water chemistry (initial):**
  - **pH:** 8.0 – 8.2
  - **D.O.:** > 6 mg/L
- **Stock solution preparation:** 1000 mg/L; noted as cloudy
- **Exposure vessels:** Not noted; solution volume 10 mL
- **Number of replicates:** 3
- **Number of organisms/replicate:** 10
- **Number of concentrations:** 6 plus a blank control
- **Water chemistry during the study:**
  - **Dissolved oxygen ranges (test and control):**
    > 6.0 mg/L
  - **pH (test and control)**
    8.0 – 8.2
  - **Test temperature range** (0 – 48 hours)
    21 - 21°C
- **Element basis:** mortality

RESULTS

Nominal concentrations: 1, 2, 3, 5, 10, 20 mg/L
Element values (95% confidence interval) calculated per replicate:
48-hour EC_{50} = 9.4 (7.4 – 12.1) mg/L
48-hour EC_{50} = 9.4 (7.3 – 12.2) mg/L
48-hour EC_{50} = 8.9 (6.7 – 11.9) mg/L
Mortality of controls: None

DATA QUALITY

Reliability: Klimisch ranking: 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 6/12/01

RS-II-14: ACUTE TOXICITY TO AQUATIC INVERTEBRATES (SALTWATER MYSID)

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, ^{1}H-HMR, ^{19}F-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: LC_{50} 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 $20^{0}/_{00}$ with well water, 0.45mm filtered.
Dilution water chemistry (during the 4-week period immediately preceding the test):
Salinity: 20 (20-20) $^{0}/_{00}$
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 ºC (control exposure)
23.8 – 24.5 ºC (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

RS-II-15: ACUTE EASTERN OYSTER SHELL DEPOSITION

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, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1025
Type: Static acute
GLP: Yes
Year completed: Study completed 1999. Report completed 2000
Species: *Crassostrea virginica*
Supplier: P. Cummins Oyster Company, Inc., Baltimore, MD
Shell grinding: Prior to test initiation, recently deposited shell at the rounded (ventral) end was removed using a small electric grinder. Care was taken to remove the shell rim uniformly to produce a smooth, rounded, blunt profile.
Analytical monitoring: PFOS measured at 0, 48, 96-hours
Exposure period: 96-hours
Statistical methods: Shell growth inhibition was calculated for each treatment group as the percent reduction in shell growth relative to mean shell growth in the negative control. The EC50 value was estimated by visual inspection of shell growth inhibition data. The shell growth data was evaluated for normality and homogeneity of variances using the Chi-Square test and Bartlett's test, respectively. Dunnett's test was used to identify treatment groups which had a statistically significant ($\leq$0.05) reduction in shell growth as compared to the control.
Test oyster age: unknown
Length: 33.8 (27.8-41.5) mm
Pretreatment: None

Test conditions:

Dilution water: Natural seawater diluted to a salinity of $20^{0}/_{00}$ with well water
Dilution water chemistry (during the 4-week period immediately preceding the test):
salinity: 21 (20-21) $^{0}/_{00}$
pH: 8.1 (8.0-8.2)
TOC: < 1.0 mg/L
Stock and test solution preparation: Primary stock prepared in dilution water at 9.1 mg/L and mixed for ~24 hours prior to use. After mixing, primary stock solution appeared clear and colorless with some white particulate material suspended throughout the solution. It was proportionally diluted with dilution water to prepare the four

additional test concentrations. All test solutions appeared clear and colorless. Due to the relatively low solubility of PFOS in natural seawater, the highest concentration attainable with this matrix is approximately 3.3 mg/L.
Concentrations dosing rate: Once
Exposure vessels: 52L polyethylene aquaria containing approximately 40L of test solution; water depth approximately 21 cm. Each chamber was continuously stirred to circulate the supplemental algae diet using an electric paddle mixer.
Feeding: Algal cells (*Thalassiosira pseudonana, Skeletonema* sp.*, Chaetoceros* sp.*, and Isochrysis* sp.) were provided to supplement naturally occurring algae and to maximize oyster growth rates during the test.
Number of replicates: one
Number of oysters per replicate: twenty
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
6.6 – 7.6 mg/L (control exposure)
6.1 – 7.7 mg/L (3.0 mg/L exposure)
pH range (0 – 96 hours)
7.6 – 8.1 (control exposure)
7.6 – 8.1 (3.0 mg/L exposure)
Test temperature range (0 – 96 hours)
22.2 – 22.3°C (control exposure)
21.8 – 22.7°C (3.0 mg/L exposure)
Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, 1.2, 2.0, 3.3, 5.5, 9.1 mg/L
Measured concentrations: <LOQ, 0.36, 0.40, 1.3, 1.9, 3.0 mg/L
Element value:
96-hour EC_{50} = >3.0 mg/L (C.I. not calculable)
96-hour NOEC = 1.9 mg/L

All element values based on mean measured concentrations

Statistical evaluation of shell growth: EC_{50} values could not be calculated due to insufficient shell growth inhibition at the highest attainable concentration.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. Samples collected at test initiation had measured values from 28 to 46% of nominal. Measured values for samples taken at 48-hours ranged from 15 to 41% of nominal. Measured values for samples taken at 96-hours ranged from <LOQ to 52% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
1.2	0.331, 0.353, 0.341, 0.429, <LOQ, <LOQ	0.36	30
2.0	0.622, 0.633, 0.299, 0.313, 0.249, 0.257	0.40	20
3.3	1.36, 1.15, 0.924, 0.878, 1.58, 1.72	1.3	39
5.5	2.42, 2.53, 2.02, 2.24, 1.45, 0.970	1.9	35
9.1	3.39, 3.44, 3.01*, 3.74, 3.57, 1.99, 2.19	3.0	33

*3 replicates analyzed at time 0

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

Shell deposition and shell growth inhibition at test termination:

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

Mortality of controls: None

CONCLUSIONS

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

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

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

RS-II-16: CHRONIC TOXICITY TO FRESHWATER INVERTEBRATES (*DAPHNIA MAGNA*)

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, ^{1}H-HMR, ^{19}F-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 _ 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 $CaCO_3$
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 ºC (negative control exposure)
19.4 – 20.1 ºC (12 mg/L exposure)
19.4 – 19.5 ºC* (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
2^{nd} 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 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
.5	20	20	0
.9	20	20	0
5.6	20	20	
12	20	18	10
24	8	0	100

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

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

Reproduction:

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

CONCLUSIONS

There were no adverse effects on survival, reproduction or growth of *Daphnia magna* exposed to the test substance at concentration ≤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

RS-II-17: CHRONIC TOXICITY TO SALTWATER INVERTEBRATES (MYSID)

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, ^{1}H-HMR, ^{19}F-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 $20^{0}/_{00}$ with well water, 0.45mm filtered.

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

Salinity: 20 (20-20) $^{0}/_{00}$

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 ºC (control exposure)
24.4 – 25.1 ºC (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
2^{nd} 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 0.0.0458 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 $\leq$ 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 ± SD, mm	Dry Weight, Mean ± SD, mg
Negative Control	36/39	6.43 ± 0.0634	0.634 ± 0.0510
0.057	44/46	6.43 ± 0.0729	0.599 ± 0.0276
0.12	36/40	6.56 ± 0.105	0.641 ± 0.0241
0.25	36/37	6.40 ± 0.0548	0.622 ± 0.0227
0.55	35/37	6.14 ± 0.0794	0.562 ± 0.00624
1.3	8/14	5.85 ± 0.178	0.436 ± 0.0441

Reproduction:

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/ Reproductive Day	Overall Mean ± SD
Negative Control	A	70	18	0.257	0.315 ± 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 ± 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 ± 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 ± 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559 ± 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/ Reproductive Day	Overall Mean ± SD
	D	56	1	0.0179	
1.3	A	22	0	-	-
	B	14	0	-	
	C	0	0	-	
	D	11	0	-	

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp exposed to potassium perfluorooctanesulfonate at concentrations $\leq$ 0.25 mg/L for 35 days. Reproduction, length and dry weight were the most sensitive biological endpoints in this study. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

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

RS-II-18: ACUTE TOXICITY TO FISH (PIMEPHALES)

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, ^{1}H-HMR, ^{19}F-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: LC_{50} 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 $CaCO_3$
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 ºC (control exposure)
21.3 – 22.3 ºC (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

RS-II-19: ACUTE TOXICITY TO FISH (PIMEPHALES)

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-Karber 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 mhos/cm (control exposure)
118 – 154 mmhos/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

RS-II-20: ACUTE TOXICITY TO FISH (PIMEPHALES)

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: LC_{50} values calculated by Trimmed Spearman - Karber.
Test fish age: 79 days.
Length and weight: Average length = 2.1 $\pm$ 0.3cm
Average weight = 0.069 $\pm$ 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 ºC

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

RS-II-21: ACUTE TOXICITY TO FISH (PIMEPHALES)

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-heptadecafluoro-, ammonium salt, CAS # 29081-56-9)

Remarks: Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

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 ºC (70-72 ºF)

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

RS-II-22: ACUTE TOXICITY TO FISH (PIMEPHALES)

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-heptadecafluoro-, ammonium salt, CAS # 29081-56-9)

Remarks: Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

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 ºC (69-70 ºF)

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

RS-II-23: ACUTE TOXICITY TO FISH (SHEEPSHEAD MINNOW)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1075
Type: Static renewal
GLP: Yes
Date completed: Study completed 2001, report completed 2002.
Species: *Cyprinodon variegatus*
Supplier: Aquatic BioSystems, Inc., Fort Collins, CO
Analytical monitoring: PFOS measured at initiation, prior to and after renewal at 24, 48, and 72 hours and at test termination (96-hours)
Exposure period: 96-hours
Statistical methods: The use of a single test concentration (at water solubility) precluded the statistical calculation of LC_{50} values.
Test fish age: Juveniles
Average Total Length and weight: 3.0 (2.4 – 3.5) cm, 0.44 (0.21 – 0.66) g
Loading: 0.29 g fish/L
Pretreatment: None

Test conditions:

Dilution water: Natural seawater, filtered and diluted to a salinity of approximately 20 parts per thousand with well water
Dilution water chemistry (during the 4-week period immediately preceding the test):
Salinity: 20 (20 – 20) parts per thousand
pH: 8.2 (8.1 – 8.3)
Stock and test solution preparation: Primary stock prepared in methanol at 40 mg/L, sonicated for approximately 20 minutes and inverted to mix prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the one test concentration. Each solution was stirred with a stainless steel whisk for approximately one minute. All test solutions appeared clear and colorless.
Solvent: Methanol
Solvent concentration (treatment and solvent control groups): 0.5 mL/L
Concentrations dosing rate: Daily static renewal
Stability of the test chemical solutions: Extremely stable

Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.1 cm.
Number of replicates: three (biotic), two (abiotic)
Number of fish per replicate: ten
Number of concentrations: One plus a negative and a solvent control, and an abiotic solution.
Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

2.8 – 7.4 mg/L (negative control exposure)
1.7 – 7.6 mg/L (solvent control exposure)
1.6 – 7.6 mg/L (15 mg/L exposure)

pH range (0 – 96 hours)

7.9 – 8.3 (negative control exposure)
7.9 – 8.3 (solvent control exposure)
7.9 – 8.3 (15 mg/L exposure)

Test temperature range (0 – 96 hours)

21.9 – 22.6ºC (negative control exposure)
22.1 – 22.9ºC (solvent control exposure)
22.2 – 23.1ºC (15 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, solvent control, 20 mg/L (biotic), 20 mg/L (abiotic)
Measured concentrations: <LOQ, <LOQ, 15, 13 mg/L
Element value:

24-hour LC_{50} = >15 mg/L (C.I. not calculable)
48-hour LC_{50} = >15 mg/L (C.I. not calculable)
72-hour LC_{50} = >15 mg/L (C.I. not calculable)
96-hour LC_{50} = >15 mg/L (C.I. not calculable)

All element values based on mean measured concentrations

Statistical Evaluation of Mortality: Element values and confidence interval could not be calculated due to lack of mortality.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 5.00 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.2. Samples collected in exposure vessels at test initiation had measured values from 78.6 to 82.0% of nominal. New samples collected at Hours 24, 48 and 72 had measured concentrations from 74.9 to 81.2 and 86.9 to 92.9, and 82.9 to 87.1% of nominal, respectively. Old samples collected at Hours 24, 48, and 72 had measured concentrations ranging from 66.6 to 76.2, 56.4 to 66.9, and 75.8 to 89.9% of nominal,

respectively. Mean measured concentrations of PFOS in samples collected at test termination were 55.6 to 68.4% of nominal. The measured concentrations of PFOS from the abiotic treatment group were slightly lower than those from the exposure treatment group. This may have been due to increased deposition of test substance at the limit of solubility, which could have resulted from the absence of the natural mixing action that was provided by the movement of the fish in the exposure treatment group.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	**Measured Replicate Values of old and new Test Solutions Respectively, mg/L[(1)]**	**Mean Measured Concentration, mg/L**	**Percent of Nominal**
Negative Control	All < LOQ	<LOQ	-
Solvent Control	All < LOQ	<LOQ	-
20 [(2)]	16.4, 15.7, 16.0; 15.2, 13.3, 15.2; 16.2, 15.7, 15.0; 13.4, 11.3, 12.7; 17.4, 18.2, 18.6; 15.2, 18.0, 16.2; 17.0, 16.6, 17.4; 13.7, 11.1, 13.4	15	75
20[(3)](abiotic)	10.9, 9.22; 16.1, 16.6; 9.62, 9.69; 16.6, 17.5; 9.01, 9.25; 17.7, 15.9; 9.84, 9.22	13	65

(1) Replicate samples are listed in this order: Day 0 (new), 24-hours (old), 24-hours (new), 48-hours (old), 48-hours (new), 72-hours (old), 72-hours (new), 96-hours (old)
(2) Triplicate samples
(3) Day 0 not measured, Duplicate samples

Biological observations after 96-hours: Fish in the negative control and solvent control appeared healthy and normal throughout the exposure period. No mortalities appeared in the 15 mg/L treatment group during the study. However, upon transfer to the new test solution at approximately 48 and 72 hours, some fish were observed swimming erratically and turning a dark color. The fish appeared normal within approximately two hours after transfer, although one fish still appeared to be discolored at test termination.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
Solvent control	0	0	0	0
15	0	0	0	0

Lowest concentration causing 100% mortality: none – mortality limit apparently greater than solubility limit
Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC_{50} for sheepshead minnow was determined to be >15 mg/L, the limit of solubility in this study. The 96-hour no mortality concentration was 15 mg/L and no observed effects concentration was <15 mg/L mg/L (1 fish out of 30 was discolored at 96-hours).

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

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

OTHER

Last changed: 1/24/02

RS-II-24: ACUTE TOXICITY TO FISH (BLUEGILL SUNFISH)

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-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1), CAS # 70225-14-8)

Remarks: Test sample is a mixture of the test substance in water (approximately 25% test substance and 75% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

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 $\pm$ 2.17 mm.
Average weight = 0.60 $\pm$ 0.15 g
Loading: 0.2 g fish / L
Pretreatment: None

Test Conditions:

Dilution water: Laboratory well water
Dilution water chemistry:
Dissolved oxygen:9.3 mg/L
Hardness: 255 mg/L as $CaCO_3$
Alkalinity: 368 mg/L as CaCO3
pH: 7.8
Conductivity: 50 mmhos/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 ºC 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 LC_{50} 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

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 Perfluorooctancesulfonate 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

RS-II-25: ACUTE TOXICITY TO FISH (FRESHWATER RAINBOW TROUT)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMRand elemental analyses techniques.

METHOD

Method: OPPTS 850.1075 and OECD 203
Type: Static acute
GLP: Yes
Date completed: Study completed 2001, report completed 2002.
Species: *Oncorhynchus mykiss*
Supplier: Thomas Fish Company, Anderson, CA
Analytical monitoring: PFOS measured at 0, 48, 96-hours
Exposure period: 96-hours
Statistical methods: LC_{50} values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test fish age: juveniles
Average Total Length and weight: 3.6 (3.4–4.0) cm, 0.34 (0.25-0.47) g
Loading: 0.23 g fish/L
Pretreatment: None

Test conditions:

Dilution water: 0.45 µm filtered moderately hard well water
Dilution water chemistry (during the 4-week period immediately preceding the test):
hardness: 130 (128-132) mg/L as $CaCO_3$
alkalinity: 177 (176-178) mg/L as CaCO3
pH: 8.3 (8.2 – 8.4)
TOC: Not given
Conductivity: 311 (310-315) µmhos/cm
Stock and test solution preparation: Primary stock prepared in dilution water at 150 mg/L and mixed for ~23 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the five test concentrations.
Concentrations dosing rate: Once
Stability of the test chemical solutions: Extremely stable
Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.5 cm.

Number of replicates: two
Number of fish per replicate: ten
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
9.4 – 10.7 mg/L (control exposure)
9.2 – 10.8 mg/L (50 mg/L exposure)
pH range (0 – 96 hours)
8.1 – 8.4 (control exposure)
8.2 – 8.4 (50 mg/L exposure)
Test temperature range (0 – 96 hours)
12.1 – 12.6ºC (control exposure)
11.8 – 12.9ºC (50 mg/L exposure)
Method of calculating mean measuredconcentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, 3.1, 6.3, 13, 25, 50 (exposure), 50 (abiotic) mg/L
Measured concentrations: <LOQ, 3.0, 6.3, 13, 25, 50, 52 mg/L
Element value:
24-hour LC_{50} = > 50 mg/L (C.I. not calculable)
48-hour LC_{50} = > 50 mg/L (C.I. not calculable)
72-hour LC_{50} = > 50 mg/L (C.I. not calculable)
96-hour LC_{50} = 22 (18 - 27) mg/L

All element values based on mean measured concentrations

Statistical Evaluation of Mortality: Element values and confidence limits for 24, 48, and 72-hours could not be calculated due to lack of mortality. Probit Analysis was used to calculate the 96-hour 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 0.200 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 101. Samples collected at test initiation had measured values from 93.2 to 103% of nominal. Measured values for the biotic samples taken at 48-hours ranged from 93.6 to 103% of nominal, while abiotic samples ranged from 105 to 106% of nominal. Measured values for biotic samples taken at 96-hours ranged from 91.4 to 105% of nominal, while the abiotic samples were 102% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
3.1	3.15, 3.02, 2.90, 3.01,2.83, 2.97	3.0	97
6.3	6.22, 6.21, 6.16, 6.43, 6.15, 6.60	6.3	100
13	13.2, 12.1, 12.7, 12.3, 13.1, 12.6	13	100
25	25.0, 25.7, 24.3, 25.7, 25.7, 26.2	25	100
50	49.7, 49.8, 51.1, 51.5, 49.6, 50.8	50	100
50 (abiotic)(1)	53.1, 52.6, 50.9, 51.0	52	104

(1) Samples taken at 48 and 96-hours only

Biological observations after 96-hours: Fish in the negative control and the 3.0 and 6.3 mg/L exposure concentration appeared normal with no mortalities or overt signs of toxicity. All surviving fish in the 13 and 25 mg/L exposures appeared normal with no overt signs of toxicity after 96-hours.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
3.0	0	0	0	0
6.3	0	0	0	0
13	0	0	0	20
25	0	0	0	50
50	0	5	35	100

Lowest concentration causing 100% mortality: 50 mg/L
Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC_{50} for rainbow trout was determined to be 22 mg/L with a 95% confidence interval of 18 –27 mg/L. The 96-hour no mortality and no effects concentration was 6.3 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

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

OTHER

Last changed: 1/24/02

RS-II-26: ACUTE TOXICITY TO FISH (SALTWATER RAINBOW TROUT)

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 of uncharacterized purity.

METHOD

Method: Standard procedures for Testing Acute Lethality of Liquid Effleunts (Environment Canada, 1980)
Type: Acute static, renewal after 48-hours
GLP: No
Year completed: 1985
Species: Rainbow Trout, *Salmo gairdneri*
Fish source: Rainbow Springs, Thamesford
Fish age at test initiation: not noted
Fish acclimation to salt water: fish gradually acclimated to increasing salinity; held at 30 parts per thousand salinity 8 days prior to test initiation.
Exposure period: 96-hours
Analytical monitoring: Dissolved oxygen, pH, conductivity
Statistical methods: Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC_{50}.
Test conditions:

Dilution water: Mississauga dechlorinated tap water amended with calcium, magnesium, sodium, and chloride to obtain 30 parts per thousand salinity
Dilution water chemistry (initial):

pH: 7.4 - 8.0
D.O.: 8.6 – 9.0 mg/L
Conductivity: 20,000 μmhos/cm

Stock solution preparation: 1000 mg/L
Exposure vessels: Not noted; solution volume 35 L
Number of replicates: 2 tests – run 4 days apart, not replicated
Number of organisms/vessel: 6
Loading: 0.75 g/L
Number of concentrations: 4 plus a blank control
Water chemistry during the studies:

Dissolved oxygen ranges

8.1 – 10.3 (control)
8.8 – 10.1 (30 mg/L)

pH ranges

7.6 – 8.2 (control)

7.3 – 8.0 (30 mg/L)
Test temperature (0 – 48 hours): 15°C
Photoperiod: 12-hours light, 12-hours dark
Element basis: mortality

RESULTS

Nominal concentrations: 5, 10, 20, 30 mg/L
Element values (95% confidence interval) calculated per replicate:
96-hour EC_{50} = 13.7 (10.7 –17.7) mg/L
96-hour EC_{50} = 13.7 (10.7 – 17.8) mg/L
Mortality of controls: 17% (1/6 in both studies)

DATA QUALITY

Reliability: Klimisch ranking: 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 6/12/01

RS-II-27: CHRONIC TOXICITY TO EARLY LIFE STAGE OF FISH (PIMEPHALES)

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, ^{1}H-HMR, ^{19}F-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 $CaCO_3$
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 ºC (control exposure)
24.3 – 24.7 ºC (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	.15	107
0.29	0.287, 0.277, 0.270, 0.289, 0.292, 0.296, 0.269, 0.266, 0.307, 0.315, 0.343, 0.341, 0.311, 0.325, 0.319, 0.313, 0.296, 0.276	0.30	103
0.57	0.571, 0.576, 0.619, 0.659, 0.597, 0.642, 0.539, 0.535, 0.608, 0.580, 0.639, 0.617, 0.646, 0.644, 0.575, 0.576, 0.545, 0.543	0.60	105
1.1	1.14, 1.13, 1.21, 1.25, 1.13, 1.23, 1.03, 1.10, 1.19, 1.24, 1.30, 1.31, 1.30, 1.31, 1.14, 1.19, 1.13, 1.09	1.2	109
2.3	2.21, 2.27, 2.52, 2.46, 2.43, 2.38, fish all dead at Day 7	2.4	104
4.6	4.56, 4.40, 4.79, 4.79, 4.46, 4.76, fish all dead at Day 7	4.6	100

Biological Observations:

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

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

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

Hatchability:

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

Larval Survival:

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

Growth:

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

CONCLUSIONS

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

RS-II-28: CHRONIC TOXICITY TO EARLY LIFE STAGE OF FISH (PIMEPHALES)

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

RS-II-29: BIOCONCENTRATION IN AND CHRONIC TOXICITY TO BLUEGILL SUNFISH

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method/guideline followed: US EPA OPPTS 850.1730 and OECD 305
Type: Flow-through exposure with flow-through depuration phase.
GLP (Y/N): Yes
Year: 2001, Report amended 2002
Species: Bluegill (*Lepomis macrochirus*)
Supplier: Osage Catfisheries, Inc., Osage Beach, Missouri
Length and weight at test termination:
Mean length = 62 mm, range 56-66 mm
Mean weight = 2.70 g, range 2.03 – 3.32 g
Loading: 0.48 g fish/L/day (based on initial loading of 90 fish per tank, using mean fish weight at the end of the study and volume of water that passed through test chamber in 24-hours).
Fish age: Approximately 7 months at test initiation
Analytical monitoring: Concentration of PFOS in water and fish.
Pretreatment: None
Number of concentrations: Two plus a negative control
Test concentrations (mean measured): Negative control, 0.086 and 0.87 mg/L
Uptake period: 62-days (0.086 mg/L exposure)
35-days (0.87 mg/L exposure – this exposure ended after 35-days due to fish mortality)
Depuration period: 56-days (0.086 mg/L exposure)
None (0.087 mg/L exposure)

Test conditions:
Dilution water: Moderately–hard well water
Dilution water chemistry:
Specific conductance: 313 (310 – 315 umhos/cm)
Hardness: 130 (128 – 132 mg/L)
Alkalinity: 178 (176 – 178)
pH: 8.1 (8.0 – 8.2)
Measured during the 4-week period immediately preceding the test.

Stock and test solution preparation: Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless.
Diluter flow rate: Approx. 6.3 volume additions per 24-hours
Exposure vessels: 104 L stainless steel aquaria filled with approximately 80 L solution.
Number of replicates: None – one vessel per concentration
Number of fish per vessel: 90
Diet: Flake food, Ziegler Brothers, Inc., Gardners, PA

Water chemistry ranges during the study:

	Neg. Control	0.086 mg/L	0.87 mg/L
Dissolved oxygen, mg/L:	6.8 – 8.6	6.8 – 8.6	6.4 – 8.2
Temperature, °C:	21.8 – 22.0	21.7 – 22.0	21.7 – 21.9
pH:	7.9 – 8.2	7.9 – 8.2	7.9 – 8.2

Photoperiod: 16 hours light and 8 hours dark with a 30 minute transition period.
Light intensity: 278 lux at surface of the negative control vessel at test initiation

Collection of tissue samples: Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed and measured. Fish then rinsed with dilution water, blotted dry again and dissected into edible and nonedible tissue fractions. The fractions were individually weighed. The head, fins and viscera were considered to be nonedible tissue. The remaining tissue, including skin was considered to be edible tissue.
Statistical methods: Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values were originally calculated from the tissue concentrations at apparent steady-state using the BIOFAC model. Upon further investigation, this model was deemed inappropriate for use with this data set. The tissue concentrations had reached a point where there were not statistically significant differences between the last three sample days. However, a plot of the data clearly shows a trend of increasing concentrations in the tissues. In addition, the BIOFAC program is not accepted as appropriate for surfactants. An amended report was issued with BCFK values calculated as outlined in the draft OPPTS 850.1730 Guidance Document. The kinetic bioconcentration factor (BCFK), uptake rate (k_1) and depuration rate (k_2) were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L. These rate constants were then used to calculate a BCFK ($BCFK = K_1/K_2$) and half-lives for clearance for each tissue type. The results from this data reanalysis are presented below.

RESULTS

Nominal concentrations: Negative control, 0.1 and 1.0 mg/L
Mean measured concentrations: < 0.05, 0.086 and 0.87 mg/L

Kinetic Bioconcentration factors (BCFK):

0.086 mg/L exposure

	Edible	Nonedible	Whole Fish
BCFK:	1124	4013	2796
Time to reach 50% clearance:	86 days	116 days	112 days

0.87 mg/L exposure

Although BCF values were calculated using the BIOFAC software, the results are not reported here. All of the fish had died or been sampled prior to achieving steady-state. As a result, the BCF values were underestimated and are not relevant.

PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L:

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	0.167, 0.155, 0.144, 0.182	0.415, 0.519, 0.417, 0.497	0.293, 0.351, 0.286, 0.363
1	0.734, 0.726, 0.631, 0.806	1.68, 1.85, 1.72, 2.07	1.26, 1.34, 1.29, 1.53
3	1.73, 2.07, 2.03, 2.11	4.59, 5.50, 5.47, 5.97	3.21, 4.04, 4.18, 4.38
7	3.73, 4.25, 4.73, 6.25	10.2, 10.6, 11.9, 15.2	7.33, 7.66, 8.73, 11.4
14	11.4, 9.07, 13.7, 12.6	27.3, 23.2, 35.3, 32.6	20.2, 16.9, 26.0, 24.6
21	11.7, 12.0, 12.9, 10.6	33.3, 22.7, 24.6, 24.4	23.3, 18.4, 19.8, 18.5
28	18.3, 13.7, 23.9, 23.1	49.4, 40.7, 65.3, 57.9	35.3, 29.2, 45.4, 44.1
35	22.6, 27.7, 23.8, 20.6	67.1, 73.3, 62.0, 59.1	46.3, 53.8, 46.6, 40.9
42	27.6, 25.3, 21.2, 27.6	64.0, 68.1, 54.4, 79.6	50.1, 49.4, 40.9, 56.3

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
49	33.3, 36.2, 39.0, 30.6	85.0, 95.1, 93.1, 77.7	62.8, 69.6, 70.8, 57.4
56	48.3, 38.9, 44.1, 38.3	122, 94.2, 73.2, 106	90.6, 71.6, 63.3, 74.8
62	42.4, 66.2, 42.2, 39.2	101, 112, 105, 96.4	77.0, 92.7, 79.6, 73.1
Depuration Day			
14	48.5, 31.8, 31.6, 42.0	124, 79.4, 81.8, 113	90.3, 60.4, 61.6, 85.3
28	26.0, 33.3, 38.7, 55.8	85.7, 95.1, 85.7, 94.8	58.2, 70.1, 68.1, 81.1
42	24.1, 31.2, 30.0, 33.0	71.7, 80.6, 78.3, 82.1	51.4, 61.4, 61.0, 62.2
56	21.1, 37.6, 32.9, 31.2	57.7, 80.3, 85.4, 84.4	41.6, 66.5, 65.8, 62.1

PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L:

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	1.46, 1.48, 1.19, 1.39	3.52, 4.37, 4.22, 4.06	2.71, 3.08, 2.84, 2.89
1	4.68, 6.59, 5.56, 5.64	11.1, 14.2, 13.3, 12.1	8.00, 10.9, 10.2, 9.47
3	17.3, 15.8, 19.0, 20.8	39.3, 42.0, 43.8, 51.8	30.5, 30.7, 34.5, 39.1
7	42.0, 44.0, 57.7, 46.8	100, 102, 102, 120	74.9, 77.0, 85.3, 89.8
14	87.1, 81.6, 90.7, 73.3	177, 207, 245, 214	141, 157, 180, 158
21	79.4, 117, 104, 102	201, 278, 246, 229	146, 210, 185, 172
28[(1)]	102, 131, 107, 133	289, 372, 320, 361	205, 267, 232, 263

[(1)] Sampling of fish stopped after Uptake Day 28 due to mortality.

Test organism mortality:

Negative control: None during the uptake phase (62 days) or depuration phase (35 days)

0.086 mg/L exposure: One fish died after 49 days and one after 59 days of exposure in the uptake phase, none during the depuration phase (total of 2.2% mortality during the study).
0.87 mg/L exposure: Mortality first noted on Day 9 and continued through Day 35 of the uptake phase at which time all of the fish had either died or had been sampled
Analytical methodology: Analyses of test solutions and fish tissues were performed at Wildlife International, Ltd. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

CONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. The BCFK values calculated for the edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were calculated to be 1124, 4013, and 2796, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were 86, 116 and 112 days, respectively.

DATA QUALITY

Reliability: Klimisch ranking**:** 1

REFERENCES

This study was conducted at Wildlife International, Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723. The report was amended and reissued June 6, 2002.

OTHER

Last changed: 6/11/02

RS-II-30: FETAX – FROG EMBRYO TERATOGENESIS ASSAY (*XENOPUS*)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS, U2723 or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: Sample obtained from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: ASTM E1439-91

Test type: Static renewal

GLP: In-life phase – no; stock solution preparation and measurement of test concentrations - yes
Year completed: 2001
Number of studies: 3

	Study 1	**Study 2**	**Study 3**
Start date:	5/15/00	5/22/00	5/22/00
End date:	5/19/00	5/26/00	5/26/00

(Study 2 and 3 set up concurrently with common stock solutions)

Analytical monitoring: PFOS measured at 0 and 96-hours

Species: *Xenopus laevis*

Source: Breeding colonies at the University of Maryland Wye Research and Education Center (UMD/WREC), Queenstown, Maryland.

Test organisms laboratory culture: Mating pairs were bred in the dark in 23.5 $\pm$ 0.5ºC UMD/WREC non-chlorinated well water at ~ 70 day intervals by injecting 400 and 800 I. U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4-6 hours after injecting HCG; egg deposition occurred 9-12 hours following HCG injection.

Age at test initiation: Embryos; normal stage 8 blastula to normal stage 11 gastrula

Loading: 25 embryos/10 mL

Pretreatment: Embryos de-jelled in a 2% L-cysteine solution, then rinsed and re-suspended in FETAX solution prior to introduction to test chambers.

Element basis: mortality, malformations (via the atlas of Bantle et al., 1991), growth

Exposure period: 96-hours

Test Conditions (all 3 studies):

Dilution water: ASTM (1998) FETAX solution
Test temperature: 24.0 ± 0.2ºC
Light levels: 60-85 foot candle fluorescent lights
Photoperiod: 12-hour light:12-hour dark
Stock and test solution preparation: A primary stock solution was prepared in FETAX medium (supplied by UM-WREC) by Wildlife International, Ltd. at 48 mg PFOS/L. The primary stock solution was mixed by sonication and stirring. After mixing, the primary stock solution was proportionally diluted with FETAX medium to prepare the six test concentrations. The six test concenetration solutions were delivered to UM-WREC prior to the start of each study.
Reference substance: 6-aminonicotinamide
Stock and reference substance solution preparation: as outlined in the ASTM (1998) protocol
Exposure vessels: Covered 60 mm glass Petri dishes containing 10 mL test solution
Number of replicates: controls – 4, treatments – 2
Number of embryos per replicate: 25
Number of concentrations: six plus a negative control plus an abiotic control at the highest concentration tested, plus two reference substance concentrations.
Renewal frequency: every 24 hours
Stability of the test chemical solutions: Extremely stable
Water chemistry during all 3 studies:

pH range (0 – 96 hours)
7.1 – 7.7 (control exposure)
7.0 – 7.6 (24 mg/L nominal exposure)

Dissolved oxygen range (0-96 hours)
7.3 – 8.4 mg/L (control exposure)
7.0 – 8.5 mg/L (24 mg/L nominal exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal PFOS concentrations: Negative control, 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg/L plus 24.0 mg/L abiotic control.

Nominal 6-aminonicotinamide concentrations: 5.5 and 2500 mg/L
Mean measured PFOS concentrations:

Study 1: <LOQ, 2.00, 2.83, 4.73, 7.90, 14.7, 24.6 mg/L;
abiotic control = 23.7 mg/L
Study 2: < LOQ, 1.91, 3.04, 4.82, 7.97, 13.3, 23.1 mg/L;
abiotic control = 23.9 mg/L

Study 3: < LOQ, 1.93, 3.27, 5.25, 8.26, 14.0, 23.9 mg/L;
abiotic control = 24.1 mg/L

PFOS element values and 95% confidence intervals, mg/L:

Study Number	96-Hr LC_{50}	96-hr EC_{50}	Minimum conc. to Inhibit Growth (MCIG)	Teratogenic Index (TI)
1	13.8 (12.4 -15.3)	12.1 (10.0 - 14.6)	Not calculable	1.1
2	17.6 (15.5 - 20.0)	17.6 (13.5 - 22.9)	7.97	1.0
3	15.3 (13.1 - 17.8)	16.8 (12.4 - 22.8)	8.26	0.9

All element values based on mean measured concentrations

Statistical methods: The Trimmed Spearman-Karber statistical procedure was used to determine the 96-hour LC_{50} for mortality and 96-hour EC_{50} for malformations. The MCIG was determined by Bonferroni's T-Test. All statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used. The teratogenic index (TI) was calculated by dividing the LC_{50} by the EC_{50}.
Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd., Easton, MD using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.240 mg/L in these studies. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.2%. Samples collected at test initiation had measured values from 112 to 141% of nominal in the first study, and in the second and third studies, from 95.8 to 117% of nominal. Measured values for samples taken at 96-hours ranged from 54.7 to 98.6% of nominal in the first study and 80.7 to 112% of nominal in the second and third studies. The samples from the abiotic 24.0 mg/L treatment group was comparable to samples from the 24.0 mg/L treatment group with the embryos present.

Summary of analytical chemistry data:

Study 1:

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
1.82	2.58, 1.42	2.00	110
3.07	3.94, 1.72	2.83	92.2
5.19	6.62, 2.84	4.73	91.1
8.64	10.7, 5.09	7.90	91.4
14.4	18.5, 10.8	14.7	102
24.0	26.9, 22.3	24.6	103
24.0 (abiotic)	not analyzed, 23.7	23.7	98.6

Study 2:

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
1.82	1.77, 2.04	1.91	105
3.07	3.59, 2.49	3.04	99.0
5.19	5.45, 4.18	4.82	92.9
8.64	8.43, 7.51	7.97	92.2
14.4	14.5, 12.1	13.3	92.4
24.0	23.0, 23.1	23.1	96.3
24.0 (abiotic)	not analyzed, 23.9	23.9	99.6

Study 3:

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
1.82	1.77, 2.08	1.93	106
3.07	3.59, 2.94	3.27	107
5.19	5.45, 5.05	5.25	101
8.64	8.43, 8.09	8.26	95.6
14.4	14.5, 13.5	14.0	97.2
24.0	23.0, 24.7	23.9	99.6
24.0 (abiotic)	not analyzed, 24.1	24.1	100

Biological observations after 96-hours:Mortality and Malformations:

	Test 1		Test 2		Test 3	
Nominal Concentration *, mg/L	Percent Mortality	Percent Malforma-tions	Percent Mortality	Percent Malforma-tions	Percent Mortality	Percent Malforma-tions
Negative Control	1.0	4.0	1.0	4.0	0	2.0
1.82	2.0	8.2	0	8.0	0	6.0
3.07	4.0	15	10	4.4	0	4.0
5.19	10	22	8.0	11	0	6.0
8.64	12	25	10	20	14	14
14.4	38	65	30	37	44	39
24.0	100	-	70	67	78	73

*Nominal concentrations used for ease of comparison table

Malformations: The most common types of malformations noted were improper gut coiling, edema, notochord abnormalities and facial abnormalities.

Growth – Mean length (mm) after 96-hours Exposure:

Nominal Concentration*, mg/L	Test 1	Test 2	Test 3
Negative Control	8.59	8.88	9.47
1.82	8.29	8.45	9.10
3.07	8.80	8.57	9.28
5.19	8.51	8.72	9.28
8.64	8.71	7.93**	8.51**
14.4	8.08	7.51**	8.11**
24.0	- (total mortality)	7.39**	7.80**

*Nominal concentrations used for ease of comparison table
** Significantly different at alpha = 0.05 (Bonferroni T-Test)

Control response: satisfactory.
Reference substance response: satisfactory at low concentration (5.5 mg/L). Did not meet ASTM (1998) criteria for high concentration (2,500 mg/L). However, results obtained at high concentration were consistent and not at variance with previous experience in this testing laboratory.
Observations: Majority of embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest test concentrations.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC_{50} range for FETAX was determined to be 13.8 – 17.6 mg/L. The 96-hour EC_{50} range was 12.1 – 17.6 mg/L. The range for Maximum Concentration to Inhibit Growth (MCIG) was 7.97 to >14.7 mg/L. The Teratogenic Index (TI) was found to be 0.9 – 1.1. This TI range indicates that potassium perfluorooctanesulfonate has a low potential to be a developmental hazard.

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

DATA QUALITY

Reliability: Klimisch ranking: 2

Although these were well-conducted studies, the in-life phases were not conducted in accordance with Good Laboratory Practices.

REFERENCES

These studies were conducted at the University of Maryland Wye Research and Education Center (UM-WREC) in Queenstown, Maryland and at Wildlife International Ltd., Easton, MD at the request of the 3M Company. Lab Request number U2723

OTHER

Last changed: 6/12/01

RS-II-31: ACUTE ORAL TOXICITY TO TERRESTRIAL INVERTEBRATES (HONEY BEE)

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 field: The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20^{o}C prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OECD Guideline 213, EPPO Guideline 170
Test type: Acute Oral
GLP: Yes
Year Completed: 2001
Species: *Apis mellifera L.*
Analytical monitoring: None – nominal concentrations
Test honey bee source: Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.
Test honey bee age at study initiation: Young adult
Test honey bee type: Worker honey bees, free of acarine, nosema and amoeba.
Varroacide treatment: None within the 4 weeks prior to test initiation.

Test conditions

Humidity: 65% $\pm$ 5%
Temperature: 25 $\pm$ 2^{o}C
Lighting: Conducted in darkness
Stock and test solutions preparation:
Test substance: Initial stock solution prepared in analytical grade acetone to a final concentration of 47.8 µg PFOS/µL (nominal concentration). Final test concentrations prepared from dilutions of this solution with 50% w/v sucrose. Resulting acetone concentration was 5%.
Reference toxicant: Primary stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Secondary stock solutions were made by diluting the primary stock solution in deionized water containing 1 g/L Triton X-100. Final test concentrations prepared from dilutions of these solutions with 50% w/v sucrose.
Stability of the test chemical solution: A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all

solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.
Exposure vessels: Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.
Feeding: During the first four hours of the test, bees provided with 50% w/v aqueous sucrose solutions containing the appropriate PFOS dose. After 4-hours, dosed sucrose removed, and bees provided with 50% w/v aqueous sucrose solutions, continuously available through the end of the exposure period.
Number of replicates: Three
Number of bees per replicate: Ten
Negative control: 50% w/v sucrose
Solvent control: 50% w/v sucrose plus 5% acetone
Reference substance: Dimethoate
Reference substance control: Triton X-100
Number of concentrations: five plus a negative and a solvent control
Dose administration: The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each group of 10 bees was offered 0.2 mL of a given test concentration or control solution. The dose was measured into a small, pre-weighed, glass feeder within the cage using a variable volume pipette. This volume of solution is equivalent to 20 µL per bee.
Dose frequency: Once, for 4 hours of exposure
Dose calculation: Feeders were weighed after removal from the cages to determine the dose consumed per bee.
Element basis: Mortality

RESULTS

Nominal concentrations: Negative control (sucrose only), acetone + sucrose control, 0.205, 0.450, 0.991, 2.17, 4.78 µg/bee
Element value and 95% confidence interval:

24-hour LD_{50} = 0.72 (0.60 – 0.85) µg/bee
48-hour LD_{50} = 0.46 (0.32 – 0.55) µg/bee
72-hour LD_{50} = 0.40 (0.33 – 0.48) µg/bee

72-hour NOEL = 0.21 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test ($p<0.05$)
Biological observations: There was significant mortality at all doses above a mean intake of 0.21 µg/bee with a steep dose response between mean intakes of 0.45 and 2.2 µg/bee.

Cumulative percent mortality:

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	3.3	3.3	3.3
0.205	0	0	6.7	10
0.450	0	20	50	60
0.991	0	70	93	97
2.17	6.7	100	100	100
4.78	30	100	100	100

Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	0	0	0
0.205	0	0	0	0
0.450	0	3.3 (S)	3.3 (K)	3.3 (K)
0.991	0	0	0	0
2.17	0	0	0	0
4.78	10 (K)	0	0	0

Control response: satisfactory
Reference toxicant response: satisfactory - dimethoate 72-hour LD_{50} = 0.11 µg/bee

CONCLUSIONS

The potassium perfluorooctanesulfonate 72-hour oral LD_{50} for the honey bee was determined to be 0.40 µg/bee with a 95% confidence interval of 0.33 – 0.48. The 72-hour no observed effect

level was 0.21 µg/bee. The dose response was steep between a mean uptake of 0.45 and 2.2 µg/bee.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

OTHER

Last changed: 5/1/01

RS-II-32: ACUTE CONTACT TOXICITY TO TERRESTRIAL INVERTEBRATES (HONEY BEE)

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 field: The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20^{o}C prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: USEPA OPPTS 850.3020 (draft), OECD Guideline 214, EPPO Guideline 170
Test type: Acute Contact
GLP: Yes
Year Completed: 2001
Species: *Apis mellifera L.*
Analytical monitoring: None – nominal concentrations
Test honey bee source: Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.
Test honey bee age at study initiation: Young adult
Test honey bee type: Worker honey bees, free of acarine, nosema and amoeba.
Varroacide treatment: None within the 4 weeks prior to test initiation.

Test conditions:

Humidity: 65% $\pm$ 5%
Temperature: 25 $\pm$ 2^{o}C
Lighting: Conducted in darkness
Stock and test solutions preparation:

Test substance: Stock solution prepared in analytical grade acetone to a final concentration of 90.4 µg PFOS/µL (nominal concentration). Final test concentrations prepared in acetone from dilutions of this solution.
Reference toxicant: Stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Final test concentrations prepared from dilutions of this solution.
Stability of the test chemical solution: A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.

Exposure vessels: Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.
Feeding: 50% w/v aqueous sucrose solution, continuously available
Number of replicates: Three
Number of bees per replicate: Ten
Negative control: Undosed
Solvent control: Acetone
Reference substance: Dimethoate
Reference substance control: Triton X-100
Number of concentrations: five plus a negative and a solvent control
Dose administration: The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each bee was dosed on the thorax with a 1 µL drop of a given test item concentration or 1µL acetone before being placed into the test chamber.
Dose frequency: Once
Element basis: Mortality

RESULTS

Nominal concentrations: Negative control, acetone control (1.0 µ/bee), 1.93, 4.24, 9.30, 20.5, 45, µg PFOS/bee
Element value and 95% confidence interval:

24-hour LD_{50} = 38.9 (28.2 – 71.2) µg/bee
48-hour LD_{50} = 10.4 (8.2 – 13.0) µg/bee
72-hour LD_{50} = 6.0 (4.7 – 7.6) µg/bee
96-hour LD_{50} = 4.78 (3.8 – 5.8) µg/bee

96-hour NOEL = 1.93 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test ($p<0.05$)

Biological observations: There was significant mortality at all doses above 1.93 µg/bee with a steep dose response between 4.24 and 9.30 µg/bee

Cumulative percent mortality:

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	3.3	3.3	3.3
Solvent Control	3.3	3.3	3.3	3.3	3.3
1.93	0	6.7	6.7	13	13
4.24	0	0	13	37	37
9.30	0	6.7	40	63	90
20.5	0	40	93	97	100
45.0	0	50	93	100	100

Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0	0
Solvent Control	0	0	0	0	0
1.93	3.3 (K)	0	0	0	0
4.24	0	0	0	0	0
9.30	0	0	0	0	3.3 (K)
20.5	0	3.3 (K)	0	3.3 (K)	0
45.0	0	3.3 (K)	3.3 (K)	0	0

Control response: satisfactory
Reference toxicant response: satisfactory - dimethoate 96-hour LD_{50} = 0.19 µg/bee

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour contact LD_{50} for the honey bee was determined to be 4.78 µg/bee with a 95% confidence interval of 3.8 – 5.8. The 96-hour no observed effect level was 1.93 µg/bee. The dose response was steep between 4.24 and 9.30 µg/bee.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

OTHER

Last changed: 5/1/01

RS-II-33: ACUTE TOXICITY TO THE EARTHWORM (*EISENIA FETIDA*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: The study was conducted using a protocol based on procedures outlined in the Organization for Economic Cooperation and Development (OECD) Guideline No. 207.
Test type: Static, artificial soil substrate
GLP: Yes
Year Completed: 2002
Species: *Eisenia fetida*
Analytical monitoring: Test substance concentrations in soil measured by LC/MS/MS at Days 0 and 14; in earthworm tissues after test termination.
Statistical methods: LC_{50} values calculated by moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan. Treatment group means were compared to control means with Dunnett's test ($\alpha = 0.05$) using SAS Version 8 to determine if significant differences from the control occurred.
Test organism source: Obtained from Worm Man's Worm Farm, R & K Trading Co., Monroe Township, NJ
Test organism age at study initiation: Adult (with clitellum)

Test conditions:

Test media: Artificial soil of the following composition:

Quartz sand	31.5 kg
Kaolin clay	9.0 kg
Sphagnum peat	4.5 kg
Calcium carbonate	1.125 kg

Artificial soil prepared in bulk by blending 70% sand, 20% kaolin clay and 10% sphagnum peat. The pH of the bulk soil prior to hydration was adjusted to 6.0 using calcium carbonate. Moisture content adjusted to ~33% by weight.

Lighting: Fluorescent lights, intensity measured as 494-775 lux. Photoperiod was 24-hours of continuous light.

Stock and test solutions preparation: The test substance was first dry mixed with the test soil. Approximately 4 kg of each test concentration and the negative control was prepared separately. The soil, test substance and deionized water were mixed together for 20 minutes prior to separation into the appropriate exposure vessel. Test soils were

prepared and held overnight at approximately 20°C prior to adding worms to allow the soil to become less fluid.

Exposure vessels: One liter Nalgene® beakers containing approximately 750 g of prepared soil, except for the 625 mg/kg (measured) concentration, where only 650 g would fit in the vessel. The beakers were covered with plastic wrap, which was perforated for air exchange.

Number of replicates: four

Number of test organisms per replicate: ten

Number of concentrations: five plus a negative control

Test media parameters:

Initial and final % moisture (controls):	33.9, 32.1
Initial and final % moisture (1042 mg/kg):	32.5, 34.5
Initial and final soil pH in water (controls):	6.9, 8.3
Initial and final soil pH in water (1042 mg/kg):	8.0, 8.3
Initial and final Temp., C° (controls):	22, 22
Initial and Final Temp., C° (1042 mg/kg):	22, 25[1]

[1] One outlier of 25°C

Element Basis: LC_{50} - mortality. NOEC - burrowing behavior, body weight and clinical signs of toxicity. At test initiation, worms were placed on the surface of the soil in each test chamber and observed for burrowing behavior. On Day 7 and Day 14, the contents of each test chamber were removed to determine the number of surviving worms and to evaluate sublethal effects. On Day 7, following observations, test soil was returned to the test chambers and the worms were placed on the soil surface in order to again observe burrowing behavior.

Method of calculating means of measured concentrations: Arithmetic mean

RESULTS

Nominal concentrations: <LOQ, 78.1, 156, 313, 625, 1250 mg/kg

Measured concentrations (Day 0): <LOQ, 77.0, 141, 289, 488, 1042 mg/kg (as dry weight)

Element values:

7-day LC_{50} (95% C.I.):	398 (289 – 488) mg/kg
7-day NOEC:	289 mg/kg
7-day LOEC:	488 mg/kg
14-day LC_{50} (95% C.I.):	373 (316 – 440) mg/kg
14-day NOEC:	77 mg/kg
14-day LOEC:	141 mg/kg

All element values were calculated using the Day 0 measured concentrations in the test soils.

Statistical Evaluation: The 7-day LC_{50} value and 95% confidence interval were calculated by binomial probability. The 14-day element values were determined using the moving average method.

Analytical Methodology: Analyses of test soils and earthworms were performed at Wildlife International Ltd. using high performance liquid chromatography with triple quadrupole mass

spectrometric detection (HPLC/MS/MS). When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components.

On Day 14, following observations and body weight determination, all surviving worms from each treatment group were placed on moistened filter paper and allowed to purge their gut contents for approximately 24 hours. The worms were then euthanized by freezing and stored frozen prior to analysis. The LOQs (limits of quantitation) in soil and earthworms were 29.3 and 50.0 mg/kg, respectively, in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 87.3% in the soil and 86.6% in the earthworm tissue. Soil samples collected at test initiation had measured values from 78.1% to 98.6% of nominal. Measured values for samples taken on Day 14 ranged from 87.5 to 103% of nominal.

Summary of analytical chemistry data for soil, Day 0:

Nominal Test Concentration, mg/kg	Measured Replicate Values, mg/kg (dry weight)	Mean Concentration, mg/kg (dry wt)	Percent of Nominal
Negative Control	All < LOQ (29.3)	<LOQ (29.3)	-
78.1	74.6, 72.7, 104, 74.7, 69.4, 66.5	77.0	98.6
156	144, 138	141	90.4
313	312, 266	289	92.3
625	526, 449	488	78.1
1250	1152, 906, 894, 930, 1405, 966	1042	83.4

Summary of analytical chemistry data for soil, Day 14:

Nominal Test Concentration, mg/kg	Measured Replicate Values, mg/kg (dry weight)	Mean Concentration, mg/kg (dry wt)	Percent of Nominal
Negative Control	All < LOQ (29.3)	<LOQ (29.3)	-
78.1	74.9, 86.2	80.6	103
156	143, 142	143	91.7
313	302, 308	305	97.4
625	532, 735	634	101
1250	1059, 1129	1094	87.5

Summary of analytical chemistry data for earthworm(1), Day 14:

Day 0 Mean Soil Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg (wet weight)	Mean tissue Concentration, mg/kg (wet weight)
Negative Control	All < LOQ (50.0)	<LOQ (50.0)
77.0	189, 201	195
141	209, 196	203
289	240, 263	252
488	1105(2)	1105(2)
1042	No surviving worms	-

(1) All surviving worms from each treatment group were placed on moistened filter paper for approximately 24 hours to purge gut contents. The entire mass of worms collected from each treatment group was composited, euthanized by freezing and stored frozen until analyzed.
(2) Results may be unreliable as limited number of worms were available for analysis

Biological observations after 96-hours: Earthworms in the negative control, and the 77.0 mg/kg treatments appeared healthy and normal throughout the test with no mortality or overt clinical signs of toxicity. On Day 7 there was 90% and 100% mortality in the 488 mg/kg group and the 1042 mg/kg group, respectively. On Day 14, the 141, 289, and 488 mg/kg groups

exhibited 7.5, 2.5 and 95% mortality, respectively. Worms in the 488 and 1042 mg/kg groups exhibited an aversion to the soil during observations of burrowing behavior on Day 0. On Day 0 after one hour, some worms in the 488 mg/kg group were not burrowed, and in the 1042 mg/kg group many remained on the soil surface. After two hours, all but one worm in the 488 mg/kg group had burrowed but in the 1042 group most worms remained out of the soil. On Day 7, all surviving worms burrowed into the soil within 15 minjutes. On Day 7, worms found dead in the 1042 mg/kg group were on the side of the test chamber, not in the soil.

Cumulative percent mortality:

Day 0 Mean Soil Concentration, mg/kg	Day 7	Day 14
Neg. Control	0	0
77.0	0	0
141	0	7.5
289	0	2.5
488	90	95
1042	100	100

Control response: Satisfactory

Average Earthworm Body Weights:

Day 0 Mean Soil Concentration, mg/kg	Day 0 weight, g	Day 14 Weight, g	Mean Total Weight Change, g
Neg. Control	0.29, 0.23, 0.23, 0.26	0.21, 0.19, 0.18, 0.20	-0.06
77.0	0.25, 0.25, 0.26, 0.25	0.20, 0.19, 0.20, 0.18	-0.06
141	0.26, 0.27, 0.23, 0.26	0.21, 0.17, 0.14, 0.20	-0.07
289	0.27, 0.25, 0.23, 0.25	0.17, 0.17, 0.16, 0.18	-0.08
488	0.24, 0.25,0.23, 0.24	0.08*	-0.17*
1042	0.25, 0.25, 0.24, 0.25	No surviving worms	-

*Statistically different ($p < 0.05$) from the control group using Dunnett's test.

CONCLUSIONS

The perfluorooctanesulfonate, potassium salt 14-day LC_{50} for the Earthworm, *Eisenia fetida,* was determined to be 373 mg/kg with a 95% confidence interval of 316 - 440 mg/kg. The 14-day no observed effect concentration was 77 mg/kg.

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

DATA QUALITY

Reliability: Klimisch ranking: 1

REFERENCES

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

OTHER

Last changed: 6/17/02

RS-II-34: DIETARY ACUTE MALLARD DUCK STUDY

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, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2
Type: Dietary acute
GLP: Yes
Year completed: 2000
Species: *Anas platyrhynchos*
Supplier: Whistling Wings, Inc., Hanover, IL
Analytical monitoring: PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.
Test phases: Acclimation – 9 days
Exposure - 5 days
Post-exposure observation – 3 or 17 days
Statistical methods: LC_{50} values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.
Test bird age: 10 days
Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors in batteries of thermostatically controlled brooding pens. Floor space of each pen measured approximately 62 X 90 cm. Ceiling height was approximately 25.5 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.
Identification: Each group of birds identified by pen number and test concentration. Individuals identified by wing bands.
Number of replicates: Six for controls, two for each treatment group
Number of ducks per replicate: five
Number of concentrations: Eight plus a negative control
Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration:

Ingredients	Percent
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Ferm (Fermatco)[1]	0.25
Salt Iodized	0.10

[1]Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix:

Vitamin or Mineral	Amount Per Ton
Vitamin D_3	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B_{12}	8 mg
Folic Acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione dimethylpyrimidinol bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 grams

Prophylaxis: None
Brooding compartment mean temperature: $38 \pm 2^{o}C$
Ambient room mean temperature: $25.2 \pm 0.7^{o}C$
Average relative humidity: $53 \pm 18\%$
Photoperiod: Sixteen hours light per day

Lighting: fluorescent lights which closely approximate noon-day sunlight; average approximately 207 lux.

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the 9.1 ppm and six from the lowest and highest concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Two verification samples from the remaining concentrations and one from the control were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Bk control, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm
Measured concentrations: <LOQ, 9.8, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm
Element value:

Dietary LC_{50} = 628 (448 – 958) ppm
No mortality concentration = 146 ppm
NOEC (body weight gain) = 36.6 ppm

All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102 - 108% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 94 – 130% of nominal.

Summary of analytical chemistry data:

Homogeneity in Avian Diet:

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
9.1	9.52, 9.70, 9.79, 8.09, 10.9, 10.5	9.8	108
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9	19.5	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in Avian Diet:

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	45.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293	274, 307	291	99
586	550, 523	537	92

Ambient Stability in Avian Diet:

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	-
9.8	12.3, 11.0	11.7	119
19.5	18.2, 19.7	19.0	97
40.2	47.9, 56.8	52.4	130
74.5	77.6, 77.9	77.8	104
174	167, 160	164	94.3
291	297, 293	295	101
537	552, 530	541	101
1196	1150, 1122	1136	95

Biological observations:

Survival and clinical observations: No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. The first deaths occurred on day 4 in the 1171 ppm treatment. Mortality occurred through Day 8 in all dose groups $\geq$ 293 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations $\leq$ 146 ppm. Birds at all concentrations $\geq$ 293 ppm displayed signs of toxicity including reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy and lower limb weakness. Birds at the 1171 ppm level also displayed prostrate posture, depression and convulsions through Day 8. Recovery with normal appearance and behavior was noted from Day 9 through test termination

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations $\leq$ 36.6 ppm. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups. There was a statistically significant ($p < 0.05$) reduction in weight gain at the 9.1 ppm level for the Day 0-5 and Day 5-8 periods. However, differences from the control group at the 9.1 ppm level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm level, and were not dose responsive. Therefore, these differences were not considered treatment related. Marked, treatment-related, concentration responsive effects on body weight was noted in concentrations > 73.2 ppm for Days 0-5; Day 5-8 post-exposure weight gain continued to be reduced at concentrations $\geq$ 293 ppm.

Feed Consumption: When compared to the control group, there was a marked reduction in feed consumption in the treatment groups $\geq$ 293 ppm throughout the study.
Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, empty crops, and empty gastrointestinal tracts. These necropsy findings were considered to be treatment related.

Percent Cumulative Mortality:

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
9.1	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0
73.2	0	0	0	0	0	0	0	0
146	0	0	0	0	0	0	0	0
293	0	0	0	0	0	0	10	20
586	0	0	0	0	10	20	30	30
1171	0	0	0	20	60	80	90	90

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

Body Weight (grams):

	Exposure Period		Recovery Period				
Nominal Concentration, ppm	**Mean Body Weight, Day 0**	**Mean Body Weight Change Day 0-5**	**Mean Body Weight Change Day 5-8**	**Mean Body Weight Change Day 8-15**	**Mean Body Weight Change Day 15-22**	**Mean Total Body Weight Change Day 8-22**	**Mean Body Weight, Day 22**
Negative Control	135	144	101	230	183	413	823
9.1	119	108	91	230	198	427	773
18.3	146	131	100	241	186	427	811
36.6	147	128	100	243	208	451	828
73.2	143	117	82	216	203	418	782
146	143	100	89	232	124	356	688
293	129	32	57	256	234	490	701
586	144	-6	36	221	219	439	613
1171	147	-37	1531	198	251	449	634

Mean Average Feed Consumption:

Nominal Concentration, ppm	Grams Feed/Bird/Days 0-5	Grams Feed/Bird/Days 6-8	Grams Feed/Bird/Days 8-15	Grams Feed/Bird/Days 15-22
Negative Control	92	125	171	180
9.1	73	117	172	198
18.3	91	132	186	204
36.6	94	125	165	179
73.2	77	101	148	173
146	105	159	159	164
293	44	63	109	132
586	36	55	114	143
1171	22	25	106	154

Gross Pathological Observations from Birds that Died in Study:

Finding	293 ppm N = 2	586 ppm N = 3	1171 ppm N = 9
Crop empty	0	2	7
Emaciated	1	1	4
G.I. Tract, primarily empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents, black and tar-like	0	0	1
Keel prominent	0	0	2
Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, grey	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4

CONCLUSIONS

The dietary LC50 value for Mallard Duck exposed to PFOS was determined to be 628 ppm with a 95% confidence interval of 448 to 958 ppm. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm. Based upon reductions in body weight gain at the 73.2 ppm test concentration, the no observed effect concentration was 36.6 ppm.

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

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: 5/1/00

RS-II-35: DIETARY ACUTE NORTHERN BOBWHITE STUDY

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

METHOD

Method: OPPTS 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2
Type: Dietary acute
GLP: Yes
Year completed: 1999 (Test), 2000 (Report)
Species: *Colinus virginianus*
Supplier: Wildlife International Ltd. Production Flock, Easton, Maryland
Analytical monitoring: Test substance concentration in standards and samples were determined by reversed-phase HPLC and mass spectroscopy. PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.
Test phases: Acclimation – 10 days
Exposure - 5 days
Post-exposure observation – 3 or 17 days
Statistical methods: LC_{50} values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.
Test bird age: 10 days
Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors in batteries of thermostatically controlled brooding pens. Each pen's floor space measured approximately 72 X 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.
Identification: Each group of birds identified by pen number and test concentration. Individuals identified by leg bands.
Number of replicates: Six for controls, two for each treatment group
Number of bobwhite per replicate: five
Number of concentrations: seven plus a negative control
Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration:

Ingredients	Percent
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Ferm (Fermatco)[1]	0.25
Salt Iodized	0.10

[1] Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix:

Vitamin or mineral	Amount Per Ton
Vitamin D_3	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B_{12}	8 mgs
Folic Acid	600 mgs
Biotin	64 mgs
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione dimethylpyrimidinol bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 grams

Prophylaxis: None
Brooding compartment mean temperature: $38 \pm 2^{o}C$
Ambient room mean temperature: $27.3 \pm 1.2^{o}C$
Average relative humidity: $31 \pm 14\%$
Photoperiod: Sixteen hours light per day

Lighting: fluorescent lights which closely approximate noon-day sunlight; average of approximately 139 lux of illumination

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the 18.3 ppm concentration and six from the 1171 ppm concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Verification samples of the other treatment groups (two samples from each) and the control (one sample) were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Bk control, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm
Measured concentrations: <LOQ, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm
Element value:

Dietary LC_{50} = 220 (164 – 289) ppm
No mortality concentration = 73.2 ppm
NOEC (body weight gain) = 73.2 ppm

All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102-107% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 101-122% of nominal.

Summary of analytical chemistry data:

Homogeneity in Avian Diet:

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9,	19.5	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in Avian Diet:

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	45.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293	274, 307	291	99
586	550, 523	537	92

Ambient Stability in Avian Diet:

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	-
19.5	19.2, 19.9	19.6	101
40.2	44.4, 53.8	49.1	122
74.5	76.4, 77.9	77.2	104
174	177, 174	176	101
291	318, 315	317	109
537	560, 665	613	114
1196	1260, 1187	1224	102

Biological observations:

Mortalities and clinical observations: One incidental mortality occurred in the control group as a result of a broken leg on the morning of Day 5. It was subsequently euthanized on Day 6. Two other birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were observed to be normal in appearance and behavior throughout the test.

The first treatment-related mortalities occurred on Day 3 in the 586 and 1171 ppm treatment groups. Mortality occurred through Day 8 in all dose groups $\geq$ 146 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations $\leq$ 73.2 ppm.

There was 11% mortality in the 146 ppm treatment group, and two additional birds displayed clinical signs of toxicity (wing droop). All other birds in this test group displayed normal appearance and behavior for the duration of the test. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 80% mortality (occurring on Days 5,6, and 7) for birds in the 293 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 100% mortality (occurring from Day 3 through Day 7) for birds in the 586 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

There was 100% mortality (occurring from Day 2 (noted on Day 3 for Day 2 afternoon) through Day 4) for birds in the 1171 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, and lower limb rigidity.

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations $\leq$ 73.2 ppm. During Days 0-5, statistically significant reductions in body weight gain or body weight loss occurred in the 146, 293, and 586 ppm treatment groups. Body weight effects could not be determined for test organisms in the 1171 ppm group due to total mortality.

Feed Consumption: No apparent treatment related effects were noted for feed consumption for birds in concentrations $\leq$146 ppm. Reduced feed consumption was noted for birds in treatment groups $\geq$ 293 ppm from Days 0-5. No treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period were observed.

Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. These necropsy findings were considered to be treatment related. The single bird euthanized from the 293 ppm treatment was found to have treatment related necropsy findings. Necropsy results for all other birds euthanized on Day 8 and Day 22 were unremarkable.

% Cumulative Mortality:

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0
73.2	0	0	0	0	0	0	0	0
146	0	0	10**	0	0	0	10	10
293	0	0	0	0	20	40	80	80
586	0	0	10	20	50	80	100	100
1171	0	0	30	100	100	100	100	100

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22
** Bird euthanized on day 3 after sustaining a broken leg.

Body Weight (grams):

	Exposure Period	Recovery Period					
Nominal Concentration(ppm)	**Mean Body Weight, Day 0**	**Mean Body Weight Change Day 0-5**	**Mean Body Weight Change Day 5-8**	**Mean Body Weight Change Day 8-15**	**Mean Body Weight Change Day 15-22**	**Mean Total Body Weight Change, Day 8-22**	**Mean Body Weight, Day 22**
Negative Control	20	+10	+8	+23	+22	45	82
18.3	21	+11	+9	+24	+23	47	87
36.6	20	+11	+8	+26	+24	50	89
73.2	20	+9	+7	+24	+20	44	79
146	20	+7*	+6**	+24	+21	45	79
293	20	-2**	-1**	+14	+20	34	55
586	20	-4**	--	--	--	--	--
1171	20	--	--	--	--	--	-

Note: Numbers may not add manually due to rounding. Values for 293 ppm treatment group are impacted by the fact that only one bird remained in that treatment group after Day 8.

*Statistically different from the control group at $p<0.05$ (Dunnett's t-test).
**Statistically different from the control group at $p=<0.01$ (Dunnett's t-test(--) = No data available due to mortality.Mean Average Feed Consumption

Nominal Concentration, ppm	Grams Feed/Bird/Days 0-5	Grams Feed/Bird/Days 6-8	Grams Feed/Bird/Days 8-15	Grams Feed/Bird/Days 15-22
Negative Control	9	10	9	13
18.3	9	11	10	12
36.6	8	12	14	15
73.2	10	13	13	15
146	9	10	11	14
293	5	9	8	9
586	6	19	--	--
1171	4	--	--	--

(--) = No data available due to mortality.Gross Pathological Observations from Birds that Died in Study

	Male, Female, and Undetermined (ppm)				
Finding	Control	146	293	586	1171
	N = 1	N = 2	N = 8	N = 10	Business Interruption = 10
Abdominal cavity, some autolysis	0	0	2	2	4
Abdominal cavity, autolysis throughout	0	0	0	1	1
Crop, empty	0	0	2	5	2
Emaciated	0	0	2	5	8
Fractured leg	1	1	0	0	0
G.I. Tract empty	0	0	1	1	0

Gizzard contents bile stained	0	0	2	5	1
Heart, anterior portion mottled white color	0	0	1	0	0
Heart, pale	0	0	0	2	1
Intestinal contents tar-like	0	0	0	2	0
Keel, prominent	0	0	1	3	10
Kidneys, pale	0	0	0	2	0
Liver, pale and mottled	0	1	0	0	0
Loss of muscle mass	0	0	4	7	9
Muscular-skeletal, pale	0	1	0	0	0
Small in stature	0	0	3	0	0
Spleen, black	0	0	0	1	0
Spleen, dark	0	0	0	0	2
Spleen, grey	0	0	0	1	0
Spleen, grey-brown	0	0	0	0	1
Spleen, pale	0	0	1	0	1
Spleen, small	0	0	0	0	1
Spleen, small and pale	0	0	0	3	0
Thin	0	0	0	4	2
Not remarkable	0	0	1	0	0

CONCLUSIONS

The dietary LC50 value for Northern Bobwhite exposed to perfluorooctanesulfonate was determined to be 220 ppm with a 95% confidence interval of 164 to 289 ppm. The slope of the concentration-response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm test concentration, the no observed effect concentration was 73.2 ppm.

Author and/or submitter: 3M Corporation, Environmental Laboratory
P.O. Box 33331
St. Paul, Minnesota, 55133

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

APPENDIX III

ROBUST SUMMARIES
OF
TOXICOLOGY, EPIDEMIOLOGY,
AND HEALTH STUDIES

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RS-III-1: 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

Subject description:

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

Health effects studied: PFOS levels in blood

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 (0.0.091-10.600); 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

RS-III-2: Clinical Chemistries, Hematology and Hormones from Voluntary Medical Surveillance of Male Fluorochemical Production Workers in 1995 and 1997.

TEST SUBSTANCE

Identity: PFOS (PFOA was also used as a surfactant at both sites.).

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 hormone).

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

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 the 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/m^2) 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.

RS-III-3: Serum Fluorochemical Levels in Sumitomo Employees.

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

RS-III-4: Descriptive Summary of Serum Fluorochemical Levels Among Employee Participants of the Year 2000 Antwerp Fluorochemical Medical Surveillance Program.

TEST SUBSTANCE

Identity: The extracts were quantitatively analyzed for PFOS (perfluorooctanesulfonate), PFOA (perfluorooctanoate, which is also known as C8 acid or POAA), PFHS (perfluorohexanesulfonate), PFOSAA (N-ethyl perfluorooctanesulfonamidoacetate), M570 (N-methyl perfluorooctanesulfonamidoacetate), PFOSA (perfluorooctanesulfonateamide) and M556 (perfluorooctanesulfonamidoacetate).

Remarks:

METHOD

Study design: Cross-sectional analysis of serum fluorochemical levels and work assignments from questionnaire data obtained from the medical surveillance examinations conducted in 2000.

Manufacturing/Processing/Use: 3M Antwerp, Belgium chemical plant

Hypothesis tested: The purpose of this report is to provide the results from the year 2000 Antwerp fluorochemical medical surveillance program. Observations were reported in relation to the production processes.

Study period: Spring 2000

Setting: Antwerp medical surveillance examinations of chemical plant employees

Total population: Approximately 340 employees were eligible to participate.

Subject selection criteria: Voluntary participation by employees

Total # of subjects in study: 258 chemical plant, research and development, and site employees participated (209 males, 49 females). Until recently, all female employees were engaged in nonproduction activities.

Comparison population: N/A

Participation rate: 76%

Subject description: Current full-time employees at the Antwerp chemical plant or current full-time employees with site-wide responsibilities at the Antwerp site which includes chemical and film plant operations.

Total number of person-years: N/A

Data collection methods: In addition to obtaining a blood sample for fluorochemical analysis, a standard battery of clinical chemistry, pulmonary function and urinalysis tests were performed on those employees who chose to participate. A work history questionnaire was also included.

Exposure period: Variable depending upon an employee's work history

Description/delineation of exposure groups/categories: Job classifications were

developed and used per the categories used on the work history questionnaire. These job titles included Building 3 operator, fluorel operator, Building 16 operator, Building 2 warehouseman, mechanic, electrician, boiler operator, process engineer, nonprocess engineer, QC/QA laboratory worker, R&D laboratory worker, supervisor and manager. Exposure categories were also defined based on production areas.

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS, PFOA, PFHS PFOSAA, M570, PFOSA and M556 using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluated versus an extracted curve from a human serum matrix. Endogenous levels of certain fluorochemical were determined in the standard serum matrix and additional fluorochemical was spiked into the matrix. The total amount of each specific fluorochemical (endogenous + spiked) was used to construct an extracted standard curve. All serum fluorochemical analyses were determined by Northwest Bioanalytical Laboratory Inc. (Salt Lake City, UT).

Statistical methods: Because the serum distributions for PFOS, PFHS, PFOA, PFOSAA, M570, PFOSA and M556 appeared log normally distributed, natural log transformations of the fluorochemicals were performed to calculate geometric means and 95% confidence intervals were calculated for the geometric mean. All employee serum values for PFOS and PFOA were above the respective lower limit of quantitation (LLOQ). There was one employee with a PFHS value below the LLOQ (0.0027 ppm) and one employee with a M570 below the LLOQ (0.0057 ppm). There were 111 employees with PFOSAA values below the LLOQ (0.006 ppm); 88 employees were below the LLOQ (0.001 ppm) for PFOSA; and 13 employees were below the LLOQ (0.0043 ppm) for M556. For statistical purposes, serum fluorochemical values that were less than the LLOQ were assumed to be at the midpoint between zero and the LLOQ. Therefore, for those employees with serum values reported at the LLOQ for PFHS, M570, PFOSAA, PFOSA and M556, they were assigned for calculations in this report the values of 0.00135 ppm, 0.00285 ppm, 0.003 ppm, 0.0005 ppm and 0.00215 ppm, respectively.

Other methodological information:

RESULTS

Describe results: Overall, the geometric mean (95% confidence interval in parenthesis) for perfluorooctanesulfonate (PFOS) was 0.44 ppm (95% CI 0.38-0.51) and for perfluorooctanoate (PFOA) was 0.33 ppm (95% CI 0.27-0.40). An analysis of the self-reported data of the workplace questionnaire in conjunction with the employees' serum fluorochemical levels suggested that the greatest potential for workplace exposure to POSF-based chemicals occurred in Building 03 and to PFOA in the glass unit in Building 16. For example production and maintenance operations in Building 03 had serum PFOS and PFOA geometric mean values of 1.11 ppm (95% CI 0.86 - 1.43) and 1.22 ppm (95% CI 1.11 - 1.48), respectively; whereas in Building 16 these values were 0.49 ppm (95% CI 0.42 - 0.59) and 1.93 ppm (95% CI 1.62 - 2.73), respectively. Administrative and laboratory workplace areas resulted in geometric mean employee serum PFOS and PFOA levels (< 0.5 ppm, on average) that were lower than those observed in the production

areas.

Study strengths and weaknesses: Besides PFOS, the study measured for 6 other fluorochemicals in employees' sera and related these data to their current work assignments. The study did not provide exposure information. The voluntary nature of medical surveillance exams raises the issue of potential volunteer bias. However, it should be not that the Antwerp findings were comparable, albeit somewhat lower, than the Decatur 2000 fluorochemical results. The latter were comparable to those reported in a 1998 random sample study of Decatur employees. The Antwerp findings were also comparable to the previous fluorochemical sera samples that were analyzed in 1997.

Research sponsors: 3M Medical Department

Consistency of results: The Antwerp employee sera findings were approximately 0.5 ppm lower, on average, than the Decatur 2000 fluorochemical results. The latter were comparable to those reported in a 1998 random sample study of Decatur employees. The Antwerp findings were also comparable to the previous fluorochemical sera samples from Antwerp employees that were analyzed in 1997.

CONCLUSIONS

A total of 258 Antwerp employees (76%) participated in the 2000 Antwerp fluorochemical medical surveillance program. The geometric mean for PFOS was 0.44 ppm (95% CI 0.38-0.51) for Antwerp employees in 2000. The year 2000 Antwerp employee sera PFOS levels were similar to the distribution reported in 1997. Administrative and laboratory workplace areas resulted in geometric mean employee serum PFOS and PFOA levels (< 0.5 ppm, on average) that were lower than those observed in the production areas.

REFERENCE

Olsen GW, Schmickler MN, Tierens JM, Logan PW, Burris JM, Lundberg JK, Mandel JH. Descriptive Summary of Serum Fluorochemical Levels Among Employee Participants of the Year 2000 Antwerp Fluorochemical Medical Surveillance Program. 3M Final Report. March 19, 2001.

RS-III-5: Descriptive Summary of Serum Fluorochemical Levels Among Employee Participants of the Year 2000 Decatur Fluorochemical Medical Surveillance Program.

TEST SUBSTANCE

Identity: The extracts were quantitatively analyzed for PFOS (perfluorooctanesulfonate), PFOA (perfluorooctanoate, which is also known as C8 acid or POAA), PFHS (perfluorohexanesulfonate), PFOSAA (N-ethyl perfluorooctanesulfonamidoacetate), M570 (N-methyl perfluorooctanesulfonamidoacetate), PFOSA (perfluorooctanesulfonateamide) and M556 (perfluorooctanesulfonamidoacetate).

Remarks:

METHOD

Study design: Cross-sectional analysis of serum fluorochemical levels and work assignments from questionnaire data obtained from the medical surveillance examinations conducted in 2000.

Manufacturing/Processing/Use: N/A

Hypothesis tested: The purpose of this report was to provide the results from the year 2000 fluorochemical medical surveillance program relating serum fluorochemical levels to workplace assignments and history. Medical results were to be reported elsewhere.

Study period: Spring, 2000

Setting: Decatur medical surveillance examinations of chemical plant employees

Total population: Approximately 500 Decatur chemical plant and site employees were eligible to voluntarily participate in the fluorochemical medical surveillance program.

Subject selection criteria: Voluntary participation

Total # of subjects in study: 263

Comparison population: N/A

Participation rate: 52%

Subject description: Current full-time employees at the Decatur chemical plant or current full-time employees with sitewide responsiblities at the Decatur site which includes chemical and film plant operations.

Total number of person-years: N/A

Data collection methods: In addition to obtaining a blood sample for fluorochemical analysis, a standard battery of clinical chemistry, pulmonary function and urinalysis tests were performed on those employees who chose to participate. A two-page work history questionnaire was also included.

Exposure period: Variable depending upon an employee's work history

Description/delineation of exposure groups/categories: A review of the employees job titles by an industrial hygienist and an epidemiologist categorized the employees' self-

reported job titles into eight job classifications: cell operators, chemical operators, engineers/laboratory workers, maintenance, mill operators, waste operators, administrative assistants and managers. These eight job classifications were similar to those used in the 1998 random sample analysis. The one difference was that crew supervisors and team leaders that were categorized in the supervisor/manager job classification in 1998 were categorized in the chemical operator category in 2000. Although the job tasks did not change, the term "secretaries" which was used as a job classification in 1998 was defined as "administrative assistants" in 2000.

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS, PFOA, PFHS PFOSAA, M570, PFOSA and M556 using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluated versus an extracted curve from a human serum matrix. Endogenous levels of certain fluorochemical were determined in the standard serum matrix and additional fluorochemical was spiked into the matrix. The total amount of each specific fluorochemical (endogenous + spiked) was used to construct an extracted standard curve. All serum fluorochemical analyses were determined by Northwest Bioanaltyical Laboratory Inc. (Salt Lake City, UT).

Statistical methods: Because the serum distributions for PFOS, PFHS, PFOA, PFOSAA, M570, PFOSA and M556 appeared log normally distributed, natural log transformations of the fluorochemicals were performed to calculate geometric means and 95% confidence intervals. All employee serum values for PFOS, PFHS, PFOA and M570 were above the respective lower limit of quantitation (LLOQ). There were 8 employees who with PFOSAA were determined to be below the LLOQ (0.006 ppm); 111 employees were below the LLOQ for PFOSA (0.001 ppm); and 13 employees were below the LLOQ for M556 (0.0043 ppm). For statistical purposes, serum fluorochemical values that were less than the LLOQ were assumed to be the midpoint between zero and the LLOQ. Therefore, employees with serum values reported at below the LLOQ for PFOSAA, PFOSA and M556, were assigned the values 0.003 ppm, 0.0005 ppm and 0.00215 ppm, respectively.

Other methodological information:

RESULTS

Describe results: Except for perfluorooctanoate (PFOA), serum fluorochemical levels were comparable to those reported in a 1998 random sample of 126 Decatur chemical plant and site employees. The log normal distribution of serum PFOS measured in 2000 ranged from 0.06 to 10.06 ppm compared to the measured PFOS range of 0.09 to 10.60 ppm in the 1998 study. The geometric mean serum PFOS in 2000 was 0.91 ppm (95% CI 0.82 - 1.02) compared to 0.94 ppm (95% CI 0.79 - 1.13) in 1998. However, the serum PFOA geometric mean increased to 1.13 ppm in 2000 (95% CI 0.99-1.30) from 0.90 ppm (95% CI 0.72-1.12) in 1998. The largest increase in serum PFOA levels was observed among cell operators whose geometric mean level was 4.12 ppm (95% CI 2.91-5.84) in 2000 compared to 1.43 ppm (95% CI 0.42-4.83) in 1998. This was likely due to the increased production of perfluorooctanoic acid that had occurred at the Decatur site since 1999. Other geometric mean values reported in 2000 were PFHS (0.18 ppm, 95% CI

0.16 - 0.21); PFOSAA (0.04 ppm, 95% CI 0.001 - 0.04); M570 (0.11 ppm, 95% CI 0.10 - 0.13); PFOSA (0.002 ppm, 95% CI 0.001 - 0.02) and M556 (0.04 ppm, 95% CI 0.03 - 0.05). Chemical operators engaged in fluorochemical production activities in Building 3 had, on average, higher serum fluorochemical levels than chemical operators and mill operators engaged in Dyneon production activities in Buildings 4, 38, 51 and 61. For example, chemical operators who worked on the monomer team in Building 3 had PFOS and PFOA geometric mean levels of 2.23 (95% CI 1.68-2.95) and 3.64 (95% CI 3.04-4.34), respectively, whereas mill operators in Building 61 had geometric mean levels of 0.69 (95% CI 0.58-0.83) and 1.32, respectively.

Study strengths and weaknesses: Besides PFOS, the study measured for 6 other fluorochemicals in the employees' serum and related it to current work assignments. The study did not provide exposure information. The voluntary nature of medical surveillance exams raises the issue of potential volunteer bias although it should be noted that the study findings were consistent with those reported in the 1998 random sample study at the Decatur location.

Research sponsors: 3M Medical Department

Consistency of results: The associations observed between serum fluorochemical levels and the chemical plant workplace were consistent with the results observed in the 1998 random sample survey of Decatur chemical plant employees.

CONCLUSIONS

The year 2000 serum PFOS levels were comparable to the distribution observed in the 1998 random sample study. The geometric mean serum PFOS level was 0.91 ppm in 2000 compared to 0.94 ppm in 1998. The highest serum PFOS value measured in 2000 (10.06 ppm) was of the same person who had the highest serum PFOS value measured in 1998 (10.60 ppm). Serum PFOA levels were higher among cell operators in 2000 due to the increased production of perfluorooctanoic acid that occurred beginning in 1999 at the Decatur chemical plant. The geometric mean level of serum PFOA was 1.43 ppm (95% CI 0.42-4.83) among the cell operators tested in 1998 compared to 4.57 ppm (95% CI 1.85-7.99) among the cell operators tested in 2000. The other serum fluorochemical levels, by major job categories, were comparable between the two time periods.

REFERENCE

Olsen GW, Logan PW, Simpson CA, Burris JM, Burlew MM, Lundberg JK, Mandel JH. Descriptive Summary of Serum Fluorochemical Levels Among Employee Participants of the Year 2000 Decatur Fluorochemical Medical Surveillance Program. 3M Final Report. March 19, 2001.

RS-III-6: Descriptive Summary of Serum Fluorochemical Levels Among 236 Building Employees.

TEST SUBSTANCE

Identity: The extracts were quantitatively analyzed for PFOS (perfluorooctanesulfonate), PFOA (perfluorooctanoate, which is also known as C8 acid or POAA), PFHS (perfluorohexanesulfonate), PFOSAA (N-ethyl perfluorooctanesulfonamidoacetate), M570 (N-methyl perfluorooctanesulfonamidoacetate), PFOSA (perfluorooctanesulfonateamide) and M556 (perfluorooctanesulfonamidoacetate).

Remarks:

METHOD

Study design: Cross-sectional analysis of serum fluorochemical levels of employees engaged in fluorochemical research.

Manufacturing/Processing/Use:

Hypothesis tested: Objective of this program was to offer employees working in fluorochemical research, Specialty Materials Markets Division Environmental Health Safety and Regulatory (EHSR) activities the opportunity to have their blood tested for seven fluorochemicals.

Study period: Fall, 2000

Setting: Occupational (research laboratory and offices)

Total population: Total population eligible was estimated at 150 employees

Subject selection criteria: Voluntary offer to employees engaged in fluorochemical research to have their blood tested for seven fluorochemicals

Total # of subjects in study: 45

Comparison population: N/A

Participation rate: Voluntary participation rate was 30%.

Subject description: Research and EHSR employees

Total number of person-years: N/A

Data collection methods: Voluntary blood collection conducted in 236 Building by a medical technician from the 3M Medical Department

Exposure period: Variable depending upon each person's work history

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS, PFOA, PFHS PFOSAA, M570, PFOSA and M556 using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluated versus an extracted curve from a human serum matrix. Endogenous levels of certain fluorochemical were determined in the standard serum matrix and additional

fluorochemical was spiked into the matrix. The total amount of each specific fluorochemical (endogenous + spiked) was used to construct an extracted standard curve. All serum fluorochemical analyses were determined by Northwest Bioanaltyical Laboratory Inc. (Salt Lake City, UT).

Statistical methods: Because the serum distributions for PFOS, PFHS, PFOA, PFOSAA, M570, PFOSA and M556 appeared log normally distributed, natural log transformations of the fluorochemicals were performed to calculate geometric means and 95% confidence intervals. All employee serum values for PFOA and PFHS were above their respective lower limit of quantitations (LLOQ). There was one employee who was determined to be below the LLOQ for PFOS (0.0368 ppm). There were 34 (76%) employees who were below the LLOQ for PFOSAA (0.0042 ppm); 15 (33%) employees were below the LLOQ for M570 (0.0037 ppm); 43 (96%) employees were below the LLOQ for PFOSA (0.001 ppm) and 40 (89%) employees were below the LLOQ for M556 (0.0038 ppm).

Other methodological information:

RESULTS

Describe results: Geometric mean serum levels for PFOS , PFOA and PFHS were (95% confidence interval in parenthesis): 0.116 ppm (0.008-0.152); 0.053 ppm (0.037-0.076); and 0.018 ppm (0.013-0.024), respectively. The majority of the employees tested for PFOSAA, M570, PFOSA and M556 had values that were below the lower limit of quantitation

Study strengths and weaknesses: Weaknesses include the low voluntary participation rates and the cross-sectional nature of the program.

Research sponsors: 3M Medical Department

Consistency of results: The serum levels of PFOS, PFOA and PFHS were consistent with the levels observed among laboratory (both QA/QC and R&D) workers at the Antwerp (Belgium) and Decatur (Alabama) manufacturing facilities.

CONCLUSIONS

The serum fluorochemical levels of Building 236 laboratory and EHSR employees were, on average, one order of magnitude lower (or more) than those reported for production workers at Antwerp and Decatur. Building 236 laboratory workers may have, on average, 2- to 4- fold higher serum PFOS levels than those levels reported in the general (non-occupational) population.

REFERENCE

Olsen GW, Mandsen DC, Burris JM, Mandel JH. Descriptive summary of serum fluorochemical levels among 236 Building employees. 3M Final Report. March 19, 2001.

RS-III-7: Detection of Fluorochemicals in 35 Lots of Commercial Sera.

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

RS-III-8: 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

RS-III-9: Analysis of PFOS from Pooled Serum of Two Commercial Laboratories.

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

RS-III-10: A Pilot Study to Identify Fluorochemicals in Children With Limited Sera for Analysis.

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.

RS-III-11: Analysis for Fluorochemicals in 39 Individual Swedish Blood Samples.

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 as 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

REFERENCE

OTHER

RS-III-12: 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 sampled 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 Netherlands 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

RS-III-13: Identification of Fluorochemicals in Human Sera. I. American Red Cross Adult Blood Donors.

TEST SUBSTANCE

Identity: . The seven fluorochemicals analyzed were perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$); N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamidoacetate (M556, $C_8F_{17}SO_2N(CH)CH_2COO$-); perfluorooctanesulfonylamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{13}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

Remarks:

METHOD

Study design: Through cooperation with six American Red Cross blood banks, 645 serum samples from adult donors (ages 20-69, equally represented of both sexes) were obtained for fluorochemical analyses. Blood bank locations were Los Angeles (CA), Portland (OR), Minneapolis-St. Paul (MN), Charlotte (NC), Hagerstown (MD) and Boston (MA). Samples were void of personal identifiers. Age, gender and location were the only known demographic factors.

Manufacturing/Processing/Use: N/A

Hypothesis tested: Descriptive analysis of seven serum fluorochemicals in adult blood donors by age and gender across six geographic locations.

Study period: 2000-2001

Setting: American Red Cross blood banks from six geographical locations in the United States (Los Angeles, CA; Portland, OR; Minneapolis-St. Paul, MN; Charlotte, NC; Hagerstown, MD; Boston, MA

Total population: Blood donors participating in each location (N = ?)

Subject selection criteria: Total samples requested per each American Red Cross blood bank was 100. (10 samples per 10 year age intervals 20-29, 30-39, 40-49, 50-59 and 60-69 for each sex.)

Total # of subjects in study: Los Angeles (males = 63, females = 62); Portland (males = 56, females = 51); Minneapolis-St. Paul (males = 50, females = 50), Charlotte (males = 47, females = 49), Hagerstown (males = 59, females = 49), Boston (males = 57, females = 52)

Comparison population: N/A

Participation rate: N/A

Subject description: Only age and gender were known for each subject.

Total number of person-years: N/A

Data collection methods: Each American Red Cross blood bank provided approximately

100 samples (see above for specifics). Allocation was done by age and gender criteria needed (described above).

Exposure period: N/A

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposures: Sera samples were extracted and quantitatively analyzed for seven fluorochemicals (PFOS, PFOSAA, M570, M556, PFOSA, PFOA and PFHS) using high-pressure liquid chromatography/electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human plasma matrix. Also presented is a calculated total organic fluorine (TOF) index. TOF was the percent of each of the seven fluorochemicals' molecular weight that was attributed to organic fluorine [PFOS (64.7%); PFHS (61.9%); PFOA (69.0%); PFOSAA (55.3%); PFOSA (64.7%); M570 (56.6%) and M556 (58.1%)] multiplied by the ppb measured for each fluorochemical and then summed across all seven fluorochemicals. Evaluation of quality control samples injected during each analytical run indicated that the reported quantitative results may have varied, on average, up to 26 percent using human plasma calibration curves for all analytes except PFOSA which may have varied on average up to 43 percent.

Statistical methods: Measures of central tendency applicable to log normally distributed data (median, geometric mean) were used for descriptive analyses. In those instances where a sample was measured below the lower limit of quantitation (LLOQ), the midpoint between zero and the LLOQ was used for calculation of the geometric mean. An assessment of this midpoint assumption and how it affected the calculation of the geometric mean was performed using the 10^{th} and 90^{th} percentile values between zero and the LLOQ for those values <LLOQ. In order to minimize parametric assumptions in the estimation of extreme percentiles of the population, a bootstrap method was used to generate confidence intervals around the empirical percentiles for serum concentrations.

Other methodological information: Twenty-four samples were split and analyzed to provide an estimate of the reliability of the analyses conducted. The analytical laboratory was blind to the identity of these split samples. These analyses were performed concurrently with all other analyses of the study to minimize experimental error.

RESULTS

Describe results: Overall, the geometric mean measured concentration of PFOS was 34.9 ppb (95% CI 33.3-36.5). The measured PFOS concentration ranged from less than the lower limit of quantitation (LLOQ) of 4.1 ppb to 1656.0 ppb. The geometric mean for PFOS was significantly higher among males (37.8 ppb; 95% CI 35.5-40.3) than females (31.3 ppb; 95% CI 30.0-34.3). No significant difference was observed with age. Charlotte (NC) had the highest geometric mean serum PFOS concentration (51.5 ppb) and Boston (MA) the lowest (29.5 ppb); however, these mean differences narrowed upon adjustment of age and gender differences. Bootstrap analyses were used to calculate a 95% tolerance limit for PFOS of 88.5 ppb with an upper 95% confidence limit of 100.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS, PFOSAA and M570. The geometric mean and 95% tolerance limits of these fluorochemicals were, on average, an order of magnitude (or more) lower than PFOS.

PFOS and PFOA were highly correlated (r = .63). PFOS had lower correlations with PFOSAA (r = .42), PFHS (r = .38) and M570 (r = .20). The number of samples with measured PFOSA and M556 concentrations below the LLOQ prohibited meaningful statistical analyses for these compounds.

Study strengths and weaknesses: As with any interpretation of data obtained from a study population, questions arise regarding the representativeness and ability to generalize the data collected. Clearly, American Red Cross blood donors are a self-selected group from the United States population. Only age and gender information was known about the donors. No information was obtained about past exposure histories to fluorochemical-related chemistries and materials. Therefore, we suspect the selection process of donors used in this study resulted in a reasonable representation of the overall blood donor population that was providing blood at the time when these donors were sampled. It was not the purpose of this study to be viewed as generalizable to the diverse U.S. general adult population.

Research sponsors: 3M Medical Department

Consistency of results: These findings were consistent with previous, albeit limited, data reported by the 3M Company that suggested the average serum PFOS concentration in human serum approximates 30 to 40 ppb.

CONCLUSIONS

The findings from this analysis of serum PFOS concentrations are consistent with those previously reported. The human data, to date, suggests the approximate average serum concentration in non-occupational adult populations may be 30 to 40 ppb with 95% of the adult population's serum PFOS concentrations below 100 ppb. Since serum PFOS concentrations likely reflect cumulative human exposure, this information will be useful for risk characterization.

REFERENCE

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH, Zobel LR. Identification of Fluorochemicals in Human Sera. III. American Red Cross Adult Blood Donors. 3M Final Report, February 25, 2002.

RS-III-14: Identification of Fluorochemicals in Human Sera. II. Elderly Participants of the Adult Changes in Thought Study, Seattle, Washington.

TEST SUBSTANCE

Identity: . The seven fluorochemicals analyzed were perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$); N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamidoacetate (M556, $C_8F_{17}SO_2N(CH)CH_2COO^-$); perfluorooctanesulfonylamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{13}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

Remarks:

METHOD

Study design: A total of 238 serum samples from elderly volunteers from a large prospective longitudinal study designed to examine cognitive function among male and female subjects, ages 65-96, in the Seattle (WA) area were obtained for fluorochemical analyses. Samples were void of personal identifiers. The only known demographic factors were: age, gender and the number of years residence in Seattle.

Manufacturing/Processing/Use: N/A

Hypothesis tested: Descriptive analysis of the serum fluorochemical concentrations in an elderly population by age and gender

Study period: Sera samples were collected in 2001.

Setting: Participants in the Adult Changes in Thought study, Seattle, WA

Total population: Total population of the Adult Changes in Thought study continues to increase as it is a longitudinal study. Total subjects enrolled exceed 4,000.

Subject selection criteria: Through cooperation with the staff of the Adult Changes in Thought (ACT) study, 238 serum samples from elderly adult donors (ages 65-96) equally represented of both sexes were obtained for analysis. Subjects were identified during an enrollment phase of this community-based prospective cohort study of dementia and normal aging conducted collaboratively between the University of Washington and Group Health Cooperative (GHC), a major health maintenance organization in Seattle, WA. Eligible individuals were those with no known history of neuropsychiatric disease or dementia. Chart reviews of these subjects' GHC medical records were conducted to confirm that the individuals did not reside in nursing homes or have a history of dementia diagnosis in their medical records. Subjects were not excluded from participation in the ACT study on the basis of common age-related chronic illnesses. Although it was desired to obtain more subjects above the age of 80, the study was truncated due to the relatively few subjects who volunteered and were eligible for this age stratum.

Total # of subjects in study: 238

Comparison population: N/A

Participation rate: N/A

Subject description: Only age, gender and years residence in Seattle area were known for each subject

Total number of person-years: N/A

Data collection methods: Serum samples were obtained during initial enrollment into the Adult Changes in Thought study

Exposure period: N/A

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposures: Sera samples were extracted and quantitatively analyzed for seven fluorochemicals (PFOS, PFOSAA, M570, M556, PFOSA, PFOA and PFHS) using high-pressure liquid chromatography/electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human plasma matrix. Evaluation of quality control samples injected during each analytical run indicated that the reported quantitative results may have varied, on average, up to 26 percent using human plasma calibration curves for all analytes except PFOSA which may have varied on average up to 43 percent. Also presented is a calculated total organic fluorine (TOF) index. TOF was the percent of each of the seven fluorochemicals' molecular weight that was attributed to organic fluorine [PFOS (64.7%); PFHS (61.9%); PFOA (69.0%); PFOSAA (55.3%); PFOSA (64.7%); M570 (56.6%) and M556 (58.1%)] multiplied by the ppb measured for each fluorochemical and then summed across all seven fluorochemicals.

Statistical methods: Measures of central tendency applicable to log normally distributed data (median, geometric mean) were used for descriptive analyses. In those instances where a sample was measured below the lower limit of quantitation (LLOQ), the midpoint between zero and the LLOQ was used for calculation of the geometric mean. An assessment of this midpoint assumption and how it affected the calculation of the geometric mean was performed using the 10^{th} and 90^{th} percentile values between zero and the LLOQ for those values <LLOQ. In order to minimize parametric assumptions in the estimation of extreme percentiles of the population, a bootstrap method was used to generate confidence intervals around the empirical percentiles for serum concentrations.

Other methodological information: Twenty-four randomly selected samples, stratified by gender, were split and analyzed to provide an estimate of the reliability of the analyses conducted. These analyses were performed concurrently with all other analyses of the study to minimize experimental error.

RESULTS

Describe results:. Overall, the geometric mean measured concentration of PFOS was 31.0 ppb (95% CI 28.8-33.4). The measured PFOS concentration ranged from less than the lower limit of quantitation (LLOQ) of 3.4 ppb to 175.0 ppb. There was no significant difference in the PFOS geometric means by sex or years residence in Seattle. Age was negatively associated with PFOS. Bootstrap analyses were used to calculate a 95% tolerance limit for PFOS of 84.1 ppb with an upper 95% confidence limit of 104.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS,

PFOSAA and M570. The geometric means and tolerance limits for these fluorochemicals were, on average, an order of magnitude (or more) lower than PFOS. There was a strong correlation between PFOS and PFOA (r = .75). PFOS had lower correlations with PFOSAA and PFHS (r = .42) and lower yet with M570 (r = .29). The number of samples with measured concentrations of PFOSA and M556 below the LLOQ prohibited meaningful statistical analysis of these compounds.

Study strengths and weaknesses: As with any interpretation of data obtained from a study population, questions arise regarding the representativeness and ability to generalize the data collected. We are unaware of any reason why the samples collected would not be representative of other enrollees in the Adult Changes in Thought Study.

Research sponsors: 3M Medical Department

Consistency of results: Previous measurements of fluorochemicals (PFOS, PFOSAA, M570, PFOSA, M556, PFOA and PFHS) in human serum samples obtained in the United States have been comparable to what was observed in these elderly samples.

CONCLUSIONS

The findings from this analysis of serum PFOS concentrations in an elderly population are consistent with those reported for adults and children. The human data, to date, suggests the approximate average serum concentration in non-occupational adult populations may be 30 to 40 ppb with 95% of the adult population's serum PFOS concentrations below 100 ppb. Since serum PFOS concentrations likely reflect cumulative human exposure, this information will be useful for risk characterization.

REFERENCE

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH, Zobel LR. Identification of Fluorochemicals in Human Sera. III. Elderly Participants of the Adult Changes in Thought Study, Seattle, Washington. 3M Final Report, February 25, 2002.

RS-III-15: Identification of Fluorochemicals in Human Sera. III. Pediatric Participants in a Group A Streptococci Clinical Trial Investigation.

TEST SUBSTANCE

Identity: . The seven fluorochemicals analyzed were perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$); N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamidoacetate (M556, $C_8F_{17}SO_2N(CH)CH_2COO^-$); perfluorooctanesulfonylamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{13}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

Remarks:

METHOD

Study design: Sera samples were obtained from a multi-center clinical trial regarding group A streptococcal infections in children ages 2-12. Samples were collected in 1994-1995 and stored frozen at -20 degrees Celsius. Each sample collected (N = 599) was analyzed for seven fluorochemicals. One sample was not analyzed.

Manufacturing/Processing/Use: N/A

Hypothesis tested: The purpose of this study was to better characterize the distribution of seven fluorochemicals in 599 pediatric samples obtained from a multi-center clinical trial of group A streptococcal infections.

Study period: Sera samples were collected in 1994-1995 and stored at -20 degrees Celsius.

Setting: There was a multi-center clinical trial of Group A streptococcal infections

Total population: Total number of children in the multi-center clinical trial was 1,131. Ages were 2-12 years who presented with signs and symptoms of acute-onset pharyngitis. All 1,131 children had positive throat cultures for group A streptococci at an initial visit.

Subject selection criteria: Serum samples were selected by age and gender groups. Because of the uncertainty regarding the population distribution of PFOS, sample size was estimated by the use of tolerance limits. Provided below is the sampling distribution that was used. Percent sampled was the highest for the younger ages and included all samples four years of age and less.

Age Group	Total N	Sampled (%)
2	27	27 (100)
3	51	51 (100)
4	81	81 (100)
5	122	100 (82)
6	146	80 (55)
7	161	60 (37)
8	131	40 (31)
9	135	40 (30)
10	109	40 (37)
11	87	40 (46)
12	81	40 (49)
Total	1131	599 (53)

Total # of subjects in study: 599 subjects (598 analyzed).

Comparison population: N/A

Participation rate: N/A

Subject description: Only age, gender and state of residence were known for each child.

Total number of person-years: N/A

Data collection methods: Sera samples were obtained during the course of the multi-center clinical trial. Samples were stored in freezers at -20 degrees Celsius.

Exposure period: N/A

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposures: Sera samples were extracted and quantitatively analyzed for seven fluorochemicals (PFOS, PFOSAA, M570, M556, PFOSA, PFOA and PFHS) using high-pressure liquid chromatography/electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human plasma matrix. Evaluation of quality control samples injected during each analytical run indicated that the reported quantitative results may have varied, on average, up to 26 percent using human plasma calibration curves for all analytes except PFOSA which may have varied on average up to 43 percent. Also presented is a calculated total organic fluorine (TOF) index. TOF was the percent of each of the seven fluorochemicals' molecular weight that was attributed to organic fluorine [PFOS (64.7%); PFHS (61.9%); PFOA (69.0%); PFOSAA (55.3%); PFOSA (64.7%); M570 (56.6%) and M556 (58.1%)] multiplied by the ppb measured for each fluorochemical and then summed across all seven fluorochemicals.

Statistical methods: Measures of central tendency applicable to log normally distributed data (median, geometric mean) were used for descriptive analyses. In those instances where a sample was measured below the lower limit of quantitation (LLOQ), the midpoint between zero and the LLOQ was used for calculation of the geometric mean. An assessment of this midpoint assumption and how it affected the calculation of the

geometric mean was performed using the 10^{th} and 90^{th} percentile values between zero and the LLOQ for those values <LLOQ. In order to minimize parametric assumptions in the estimation of extreme percentiles of the population, a bootstrap method was used to generate confidence intervals around the empirical percentiles for serum concentrations.

Other methodological information: .An analysis of the reliability of the assay was conducted after the original samples were analyzed. The laboratory was blind to the identity of these samples as they related to the original values reported. Triplicate samples were analyzed for the highest one percent of the measured concentrations of PFOS, PFOA and PFHS. If there was insufficient serum sample left for analysis, the next highest sample was included for analysis. A 20 percent random sample of the next highest nine percent samples was also conducted but with only a single measurement. Finally, a five percent sample was randomly chosen of the remaining 90 percent of all samples. This five percent sample was also analyzed only once. Altogether, there were 62 samples reanalyzed representing sera from 44 unique children.

RESULTS

Describe results: Overall, the geometric mean measured concentration of PFOS was 37.5 ppb (95% CI 33.3-36.5). The measured PFOS concentrations ranged from 6.7 ppb to 515.0 ppb. Male children had a significantly ($p < .01$) higher geometric mean serum PFOS level compared to female children [male children geometric mean = 40.1 ppb (95% CI 37.7-42.6) vs female geometric mean = 35.2 ppb (95% CI 33.3-37.2)]. Bootstrap analysis was used to calculate a mean 95% tolerance limit of 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS, PFOSAA and M570. A unique finding observed in these pediatric data that was not observed in the adult or elderly data reported elsewhere, were the higher 95% tolerance limit mean concentrations for PFHS (64.5 ppb) and M570 (11.9 ppb) with upper 95% confidence limits of 80.6 ppb and 14.8 ppb, respectively. It is unlikely that these findings are a consequence of analytical systematic error between these three studies. There was a strong correlation between PFOS and both PFOA ($r = .70$) and PFHS ($r = .66$) with lower correlations with PFOSAA ($r = .43$) and M570 ($r = .42$). The number of samples with measured concentrations of PFOSA and M556 less than the LLOQ prohibited meaningful statistical analyses for these compounds.

Study strengths and weaknesses: As with any interpretation of data obtained from a study population, questions arise regarding the representativeness and ability to generalize the data collected. The overall population from which these samples came from is not unique because group A streptococcal infections are quite common in children. The samples selected were likely representative of the samples collected during the multi-center trial.

Research sponsors: 3M Medical Department

Consistency of results: Previous measurements of human serum samples obtained in the United States have been comparable to what was observed in these pediatric samples except for the higher measured concentrations for PFHS and M570.

CONCLUSIONS

The findings from this analysis of serum PFOS concentrations in children are consistent

with those previously reported. The human data, to date, suggests the approximate average serum concentration in non-occupational adult populations may be 30 to 40 ppb with 95% of the adult population's serum PFOS concentrations below 100 ppb. Since serum PFOS concentrations likely reflect cumulative human exposure, this information will be useful for risk characterization.

REFERENCES

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH, Zobel LR. Identification of Fluorochemicals in Human Sera. III. Pediatric Participants in a Group A Streptococci Clinical Trial Investigation. 3M Final Report, February 25, 2002.

RS-III-16: Identification of Fluorochemicals in Human Tissue.

TEST SUBSTANCE

Identity: PFOS (perfluorooctanesulfonate, $C_8F_{17}SO_3^-$); PFOA (perfluorooctanoate, $C_7F_{15}CO_2^-$); PFOSA (perfluorooctanesulfonamide, $C_8F_{17}SO_2NH_2$); PFHS (perfluorohexanesulfonate, $C_6F_{13}SO_3^-$)

Remarks:

METHOD

Study design: Prevalence study of available donor tissue.

Manufacturing/Processing/Use: None

Hypothesis tested: The purpose of this study was to determine the concentration of PFOS (and other fluorochemicals) in human liver and compare to a donor's serum levels. The hypothesis investigated was to determine whether the 1:1 serum ratio observed in a 6 month primate feeding study also occurred in individual, non-occupationally exposed humans whose serum PFOS levels were at much lower concentrations.

Study period: March 22, 1999 through June 15, 2001

Setting: Donor samples were obtained from the International Institute for the Advancement of Medicine (IIAM). IIAM is a non-profit organization whose purpose is to facilitate the placement of non-transplantable human organs and tissues for biomedical research and education. Upon acceptable donor qualifications, IIAM obtained 5 ml of blood and 10 grams of liver and then froze the samples until shipment to the 3M Medical Department.

Total population: A total of 31 donor samples (16 males, 15 females) were obtained over an 18 month time period.

Subject selection criteria: Criteria for donor acceptance are outlined in IIAM protocols.

Total # of subjects in study: There were 31 donors.

Comparison population: Not applicable

Participation rate: Not applicable

Subject description: Average age of the male and female donors was 50 years (S.D. 15.6, range 5 – 69 years) and 45 years (S.D. 18.5, range 13-74 years), respectively. Causes of death were intracranial hemorrhage (n = 16, 52%), motor vehicle accidents (n = 7, 23%), head trauma (n = 4, 13%), brain tumor (n = 2, 6%), drug overdose (n = 1, 3%) and respiratory arrest (n = 1, 3%). All donors were tested for HIV-1 and HIV-2 antibodies, hepatitis B surface antigen, hepatitis C antibody, HTLV-1 antibody, syphilis, and hepatitis B core antibody (IgG plus IgM). In addition, one or more of the following tests were sometimes done: CMV antibody, hepatitis B surface antibody, hepatitis B core IgM and HIV p24 antigen. Results that suggested the donor was infectious precluded the distribution of the tissue.

Total number of person-years: Not applicable

Data collection methods: Upon acceptable donor qualifications, IIAM obtained 5 ml of blood and 10 grams of liver and then froze the samples until shipment to the 3M Medical Department. IIAM notified the 3M Medical Department of potential donor tissue approximately twice a month over an 18 month period..

Exposure period: Not applicable

Description/delineation of exposure groups/categories: Not applicable

Measured or estimated exposures: All samples were analyzed for quantitative determination of PFOS, PFOSA, PFOA and PFHS. Sera and liver samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively assayed using high performance liquid chromatography electrospray tandem mass spectrometry (HPLC-ESMSMS) and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the analysis. These matrix spike studies indicated that the data can be considered to be accurate to within one standard deviation of the average fortified sample recovery. For example, the average fortified sample recovery of PFOS from human sera was 89% (S.D. 21%). The average fortified sample recovery of PFOS from human liver was 78% (S.D. 24).

Statistical methods: Serum fluorochemical levels determined to be less than the limit of quantitation (< LOQ) were assigned a value midpoint between zero and the LOQ. Measures of central tendency and its variation were calculated for males and females. A liver to serum ratio was calculated for each individual and each gender-specific group. In order to study the maximum liver:serum ratio for PFOS, the average values were adjusted to accommodate the upper (liver) and lower (sera) quantitative limits described by the analytical accuracy.

Other methodological information:

RESULTS

Describe results: Mean serum PFOS level was 17.7 ng/ml (95% CI 13.0-22.5; range <6.1 – 58.3 ng/mL) for the average of the 24 serum donors' samples analyzed. The geometric mean was 14.7 ng/mL (95% CI 11.1-19.4). Fifteen of the 30 liver donors had liver PFOS results at <LOQ and thus they were assigned a value midpoint between zero and the LOQ. The mean liver PFOS was 18.8 ng/g (95% 14.1-23.5; range <3.7 – 57.0 ng/g) for the average of the 30 liver donors' samples analyzed. The geometric mean for liver PFOS was 15.2 ng/g (95% CI 11.9-19.6). Mean PFOS levels between male and female donors for serum (male 18.2 ng/mL; female = 17.2 ng/mL) or liver (male = 19.2 ng/g; female 18.4 ng/g) were similar. No associations were observed between measured PFOS levels and age. A ranked distribution of the average PFOS serum and liver data for each of the 23 paired samples showed good correlation (Spearman's Rho - 0.41, $p < .05$). In these 23 pairs, the mean liver:serum ratio was 1.3:1 (95% CI 0.9:1 - 1.7:1). Assuming the variation of the analytical error is one standard deviation, the mean liver:serum ratio ranged from a minimum of 0.8:1 (95% CI 0.5 - 1.0) to a maximum of 2.1:1 (95% CI 1.5 - 2.8). Assuming a two standard deviation difference, the mean liver:serum ratio ranged from a minimum of 0.5:1 (95% CI 0.3 - 0.6) to a maximum of 3.4:1 (95% CI 2.3:1 - 4.4:1). The levels of PFOSA, PFHS and PFOA were determined in the samples but

liver:serum ratios for these analytes were not estimated as 90% of the liver samples were determined to be <LOQ.

Study strengths and weaknesses: Refinement of the human liver:serum ratio, even if a larger donor pool was available, may be unlikely without more precise analytical techniques that can consistently measure PFOS at lower levels as well as the other fluorochemical analytes that were measured in this study. Although the study data were limited by the number of donors (raising the question about how representative was the sample set), it should be noted that the average serum PFOS levels determined were comparable to those reported elsewhere.

Research sponsors: 3M Medical Department

Consistency of results: The purpose of this study was to estimate a mean liver:serum ratio in human donors from a nonoccupationally exposed population. The study data indicated that at non-occupationally exposed population serum levels (estimated to be approximately 30 ng/mL), the mean liver:serum ratio for PFOS was 1.3:1 (95% CI 0.9-1.7). This ratio is comparable to those reported in a six-month primate feeding study.

CONCLUSIONS

This study data, as well as the data from a six month primate study, would suggest that a liver:serum ratio that approximates 1:1 for the non-occupationally exposed human population may be a reasonable assumption to use in a risk characterization assessment of PFOS. Assuming the variation of the analytical error is one standard deviation, this ratio could range from 0:8 (95% CI 0.5 - 1.0) to a maximum of 2:1 (95% CI 1.5 - 2.8).

REFERENCE

Olsen GW, Hansen KJ, Clemen LA, Burris JM, Mandel JH. Identification of fluorochemicals in human tissue. 3M Final Report. June 25, 2001.

RS-III-17: A Cross-Sectional Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) in Relation to Clinical Chemistry, Thyroid Hormone, Hematology and Urinalysis Results from Male and Female Employee Participants of the 2000 Antwerp and Decatur Fluorochemical Medical Surveillance Program.

TEST SUBSTANCE

Identity: The extracts were quantitatively analyzed for PFOS (perfluorooctanesulfonate), PFOA (perfluorooctanoate), PFHS (perfluorohexanesulfonate), PFOSAA (N-ethyl perfluorooctanesulfonamidoacetate), M570 (N-methyl perfluorooctanesulfonamidoacetate), PFOSA (perfluorooctanesulfonamide) and M556 (perfluorooctanesulfonamidoacetate).

Remarks: 3M offers on a voluntary basis a periodic medical surveillance program to its production and non-production fluorochemical employees. This report is the most recent analysis for the Antwerp and Decatur manufacturing sites. The previous analysis was done in 1997.

METHOD

Study design: Cross-sectional analysis of serum fluorochemical levels and hematology, clinical chemistry and thyroid hormones obtained during fluorochemical medical surveillance exams conducted in 2000 at Antwerp (Belgium) and Decatur (Alabama) manufacturing sites. Urinalyses were also conducted on Decatur employees.

Manufacturing/Processing/Use: 3M Antwerp (Belgium) and Decatur (Alabama) manufacturing sites

Hypothesis tested: The purpose of this report is to provide the results from the year 2000 Antwerp and Decatur fluorochemical medical surveillance program in relation to employees' measured serum fluorochemical concentrations.

Study period: Spring 2000

Setting: Antwerp and Decatur medical surveillance examinations of chemical plant employees

Total population: Approximately 340 employees were eligible to participate in Antwerp and 500 employees were eligible for participation in Decatur.

Subject selection criteria: Voluntary participation by employees

Total # of subjects in study: Antwerp: 255 chemical plant, research and development, and site employees participated (206 males, 49 females). Until recently, all female employees were engaged in nonproduction activities. Decatur: 263 chemical plant, research and development and site employees participated (215 male, 48 female)

Comparison population: N/A

Participation rate: Antwerp (75%); Decatur (52%)

Subject description: Current full-time employees at the Antwerp and Decatur chemical plants and current full-time employees with site-wide responsibilities at both sites.

Total number of person-years: N/A

Data collection methods: A standard set of hematological and clinical chemistry tests were analyzed. These included the following hematological tests: hematocrit (percent), hemoglobin (gm/dl), red blood cells (RBC, 1000/mm^3), white blood cells (WBC, 1000/ mm^3) and platelet count (1000/ mm^3); and the following clinical chemistry tests: alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dl), blood urea nitrogen (BUN, mg/dl), serum creatinine (mg/dl), blood glucose (mg/dl), cholesterol (mg/dl), high density cholesterol (HDL, mg/dl) and triglycerides (mg/dl). Urinalyses were only assessed for Decatur employees via the standard urine microstick analysis, which tested for urine glucose, albumin and red blood cells. Six thyroid hormones were also assayed: thyroid stimulating hormone (TSH; µIU/ml); serum thyroxine (T4; µg/dL); free thyroxine (free T4; ng/dL); serum triiodothyronine (T3; pg/mL); thyroid hormone binding ratio (THBR, %, previously referred to as T3 Uptake) and free thyroxine index (FTI). Urinalyses were performed on Decatur employees.

Exposure period: Variable depending upon an employee's work history.

Description/delineation of exposure groups/categories: Employees were categorized into quartile distribution of measured serum PFOS concentrations.

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS, PFOA, PFHS PFOSAA, M570, PFOSA and M556 using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluated versus an extracted curve from a human serum matrix. Endogenous levels of certain fluorochemical were determined in the standard serum matrix and additional fluorochemical was spiked into the matrix. The total amount of each specific fluorochemical (endogenous + spiked) was used to construct an extracted standard curve. All serum fluorochemical analyses were determined by Northwest Bioanaltyical Laboratory Inc. (Salt Lake City, UT). Each analytical run included duplicate calibration standards at six or more concentrations covering the lower to upper limit of quantitation. For all analytes except PFOSA, at least three-fourths of the calibrations standard's back-calculated concentrations were within ± 15% (± 20% for LLOQ) of their individual target concentrations. For PFOSA, three-fourths of the calibration standard's back-calculated concentrations were ± 20% (±25% for LLOQ) of their individual target concentrations. Each analytical run also included low, medium and high QA samples in duplicate. The measured concentrations of at least two-thirds of all analytical QCs must have been within ±20% of their target concentration (±25% for PFOSA). A total organic fluorine index (TOF) was also determined by calculating the percent of each specific fluorochemical's molecular weight that was attributed to organic fluorine and multiplied by the ppm measured for each fluorochemical and then summed across all seven fluorochemicals.

Statistical methods: Statistical analyses were conducted on the entire surveillance population as well as subgroups by gender, production worker (yes/no) and location. Univariate analyses categorized mean levels by serum PFOS quartile distributions.

Multivariable regression was used to analyze the clinical chemistry and thyroid hormones as dependent variables in relation to the independent effects of PFOS, PFOA or TOF adjusted for several demographic variables (age, body mass index, number of alcoholic drinks per day, cigarettes smoked per day and years worked).

Other methodological information: There were important confounding differences between Antwerp and Decatur employees. Antwerp workers tended to be younger, had lower BMI's and drank more alcoholic beverages per day. As a result of these differences, Antwerp employees, compared to Decatur employees, had higher mean HDL and bilirubin levels and lower hepatic enzymes and serum triglyceride levels.

RESULTS

Describe results: Mean serum PFOS levels for Antwerp production and non-production male workers were 1.16 and 0.42 ppm, respectively. Among Decatur production and non-production male workers, their mean serum PFOS levels were 1.63 and 0.73 ppm, respectively. Mean serum PFOA levels for Antwerp male production and non-production workers were 1.28 and 0.34 ppm, respectively. Among Decatur male production and non-production workers, their mean serum PFOA levels were 2.34 and 0.59 ppm, respectively. The mean PFOS and PFOA levels for all Antwerp female employees (12% production, 88% nonproduction) were 0.13 ppm and 0.07 ppm, respectively. The mean PFOS and PFOA levels for all Decatur female employees (63% production, 37% nonproduction) were 0.93 and 1.23 ppm, respectively. Separate reports have been written which analyzed the employees' serum levels in relation to their job and building location work assignments as obtained from a self-reported work history questionnaire.

There was a modest positive association between PFOS or PFOA with cholesterol as well as a stronger positive association between PFOA and triglycerides. These associations were inconsistent with the known toxicological evidence that has shown the hypolipidemic (not hyperlipidemic) effect of PFOS (in rats and primates) and PFOA (in rats but no effect in primates) at dosages that produced serum PFOS or PFOA levels higher than those measured in this population. Therefore, it is unlikely the observed positive associations between PFOS or PFOA and lipids are causal. Because of the potential confounding positive association with serum triglycerides, this variable was added to the hepatic clinical chemistry models as an independent variable. In these models, no significant associations were observed with PFOS, PFOA or TOF in relation to alkaline phosphatase, GGT, AST, ALT or total bilirubin. Although T3 was positively associated with PFOA, no other thyroid hormones were associated with PFOS, PFOA or TOF; thus there is unlikely a causal explanation (e.g., hypothyroidism or hyperthyroidism) for this positive T3 association with PFOA. Hematological and urinalysis results were unremarkable.

Study strengths and weaknesses: Number of participants in the 2000 fluorochemical medical surveillance exams was the largest ever for either of these manufacturing sties. Limitations of the study include its cross-sectional design, the voluntary participation rates (e.g., 50% in Decatur) and the lower concentrations of serum PFOS and PFOA measured among these employees compared with those suspected to cause effects in laboratory animals.

Research sponsors: 3M Medical Department

Consistency of results: These findings were consistent with previously published data from the 1995 and 1997 medical surveillance program data from the Antwerp and Decatur manufacturing sites. Serum PFOS measurements among Decatur employees were consistent with a random sample analysis performed in 1998.

CONCLUSIONS

The results from the 2000 fluorochemical medical surveillance program, in conjunction with prior analyses of the 1995 and 1997 fluorochemical medical surveillance programs, continue to suggest that Antwerp and Decatur fluorochemical production and non-production employees do not have significant changes in serum cholesterol, lipoproteins or hepatic enzymes that are consistent with toxicological findings observed in laboratory animals. The earliest clinical effect (lowering of serum cholesterol) in laboratory animals (cynomolgus monkeys) occurred at serum PFOS concentrations above 100 ppm which is 10 times higher than the highest serum PFOS concentrations measured in the workers (average ≅ 1 - 2 ppm, range 0.1 - 10 ppm) during the course of the 2000 fluorochemical medical surveillance program.

REFERENCE

Olsen GW, Burlew JM, Burris JM, Mandel JH. A Cross-Sectional Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) in Relation to Clinical Chemistry, Thyroid Hormone, Hematology and Urinalysis Results from Male and Female Employee Participants of the 2000 Antwerp and Decatur Fluorochemical Medical Surveillance Program. 3M Final Report. October 11, 2001.

RS-III-18: A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee participants of the 1994/95, 1997 and 2000 Fluorochemical Medical Surveillance Program.

TEST SUBSTANCE

Identity: PFOS (perfluorooctanesulfonate) and PFOA (perfluorooctanoate)

Remarks: 3M offers on a voluntary basis a periodic medical surveillance program to its production and non-production fluorochemical employees. This is the first report that conducted a longitudinal assessment of Antwerp and Decatur employees who participated in the 2000 fluorochemical medical surveillance program and at least one prior program in either 1994/95 or 1997.

METHOD

Study design: A longitudinal analysis was conducted of serum PFOS, PFOA and TOF (calculated total organic fluorine index) levels and lipid and hepatic clinical chemistry tests for participants of the 1994/95, 1997 and 2000 fluorochemical medical surveillance programs at the Antwerp (Belgium) and Decatur (Alabama) manufacturing sites.

Manufacturing/Processing/Use: 3M Antwerp (Belgium) and Decatur (Alabama) manufacturing sites

Hypothesis tested: The purpose of this report is determine whether a trend could be detected between serum PFOS and PFOA and changes in lipid or hepatic clinical chemistry tests

Study period: 1994 through 2000

Setting: Antwerp and Decatur medical surveillance examinations of chemical plant employees

Total population: There were 206 Antwerp male employees and 215 Decatur male employees who participated in the 2000 fluorochemical medical surveillance program who were initially eligible for this longitudinal assessment dating back to 1994/95.

Subject selection criteria: Male employees who participated in the 2000 fluorochemical medical surveillance program for Antwerp and Decatur and at least one of the two previous program years (1994/95 and/or 1997). [Note: The Decatur fluorochemical medical surveillance program was done in 1994 and 1997; the Antwerp fluorochemical medical surveillance program was done in 1995 and 1997 (thus the 994/95 and 1997 notation).]

Total # of subjects in study: A total of 175 male employees voluntarily participated in the 2000 program and at least one of the two previous program years. A total of 106 (61 percent) of the 175 employees participated in the 1994/95 program and 110 (63 percent) of the 175 participated in the 1997 program. Of these 175 employees, a total of 41 (24 percent) participated in all three years (Antwerp = 21, Decatur = 20), 65 (37 percent) participated in 1994/95 and 2000 (Antwerp = 45, Decatur = 20) and 69 (39 percent) participated in 1997 and 2000 (Antwerp = 34, Decatur = 35). There were insufficient

numbers of female employees to conduct any meaningful longitudinal assessment. Only 14 female employees participated in the 2000 fluorochemical medical surveillance program and at least one of the previous program years.

Comparison population: N/A

Participation rate: N/A

Subject description: Voluntary participants of a fluorochemical medical surveillance program offered to employees at the 3M Antwerp and Decatur manufacturing sites

Total number of person-years: N/A

Data collection methods: A standard set of clinical chemistry tests were analyzed by the same hospital laboratory during 1994/95, 1997 and 2000 for both manufacturing sites. These tests included the following: alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dl), cholesterol (mg/dl), high density cholesterol (HDL, mg/dl) and triglycerides (mg/dl). Most reference ranges remained relatively constant over time except for ALT. In each surveillance year four potential confounding factors were also determined: age, BMI, number of alcohol drinks consumed per day, and number of cigarettes smoked per day.

Exposure period: Variable depending upon an employee's work history.

Description/delineation of exposure groups/categories: Measured serum PFOS and PFOA concentrations were used as continuous variables.

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure in each fluorochemical medical surveillance year and analyzed by mass spectrometry. However, a different laboratory was involved in each year. The extracts were quantitatively analyzed for PFOS and PFOA. A total organic fluorine index (TOF) was also determined by calculating the percent of the molecular weight of PFOS and PFOA that was attributed to organic fluorine and multiplied by the ppm measured for each fluorochemical and then summed across the two fluorochemicals.

Statistical methods: The continuous outcomes of lipid and hepatic clinical chemistry tests were evaluated as repeated measures incorporating the random subject effect fitted to a mixed model by the MIXED procedure in the SAS statistical package. Restricted maximum likelihood estimates of variance parameters were computed. Adjusted regression models were built by introducing all covariates (age, BMI, alcohol drinks, cigarettes) and testing the covariance structure. Significant coefficients were defined when the p value was $< .05$.

Other methodological information: Historically, there were important confounding differences between Antwerp and Decatur employees. Antwerp workers have tended to be younger, had lower BMI's and drank more alcoholic beverages per day. As a result of these differences, Antwerp employees, compared to Decatur employees, have had higher mean HDL and bilirubin levels and lower hepatic enzymes and serum triglyceride levels. These differences were observed among the 175 study subjects.

RESULTS

Describe results: There was a positive association between PFOA and serum cholesterol and triglycerides over time but not with PFOS. This association was limited to the Antwerp employees and, in particular, the 21 Antwerp employees who participated in all three surveillance years. This positive association between PFOA and serum lipids is opposite the inconsistent toxicological evidence that suggested a possible hypolipidemic effect of PFOA in rodents and no effect in primates. Adjusting for potential confounders, there were no temporal changes associated with the fluorochemical tests, PFOS, PFOA and TOF, and the hepatic clinical chemistry tests.

Study strengths and weaknesses: This is the first longitudinal assessment of the 3M fluorochemical medical surveillance program. Limitations of this analysis included the number of employees with three years of surveillance data (only 24% of the 175 subjects), the inability to analyze temporal changes due to small numbers in female employees, the use of different laboratories and the associated systematic (experimental error) with each fluorochemical assay for the three surveillance program years and the lower levels of serum PFOS and PFOA measured in each program year among these employees compared with those that cause effects in laboratory animals.

Research sponsors: 3M Medical Department

Consistency of results: These findings were consistent with previous cross-sectional data from the 1994/95, 1997 and 2000 fluorochemical medical surveillance program data from the Antwerp and Decatur manufacturing sites which have not shown associations consistent with toxicological evidence known about PFOS or PFOA.

CONCLUSIONS

The findings from this longitudinal analysis of 175 male employees who participated in the 2000 fluorochemical medical surveillance program and at least one other fluorochemical medical surveillance program conducted in 1994/95 or 1997 continue to suggest that Antwerp and Decatur fluorochemical employees do not have significant changes in serum cholesterol, lipoproteins or hepatic enzymes that are consistent with toxicological findings observed in laboratory animals. The earliest clinical effect (lowering of serum cholesterol) in laboratory animals (cynomolgus monkeys) occurred at serum PFOS concentrations above 100 ppm which is 10 times higher than the highest serum PFOS concentrations measured in the workers (average ≅ 1 - 2 ppm, range 0.1 - 13 ppm) during the three different years (1994/95, 1997 and 2000) that the fluorochemical medical surveillance program was offered to employees.

REFERENCE

Olsen GW, Burlew MM, Burris JM, Mandel JH. A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee participants of the 1994/95, 1997 and 2000 Fluorochemical Medical Surveillance Program. 3M Final Report. October 11, 2001.

RS-III-19: Retrospective Cohort Mortality Study of the 3M Decatur Plant.

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:

	Males	Females
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

Health effects studied: Mortality

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.

OTHER

RS-III-20: Mortality Study of Workers Employed at the 3M Decatur Facility.

TEST SUBSTANCE

Identity: POSF (perfluoroctanesulfonyl fluoride); PFOS (perfluorooctanesulfonate); PFOA (perfluorooctanoate)

Remarks:

METHOD

Study design: Retrospective cohort mortality study

Manufacturing/Processing/Use: POSF-based chemistry

Hypothesis tested: The objective of this study was to determine whether occupational exposure to fluorochemicals exposure, particular to perfluorooctanesulfonyl fluoride-(POSF $C_8F_{17}SO_2F$) based fluorochemicals, are related to mortality of employees of the 3M manufacturing plant located in Decatur, Alabama.

Study period: The cohort consisted of employees who had worked at least one year at the Decatur plant from March 1961 through December 31, 1998. Vital status was ascertained through December 31, 1998.

Setting: Occupational setting. The 3M manufacturing site is located in Decatur, Alabama. The chemical plant is approximately 300 yard away from the film plant.

Total population: 2,083 employees were eligible for the study

Subject selection criteria: Eligible employees had to be employed at least one year at the 3M Decatur manufacturing site.

Total # of subjects in study: There were a total of 2,083 persons (1730 males, 83%; 353 females, 17%). There were 1,271 individuals (1030 males, 241 females) who were classified as ever employed in a high or low exposure job (see below) in the chemical plant and 812 film plant workers (700 males, 112 females) who were considered to have no or minimal workplace fluorochemical exposure.

Comparison population: U.S. population, Alabama population, and 23 regional counties as were selected for the original mortality study that was conducted five years prior to this study.

Participation rate: A death certificate could not be found for only 6 cases.

Subject description:

	High exposed	Low exposed	Non-exposed	Total
Mean age at follow-up	49.9	53.6	51.5	51.1
Mean years at Decatur	16.4	13.3	13.7	14.9
Deaths	65	27	53	145

Total number of person-years:

	High exposed	Low exposed	Non-exposed	Total
Person-years	21,867	6,823	20,304	50,970

Data collection methods: The cohort was enumerated using employment records from the Decatur facility. A line by line abstraction and computerization of the data were undertaken by the 3M Medical Department. Workers who accrued at least one year of cumulative employment at Decatur were eligible for inclusion in the cohort. Because the previous study had only abstracted selected dates from the work history record (not the entire record), the newly enumerated cohort was linked to records from the original cohort to ensure completeness. Discrepancies identified in the records were resolved using TRW/Experian, a credit reporting agency, and the Social Security Administration (SSA) service for epidemiologic research studies. SSA was used to identify deaths prior to 1979; the National Death Index was used to identify deaths since 1979. Copies of death certificates were requested from the state of record.

Exposure period: Potential exposure period was March 1, 1961 through December 31, 1998 for those eligible (one year employment duration).

Description/delineation of exposure groups/categories: Exposure categories were based on job titles and serum PFOS levels measured in a 1998 study which randomly sampled employees at the chemical and film plants. In this random sample study of employee PFOS serum levels, chemical plant jobs were categorized into eight categories. The highest geometric mean level of serum PFOS was observed in cell operators (1.97 ppm) followed by the waste operators (1.50), chemical operators (1.48 ppm), and maintenance workers (1.30 ppm). Because production has been relatively consistent over time through 1997, a straightforward exposure matrix was developed based on the work history records of the study cohort. With the knowledge of the major job-specific serum PFOS levels, a 3M industrial hygienist and epidemiologist assigned a unique job and department combination in the work history records to one of the following 3 major exposure categories:

1. no workplace exposure to POSF (perfluorosulfonyl fluoride)-based fluorochemicals;
2. low potential workplace exposure to POSF-based fluorochemicals (includes such jobs as engineers, quality control technicians, environmental, health and safety workers, administrative assistants and managers);
3. high potential workplace exposure to POSF-based fluorochemicals (includes cell operators, chemical operators, maintenance workers, mill operators, waste operators and crew supervisors.

Measured or estimated exposures: There was no estimation of environmental exposures to develop the exposure matrix. This matrix was based on work history records and serum PFOS levels measured in workers, especially the 1998 random employee serum PFOS sample study.

Statistical methods: Standardized Mortality Ratios (SMRs) were computed for all cause and specific causes of death. SMRs and 95% confidence intervals were computed using the PC Life Table Analysis System (PCLTAS) software developed by the National Institutes of Occupational Safety and Health (NIOSH). Causes of death potentially related to fluorochemical exposure were analyzed by duration of employment in the three fluorochemical exposure subgroups.

Other methodological information:

RESULTS

Describe results: The all cause and cause specific mortality rates for the entire cohort were lower than expected compared to the general population of Alabama; 145 observed and 230 expected (SMR=0.63, 95% CI=0.53-0.74). A similar pattern was observed for all deaths from cancer; 39 observed, 54 expected (SMR=0.72, 95% CI = 0.51-0.98). The all cause and all cancer causes of death were fewer than expected for the high exposure, low exposure, and nonexposed subcohorts. When restricted to workers who accrued at least one year of employment in the high or low exposure subgroups, the standardized mortality ratios for all causes of death and all malignant neoplasms were well below unity.

Five deaths from cirrhosis of liver were identified in the entire cohort, two of which occurred in the high exposure group; however this did not exceed the number expected. Two deaths from liver cancer were observed in the entire cohort, with 1.24 expected (SMR=1.61, 95% CI=0.20-5.82). One liver cancer was observed in the sub-cohort employed in a high exposure job for at least a year (0.50 expected), and the other held a low exposure job for at least one year. The SMR for liver cancer among workers who held high or low exposure jobs for at least one year was 3.08 (95% CI 0.37-11.10).

Three deaths were attributed to malignant neoplasms of the bladder (0.62 expected in the entire cohort, SMR = 4.81, 95% CI = 0.99-14.05). The workers who died from bladder cancer were in the sub-cohort that worked in jobs with high exposure for at least one year (0.19 expected, SMR = 16.12, 95% CI = 3.32-47.41). All three cases of bladder cancer were male employees and each had worked in high exposure jobs for at least 5 years. The results for bladder cancer in relation to fluorochemical exposure did not change when the reference population was the local counties rather than the entire state of Alabama. Because mortality from bladder cancer is relatively low with annual incidence

rates five to ten times greater than mortality rates, depending upon age and gender, it is therefore likely that this mortality study did not ascertain all cases of bladder cancer in this employee population. Based on rates of bladder cancer incidence from the NCI SEER program, 6.9 incident cases of bladder cancer would be expected in this population during the 1961-1997 follow-up period. Because the population understudy was not covered by a cancer registry system, ascertaining additional cases would require direct surveys with living cohort members.

Study strengths and weaknesses: The study provides a comprehensive assessment of the mortality experience of Decatur employees from 1961-1997. As like all retrospective cohort mortality study designs, its research value is minimized if outcomes of interest have high survivorship. Additional limitations include the limited number of person-years for analysis purposes.

Research sponsors: University of Minnesota Division of Environmental and Occupational Health

Consistency of results: Overall, the findings of lower all cause and all cancer SMRs were consistent with what was reported in the initial mortality study of this occupational population. In the original mortality study, there was one bladder cancer death. Two deaths have occurred since 1991 which were incorporated in this study.

CONCLUSIONS

Workers employed in jobs with high exposure to POSF-fluorochemicals at the Decatur chemical plant had an increased risk of death from bladder cancer. However, it is not clear whether these cases can be attributed to fluorochemical exposure, another, undetermined occupational exposure, non-occupational factors or chance. A review of potential exposure to known occupational bladder carcinogens at the Decatur site was recommended. The relatively young age and small size of the cohort currently precludes a detailed analysis by exposure, particularly for less common diseases. To further evaluate the health of this cohort in reference to the risk of liver and bladder cancer, and continue to develop the understanding of the toxicology of fluorochemicals, additional follow-up and monitoring of this cohort was recommended.

REFERENCE

Alexander BH. Mortality study of workers employed at the 3M Decatur facility. University of Minnesota Final Report. April 26, 2001.

RS-III-21: Urine Solubility Study.

Title: Solubility study of perfluorooctanesulfonate (PFOS), SD-018 and Perfluorooctanesulfonamide (FOSA), SE-027 in male/female human urine

TEST SUBSTANCES

Identity: A) Perfluorooctanesulfonate (PFOS), purity 86.9%, sourced as 98-0211-0888-5 (3M Laboratory ID No. SD-018) B) Perfluorooctanesulfonamide (FOSA), purity not specified, sourced from R. Buckanin (3M Laboratory ID No. SE-027) C) Normal human male and female urine, sourced from Lampire (3M Laboratory ID No. TN-A 4822)

METHODS

The solubility determinations of PFOS (SD-018) and FOSA (SE-027) in human urine were performed according to the following methods:

A) 3M Environmental Laboratory methods ETS-8-170.1 "Solubility Screen Test: Approximate Solubility of a Test Substance in Various Solvents"
B) 3M Environmental Laboratory methods ETS-8-172.1 "Shake Flask Method: Solubility of a Test Substance in Various Solvents" as adapted from U.S.E.P.A. OPPTS 830.7840 "Water Solubility: Column Elution Method; Shake Flask Method" and OECD Guideline 105 "Water Solubility"
C) 3M Environmental Laboratory methods ETS-8-155.0 "Analysis of Perfluorooctanesulfonate or Other Fluorochemicals in Waste Stream or Water Extracts Using HPLC-Electrospray/Mass Spectrometry."

Method ETS-8-170.1 was used to determine solubility range of test substances in human urine by adding incremental amounts of urine to the test substances until a qualitative determination of solubility point was made.

Method ETS-8-172.1 was used for the quantitative determination of PFOS and FOSA solubility in urine.

Urine (approximately 5 ml) was added to the 24 plastic 15 mL centrifuge tubes. For solubility determination, 10 mg of PFOS was added to each of nine plastic 15 mL centrifuge tubes and 10 mg of FOSA was added to each of nine plastic 15 mL centrifuge tubes. To these 18 centrifuge tubes and three additional method blank centrifuge tubes, approximately 5 mL of urine was added. The tubes were placed in an orbital shaker bath at 30 °C. Three tubes for each compound and one method blank were removed after 24, 48 or 72 hours, allowed to equilibrate for 24 hours, centrifuged., prepared for analysis and analyzed.

RESULTS

The solubility of PFOS SD-018 in urine was determined to be 305 µg/mL at 23-24 °C. The solubility of FOSA SE-027 in urine was determined to be 2.68 µg/mL at 23-24 °C.

RS-III-22: An Epidemiologic Analysis of Episodes of Care of 3M Decatur Chemical and Film Plant Employees, 1993-1998.

TEST SUBSTANCE

Identity: perfluoroctanesulfonyl fluoride (POSF)-related chemistry; perfluorooctanesulfonate (PFOS); and perfluorooctanoate (PFOA).

Remarks: A health care episode is defined as a series of related events with a beginning, an end, and a course, all related to a particular health problem that exists continuously for a delineated period of time. The episode of care concept does not translate into a well-defined epidemiologic endpoint as it may include incident cases, prevalent cases, tentatively diagnosed cases and misclassified cases that are the routine consequence of the differential diagnoses that individuals may undergo in the course of disease diagnosis, treatment and management. Nevertheless, the episode of care concept may be a useful screening method for the potential risk of diseases and/or conditions that do not lead to traditional occupational epidemiologic study endpoints such as mortality, or morbidity outcomes that would be difficult to assess without formal investigations involving comprehensive medical record reviews to validate the diagnoses.

METHOD

Study design: Retrospective cohort episodes of care analysis

Manufacturing/Processing/Use: 3M Decatur chemical and film plants.

Hypothesis tested: The purpose of this study was to compare the observed to expected episodes of care experience from 1993 through 1998 of 652 chemical employees to the observed to expected episode of care experience of 659 film plant employees at the 3M Decatur manufacturing site. Based on toxicology and epidemiology research conducted, to date, on the POSF-based and PFOA chemistry, episodes of care that were considered *a priori* interests in this study included liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, gastrointestinal disorders of the liver and biliary tract, and reproductive, pregnancy, congenital and perinatal disorders.

Study period: January 1, 1993 through December 31, 1998

Setting: Occupational setting.

Total population: 1,311 employees

Subject selection criteria: The initial study population consisted of all full-time and inactive employees at the Decatur site as identified in the 3M Epidemiology's work history database for Decatur as of January 1, 1993. There was a one-year eligibility employment criterion to be included in this database. All employees hired subsequently were included in the study as long as they met the criterion to be eligible for this database. Employees hired, terminated (voluntary or involuntary termination) or died while employed from the company during the study (1/1/93 through 12/31/98) had their Decatur episodes of care experience limited to their Decatur time of employment. Full-time active employees who retired during the study period continued to have their episodes of care experience examined through the end of study; Medicare, however,

becomes the employee's primary provider upon the employee's 65th birthday and thus Ingenix did not have most of these Medicare claims. Similarly, claim records for employees who went on long term disability (LTD) were covered primarily by Medicare after 18 months of LTD status. There were some employees (n < 40) who chose HMO coverage between 1996 and 1998. HMO records were not incorporated in the Ingenix database coverage for the Decatur site. Altogether, there were 1,311 Decatur employees (97%) eligible for the episode of care analysis during the 1993 - 1998 time period.

Total # of subjects in study: 652 chemical workers and 659 film plant workers

Comparison population: Two normative databases were used to calculate the expected number of claims: 1) the entire U.S. 3M population health claims experience from 1993-1998 excluding the Cottage Grove and Cordova sites due to their fluorochemical production activities; and 2) the U.S. 3M population health claims experience from 1993-1998 excluding employees at the St. Paul, Woodbury, Cottage Grove, and Cordova sites. The latter database, was identified as the 3M manufacturing plant normative database, and it provided a comparison database that was more representative of 3M manufacturing plant employees, in general, as it excluded the St. Paul 3M Center corporate and research employees. For each of the four comparison group's chemical and film plant populations, the observed and expected number of episodes of care experience for the CCG's Specialty Category, Category and Class descriptions were calculated. However, it is the ratio of the two plant indirect standardized ratios for each comparison group, which was defined as the Risk Ratio Episodes of Care (RRE_pC), that provided the measure of risk between the two study populations (chemical and film). That is, the ratio of the observed to expected episodes of care experience in the chemical plant cohort was compared to the observed to expected episodes of care experience in the respective film plant cohort for 400 diseases or conditions as identified in the Ingenix computer software grouper called the Clinical Care Group.

Participation rate: 100% of those eligible

Subject description: See above subject selection criteria

Total number of person-years: N/A

Data collection methods Entire work history data for all employees were computerized as part of the Decatur mortality study. Investigators then combined six years of health claims data for those cohorts (comparison groups A - D) as identified from the work history databases. The Ingenix Employer Group then combined their six years of health claims data for these cohorts to be analyzed by the Clinical Care Group (CCG) software. The CCG software involves a comprehensive grouping of all visits (inpatient, outpatient), procedures, ancillary services and prescription drugs used in the diagnosis treatment and management of more than 400 diseases or conditions. The software code constructs an episode of care around the index-eligible record by searching backward and forward in time for records that are related to the disease in question. CCG software provides a hierarchical disease/condition classification scheme. There were 20 Specialty Categories that were subdivided into 103 Categories. The Categories were then further subdivided into 442 Class descriptions.

Exposure period: Variable depending upon employee work history

Description/delineation of exposure groups/categories: : Each employee's work history record was examined to determine whether the employee: 1) ever worked in the chemical plant, film plant or both; 2) worked in the chemical plant, film plant or both during the study time period of January 1, 1993 through December 31, 1998; and 3) worked continuously in the chemical plant or film plant for the entire 10 years prior to the onset of the study time period, January 1, 1993 ('long-term' workers). For those employees who had work experience in both the chemical and film plants, records were reviewed to identify which plant each employee primarily worked at in his or her career at the 3M Decatur site. Both chemical and film plant employees were categorized in the analyses as to whether they have, or have not, worked in both plants. The majority of film plant employees with prior work experience in the chemical plant worked at the chemical plant for a brief period of time (1 to 3 months) and this usually occurred during the first year of their employment. These employees were categorized as film plant employees. Likewise, the majority of chemical plant employees with prior work experience in the film plant worked at the film plant for a brief period of time and this usually occurred during the first year of their employment. These employees were categorized as chemical plant employees. Employees who had site-wide responsibilities (those who may work daily in both chemical and film plants, such as environmental, health and safety specialists) were assigned to the chemical plant because of their greater likelihood of exposure to POSF-based chemicals than those employees who only worked in the film plant. Each employee was assigned a job title that described the person's usual job activity while a Decatur employee. These job titles were: boiler operator, environmental health and safety specialist, engineer, mill operator, maintenance, office worker, operator (e.g., cell, chemical), quality control worker, shipping clerk and supervisor. Based on the findings from the Decatur serum fluorochemical assessment study three subcohort groups were categorized as to their potential for high, low and minimum/nonexposed POSF-based exposure.. These were the same categorizations used in the Decatur retrospective cohort mortality study. For example, cell operators, chemical operators and maintenance workers at the chemical plant were categorized as high exposure. Engineers and laboratory workers at the chemical plant were categorized as low exposure. Film plant workers were categorized as minimal/nonexposed.

Four study employee comparison groups were then identified for the episodes of care analyses. The rationale for these four categories was to increase the likelihood of the chemical plant cohort to have long-term high fluorochemical exposure jobs. These four comparisons were:

> Group A Comparison: all chemical plant employees with or without prior film plant experience (n = 652) compared to all film plant employees with or without a prior chemical plant experience (n = 659).
>
> Group B Comparison: all chemical plant employees who never worked in the film plant (n = 388) compared to all film plant employees who never worked in the chemical plant (n = 424). Group B is a subset of Group A.

Group C Comparison: all high (defined above) fluorochemical exposure chemical plant employees (n = 498) compared to their job counterparts (considered least exposed) in the film plant, (n = 490). Group C is a subset of Group A.

Group D Comparison: all long-term (defined above), high fluorochemical exposure chemical plant workers (n = 211) compared to their job counterparts (considered least exposed) in the film plant (n = 345). Group D is a subset of Group C, which is a subset of Group A .

Measured or estimated exposures: None. Serum PFOS results from a random sample of Decatur employees conducted in 1998 were used to help construct the exposure groups as described above.

Statistical methods: For each normative comparison group, an observed to expected ratio was calculated using indirect standardization techniques for each of the chemical and film plant populations. In other words, an observed to expected ratio was calculated for the chemical plant and likewise for the film plant. The direct comparison of two indirect standardized ratios is an unbiased estimator of a risk ratio if there are similar age-specific structures between the two comparison populations. In this study, this estimate was termed the Risk Ratio Episodes of Care (RRE_pC). Because the chemical and film plant cohorts had slightly different age structures used for the indirect standardization, the ratio of the two indirect standardized ratios for the chemical and film plant populations was not necessarily directly comparable. Therefore, an additional analysis was conducted which compared the ratio of the two indirect standardized ratios, corrected for their age structure. This estimate was termed the Risk Ratio Episodes of Care corrected ($RRE_pC_{corrected}$). Ninety-five percent confidence intervals were provided for RRE_pC. Ninety-five percent confidence intervals were not provided for $RRE_pC_{corrected}$ as its method of calculation was not readily apparent. Since the age structures of the chemical and film plant populations differed only slightly, the RRE_pC and its 95% confidence intervals were used as a measure of estimation of the risk ratio.

Other methodological information: Several methodological issues need to be considered when using an episode of care software product. Most importantly, among the various software programs there is no uniform approach to the software construction (i.e., grouping) of an episode of care. Thus, types and counts of episodes of care may differ by the software used. An episode of care may begin with a clear diagnosis by a provider; some providers, however, base an initial diagnosis on the patient's signs and symptoms whereas others assign a diagnosis only after a confirmed procedure. It is possible that two different diagnoses may be assigned to the same episode. A diagnosis that is categorized as confirmed, may in fact be tentative, or even incorrect (i.e., only a rule-out diagnosis). Thus, from an epidemiologic perspective an episode of care could represent any and all of the incident cases (newly diagnosed), prevalent cases (existing cases) and/or misclassified cases (both directions, false positive and false negative). Therefore, it is important that the term episode of care not be confused with incidence or prevalence as it can be a measure of both and also have an undetermined degree of misclassification. It is best to view episodes of care as its own unique metric. Second, there is variability in the comprehensiveness of the services that are included in the construction of the health care episode. Generally inpatient and outpatient services are

considered but other services including prescriptions and laboratory work-up may, or may not, be included. Third, the endpoint of an episode can also vary among these software programs. An endpoint can be a predetermined time period for a defined condition or it may be determined through the use of "clean periods" or "clean windows" which are defined as gaps in time between health care services necessary to separate possible recurring episodes. Finally, the clinical flexibility of the algorithm may differ depending upon the software program.

RESULTS

Describe results: The overall episodes of care experience was comparable between chemical and film plant employees for most diseases and conditions. Where increased RRE_pCs were observed, they were often attributed to a deficit of observed episodes of care in the film plant as much as any observed excess of episodes of care in the chemical plant. These high RRE_pC values often had very wide 95% confidence intervals which did not exclude, or barely excluded, the null value. Of the *a priori* concerns, only one liver cancer episode of care, which was from a film plant employee, was observed. RRE_pCs did not exclude the null hypothesis ($RRE_pC = 1.0$ within 95% confidence interval) for a variety of liver disorders, thyroid and lipid metabolism disorders and reproductive, pregnancy, congenital and perinatal disorders. There was an association with cholelithiasis with acute cholecystitis ($RRE_pC = 8.6$, 95% confidence interval 1.1-381); this may have been due, in part, to fewer expected number of episodes of care in the film plant. The RRE_pC for other biliary tract disorders did not exclude the null hypothesis in the confidence interval.

Because of a previously reported increased mortality risk for bladder cancer among the chemical plant employees, particular attention was given to those episodes of care which involved the urogenital tract. One episode of care for bladder cancer was reported for a film plant employee who had never worked in the chemical plant. There was an increased risk in episodes of care among chemical plant employees for lower urinary tract infections ($RRE_pC = 1.3$, 95% confidence interval 1.0-1.6). This increased risk of episodes of care was greater among the long-term high exposure chemical plant workers ($RRE_pC = 2.2$, 95% CI 1.4-3.3). There was also a greater percentage of these long-term high exposure chemical plant workers who had recurring lower urinary tract infections (59%) than film plant workers (31%). It is not known whether this reoccurrence is a result of occupational exposure(s) or nonoccupational-related factors. It was noted that the prevalence of episodes of care for lower and unspecified urinary tract infections (number of unique individuals divided by population at risk) was comparable between the chemical (9.5%) and film (9.9%) plant employees.

Other associations observed, that were not *a priori* concerns, among all study subjects included increased RRE_pCs for cancers and benign growths ($RRE_pC = 1.3$, 95% CI 1.1-1.6) that was contributed to by increased RRE_pCs (which did not exclude the null hypothesis) for benign colonic polyps ($RRE_pC = 1.4$, 95% CI 0.9-2.1), malignant neoplasms of the colorectal tract ($RRE_pC = 5.4$, 95% CI 0.5-265), malignant neoplasms of the rectum ($RRE_pC = 1.8$, 95% CI 0.3-12), malignant neoplasms of the prostate ($RRE_pC = 7.7$, 95% CI 0.9-364) and benign neoplasms of the skin ($RRE_pC = 1.3$, 95% CI 0.9-1.7).

Study strengths and weaknesses: An important strength of this study was the comparison of two local study groups: one likely to have been exposed to POSF-based chemicals and a reference group of employees from the film plant who were unlikely to have been exposed to fluorochemicals. This study design limited the influence of regional differences in diagnostic and therapeutic patterns as well as regional differences in patterns of morbidity. Another strength of this study was its ability to further subdivide the exposed (fluorochemical) group into subcohorts of higher potential for exposure and for longer periods of time. A third strength of the study is the use of a metric, episodes of care, which likely was comprehensive for all health outcomes experienced by the study subjects from 1993 and 1998. Study limitations include the fact that the concept of an episode of care does not readily translate into a well-defined epidemiologic metric (incidence or prevalence data). Positive or negative associations can therefore be difficult to interpret. The study time period was only six years. Additional data were not available for analysis purposes. Former employees were not part of this analysis as they would not be covered by the 3M health insurance system.

Research sponsors: 3M Medical Plan and the Ingenix Employer Group

Consistency of results: The findings from this study should be considered in context with all other toxicology and epidemiology research regarding perfluoroctanesulfonyl fluoride-related chemistry.

CONCLUSIONS

This study compared the episode of care experience of 652 chemical employees to the episode of care experience of 659 film plant employees at the 3M Decatur manufacturing site from 1993-1998. Although the analyses of episodes of care does not translate into a well-defined epidemiologic measure, it can be used as a screening method to determine if a study population could be at an increased risk for a disease or condition. In this study the overall episodes of care experience was comparable between chemical and film plant employees for most Specialty Categories, Categories and Class diseases and conditions. Of *a priori* concerns, there were not positive associations that excluded the null hypothesis for malignant neoplasm of the liver, liver disorders, thyroid and lipid metabolism disorders and reproductive, pregnancy, congenital and perinatal disorders. An episode of care greater than expected occurred for cholelithiasis with acute cholecystitis which was due, in part, to fewer observed than expected number of episodes of care in the film plant. Other biliary tract disorders did not exclude the null hypothesis in the confidence interval.

Because of an increased mortality risk for bladder cancer among the chemical plant employees, the study also focused particular attention on episodes of care which involved the urogenital tract. There was one episode of care for bladder cancer reported for a film plant employee who had never worked in the chemical plant. There was a greater increased risk in episodes of care among chemical plant employees for lower urinary tract infections but this was largely due to increased percentages of these employees having recurring episodes of care, rather than a greater prevalence of individuals having episodes of care. This increased risk of episodes of care was greater among the long-term high exposure chemical plant workers. It is not known whether this recurrence is a result of occupational exposure or nonoccupational-related factors. Other associations observed,

that were not *a priori* concerns, included increased risk of episodes of care for benign colonic polyps and malignant neoplasms of the colorectal tract as well as malignant neoplasms of prostate. Whether these non *a priori* associations have a biological rationale is questionable as other toxicologic and epidemiologic research does not offer support in relation to the serum PFOS and/or PFOA levels measured in Decatur chemical plant employees. Additional investigations would be required to determine whether an increase in incidence or prevalence actually exists based on the episodes of care reported.

REFERENCE

Olsen GW, Burlew MM, Hocking BB, Skratt JC, Burris JM, Mandel JH. An Epidemiologic Analysis of Episodes of Care of 3M Decatur Chemical and Film Plant Employees, 1993-1998. 3M Final Report. May 18, 2001.

RS-III-23: Absorption of FC-95-14C in Rats after a Single Oral Dose.

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: Cp = 15.65e^(-0.00387t) where Cp 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.

RS-III-24: 28-Day Percutaneous Absorption Study with FC-95 in Albino Rabbits.

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 LD_{50} 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.

RS-III-25: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-14C.

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 exsangination, 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 (<= 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

RS-III-26: Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium [14C]Perfluorooctanesulfonate.

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 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.

RS-III-27: 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: Potassium perfluorooctanesulfonate (KPFOS, FC-95, Lot 217, 86.9 % pure) was provided by 3M Company, Specialty Materials Manufacturing Division (St Paul, MN). Impurities included: lesser homologs (C4-C7) at 8.41%; impurities by NMR at 1.93%; metals (calcium, magnesium, sodium, nickel, and iron) at 1.45%; inorganic fluoride at 0.59%; perfluorooctanoic acid at 0.33%; nonofluoropentanoic acid at 0.28%; and, heptafluorobutyric acid at 0.1%.

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); Interim histopathology

GLP: Yes

Year: Report issued January 2, 2002

Species/Strain: Rat; Crl:CD ® (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, with subchronic observations at 4, 14 and 52 weeks

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

Method Detail:

Diet Preparation and Analysis

Dietary concentrations were based on the test material as supplied. Before initiation of treatment, diets of 0.5, 1, 2, and 20 ppm concentrations were prepared for stability and homogeneity analyses. Diets were prepared at least once every 4 weeks during the treatment phase of the study. Control animals received basal diet (PMI Nutrition International Certified Rodent Diet 5002 meal, St. Louis, MO) prepared with acetone (Spectrum Chemical Mfg. Corp., New Brunswick, NJ and Gardena, CA, Lot Nos. LH0253 and NS0231). The appropriate amount of certified diet was weighed and mixed

with an appropriate volume of acetone. Diet for each dose level was prepared independently using a base diet premix. Base diet was prepared by weighing a specified amount of certified diet into a labeled container. The required amount of test material was weighed and transferred into a labeled container and the appropriate volume of acetone (Spectrum Chemical Mfg. Corp., New Brunswick, NJ and Gardena, CA, Lot Nos. LH0253 and NS0231) was added to the container and mixed manually. Additional volume of acetone was added as necessary until the test material dissolved. To prepare a premix, the certified diet was transferred into a labeled Hobart® mixing bowl. The test material and acetone were added to the mixing bowl, overlaid with a portion of diet from the mixing bowl, and thoroughly mixed. A portion of diet from the mixing bowl was transferred to a second mixing bowl, mixed manually to recover residual test material, and returned to the first mixing bowl. The contents of the mixing bowl were thoroughly mixed. For each dose level, a specified amount of certified diet was weighed into a labeled container and a specified amount of base diet was weighed for each dietary concentration. A pocket was formed in the feed in the mixing bowl and the amount of base diet was transferred to the pocket. The contents of the mixing bowl were thoroughly mixed for 15 minutes. The diets were stored at room temperature in covered containers until dispensed into feeding jars.

Analyses for the concentration of test material in the dose preparations were done on samples taken directly from the mixing bowl by using an analytical method, supplied by the 3M and validated by Covance (Method MP-M383-MA). These methods have been described previously (Hansen et al., 2002; Seacat et al., 2002). Dose preparations were extracted using a validated ion-pairing extraction method and analyzed by high-pressure liquid chromatography/tandem mass spectroscopy (HPLC-MS/MS) for homogeneity, stability and concentration. For dose confirmation, samples (approximately 100 g each) from all dose preparations were analyzed in duplicate. Homogeneity was determined from the dose preparations mixed pretest and from preparations mixed for Week 1. One sample (approximately 100 g each) from the top, middle, and bottom of the dose preparations were collected, divided into three subsamples for extraction and analysis, and analyzed for test material content. All samples were stored at room temperature until analyzed within 7 days of mixing. To evaluate the stability of the test material in the carrier, one set of samples (approximately 100 g each) were taken from the preparations mixed pretest, stored at room temperature for 28 days, then analyzed. Homogeneity samples collected from the middle of the pretest dose preparations were analyzed within 2 days of mixing and used as the baseline value. An additional set of samples (approximately 100 g each) were taken from the preparations mixed for Week 1, stored at room temperature for 33 days, then analyzed. Homogeneity samples collected from the middle of the dose preparations for Week 1 were analyzed on the day of mixing and used as the baseline value.

Animals and Husbandry

Male and female Crl:CD®(SD) IGS BR rats were purchased from Charles River Laboratories Inc (Raleigh, NC). The rats were quarantined for 13 days and evaluated for weight gain and any gross signs of disease or injury. Selection of animals for the study was based on acclimation data. The rats were approximately 41 days old at the beginning of the study, and weight ranges for males and females were 135 to 226 g for males and

128 to 182 g.

Animal rooms were maintained at a temperature of 22 ± 4°C, a relative humidity of 50 ± 20 %, and a 12-hour light/dark cycle in an AAALAC approved facility (Covance Laboratories, Madison, WI). After acclimatization, rats were housed individually in stainless steel cages. Some animals were placed in polycarbonate cages during the study when health problems dictated. Food was provided *ad libitum*, except when animals were fasted. Water was provided *ad libitum*, and samples of the water are analyzed for specified microorganisms and environmental contaminants.

Dosing

Target diet concentrations were 0, 0.5, 2.0, 5.0, or 20 ppm KPFOS, and the dose preparations were administered *ad libitum*. Rats were assigned to six groups as presented in Table 1. Groups of 50 rats per sex per treatment level were fed control diet or diet containing 0.5, 1, 2, 5 or 20 ppm KPFOS feed *ad libitum* for up to 104 weeks to evaluate chronic toxicity and potential carginogenicity. Additional rats were added for evaluations at 4, 14 and 52 weeks. Five rats per sex per group (except the 20 ppm dose-group recovery animals) were sacrificed after 14 weeks of dosing to evaluate sub-chronic toxicity and mechanistic endpoints, and this sub-chronic and mechanistic evaluation has been previously reported (Seacat et al., 2002). Five animals per sex per in the control group and the 20 ppm dose group were sacrificed after 52 weeks of dosing to evaluate chronic toxicity at one year. The 20 ppm dose level recovery group (Group 6 in Table 1) was placed on control diet after 52 weeks of treatment.

Table 1. Dose Groups and Number of Animals

Group	Number of Animals		Dietary Levels (ppm T-6295)
	Male	Female	
1 (Control)[a, b, c]	70	70	0
2 (Low)[b]	60	60	0.5
3 (Mid)[b]	60	60	2.0
4 (Mid-High)[b]	60	60	5.0
5 (High)[b, c]	70	70	20.0
6 (High Recovery)[d]	40	40	20.0

[a] The control animals received the control diet (basal diet with acetone).

[b] Five animals/sex in Groups 1 through 5 were sacrificed at Weeks 4 and 14 for hepatocellular proliferation rate measurements, biochemical analyses (palmitoyl-CoA oxidation), and histopathology (Week 14 only).

[c] Ten animals/sex in Groups 1 and 5 were designated as interim sacrifice animals. These

animals were sacrificed after at least 52 weeks of treatment.

[d] Animals in Group 6 were treated for at least 52 weeks, then treatment was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 52 weeks posttreatment. During recovery, the animals received basal diet only.

Clinical Observations

The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity, and findings were recorded as they were observed. At least once prior to treatment and weekly thereafter, each animal was removed from its cage and examined; abnormal findings or an indication of normal was recorded. Body weight data were collected weekly through Week 17, once every 4 weeks thereafter, and at Week 105. Food consumption data were collected weekly for the first 16 weeks and once every 4 weeks thereafter. Food efficiency (g wt gain/g food consumed) and mean daily intake of KPFOS (mg/kg/day) were calculated from body weight, food consumption data and feed analysis data. In addition, the time of onset, location, size, appearance, and progression of each grossly visible or palpable mass were recorded.

Clinical Chemistry, Hematology, and Urinalysis

During Weeks 4, 14, 27, and 53, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry tests from 10 animals/sex in all dose groups except the 20 ppm recovery group. Blood was collected for cholesterol and triglyceride determinations from all animals prior to the terminal sacrifice during Week 105 for all groups except 2 ppm dose-group females the 20 ppm recovery group, which were sacrificed in Weeks 103 and 106, respectively. For blood collection, overnight fasted (approximately 16 hours) rats were bled in random order. Approximately two ml of whole blood was drawn from the jugular vein. Serum samples were obtained for clinical chemistry by centrifugation of blood allowed to clot in tubes without anticoagulant. The anticoagulant was potassium EDTA for hematology tests. Blood films were also prepared for animals at the terminal and recovery sacrifices. For urinalysis, animals were fasted overnight, and urine was collected chilled overnight (approximately 16 hours) before blood sampling.

Hematology included determination of hemoglobin; hematocrit; mean corpuscular volume; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration; and, blood cell morphology in addition to blood cell counts which included: erythrocytes; platelets; leukocytes, and differential (segmented neutrophils; lymphocytes; monocytes; eosinophils; and, basophils). Reticulocyte smears were made and held for possible future examination.

The clinical chemistry parameters evaluated were made using a Kinetic/Hitachi® 704,911 instrument (Roche Diagnostics) according to the manufacturer's directions and included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), glucose (GLU), blood urea nitrogen (UN), calcium (CALC), phosphate (PHOS), total bilirubin (TBILI), cholesterol (CHOL), creatinine (CREAT), total protein (PROT), albumin (ALB), globulin (GLOB), sodium (Na), potassium (K),

and chloride (Cl).

Routine urinalysis included volume (graduated cylinder), specific gravity (AO/TS refractometer) and microscopic examination of urinary sediment by standard methods (Anon, 1968). The urinary pH, protein, bilirubin, blood, urobilinogen, ketones, glucose, sodium and potassium were measured with Mulitistix according to manufacturer's instructions (Bayer Diagnostics, Tarrytown, NY).

Necropsy and Postmortem Examination

At necropsy, macroscopic observations were recorded, and tissues were placed in appropriate fixative. The necropsy included a macroscopic examination of the external features of the carcass; all external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

During Week 53, 10 animals/sex/group from the control and 20 ppm dose groups were fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied in random order. The liver was weighed and liver samples were collected and frozen for analysis. Macroscopic observations were recorded, selected organs were weighed, and selected tissues collected and preserved. The following organs (when present) were weighed; paired organs were weighed separately: adrenal (2); brain; kidney (2); liver; lung; ovary (2); spleen; testes; thyroid (2) with parathyroid; uterus with cervix. A blood film was taken for each animal sacrificed at the Week 53 interim sacrifice. Organ-to-body weight percentages and organ-to-brain weight ratios were calculated.

A necropsy was done on each animal that died or was sacrificed at an unscheduled interval, but organ weights were not recorded. Animals sacrificed at unscheduled intervals were anesthetized with carbon dioxide, weighed, and exsanguinated.

After at least 104 weeks of treatment (Week 103 for females in Group 3), the remaining animals were fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied. Animals were necropsied in random order. Macroscopic lesions, liver, lung, kidneys, pancreas, thyroid, testes, and mammary gland (females), and urinary bladder were examined for animals in Groups 2, 3, 4, and 6 that were sacrificed during Weeks 103, 105, and 106. Surviving females in Group 3 were sacrificed and necropsied during Week 103 because of reduced survival. The remaining animals in Groups 1 through 6 were sacrificed and necropsied during Weeks 105 and 106. After at least 52 weeks of treatment and 52 weeks without treatment, the remaining animals in Group 6 were fasted overnight, anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied. A blood film was taken as part of the necropsy procedures at the terminal and recovery sacrifices. Bone marrow smears from the femur of each animal at scheduled sacrifices were prepared, stained with Wright's stain, and retained for possible examination. At the terminal and recovery sacrifices, sections of the heart and liver were preserved in 2.0% parafomaldehyde/2.5% gluteraldehyde in 0.1M phosphate buffer, processed, and embedded in epoxy resin for possible future electron microscopy from 10 animals/sex in Groups 1, 4, 5, and 6.

The following tissues were collected and preserved in 10% neutral-buffered formalin: adrenal (2); brain; cecum; cervix; colon; duodenum; epididymis (2); esophagus; eye (2);

femur with bone marrow (articular surface of the distal end); Harderian gland; heart; ileum; jejunum; kidney (2); lesions; liver; lung with mainstem bronchi; lymph node (mesenteric); mammary gland (females only); ovary (2); pancreas; pituitary; prostate; rectum; salivary gland [mandibular (2)]; sciatic nerve; seminal vesicle (2); skeletal muscle (thigh); skin; spinal cord (cervical, thoracic, and lumbar); spleen; sternum with bone marrow; stomach; testis (2); thymus; thyroid (2) with parathyroid; trachea; urinary bladder; uterus; vagina.

Histopathology

Microscopic examinations were done on selected tissues (adrenals, brain, eyes, kidney, liver, mesenteric lymph node, pancreas, spleen, testes, and ovaries) from the animals necropsied during Week 14 and on all tissues from the animals in Groups 1 and 5 that were necropsied during Weeks 53, 105, and 106. In addition, microscopic examinations were done on the tissues from animals that died or were sacrificed due to poor health. Also, urinary bladder was examined microscopically from all terminally sacrificed animals in Groups 2, 3, and 4, and from all animals at the recovery sacrifice. Liver sections from five animals/sex/group from Groups 1 and 5 were stained with Oil Red "O" and examined microscopically. In addition, liver samples from five animals/sex/group were processed and evaluated by electron microscopy. Liver samples were obtained from each of the 20 rats during necropsy at terminal sacrifice for examination by transmission electron microscopy (TEM). Liver samples were assessed for ultrastructural changes that may be related to chronic toxicity of the test material when administered in the diet.

Tissues (as appropriate) from each animal in Groups 1, 5, and 6 sacrificed at the Week 53 interim sacrifice were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Tissues (as appropriate) from each animal in Groups 1 and 5 sacrificed at the terminal sacrifice and any animals that died on test or were sacrificed at unscheduled intervals were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Lesions, liver, lungs, kidneys, pancreas, thyroid, testes, mammary glands (females), and urinary bladder from each animal in Groups 2, 3, 4, and 6 sacrificed at the terminal and recovery sacrifices were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically (see Protocol Deviations for exceptions). Parathyroids were processed with the thyroids, but were not examined.

At the Week 53 sacrifice, BrdU immunohistochemistry was performed by PAI on the livers and duodenums from five animals/sex/group in Groups 1 and 5 that received BrdU. In addition, sections of the liver and duodenums from these animals were stained with hematoxylin and eosin and examined microscopically.

A pathology peer review was conducted by a pathologist designated by the sponsor.

Liver sections from five animals/sex/group from Groups 1 and 5 were stained with Oil Red "O" stain and examined microscopically.

Liver sections in epoxy blocks for five animals/sex/group from Groups 1 and 5 were processed and evaluated by electron microscopy by Pathology Associates International (report included in Appendix 8).

Serum and Liver PFOS Analysis

Serum and liver samples collected for PFOS determination during Week 53 and at terminal sacrifice were flash frozen in liquid nitrogen, stored at -60 to -80°C and shipped on dry ice to 3M Environmental Laboratory (Saint Paul, MN). Analysis of PFOS levels by HPLC-MS/MS was performed according to published methods (Hansen et al., 2001). Quantitation of PFOS was based on the comparison of a single ion peak area to the response of two standard curves, with mid-level calibration checks analyzed every 5-10 samples. Based on the precision and accuracy determined from repeat injections of the standard curves, results were considered quantitative to ± 30%.

During Week 53, blood samples (approximately 2 mL) were collected from five animals/sex/group in Groups 1 and 5 (from animals selected for interim sacrifice) and from all remaining animals at terminal and recovery sacrifices. Animals were fasted overnight. Blood was collected from a jugular vein, allowed to clot at room temperature, and centrifuged. Samples were collected without anticoagulant. Serum was harvested and stored in a freezer, set to maintain -60 to -80°C, until sent to the sponsor for analyses of PFOS levels and metabolites. Analysis of these samples is the responsibility of the sponsor; results are not included in this report.

Hepatic Cell Proliferation

Seven days before the Week 53 interim sacrifice, osmotic pumps (ALZET® Model 2ML1) were surgically implanted in five animals/sex/group from Groups 1 and 5. The osmotic pumps were preloaded with approximately 2 mL of bromodeoxyuridine (BrdU) at a concentration of 20 mg/mL. The animals were anesthetized by administration of acepromazine-ketamine-xylazine, and one pump/animal was aseptically inserted subcutaneously (dorsal surface). The incision was closed with wound clips, and the animals were monitored during clinical observations until the time of sacrifice to ensure that there were no clinical signs of infection. One Group 5 male implanted with the osmotic pump was sacrificed before the Week 53 sacrifice.

Hepatocellular cell proliferation was assessed by bromodeoxyuridine incorporation.

Palmitoyl CoA Oxidase

Samples of the right lateral lobe of the liver were flash-frozen in liquid nitrogen at necropsy and stored at -70°C until analyzed for palmitoyl CoA oxidase (PCoAO) activity as an indication of peroxisome proliferation (Lazarow, 1981).

Data Collection and Processing

Each animal was assigned a temporary number upon arrival. Before initiation of treatment, a microchip identification device was implanted into each animal. After randomization for placement on test, each animal was assigned a permanent number, and the microchip was coded with the permanent number. All data for an animal were recorded under these numbers.

The Path/Tox application, supplied by Xybion Medical Systems Corporation, was used for the direct on-line capture of in-life toxicology and anatomic pathology data. The Randomization and Data Extension Systems (RADES) and Automatic Form and Label Generation System (AFLGS) applications were used in conjunction with the Path/Tox

system to randomize animals and produce labels and forms, respectively. The Talisman application was used for the dose preparation information, and the Millennium system was used for the collection of dose analysis data, and the Clinical application was used for the collection of clinical pathology data.

Data Analysis and Statistics

Animals were assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment group. At the time of randomization, the weight variation of the animals did not exceed ± 2 standard deviations of the mean body weight for each sex. Mean body weights per group were analyzed using Levene's test for homogeneity of variance at the 5.0% probability level and were found to be homogeneous.

Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. One-way analysis of variance (ANOVA (Winer, 1971)) was used to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, palmitoyl CoA oxidase activities, and organ weight data (Week14 and 53 interim sacrifices only).

ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's t-test (Dunnett, 1964) was used for pairwise comparisons between treated and control groups.

Group comparisons (Groups 2 through 6 versus Group 1) were evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment were analyzed statistically.

Evaluations of trend and heterogeneity of survival data were performed using the Cox-Tarone binary regression method using the National Cancer Institute (NCI) Life Table Package (Thomas, Breslow, and Gart, 1977). Week 105 was treated as the end of the study in the NCI package for both sexes. Animals that were sacrificed at scheduled intervals were censored in the analysis. Continuity-corrected one-sided tail probabilities for trend and two-sided tail probabilities for group comparisons were evaluated at the 5.0% significance level.

Nonneoplastic lesions were chosen for statistical analyses if the incidence in at least one treated group (Groups 2 through 5) was increased or decreased by at least two occurrences over the control group (Group 1). The high recovery group (Group 6) was selected based on the incidence rate (%) if it was increased or decreased by at least two as compared to the control or high-dose (20.0 ppm) group. When there was only one incidence in the high-recovery and none in the other groups, that case was excluded from the statistical evaluation. These were analyzed by the Cochran-Armitage test for trend and the Fisher-Irwin exact test for control versus treatment comparisons (Thakur, Berry, and Mielke, 1985). One-sided tail probabilities for trend and group comparisons were evaluated at the 5.0% significance level and are reported in Text Tables 3 and 4 for males and females, respectively.

Neoplastic lesions were chosen for statistical analyses using the same criteria as in the case of nonneoplastic lesions. The incidental tumors (i.e., tumors that were not assigned

to be the cause of death of the animals by the study pathologist) were analyzed by logistic regression of tumor prevalence (Dinse and Lagakos, 1983). The logistic regression model included linear and quadratic terms in age (week of death), but thequadratic term was eliminated if there was a lack of convergence. The fatal and palpable (superficial) tumors were analyzed by Cox-Tarone binary regression method using the death time or the first palpation time (as applicable) as a surrogate for the tumor onset time. In the case of any particular tumor type where the study pathologist assigned the tumor in question being the cause of death of a subset of the animals and the rest of the animals were assumed to be dead of other competing risks, IARC-type (Peto et al, 1980) cause of death analysis was performed. Specifically, the subset of the tumors which were assigned to be the cause of death by the study pathologist was analyzed by Cox-Tarone logistic regression under life table techniques. The subset, which was considered incidental by the pathologist, was analyzed by logistic regression of tumor prevalences. Tumor types whose cause of death was undetermined, were treated as incidental for statistical evaluation. The score statistics and their respective variances from the above tests were then used to compute the combined evidence as described by Gart et al. (1986). If one tumor in the group belonged to one of the two categories (fatal and incidental) in a test was combined with the other category for the purpose of statistical analyses. In addition, in the cases where there was lack of convergence for the asymptotic test of the logistic regression method, the exact probability of the significance was obtained by using LogXact-Turbo (1993) and combined with the probability of Cox-Tarone test, if necessary.

The benign and malignant neoplastic incidences were evaluated both separately and combined where appropriate. The criteria for combination were based on the work of McConnell et al. (1986). The incidences of multiple-organ findings such as hemangioma, hemangiosarcoma, lipoma, liposarcoma, fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, endometrial stromal polyp, and endometrial stromal sarcoma were counted by animal, not by tissue type. They were evaluated statistically if they met the selection criterion for the analysis. The statistical results for these cases may be biased because not all the animals were examined for every tissue. One-sided trend and pairwise group comparisons were evaluated at the 5.0% significance level and are shown in Text Tables 5 and 6 for males and females, respectively.

In the nonneoplastic analyses, animals sacrificed early (Week 4) were excluded from the denominators. In the statistical evaluations of the neoplastic lesions, animals sacrificed early (during or before Week 14) were discounted from the number of animals at risk. In the neoplastic analyses, the low- (0.5 ppm), mid-(2.0 ppm), mid-high, and high recovery groups that did not have complete histopathology examinations were excluded from statistical analyses.

RESULTS

Results of subchronic evaluations at 4 and 14 weeks are covered in:

> Seacat, A M, Thomford, P J, Hansen, K J, Clemen, L A, Eldridge, S R, Elcombe, C R and Butenhoff, J L (2002) Sub-Chronic Dietary Toxicity of Potassium Perfluorooctanesulfonate in Rats. *Toxicology* (in press).

REMARKS

Dose Analysis:

Results of the homogeneity, stability, and dose preparation analyses are in Tables 1 through 3.

Several homogeneity and stability analyses were conducted on each of the several mixes in an attempt to resolve issues related to the inherent variability and lack of sufficient sensitivity in the analytical method for measuring PFOS in feed. The homogeneity results indicate that the mixing procedure produced a homogeneous distribution of the test material in the dose preparations; although variability generally appeared slightly greater at the lower dietary concentrations (Table R1).

Table R1: Mean values of the homogeneity analyses for the pre-dosing mixes, % of nominal concentration

Mix Date	0.5	2	5	20
Homogeneity				
March 18, 1998	119-135%	-	-	107-118%
April 16, 1998	96.2-122%	98.5-107%	97.6-101%	90.0-101%
April 22, 1998	-	-	-	93.5-103%
June 18, 1998	91.0-175%	111-130%	99.6-102%	90.5-97.0%
Stability				
March 18, 1998 (28 days)	78.5	76.7	79.8	88.1
April 16, 1998 (33 days)	112.3	119.6	113.9	126.7

The mean concentrations of the dose preparation analyses for all levels ranged from 44.4-635% of the theoretical concentrations (including the reassay and retention sample analyses). The overall mean concentrations within each dose level ranged from 102.3 to 108.7. Inherent variability and lack of sufficient sensitivity at low levels in the analytical method for measuring PFOS resulted in homogeneity, stability, and routine analysis data that were in many cases outside of the standard limits of +/- 15%.

Observation of Animals:

Survival after 104 weeks of treatment was 22.0, 22.0, 34.0, 50.0, 45.0, and 27.0% for males in Groups 1, 2, 3, 4, 5, and 6, respectively, and 50.0, 30.0, 20.0 (after 102 weeks), 34.0, 52.0, and 48.0% for females in Groups 1, 2, 3, 4, 5, and 6, respectively. There was a significant increase trend in survival that occurred in the males that and was due to significant increases in survival in mid-high- (5.0 ppm) and high-dose (20.0 ppm) groups

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

There were no clinical observations attributed to administration of the test material. The types of clinical observations noted are commonly observed in laboratory rats as they age on long term safety studies.

There was no effect of the test material on the incidence of palpable masses.

Body Weight, Body-Weight Chgange and Feed Consumption:

Body weight and body weight change data are illustrated in Figures 3 and 4 and summarized in Tables 7 and 8; individual body weight data are in Appendix 3.

Males given 20 ppm (Groups 5 and 6) had significantly lower mean body weights compared to animals in the control group during Weeks 9 through 37. During recovery the mean body weights for Group 6 males were similar to mean body weights for Group 5 males. Females in Group 5 given 20 ppm had significantly lower body weights compared to animals in the control group during Weeks 3 through 101. Mean body weights for females in Group 6 given 20 ppm were statistically lower than mean body weights for control females during Weeks 3 through 61, but their mean body weights became nearer to the weights of the control females when placed on recovery. At Week 105, mean body weights for surviving males were 98, 108, 103, 101, and 96% of controls for the low-, mid-, and high-dose groups (Groups 5 and 6), respectively. At Week 105, mean body weights for surviving females were 101, 107 (Week 101), 100, 86, and 105% of controls, for the low-, mid-, and high-dose groups (Groups 5 and 6), respectively.

Overall mean body weight change was statistically significantly lower for males given 20 ppm diets for Weeks 1 through 15 and Weeks 15 through 29. Mean body weight change was statistically significantly lower for females given 20 ppm for Weeks 1 through 15 and Weeks 29 through 57 (Group 5 only). Mean body weight changes for males and females in Group 6 were significantly increased during the several weeks of recovery. Animals at lower dose levels occasionally had statistically significant decreases in body weight gain; however, these occurrences were inconsistent over time between sexes and were not clearly dose-related.

Feed Consumption

Although not always statistically significant, males given 20 ppm tended to consume less food during Weeks 1 through 24. Food consumption was similar for males given 20 ppm compared to animals given the control material during Weeks 28 through 104. Statistically significantly lower food consumption was noted for females given 20 ppm during Weeks 2 through 36. Food consumption for males and females was similar in all the other treated groups compared to animals given the control material.

Test Material Consumption

Test material consumption data are summarized in Table 10. Individual test material

consumption data are presented in Appendix 4.

Animals were fed diets containing 0, 0.5, 2, 5, or 20 ppm T-6295. The amount of test material consumed by animals on a mg/kg of body weight/day basis was as follows.

Group	Dietary Concentrations (ppm)	Mean Achieved Dose Levels - Range (mg/kg body weight/day)	
		Males	Females
2	0.5	0.015 - 0.057	0.015 - 0.052
3	2.0	0.064 - 0.226	0.073 - 0.213
4	5.0	0.153 - 0.570	0.186 - 0.559
5	20.0	0.643 - 2.205	0.838 - 2.149
6	20.0	0.732- 2.336	1.047 - 2.160

Clinical Pathology:

Dietary administration of PFOS for approximately 53 weeks was associated with mildly higher urea nitrogen for males and females fed 5 or 20 ppm; mildly lower glucose for males and females fed 20 ppm; mildly to moderately lower cholesterol for males and females fed 20 ppm; and mildly higher alanine aminotransferase for males fed 20 ppm. None of these effects were considered adverse. The effect on cholesterol was no longer apparent after 104 weeks of treatment. There was no effect on hepatic palmitoyl-CoA oxidase activity.

Anatomic Pathology:

At the Week 53 interim sacrifice, terminal body weights were significantly decreased in the females given 20 ppm. In the males, absolute and relative liver weights were increased in the group receiving 20 ppm. In addition, absolute and relative spleen weights were decreased in males given 20 ppm. There were no clear or consistent gross observations at the Week 53 interim sacrifice that could be attributed to the administration of the test material. At the Week 53 interim sacrifice, centrilobular hepatocytic hypertrophy and vacuolation was increased in incidence and severity in the males given 20 ppm. Generally, in the females given 20 ppm, only centrilobular hypertrophy was seen, and the change was less severe than that noted in the males. In addition, minimal to slight centrilobular hepatocytic pigment was found in the females given 20 ppm. There were no other histomorphologic changes that could be associated with the administration of the test material. Liver findings in several unscheduled deaths given 20 ppm resembled those seen in animals sacrificed at Week 53.

In the unscheduled sacrifices between Weeks 54 and 105, animals given 20 ppm had increased hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment were noted. Increased hepatocellular centrilobular hypertrophy was seen in animals given 5 ppm.

At the terminal sacrifice, the livers of animals given 5 or 20 ppm exhibited a slight increase in macroscopic findings, including enlarged, mottled, diffuse darkened, or focally lightened. Hepatotoxicity, characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in the animals given 5 or 20 ppm. An increase in eosinophilic clear cell altered foci and cystic hepatocellular degeneration was noted in the males given 2, 5, or 20 ppm.

The data suggests that the hepatotoxicity did not persist in the recovery-sacrifice animals.

A significant positive trend was noted in the incidences of liver hepatocellular adenoma for males. This was associated with a significant increase in the high-dose (20.0 ppm) group over the control. The high-dose recovery (20.0 ppm) group animals in this case exhibited a significantly decreased incidence when compared to that of the high-dose (20.0 ppm) group. Significantly increased incidences were observed for thyroid follicular cell adenoma in the high-dose recovery (20.0 ppm) group when compared to either the control or the high-dose (20.0 ppm) group. There was also a significant increase in the combined (adenoma and carcinoma) thyroid follicular cell tumors in the high-dose recovery (20.0 ppm) group animals as compared to that of the high-dose (20.0 ppm) group. None of these cases were associated with any significant trend. Thyroid follicular cell carcinoma did not show any significant effect.

There were no other significant neoplastic findings in the males.

Significant positive trends were observed in the incidences of hepatocellular adenoma and hepatocellular combined adenoma and carcinoma in the females. These cases were associated with significant increases in the high-dose (20.0 ppm) group as compared to the control. All these tumors were incidental and noted at the terminal sacrifice. A marginally significant increase for thyroid follicular cell adenoma and carcinoma combined was observed in the mid-high (5.0 ppm) group but not in the high-dose (20.0 ppm) group as compared to the control group. In fact, thyroid C-cell adenoma and thyroid C-cell combined adenoma and carcinoma actually showed significant decreases in the high-dose (20.0 ppm) group as compared to the control. There were significant negative trends in the incidences of mammary fibroadenoma and mammary combined adenoma and fibroadenoma. These cases were associated with significantly lower incidences in the respective high-dose (20.0 ppm) group when compared to that of the control. However, the low-dose (0.5 ppm) group animals for these cases exhibited significant increases over the control. A significant decreased incidence was found for the high-dose recovery (20.0 ppm) group in the incidences of mammary carcinoma as compared to the high-dose (20.0 ppm) group. There was no other significant group effect in mammary carcinoma. There was a marginal significant negative trend for the mammary combined fibroadenoma/adenoma/carcinoma. However, the low- (0.5 ppm) and mid-high (5.0 ppm) groups for this case exhibited significantly increased incidences over the control.

There were no other significant neoplastic findings in the females.

Cell Proliferation Results:

There were no statistically significant increases in cell proliferation as measured by

PCNA at weeks 4 and 14, or by bromodeoxyuridine (BrdU) immunohistochemistry at week 53. However, two out of five females in each of the mid-high and high dose groups showed a very mild, but biologically significant response (i.e. had a proliferative index that was twice the mean of the controls and greater than that of the highest control) at week 4. The cell proliferation response at week four was transient in nature as it was not observed at week 14, and was considered to be test compound-related.

Electron Microscopy Results:

Liver samples were obtained from each of the 20 rats during necropsy at terminal sacrifice for examination by transmission electron microscopy (TEM). Liver samples were assessed for ultrastructural changes that may be related to chronic toxicity of the test material when administered in the diet. Electron microscopic evaluation identified mild to moderate changes related to treatment. Mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy were the prominent features found to be different between the high dose group and the control animals. Other changes included a slight increase in the amount of glycogen in treated animals compared with controls.

Characterization of Tumor Incidence Outcome:

In males, hepatocellular adenoma was increased for trend ($p = 0.0276$) and pair-wise against control for the 20 ppm dose group (7/60 20 ppm vs. 0/60 control, $p = 0.0456$). Hepatocellular adenoma was not observed in the 20 ppm recovery group, which was significant compared to the 20 ppm dose group (0/40 20 ppm recovery group vs. 7/60 200 ppm dose group, $p = 0.0240$). Overall, the response in terms of hepatocellular adenoma can be considered weak at the 20 ppm dose level.

Thyroid follicular cell adenoma was increased pair-wise against control for the 20 ppm recovery group (9/39 20 ppm recovery group vs. 3/60 control, $p = 0.0280$). Although this finding is outside the range of recent historical control values in this laboratory, there were no other indications of thyroid abnormality.

In females, hepatocellular adenoma was increased for trend ($p = 0.0153$) and pair-wise against control for the 20 ppm dose group (5/60 20 ppm dose group vs. 0/60 control, $p = 0.0386$). Overall, the response in terms of hepatocellular adenoma can be considered weak at the 20 ppm dose level. The only hepatocellular carcinoma observed in the study was at the high dose. Hepatocellular adenoma/carcinoma was increased for trend ($p = 0.0057$) and pair-wise against control for the 20 ppm dose group (6/60 20 ppm vs. 0/60 control, $p = 0.0204$).

Mammary fibroadenoma was decreased for trend ($p = 0.0152$) and pair-wise against control for the 20 ppm dose group (11/60 20 ppm vs. 20/60 control, $p = 0.0235$).

RS-III-28: Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats.

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 Sponsor for analysis.

On days 1 to 4 of the 43-day premating 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

RS-III-29: Absorption and Biotransformation of N-EtFOSE and Tissue Distribution and Elimination of Carbon-14 After Administration of N-EtFOSE-^{14}C in Feed.

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 μCi/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-^{14}C 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	Carcass
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.

RS-III-30: Interim Report #2. Determination of Serum Half-Lives of Several Fluorochemicals.

TEST SUBSTANCE

Identity: The extracts were quantitatively analyzed for PFOS (perfluorooctanesulfonate), PFOA (perfluorooctanoate) and PFHS (perfluorohexanesulfonate).

Remarks: Four other fluorochemicals were initially analyzed in this study but their values approximated the lower limit of quantitation and were therefore discontinued for analysis.

METHOD

Study design: The overall research design is a prospective study that obtains multiple serial blood samples from 27 retirees throughout the course of a five-year period. The results from this interim report are at 18 months and are restricted to nine subjects. Each of four sample time periods (t_0, t_1, t_2 and t_3) were analyzed in triplicate at the same time to minimize experimental error.

Manufacturing/Processing/Use:

Hypothesis tested: The purpose of this study is to determine the serum half-life of PFOS, PFOA and PFHS in 27 retirees from 3M manufacturing sites (Decatur N = 24; Cottage Grove N = 3).

Study period: 1998 - 2004

Setting: Retirees provide semi-annual blood samples (annual after the second year)

Total population: N = 27 (24 males, 3 females)

Subject selection criteria: Retirees were invited to participate based on having prior work assignments in the chemical division. These participants were eligible for study selection if they retired from the Decatur chemical plant between 1995 and 1998. Of the 34 initially identified eligible subjects, 24 agreed to participate in the study. In addition, three retirees from the Cottage Grove manufacturing site were invited to participate.

Total # of subjects in study: 27

Comparison population: N/A

Participation rate: Voluntary participation rate was 71%.

Subject description: Retirees from Decatur and Cottage Grove fluorochemical manufacturing plants

Total number of person-years: N/A

Data collection methods: Voluntary blood collection conducted on a semi-annual, and then annual basis

Exposure period: Variable depending upon each person's work history prior to retirement

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposures: Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS, PFOA and PFHS using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluated versus an extracted curve from a human serum matrix. Endogenous levels of certain fluorochemical were determined in the standard serum matrix and additional fluorochemical was spiked into the matrix. The total amount of each specific fluorochemical (endogenous + spiked) was used to construct an extracted standard curve. All serum fluorochemical analyses were determined by Northwest Bioanaltyical Laboratory Inc. (Salt Lake City, UT).

Statistical methods: In an effort to minimize experimental error including systematic and random error in the analytical method, this interim report provides analysis on the estimated serum half-lives of PFOS, PFOA and PFHS on only nine subjects. Each had their serum from four time periods (t_0, t_1, t_2, t_3) measured in triplicate, with all four time-points analyzed in the same analytical run. This approach allowed for statistical evaluation of the precision of the measurement and assured that all experimental biases equally affected each sample used for half-life determination. Serum half-lives were calculated for these nine subjects using a one-compartment model.

Other methodological information:

RESULTS

Describe results: The mean serum PFOS, PFOA and PFHS values at study initiation (t0) were 0.9 ppm (range 0.1 - 3.5 ppm; SD = 1.1), 0.7 ppm (range 0.06 - 1.8 ppm; SD = 0.6), and 0.31 ppm (range 0-.02-1.3 ppm; SD = 0.4), respectively. The mean serum half-life for PFOS was 8.7 years (range 2.3 - 21.3 years; SD = 6.1). The mean serum half-life for PFOA was 4.4 years (range 1.5 -13.5 years; SD = 3.5). The mean serum half-life for PFHS was - 2.3 years (range -47.6 -30.1 years; SD = 23.1).

Study strengths and weaknesses: This interim report provides data on only 9 of the 27 subjects included in this study. Additional limitations include the following: 1) no effort was made to determine or control for retiree re-exposure to PFOS, PFOA or PFHS during the study time-period although retirees were not present in the production plant; 2) because PFOS is a metabolic product of compounds known to be present in the subject's blood it is possible that PFOS is being produced in the body through metabolic conversion of other PFOS-related materials in the body. Both exposure to, and metabolic product conversion of target analytes will lead to artificially long half-life estimations.

Research sponsors: 3M Medical Department

Consistency of results: These data are consistent with other reported, albeit sparse, observations that the serum half-life of PFOS and PFOA in humans is long (years) although the precision of the estimate remains quite variable. The serum half-life of PFHS is unexplainable at this time.

CONCLUSIONS

Half-life estimates for both PFOS and PFOA are estimated in several years although the precision of these estimates is not ideal due to the few subjects and period of time analyzed. Further data analyses will occur upon collection of several more years of samples from the 27 retirees (not just the nine that were used in this interim report).

REFERENCE

Burris HM, Lundberg JK, Olsen GW, Simpson C, Mandel JH. Determination of serum half-lives of several fluorochemicals. 3M Interim Report #2. January 11, 2002.

RS-III-31: An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat.

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

RS-III-32: Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD_{50}) Study in Rats.

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 (LD_{50}) 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.

RS-III-33: Ninety Day Study in Rats [PFOS].

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 or 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

RS-III-34: First Ninety-Day Rhesus Monkey Toxicity Study [PFOS].

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

RS-III-35: Second Ninety-Day Rhesus Monkey Toxicity Study [PFOS].

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.

RS-III-36: 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 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 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

RS-III-37: Rat Teratology Study T-3351 Final Report – Project No. 154-160.

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 CO_2 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 sternebrae 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

RS-III-38: Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits – 3M T-6295.10, Argus Research Laboratories Study Number 418-012.

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate (PFOS), CAS No. 2795-39-3

Remarks: PFOS – Lot 217, 98.4% pure (SMD Analytical Request 53030) Analytical Documentation filed along with final report. Note: Same lot as used in two-year rat PFOS carcinogenicity study (T-6295, Covance 6329-183).

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): The requirements of the International Conference on Harmonization (ICH) Harmonized Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal products, stages C and D of the reproductive process in a non-rodent species were used as the basis for study design (U.S. Food and Drug Administration, 1994. Federal Register, September 22, 1194, Vol. 59, No. 183).

GLP (Y/N): The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (MHW) and the European Economic Community (EEC). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study.

Year study performed: 1999

Species/Strain: New Zealand White rabbits

Number of animals per dose: 22

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 Caesarean-sectioning were evaluated using the procedures described for the Kruskal-Wallis Test.

Remarks – Detail and discuss any significant protocol parameters and deviations: Timed-pregnant New Zealand White rabbits (obtained from Covance Research Products, Inc.), 22 per group, were given doses of 0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day PFOS in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weights. The does were observed twice daily for viability, and clinical observations were recorded 1 hour prior to and after dosing during the treatment period and once daily during the post-treatment period (i.e. gestation days 20-29). Maternal body weights were recorded on gestation days 0 and 6-29; food consumption was recorded daily throughout the study. On gestation day 29, the does were euthanized; a gross necropsy of the thoracic, abdominal and pelvic viscera was conducted and the number of corpora lutea in each ovary was recorded. The uteri were examined for number and distribution of implantations, live and dead fetuses, and early and late 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≤0.01 in the 2.5 mg/kg/day dosage group). Average body weights were significantly reduced (p≤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≤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≤0.05 and p≤ 0.01, respectively) in the 2.75 and 3.75 dosage groups. Significant delays (p≤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. Ceasarean-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

RS-III-39: Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats – Argus Research Laboratories Study Number 6295.9, 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. F1 rats selected for mating and rearing of the F2 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 Ceasarean-sectioning, natural delivery, preweaning reflex/physical developmental data and postweaning behavorial 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 below 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 F_0 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.

F_1 Generation:

Since F_1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosages groups, only the 0.1 and 0.4 mg/kg/day dosage groups were carried into the second generation. Twenty-five F_1 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 F_1 generation male rats were sacrificed after mating necropsied and evaluated as described in the F0 generation. All F_1 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 F_2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F_1 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 F_0, F_1, and F_2: The NOAEL for the F_0 generation males and females = 0.1 mg/kg/day, the lowest dose tested. The NOAEL for the F_1 generation = 0.4 mg/kg/day, the highest dose tested. The NOAEL for the F_2 generation = 0.4 mg/kg/day, the highest dose tested.

LOAEL (dose and effect) – for F_0, F_1, and F_2: The LOAEL for the F_0 generation males and females = 0.4 mg/kg/day, based on reductions in body weight gain and food consumption. The LOAEL for the F_1 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 F_2 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:

F_0 /F_1. In F_0 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 F_0 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 F_0 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 F_1 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. F_1 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.

F_1/F_2: 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/say. 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 F_1 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 F_2 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:

F_0 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.

F_0 generation female animals: Significant increases ($p \leq 0.05$ or $p \leq 0.01$) in localized alopecia were observed in the 0.4, 1.6, and 3.2 mg/kg/day dosages groups. Significant reductions in body weight 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.

F_1 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.

F_1 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.

F_2 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:

F_0 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.

F_1 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 F_1 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.

F_2 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 F_2 pups were unremarkable.

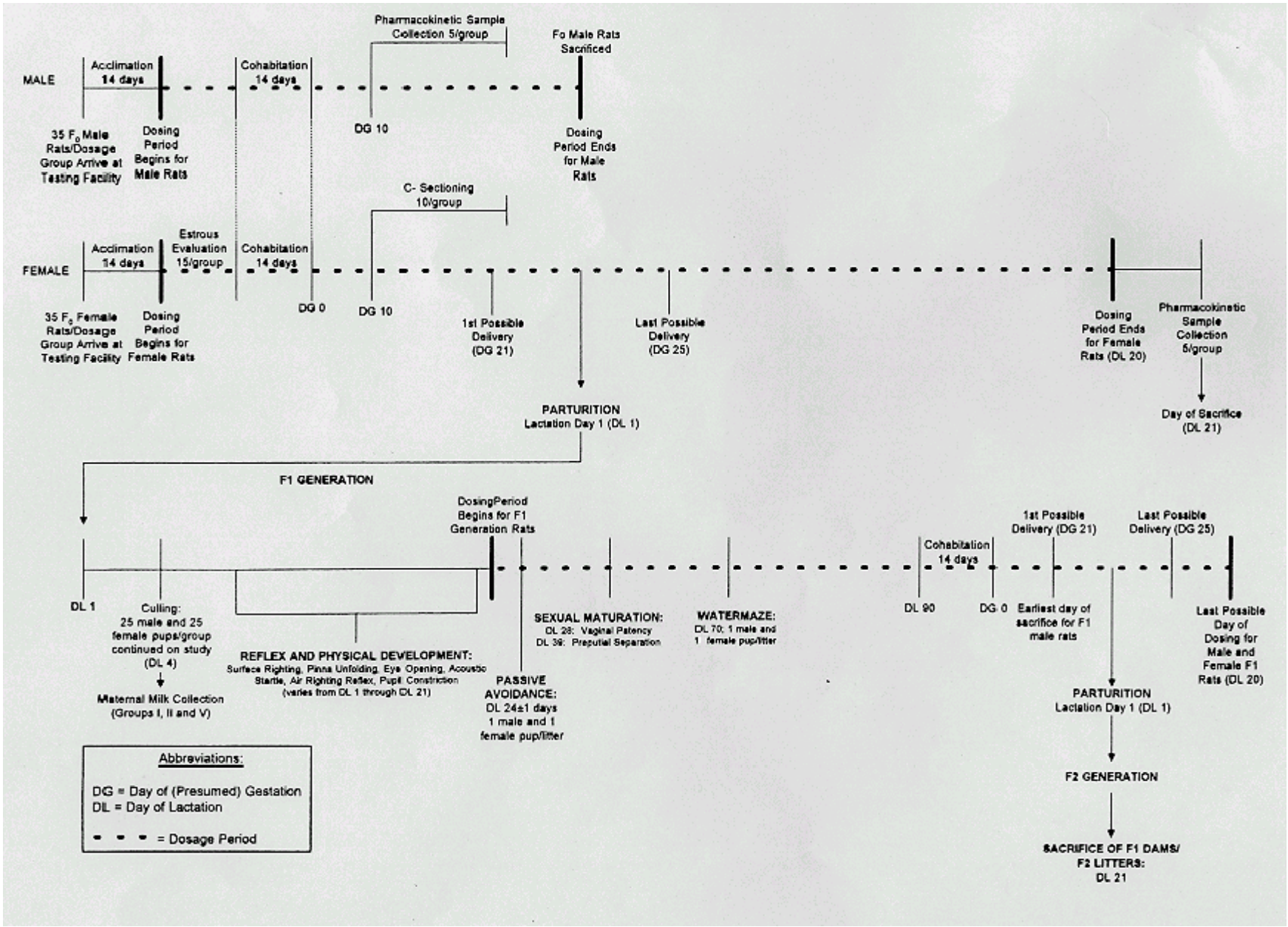
CONCLUSIONS

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".


Pharmacokinetic Sample Collection 5/group
Fo Male Rats Sacrificed
MALE
Acclimation 14 days
Cohabitation 14 days
35 F0 Male Rats/Dosage Group Arrive at Testing Facility
Dosing Period Begins for Male Rats
DG 10
Dosing Period Ends for Male Rats
C- Sectioning 10/group
FEMALE
Acclimation 14 days
Estrous Evaluation 15/group
Cohabitation 14 days
35 F0 Female Rats/Dosage Group Arrive at Testing Facility
Dosing Period Begins for Female Rats
DG 0
DG 10
1st Possible Delivery (DG 21)
Last Possible Delivery (DG 25)
Dosing Period Ends for Female Rats (DL 20)
Pharmacokinetic Sample Collection 5/group
Day of Sacrifice (DL 21)
PARTURITION
Lactation Day 1 (DL 1)
F1 GENERATION
DosingPeriod Begins for F1 Generation Rats
1st Possible Delivery (DG 21)
Last Possible Delivery (DG 25)
Cohabitation 14 days
DL 1
Culling: 25 male and 25 female pups/group continued on study (DL 4)
Maternal Milk Collection (Groups I, II and V)
REFLEX AND PHYSICAL DEVELOPMENT:
Surface Righting, Pinna Unfolding, Eye Opening, Acoustic Startle, Air Righting Reflex, Pupil Constriction
(varies from DL 1 through DL 21)
PASSIVE AVOIDANCE:
DL 24±1 days
1 male and 1 female pup/litter
SEXUAL MATURATION:
DL 28: Vaginal Patency
DL 39: Preputial Separation
WATERMAZE:
DL 70; 1 male and 1 female pup/litter
DL 90
DG 0
Earliest day of sacrifice for F1 male rats
Last Possible Day of Dosing for Male and Female F1 Rats (DL 20)
PARTURITION
Lactation Day 1 (DL 1)
F2 GENERATION
SACRIFICE OF F1 DAMS/ F2 LITTERS:
DL 21
Abbreviations:
DG = Day of (Presumed) Gestation
DL = Day of Lactation
= Dosage Period

RS-III-40: 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.

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	female 0.171
	male 0.0244	male 0.665
0.1:	female 5.28	female 14.8
	male 10.5	male 84.9
0.4:	female 18.9	female 58.0
	male 45.4	male 176
1.6:	female 82	female 184
	male 152	male 323
3.2:	female NR*	female NR*
	male 273	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

RS-III-41: Oral (Gavage) Cross-Fostering Study of PFOS in Rats.

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate (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 0.32 mg/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	Total	Percent	Litters	Pup
Gestation	Lactation	Dead	Pups	Mortality	Affected	Weight
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

Pup Exposure Regimen		Nursing Dams	Litters
Gestation	Lactation	Mean	Pooled Mean
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.

RS-III-42: One Generation Reproduction Study of PFOS - Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats.

TEST SUBSTANCE

Identity: Potassium Perfluorooctanesulfonate (KPFOS), CAS No. 2795-39-3, 3M Company.

Remarks: The test article, FC-95 (lot 217) was received on August 25, 2000 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. Purity is 98.9 %. The report will contain a certificate of analysis. Potassium perfluorooctanesulfonate (KPFOS) was administered; however, for simplification, KPFOS and the dissociated anion, perfluorooctanesulfonate (PFOS), will both be referred to as PFOS throughout this document.

CONTROL ARTICLES

Identity: Mevalonic acid lactone, Lots 070K26603 and 080K2618, Sigma Chemical Company. Cholesterol, Lot 119H0218, Sigma Chemical Company. Tween® 80, J.T.Baker.

Remarks: Mevalonic acid lactone was received on August 1, 2000 and September 21, 2000 and stored frozen. Cholesterol was received on July 20, 2000 and stored at room temperature. Tween® 80 was received on March 7, 2000, and stored at room temperature.

METHOD

Method/guideline followed: 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: Investigative, mechanistic one-generation reproduction study

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 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: 2000 - 2001

Species/Strain: Rat Crl:CD® (SD) IGS BR VAF/Plus® (Sprague-Dawley)

Sex (males/females/both): Both (males were not dosed and were only used for breeding)

Number of animals per dose: 20 - 29 females per group

Route of administration: Oral (gavage)

Dosing regimen: Female rats were given the test article, control article and/or vehicle daily beginning 42 days before cohabitation and continuing through gestation day 20 or lactation day 4, depending on whether rats were assigned to caesarean sectioning on

gestation day 21 or sacrifice on lactation day 5, respectively. Rats in the process of delivering were not treated. The F1 generation pups were not given test article directly; however, exposure occurred during gestation (*in utero)* and via milk during the lactation period. Male rats were used for breeding purposes only and were not treated.

Doses: 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 mg/kg/day (PFOS alone); 1.6 and 2.0 mg/kg/day (PFOS + 1000 mg/kg/d mevalonic acid lactone as two 500 mg/kg/day doses); 1.6 and 2.0 mg/kg/day (PFOS + 500 mg/kg/day cholesterol); 1000 mg/kg/day mevalonic acid lactone (given in two 500 mg/kg/day doses); 500 mg/kg/day cholesterol; 5 ml/kg 0.5 % Tween 80 (vehicle control). Dosage volumes were adjusted to 5 ml/kg.

Statistical methods used: Vehicle control data was first compared to mevalonic acid lactone and cholesterol control data. Then, vehicle control data was compared to PFOS-only dosed groups. Mevalonic acid lactone control data was compared to dose groups also supplemented with mevalonic acid lactone. Cholesterol control data was compared to dose groups also supplemented with cholesterol. 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 Ceasarean-sectioning and natural delivery involving discrete data (number of corpora lutea, number of pups per litter) were evaluated by the Kruskal-Wallis Test.

Remarks – Detail and discuss any significant protocol parameters:

F_0 Generation:

Female rats were used for this study. Due to the size and complexity of the study design, the rats were received in two shipments designated as replicate 1 and 2, respectively. The rats were randomly assigned to 13 dosage groups detailed below.

Dosage Group	Identification	Number of Rats			
		Total	Replicate 1 C-sectioning	Replicate 1 Natural Delivery	Replicate 2 Natural Delivery
1	Tween 80 Control	28	9	5	14
2	Mevalonic acid (MA) control	28	8	6	14
3	1.6 mg/kg PFOS + MA	29	8	6	15
4	2.0 mg/kg PFOS + MA	28	8	6	14
5	Cholesterol (Chol) Control	28	8	6	14

6	1.6 mg/kg PFOS + Chol.	28	8	6	14
7	2.0 mg/kg PFOS + Chol.	28	8	6	14
8	0.4 mg/kg PFOS	20	NA	10	10
9	0.8 mg/kg PFOS	20	NA	10	10
10	1.0 mg/kg PFOS	20	NA	10	10
11	1.2 mg/kg PFOS	20	NA	10	10
12	1.6 mg/kg PFOS	28	8	6	14
13	2.0 mg/kg PFOS	28	8	6	14

The first eight females per dosage group in Groups 1-7, 12, and 13 of replicate 1 with a confirmed date of mating were assigned to caesarean sectioning on day 21 of gestation. The remaining females were permitted to naturally deliver litters. Dosing began 42 days prior to cohabitation and continued until day 20 of gestation for rats assigned to caesarian sectioning or day 4 of lactation for rats assigned to natural delivery. Rats assigned to natural delivery and not producing a litter were dosed until gestation day 24.

Viability observations were made twice daily and rats were observed for clinical signs of treatment effect daily prior to dosage administration, approximately one-hour after the second dosage and at the end of the working day. Body weights were taken weekly to cohabitation and daily through presumed gestation, and on days 1 and 5 of lactation. Mating was confirmed by vaginal smear or observation of a vaginal plug.

Rats assigned to natural delivery were observed for adverse clinical signs during parturition. Other indices examined were duration of gestation, pup viability at birth, fertility index, gestation index, number of offspring per litter, number of implantation sites, general condition of dam and litter during the post-partum period, and lactation day 5 viability index.

Eight rats from groups 1-7, 12, and 13 of replicate 1 were sacrificed on gestation day 21 and caesarian sectioned. Number of corpora lutea in each ovary was recorded. The uterus was examined for pregnancy, number of implantation sites, live and dead fetuses, and early and late resorptions. Placentae were examined for size, color, and shape. Blood samples were collected, the liver was excised, and liver weight was recorded. A sample of liver (right lateral lobe) was flash frozen in liquid nitrogen and remained frozen. The median liver lobe was frozen. A section of the remaining portion of liver was fixed in glutaraldehyde.

Clinical chemistry parameters observed were cholesterol, glucose, LDL-direct, HDL-direct, triglycerides, total and free thyroxin, total and free triiodothyronine, and thyroid stimulating hormone. Milk cholesterol was determined in those dams allowed to litter. In animals supplemented with mevalonic acid lactone, plasma mevalonate was also determined.

Liver and serum PFOS concentrations were determined in selected groups. Individual samples were used.

F_1 Generation:

Litter observations were made for number and sex of pups, stillborn, live births, and gross alterations. Viability of pups was observed twice daily. Pups were counted once daily. Pooled litter weights were taken through day 5 of lactation.

Fetuses taken by caesarian section on day 21 of gestation were pooled by litter and litter body weights were recorded. A blood sample and liver were collected from each fetus. One fetal liver from each litter was fixed in glutaraldehyde. The remaining fetal livers in each litter were divided into three samples of equal number. Two of these were frozen. The third was flash frozen in liquid nitrogen and remained frozen.

Pups were sacrificed on day 5 of lactation and examined for gross lesions. Necropsy included a single cross section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. Blood samples were collected from each pup. In replicate 1, blood samples were pooled per litter. In replicate 2, two litters per sample from each dose group were pooled. Liver was collected from each pup, pooled by sex per litter, and the pooled liver weight was recorded. One liver per litter pool was fixed in glutaraldehyde. The remaining fetal livers in each litter were divided into three samples of equal number. Two of these were frozen. The third was flash frozen in liquid nitrogen and remained frozen. The hearts were collected from the first two male and two female pups from each litter and fixed in glutaraldehyde. Thyroids were excised from each pup, pooled by sex per litter and retained.

Clinical chemistry parameters observed were cholesterol, glucose, LDL-direct, HDL-direct, triglycerides, total and free thyroxin, total and free triiodothyronine, and thyroid stimulating hormone. In those animals supplemented with mevalonic acid lactone, plasma mevalonate was also determined.

Liver and serum PFOS concentration was determined in selected groups. Pooled samples were used.

Selected thyroid and heart tissues were examined by light microscopy.

RESULTS

Mevalonate and cholesterol rescue sub-study results:

For fetuses and gestation day 21 dams, all clinical chemistry parameters remained at or above control levels with the exception of triglycerides (TRIG), which were decreased in fetuses in the MAL-supplemented 2 mg/kg dose group. On lactation day 5, dams had decreased CHOL in all PFOS-treated groups and decreased TRIG in the PFOS-only and CHOL-supplemented PFOS groups. Pups in the CHOL-supplemented 2 mg/kg/d group had decreased TRIG. MAL or CHOL supplementation was unable to prevent or mitigate the perinatal mortality. Viability indices for the Tween 80 vehicle control, CHOL/vehicle control, MAL/vehicle control, 1.6 mg/kg/d PFOS, 1.6 mg/kg/d PFOS + MAL, 1.6 mg/kg/d PFOS + CHOL, 2.0 mg/kg/d PFOS, 2.0 mg/kg/d PFOS + MAL, and 2.0 mg/kg/d PFOS + CHOL were 97, 99, 98, 49, 41, 42, 17, 1, and 14 percent, respectively. Results suggest that the observed perinatal mortality is not a result of hypolipidemia in the perinatal period.

Dose-response sub-study results:

Significant decreases in cholesterol and increases in LDL were observed in all fetuses from the 1.6 and 2.0 mg/kg/d PFOS dose groups on day 21 of gestation. On day 5 of lactation, dams had significantly decreased serum cholesterol at all dose levels, significantly decreased serum triglycerides in the 1.6 and 2.0 mg/kg/d dose groups, and significantly increased serum glucose in the 2.0 mg/kg/d dose group. Although T4 (free and total) appeared to be decreased when measured by RIA, free T4 was normal across all dose groups (dams) when measured using equilibrium dialysis. T3 was low at 1.2 mg/kg/d and above in dams but not fetuses and pups. TSH was not elevated and thyroid histology was normal. Dams in the 0.8 mg/kg/d and higher dose groups showed an increase in relative liver weight, a decrease in body weight gain, a decrease in feed consumption, and a slight decrease in the duration of gestation. Pup growth and viability was significantly decreased at 1.2 mg/kg/d and higher. Viability indices for pups from the 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/d groups were 97, 98, 93, 89, and 82, 49, and 17 percent, respectively. The no-observed-effect level for pup growth and viability was 1.2 mg/kg/day maternal dose.

Serum and Liver PFOS:

Serum and liver PFOS concentrations are detailed in tables 17-19. Serum PFOS concentrations on gestation day 21 equaled 0.007 ± 0.003 ug/ml in control dams and were below the limit of quantitation (0.005 ug/ml) in the control fetuses. Dams and fetuses in the 1.6 mg/kg dose group had serum levels of approximately 142 ug/ml. Serum PFOS levels in the 2.0 mg/kg dose group equaled 125 ± 19.8 ug/ml in the dams and 170 ± 38.1 ug/ml in the fetuses. Serum PFOS levels were approximately equal in dams and pups at lactation day 5 and increased in a dose response manner. Liver PFOS levels in dams at lactation day 5 were approximately equal to the lactation day 5 serum levels. Liver PFOS levels in the pups at lactation day 5, however, were approximately twice as high as the pup serum levels and the dam liver levels. Serum PFOS levels in the supplemented dose groups were approximately equal in dams and pups and increased in a dose response manner at both time points. Liver PFOS was not measured in the supplemented dose groups.

Histology:

Light microscopy was performed on hearts and thyroids from lactation day 5 pups in the 0.0, 0.4, 1.6, and 2.0 mg/kg/day PFOS only dose groups. No abnormalities were observed in any of the tissues.

CONCLUSIONS

The final report is not available at the time of this review. Based on the data currently available from an audited draft final report, co-administration of mevalonic acid lactone or cholesterol was unable to prevent or mitigate adverse effects experienced by the dam, fetus, or pup in prior reproduction studies. Perinatal mortality is not a result of cholesterol deficiency in the perinatal period. The relationship of apparent reductions in thyroxin and triiodothyronine in treated rats and their offspring to the observed effects is unclear and the subject of further investigation.

NOEL/NOAEL: The NOEL for the F_0 generation females = 0.4 mg/kg/day, the lowest dose tested. For dams, the 0.8 mg/kg/day dosage and higher dosages caused an increase in relative liver weight, decreased body weight gain during gestation and lactation and decreased relative and absolute feed consumption during gestation and lactation. The duration of gestation was also decreased at 0.8 mg/kg/day. The NOEL for viability and growth in the F1 pups is 1.2 mg/kg/day.

REFERENCE

Hoberman, A.M., and York, R.G.. Argus Research Laboratories, Inc. Argus Study Number: 418-018, Sponsor Study Number: 6295.25.

Table 1: Maternal Body Weight and Feed Consumption - Dose Response.

F1 Body Weight (g)	Tween 80 Control	0.4 mg/kg/day PFOS	0.8 mg/kg/day PFOS	1.0 mg/kg/day PFOS	1.2 mg/kg/day PFOS	1.6 mg/kg/day PFOS	2.0 mg/kg/day PFOS
Maternal Body Weight (g)							
Pre-mating Day 42	265.6 ± 21.0	276.0 ± 16.8	266.0 ± 19.5	269.2 ± 21.2	264.6 ± 16.7	260.2 ± 15.5	255.1 ± 17.5
Gestation Day 1	283.3 ± 20.4	291.2 ± 18.5	281.1 ± 23.3	282.6 ± 21.1	275.4 ± 17.5	272.6 ± 16.8	265.7 ± 19.9**
Gestation Day 7	298.9 ± 20.0	308.4 ± 19.2	292.5 ± 21.7	294.3 ± 19.9	286.1 ± 19.2	283.6 ± 16.6*	276.5 ± 17.6**
Gestation Day 14	326.8 ± 22.6	337.6 ± 21.2	323.4 ± 27.3	326.6 ± 17.9	316.2 ± 19.4	310.4 ± 18.2*	302.9 ± 19.0**
Gestation Day 21	410.6 ± 31.1	421.2 ± 28.9	408.5 ± 25.3	408.6 ± 22.2	394.5 ± 31.7	398.3 ± 26.1	397.5 ± 30.2
Lactation Day 1	301.3 ± 26.0	311.0 ± 19.9	304.0 ± 24.6	304.4 ± 15.5	298.1 ± 20.1	298.2 ± 20.0	291.0 ± 21.2
Lactation Day 5	318.7 ± 28.5	326.7 ± 21.6	308.3 ± 42.0	309.5 ± 19.8	305.6 ± 23.2	298.6 ± 19.8	283.8 ± 7.5*
Maternal Absolute Feed Consumption (g/day)							
Pre-mating Days 1-42	18.9 ± 1.8	19.6 ± 1.8	19.0 ± 1.8	19.4 ± 1.8	18.6 ± 1.3	18.7 ± 1.6	17.8 ± 1.3*
Gestation Days 0-7	22.3 ± 2.2	22.7 ± 2.3	20.6 ± 2.4*	20.7 ± 2.0*	19.6 ± 1.9**	19.0 ± 2.1**	18.6 ± 2.2**
Gestation Days 7-14	22.9 ± 2.2	23.2 ± 1.6	22.7 ± 2.5	21.7 ± 2.7	22.0 ± 2.1	21.2 ± 2.7	21.0 ± 4.0*
Gestation Days 14-21	24.3 ± 2.2	24.5 ± 2.5	25.1 ± 2.2	25.0 ± 2.5	24.7 ± 2.6	25.2 ± 2.3	24.4 ± 2.8
Lactation Days 1-5	28.8 ± 4.7	29.3 ± 9.7	24.0 ± 6.1	25.5 ± 8.7	23.7 ± 5.2	19.4 ± 5.8**	17.2 ± 7.3**
Maternal Relative Feed Consumption (g/kg/day)							
Pre-mating Days 1-42	77.2 ± 4.6	77.6 ± 5.8	76.7 ± 5.3	78.8 ± 6.3	75.4 ± 4.0	75.8 ± 4.2	73.4 ± 3.8*
Gestation Days 0-7	77.2 ± 5.7	76.3 ± 5.0	72.2 ± 5.4*	72.3 ± 7.2*	70.3 ± 5.2**	68.6 ± 5.4**	69.0 ± 6.6**
Gestation Days 7-14	73.4 ± 4.2	72.2 ± 4.1	74.0 ± 4.2	70.6 ± 8.3	73.7 ± 6.8	71.8 ± 6.3	72.7 ± 11.4
Gestation Days 14-21	66.8 ± 5.4	65.1 ± 6.8	68.8 ± 4.3	68.1 ± 9.0	68.9 ± 5.8	72.1 ± 5.8*	70.7 ± 7.5
Lactation Days 1-5	92.8 ± 13.7	92.3 ± 32.1	77.5 ± 15.0*	83.0 ± 29.6*	78.5 ± 15.3*	65.1 ± 18.9**	60.7 ± 26.6

* = significantly different from control at $p \leq 0.05$; ** $p \leq 0.01$

Table 2: Maternal Body Weight and Feed Consumption - Rescue:

Parameter	Tween 80 Control	MA Control	Chol. Control	1.6 mg/kg/day PFOS	1.6 mg/kg/day PFOS +MA	1.6 mg/kg/day PFOS +Chol.	2.0 mg/kg/day PFOS	2.0 mg/kg/day PFOS +MA	2.0 mg/kg/day PFOS +Chol.
Maternal Body Weight (g)									
Pre-mating Day 42	265.6 ± 21.0	267.6 ± 17.4	275.5 ± 19.7	260.2 ± 15.5	261.3 ± 23.1	257.6 ± 16.4**	255.1 ± 17.5	266.4 ± 16.9	257.9 ± 14.9**
Gestation Day 1	283.3 ± 20.4	283.0 ± 18.8	292.6 ± 22.7	272.6 ± 16.8	271.5 ± 22.3	268.2 ± 19.4**	265.7 ± 19.9**	275.7 ± 17.5	267.1 ± 18.3**
Gestation Day 7	298.9 ± 20.0	296.0 ± 17.9	309.8 ± 24.0	283.6 ± 16.6*	280.0 ± 22.3**	281.5 ± 20.5**	276.5 ± 17.6**	284.7 ± 19.9*	278.4 ± 17.7**
Gestation Day 14	326.8 ± 22.6	322.8 ± 18.8	340.7 ± 25.1^	310.4 ± 18.2*	308.4 ± 26.9	310.1 ± 23.8**	302.9 ± 19.0**	312.2 ± 21.6	305.4 ± 21.0**
Gestation Day 21	410.6 ± 31.1	403.5 ± 24.8	428.0 ± 31.1^	398.3 ± 26.1	398.2 ± 34.1	392.0 ± 31.6**	397.5 ± 30.2	403.4 ± 35.1	394.5 ± 29.0**
Lactation Day 1	301.3 ± 26.0	299.3 ± 24.8	312.4 ± 24.3	298.2 ± 20.0	301.4 ± 28.6	294.8 ± 24.8*	291.0 ± 21.2	300.8 ± 20.0	295.4 ± 22.6*
Lactation Day 5	318.7 ± 28.5	319.5 ± 18.5	328.0 ± 31.9	298.6 ± 19.8	315.8 ± 23.4	303.9 ± 19.0*	283.8 ± 7.5*	297.0 ± 14.1	280.8 ± 15.6**
Maternal Absolute Feed Consumption (g/day)									
Pre-mating Days 1-42	18.9 ± 1.8	19.8 ± 1.7	19.7 ± 1.7	18.7 ± 1.6	18.5 ± 2.0**	18.3 ± 1.2**	17.8 ± 1.3*	18.7 ± 1.5*	18.4 ± 1.6**
Gestation Days 0-7	22.3 ± 2.2	21.6 ± 1.8	22.9 ± 2.9	19.0 ± 2.1**	18.2 ± 2.3**	18.9 ± 2.2**	18.6 ± 2.2**	19.1 ± 2.7**	18.3 ± 2.0**
Gestation Days 7-14	22.9 ± 2.2	23.2 ± 2.1	24.3 ± 2.7	21.2 ± 2.7	20.9 ± 1.9**	21.2 ± 2.1**	21.0 ± 4.0*	20.8 ± 2.0**	20.8 ± 1.8**
Gestation Days 14-21	24.3 ± 2.2	24.4 ± 2.5	25.4 ± 2.9	25.2 ± 2.3	25.0 ± 2.4	24.6 ± 2.1	24.4 ± 2.8	23.8 ± 3.6	25.0 ± 2.3
Lactation Days 1-5	28.8 ± 4.7	28.4 ± 5.3	31.6 ± 5.1	19.4 ± 5.8**	20.2 ± 5.6**	19.9 ± 6.1**	17.2 ± 7.3**	13.9 ± 2.2**	14.6 ± 3.5**
Maternal Relative Feed Consumption (g/kg/day)									
Pre-mating Days 1-42	77.2 ± 4.6	80.3 ± 4.5^	78.6 ± 4.7	75.8 ± 4.2	75.7 ± 5.3**	75.1 ± 2.8**	73.4 ± 3.8*	7.4 ± 4.7**	75.5 ± 5.7*
Gestation Days 0-7	77.2 ± 5.7	75.4 ± 6.1	76.7 ± 7.8	68.6 ± 5.4**	66.3 ± 6.4**	69.1 ± 6.0**	69.0 ± 6.6**	68.4 ± 8.2**	67.5 ± 7.2**
Gestation Days 7-14	73.4 ± 4.2	74.9 ± 6.0	74.8 ± 6.7	71.8 ± 6.3	71.5 ± 3.4*	71.8 ± 5.1	72.7 ± 11.4	70.1 ± 6.4**	71.8 ± 5.3
Gestation Days 14-21	66.8 ± 5.4	67.9 ± 4.8	66.9 ± 6.4	72.1 ± 5.8*	71.8 ± 5.9	71.2 ± 4.4*	70.7 ± 7.5	68.6 ± 8.4	72.6 ± 6.0**
Lactation Days 1-5	92.8 ± 13.7	92.6 ± 19.5	98.4 ± 11.5	65.1 ± 18.9**	65.3 ± 19.1**	66.5 ± 20.1**	60.7 ± 26.6	46.9 ± 6.2**	52.0 ± 14.7**

* = significantly different from appropriate control at $p \leq 0.05$; ** $p \leq 0.01$
^ = significantly different from Tween 80 control group $p \leq 0.05$

Table 3:

Gestation Day 21 Dams – Clinical Chemistry Values (mg/dl) – Dose Response					
	Cholesterol	Glucose	LDL- Dir	HDL- Dir	Trig
Tween 80 Control	84.3 ± 13.3 (8)	98.9 ± 11.7 (8)	7.1 ± 5.0 (8)	43.3 ± 19.0 (8)	313.5 ± 233.6 (8)
1.6 mg/kg PFOS	78.1 ± 10.5 (8)	104.9 ± 7.8 (8)	10.8 ± 4.4 (8)	43.5 ± 11.2 (8)	194.3 ± 65.2 (8)
2.0 mg/kg PFOS	72.3 ± 6.3 (8)	96.3 ± 18.0 (8)	11.3 ± 3.6 (8)	41.0 ± 11.7 (8)	201.0 ± 87.5 (8)
* Significantly different from control group; $p \leq 0.05$ ** Significantly different from control group; $p \leq 0.01$					

Table 4:

Gestation Day 21 Fetuses – Clinical Chemistry Values (mg/dl) – Dose Response					
	Cholesterol	Glucose	LDL-DIR	HDL-DIR	Trig
Tween 80 Control	50.8 ± 8.0 (8)	63.8 ± 5.9 (8)	27.3 ± 8.0 (8)	13.0 ± 1.9 (8)	34.4 ± 5.3 (8)
1.6 mg/kg PFOS	61.3 ± 8.5* (8)	76.1 ± 11.8 (8)	44.9 ± 7.5** (8)	14.5 ± 1.5 (8)	27.9 ± 8.2 (8)
2.0 mg/kg PFOS	61.5 ± 8.0* (8)	73.9 ± 15.5 (8)	45.4 ± 9.8** (8)	14.0 ± 1.2 (8)	27.0 ± 9.8 (8)
* Significantly different from control group; $p \leq 0.05$ ** Significantly different from control group; $p \leq 0.01$					

Table 5:

Lactation Day 5 Dams – Clinical Chemistry Values (mg/dl) – Dose Response

	Cholesterol (blood)	Cholesterol (milk)	Glucose	LDL- Dir	HDL- Dir	Trig
Tween 80 Control	77.6 ± 15.2 (19)	96.7 ± 53.9 (19)	166.1 ± 28.4 (19)	4.0 ± 3.0 (19)	58.2 ± 20.1 (19)	152.4 ± 97.6 (19)
0.4 mg/kg PFOS	65.0 ± 13.2** (20)	71.1 ± 62.8 (20)	169.6 ± 26.7 (20)	3.2 ± 2.3 (20)	51.2 ± 17.2 (20)	130.1 ± 78.3 (20)
0.8 mg/kg PFOS	59.1 ± 13.3** (17)	68.2 ± 52.6 (17)	163.0 ± 17.3 (17)	3.2 ± 2.1 (17)	47.5 ± 14.6 (17)	127.7 ± 66.5 (17)
1.0 mg/kg PFOS	58.5 ± 11.8** (19)	59.8 ± 51.2 (18)	171.9 ± 19.9 (19)	3.7 ± 1.9 (19)	49.0 ± 11.0 (19)	101.2 ± 52.7 (19)
1.2 mg/kg PFOS	59.9 ± 11.7** (18)	46.8 ± 41.5 (18)	172.5 ± 17.7 (18)	2.8 ± 2.2 (18)	48.9 ± 16.2 (18)	141.1 ± 74.3 (18)
1.6 mg/kg PFOS	56.6 ± 14.0** (17)	64.6 ± 30.4 (13)	178.7 ± 19.0 (17)	3.6 ± 2.6 (17)	49.5 ± 10.3 (17)	92.2 ± 58.9* (17)
2.0 mg/kg PFOS	59.6 ± 10.2** (19)	74.0 ± 41.7 (5)	189.2 ± 32.2** (19)	4.6 ± 1.8 (19)	53.1 ± 9.4 (19)	85.5 ± 35.0** (19)

* Significantly different from control group; $p \leq 0.05$
** Significantly different from control group; $p \leq 0.01$

Table 6:

Lactation Day 5 Pups – Clinical Chemistry Values (mg/dl) – Dose Response

	Cholesterol	Glucose	LDL- Dir	HDL- Dir	Trig
Tween 80 Control	112.5 ± 10.6 (12)	255.8 ± 61.8 (12)	33.5 ± 8.7 (12)	29.8 ± 7.9 (12)	214.2 ± 51.8 (12)
0.4 mg/kg PFOS	111.9 ± 11.3 (15)	244.5 ± 60.1 (15)	29.1 ± 7.4 (15)	32.7 ± 6.9 (15)	234.9 ± 95.0 (15)
0.8 mg/kg PFOS	114.8 ± 11.1 (13)	220.3 ± 56.2 (13)	31.5 ± 7.6 (13)	38.1 ± 11.0 (13)	179.2 ± 66.5 (13)
1.0 mg/kg PFOS	113.1 ± 16.8 (14)	210.0 ± 47.0 (14)	29.4 ± 5.3 (14)	36.3 ± 6.6 (14)	197.8 ± 64.4 (14)
1.2 mg/kg PFOS	124.7 ± 23.4 (14)	217.2 ± 60.6 (14)	34.8 ± 15.0 (14)	31.9 ± 5.8 (14)	230.3 ± 78.5 (14)
1.6 mg/kg PFOS	132.3 ± 33.9 (6)	222.0 ± 20.8 (6)	40.3 ± 20.1 (6)	36.0 ± 7.0 (6)	231.8 ± 52.7 (6)
2.0 mg/kg PFOS	128.0 ± 38.3 (3)	229.3 ± 17.2 (3)	44.7 ± 31.6 (3)	34.3 ± 4.0 (3)	213.0 ± 56.3 (3)

* Significantly different from control group; $p \leq 0.05$
** Significantly different from control group; $p \leq 0.01$

Table 7:

Gestation Day 21 Dams – Clinical Chemistry Values (mg/dl) - Rescue

	Cholesterol	Glucose	LDL-DIR	HDL-DIR	Trig	Mevalonic acid
Tween 80 Control	84.3 ± 13.3 (8)	98.9 ± 11.7 (8)	7.1 ± 5.0 (8)	43.3 ± 19.0 (8)	313.5 ± 233.6 (8)	47.7 ± 63.8 (8)
MA Control	92.5 ± 14.6 (8)	108.5 ± 11.9 (8)	2.9 ± 3.6 (8)	32.4 ± 20.0 (8)	453.9 ± 307.8 (8)	1663.8 ± 415.6^^ (8)
Chol. Control	89.6 ± 19.6 (8)	100.4 ± 20.2 (8)	8.5 ± 5.2 (8)	28.0 ± 11.5 (8)	348.8 ± 218.6 (8)	Not evaluated
1.6 mg/kg PFOS	78.1 ± 10.5 (8)	104.9 ± 7.8 (8)	10.8 ± 4.4 (8)	43.5 ± 11.2 (8)	194.3 ± 65.2 (8)	20.6 ± 5.7 (8)
1.6 mg/kg PFOS + MA	91.0 ± 21.4 (8)	108.1 ± 15.4 (8)	11.8 ± 9.0** (8)	35.1 ± 12.1 (8)	305.9 ± 85.1 (8)	1369.8 ± 240.1 (8)
1.6 mg/kg PFOS + Chol.	79.3 ± 9.4 (8)	98.5 ± 18.5 (8)	9.9 ± 3.9 (8)	41.0 ± 13.6 (8)	191.9 ± 79.3 (8)	Not evaluated
2.0 mg/kg PFOS	72.3 ± 6.3 (8)	96.3 ± 18.0 (8)	11.3 ± 3.6 (8)	41.0 ± 11.7 (8)	201.0 ± 87.5 (8)	86.2 ± 177.0 (8)
2.0 mg/kg PFOS + MA	88.9 ± 23.1 (8)	106.0 ± 14.0 (8)	9.6 ± 5.0* (8)	47.8 ± 16.2 (8)	243.6 ± 270.8 (8)	1482.5 ± 521.9 (8)
2.0 mg/kg PFOS + Chol.	77.9 ± 11.5 (8)	106.4 ± 17.9 (8)	12.0 ± 8.6 (8)	46.6 ± 18.4 (8)	200.9 ± 190.1 (8)	Not evaluated

* Significantly different from appropriate control group; $p \leq 0.05$; ** $p \leq 0.01$ Mevalonic acid lactone (MA)
^ Significantly different from Tween 80 control group; ^^ $p \leq 0.01$ Cholesterol (Chol.)

Table 8:

Gestation Day 21 Fetuses – Clinical Chemistry Values (mg/dl) - Rescue

	Cholesterol	Glucose	LDL-Dir	HDL-Dir	Trig	Mevalonic acid
Tween 80 Control	50.8 ± 8.0 (8)	63.8 ± 5.9 (8)	27.3 ± 8.0 (8)	13.0 ± 1.9 (8)	34.4 ± 5.3 (8)	54.5 ± 40.5 (7)
MA Control	53.1 ± 5.5 (8)	63.4 ± 15.0 (8)	29.4 ± 5.3 (8)	13.9 ± 1.2 (8)	36.4 ± 8.7 (8)	18971.4 ± 5681.5^^ (7)
Chol. Control	45.1 ± 4.3 (8)	70.4 ± 7.9 (8)	24.1 ± 5.1 (8)	12.4 ± 0.9 (8)	31.8 ± 7.6 (8)	Not evaluated
1.6 mg/kg PFOS	61.3 ± 8.5* (8)	76.1 ± 11.8 (8)	44.9 ± 7.5** (8)	14.5 ± 1.5 (8)	27.9 ± 8.2 (8)	67.0 ± 53.0 (8)
1.6 mg/kg PFOS + MA	68.3 ± 13.4** (8)	69.6 ± 6.2 (8)	48.3 ± 8.1** (8)	16.1 ± 3.0 (8)	31.7 ± 7.1 (8)	16562.5 ± 7160.4 (8)
1.6 mg/kg PFOS + Chol.	59.1 ± 11.8** (8)	77.4 ± 19.8 (8)	43.9 ± 5.4** (8)	14.4 ± 1.6* (8)	25.4 ± 13.6 (8)	Not evaluated
2.0 mg/kg PFOS	61.5 ± 8.0* (8)	73.9 ± 15.5 (8)	45.4 ± 9.8** (8)	14.0 ± 1.2 (8)	27.0 ± 9.8 (8)	104.8 ± 73.9 (8)
2.0 mg/kg PFOS + MA	63.1 ± 11.7 (8)	74.5 ± 12.3 (8)	46.9 ± 9.4** (8)	15.5 ± 1.9 (8)	25.1 ± 8.1** (8)	20175.0 ± 2830.8 (8)
2.0 mg/kg PFOS + Chol.	64.0 ± 10.3** (8)	70.3 ± 10.3 (8)	45.6 ± 8.0** (8)	14.4 ± 1.7* (8)	30.0 ± 5.7 (8)	Not evaluated

* * Significantly different from appropriate control group; p ≤ 0.05; ** p ≤ 0.01 Mevalonic acid lactone (MA)
^ Significantly different from Tween 80 control group; ^^ p ≤ 0.01 Cholesterol (Chol.)

Table 9:

Lactation Day 5 Dams – Clinical Chemistry Values (mg/dl) - Rescue

	Cholesterol (blood)	Cholesterol (milk)	Glucose	LDL- Dir	HDL- Dir	Trig	Mevalonic acid
Tween 80 Control	77.6 ± 15.2 (19)	96.7 ± 53.9 (19)	166.1 ± 28.4 (19)	4.0 ± 3.0 (19)	58.2 ± 20.1 (19)	152.4 ± 97.6 (19)	31.0 ± 19.8 (18)
MA Control	80.5 ± 15.6 (18)	81.4 ± 52.4 (17)	174.3 ± 39.2 (18)	3.7 ± 2.9 (18)	63.7 ± 18.1 (18)	135.6 ± 58.6 (18)	201.4 ± 135.9^^ (18)
Chol. Control	75.9 ± 17.5 (17)	80.2 ± 57.9 (17)	168.8 ± 29.2 (17)	3.8 ± 3.0 (17)	55.6 ± 19.1 (17)	146.1 ± 64.8 (17)	Not evaluated
1.6 mg/kg PFOS	56.6 ± 14.0** (17)	64.6 ± 30.4 (13)	178.7 ± 19.0 (17)	3.6 ± 2.6 (17)	49.5 ± 10.3 (17)	92.2 ± 58.9* (17)	32.2 ± 20.9 (16)
1.6 mg/kg PFOS + MA	63.6 ± 14.6** (18)	70.5 ± 46.2 (12)	174.9 ± 24.1 (18)	3.4 ± 2.0 (18)	54.2 ± 12.1 (18)	103.3 ± 43.5 (18)	1123.0 ± 2000.2* (18)
1.6 mg/kg PFOS + Chol.	56.2 ± 12.2** (20)	81.9 ± 52.5 (12)	170.2 ± 20.8 (20)	4.4 ±2.8 (20)	48.6 ± 11.1 (20)	87.2 ± 32.1** (20)	Not evaluated
2.0 mg/kg PFOS	59.6 ± 10.2** (19)	74.0 ± 41.7 (5)	189.2 ± 32.2** (19)	4.6 ± 1.8 (19)	53.1 ± 9.4 (19)	85.5 ± 35.0** (19)	29.6 ± 14.4 (15)
2.0 mg/kg PFOS + MA	67.3 ± 11.2** (19)	188.0 ± 114.6* (2)	187.6 ± 30.9 (19)	3.7 ± 3.1 (19)	55.0 ± 10.1 (19)	118.7 ± 50.7 (19)	18662.4 ± 70304.8* (19)
2.0 mg/kg PFOS + Chol.	58.0 ± 15.2** (19)	57.8 ± 3.8 (4)	190.6 ± 28.5* (19)	4.9 ± 2.4 (19)	50.9 ± 13.3 (19)	85.0 ± 36.1** (19)	Not evaluated

* Significantly different from appropriate control group; $p \leq 0.05$; ** $p \leq 0.01$
^ Significantly different from Tween 80 control group; ^^ $p \leq 0.01$

Mevalonic acid lactone (MA)
Cholesterol (Chol.)

Table 10:

Lactation Day 5 Pups – Clinical Chemistry Values (mg/dl) - Rescue

	Cholesterol	Glucose	LDL- Dir	HDL- Dir	Trig	Mevalonic acid
Tween 80 Control	112.5 ± 10.6 (12)	255.8 ± 61.8 (12)	33.5 ± 8.7 (12)	29.8 ± 7.9 (12)	214.2 ± 51.8 (12)	22.6 ± 5.3 (9)
MA Control	115.4 ± 7.6 (12)	241.4 ± 88.4 (12)	35.3 ± 7.5 (12)	35.4 ± 9.0 (12)	193.2 ± 69.9 (12)	335.3 ± 229.2^^ (11)
Chol. Control	117.8 ± 9.1 (12)	263.8 ± 56.4 (12)	26.8 ± 12.2 (12)	28.7 ± 7.7 (12)	321.3 ± 208.8 (12)	Not evaluated
1.6 mg/kg PFOS	132.3 ± 33.9 (6)	222.0 ± 20.8 (6)	40.3 ± 20.1 (6)	36.0 ± 7.0 (6)	231.8 ± 52.7 (6)	20.6 ± 4.0 (4)
1.6 mg/kg PFOS + MA	157.0 ± 23.7** (7)	273.3 ± 65.1 (7)	50.8 ± 20.0* (7)	33.0 ± 4.9 (7)	302.3 ± 62.7** (7)	270.8 ± 85.8 (7)
1.6 mg/kg PFOS + Chol.	130.0 ± 18.5 (8)	247.8 ± 48.9 (8)	41.0 ± 11.4* (8)	39.2 ± 3.6** (8)	232.9 ± 74.4 (8)	Not evaluated
2.0 mg/kg PFOS	128.0 ± 38.3 (3)	229.3 ± 17.2 (3)	44.7 ± 31.6 (3)	34.3 ± 4.0 (3)	213.0 ± 56.3 (3)	(0)
2.0 mg/kg PFOS + MA	(0)	(0)	(0)	(0)	(0)	195.2 ± 203.4 (2)
2.0 mg/kg PFOS + Chol.	134.8 ± 26.1 (5)	223.2 ± 20.2 (5)	55.2 ± 22.5** (5)	47.2 ± 12.8** (5)	120.4 ± 36.6* (5)	Not evaluated

* Significantly different from appropriate control group; $p \leq 0.05$; ** $p \leq 0.01$ Mevalonic acid lactone (MA)
^ Significantly different from Tween 80 control group; ^^ $p \leq 0.01$ Cholesterol (Chol.)

Table 11:

Gestation Day 21 Dams – Thyroid Hormone Values – Dose Response					
	Total T4 (ug/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)	Free T4 (ng/dl)
Tween 80 Control	0.584 ± 0.482 (8)	74.0 ± 21.2 (8)	0.579 ± 0.209 (8)	1.82 ± 0.79 (8)	0.299 ± 0.092 (8)
1.6 mg/kg PFOS	0.000 ± 0.000** (8)	47.5 ± 10.3** (8)	0.140 ± 0.117** (7)	2.35 ± 1.26 (7)	0.066 ± 0.038** (8)
2.0 mg/kg PFOS	0.000 ± 0.000** (8)	42.4 ± 8.3** (8)	0.157 ± 0.161** (7)	1.51 ± 0.97 (7)	0.055 ± 0.023** (8)
* Significantly different from control group; $p \leq 0.05$ ** Significantly different from control group; $p \leq 0.01$					

Table 12:

Gestation Day 21 Fetuses – Thyroid Hormone Values – Dose Response					
	Total T4 (ug/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)	Free T4 (ng/dl)
Tween 80 Control	0.000 ± 0.000 (7)	0.423 ± 0.845 (8)	(0)	(0)	0.050 ± 0.000 (1)
1.6 mg/kg PFOS	0.000 ± 0.000 (8)	0.000 ± 0.000 (8)	0.000 ± 0.000 (1)	(0)	0.000 ± 0.000** (3)
2.0 mg/kg PFOS	0.000 ± 0.000 (8)	0.925 ± 1.147 (6)	(0)	1.160 ± 0.000 (1)	0.000 ± 0.000** (5)
* Significantly different from control group; $p \leq 0.05$ ** Significantly different from control group; $p \leq 0.01$					

Table 13:

Lactation Day 5 Dams – Thyroid Hormone Values – Dose Response

	Total T4 (ug/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)	Free T4 (ng/dl)
Tween 80 Control	1.454 ± 0.660 (19)	74.7 ± 19.0 (19)	0.638 ± 0.297 (19)	1.63 ± 0.99 (18)	0.788 ± 0.219 (19)
0.4 mg/kg PFOS	0.808 ± 0.414 (10)	72.9 ± 13.5 (10)	0.698 ± 0.246 (10)	1.14 ± 0.23 (9)	0.462 ± 0.114** (10)
0.8 mg/kg PFOS	0.596 ± 0.443* (8)	63.8 ± 6.7 (8)	0.588 ± 0.152 (8)	1.44 ± 0.92 (7)	0.279 ± 0.060** (8)
1.0 mg/kg PFOS	0.728 ± 0.243** (9)	62.3 ± 13.2 (9)	0.552 ± 0.230 (9)	1.11 ± 0.52 (8)	0.277 ± 0.095** (9)
1.2 mg/kg PFOS	0.283 ± 0.320** (18)	52.9 ± 15.0** (18)	0.411 ± 0.265* (18)	1.45 ± 1.03 (17)	0.260 ± 0.122** (18)
1.6 mg/kg PFOS	0.272 ± 0.172** (17)	47.0 ± 20.0** (17)	0.256 ± 0.230** (17)	1.67 ± 0.77 (17)	0.253 ± 0.067** (17)
2.0 mg/kg PFOS	0.235 ± 0.147** (19)	53.3 ± 17.3** (19)	0.353 ± 0.236** (18)	1.53 ± 0.68 (18)	0.259 ± 0.078** (18)

* Significantly different from control group; $p \leq 0.05$
** Significantly different from control group; $p \leq 0.01$

Table 14:

Lactation Day 5 Pups – Thyroid Hormone Values – Dose Response					
	Total T4 (ug/dl)	Total T3 (ng/dl)	Free T3 (pg/ml)	TSH (ng/ml)	Free T4 (ng/dl)
Tween 80 Control	0.534 ± 0.221 (12)	54.4 ± 18.0 (12)	0.039 ± 0.086 (9)	1.11 ± 0.17 (8)	0.090 ± 0.024 (11)
0.4 mg/kg PFOS	0.000 ± 0.000** (10)	55.8 ± 18.9 (9)	0.016 ± 0.036 (5)	(0)	0.018 ± 0.009** (8)
0.8 mg/kg PFOS	0.000 ± 0.000** (8)	48.8 ± 17.7 (8)	0.000 ± 0.000 (5)	(0)	0.014 ± 0.018** (7)
1.0 mg/kg PFOS	0.017 ± 0.050** (9)	47.7 ± 8.6 (7)	0.000 ± 0.000 (4)	2.36 ± 0.00** (1)	0.016 ± 0.010** (7)
1.2 mg/kg PFOS	0.005 ± 0.014** (13)	44.7 ± 21.7 (11)	0.003 ± 0.009 (10)	1.01 ± 0.25 (5)	0.011 ± 0.009** (11)
1.6 mg/kg PFOS	0.005 ± 0.012** (6)	32.7 ± 7.9 (6)	0.004 ± 0.009 (5)	1.45 ± 0.34* (5)	0.010 ± 0.006** (6)
2.0 mg/kg PFOS	0.000 ± 0.000** (3)	32.8 ± 12.2 (3)	0.050 ± 0.087 (3)	1.50 ± 0.00 (1)	0.010 ± 0.010** (3)
* Significantly different from control group; $p \leq 0.05$ ** Significantly different from control group; $p \leq 0.01$					

Table 15: Natural Delivery and Litter Observations - Dose Response

Parameter	Units	Tween 80 Control	0.4 mg/kg/day PFOS	0.8 mg/kg/day PFOS	1.0 mg/kg/day PFOS	1.2 mg/kg/day PFOS	1.6 mg/kg/day PFOS	2.0 mg/kg/day PFOS
F1 Natural Delivery Observations								
Pregnant	N (%)	19 (100.0)	20 (100.0)	17 (85.0)	19 (95.0)	18 (90.0)	17 (85.0)	19 (95.0)
Delivered Litters	N (%)	19 (100.0)	20 (100.0)	17 (100.0)	19 (100.0)	(100.0)	17 (100.0)	19 (100.0)
Maternal Body Weight at Delivery	Grams	410.6 ± 31.1	421.2 ± 28.9	408.5 ± 25.3	408.6 ± 22.2	394.5 ± 31.7	398.3 ± 26.1	397.5 ± 30.2
Duration of Gestation	Days	22.9 ± 0.3	22.6 ± 0.5	22.5 ± 0.5*	22.4 ± 0.6**	22.3 ± 0.5**	22.0 ± 0.0**	22.2 ± 0.4**
Dams with Stillborn Pups	N (%)	4 (21.0)	8 (40.0)**	2 (11.8)	1 (5.3)*	1 (5.6)*	1 (5.9)*	1 (5.3)*
Dams with No Liveborn Pups	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dams with All Pups Dying During Days 1-5 Postpartum	N	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)	4 (23.5)	14 (73.7)**
F2 Litter Observations								
Litters with ≥ 1 Liveborn Pup	N	19	20	17	19	18	17	19
Total Pups Delivered in Group	N	265	300	247	287	253	231	252
Mean Pups Delivered per Litter	N	13.9 ± 2.6	15.0 ± 2.3	14.5 ± 2.3	15.1 ± 2.3	14.0 ± 2.9	13.6 ± 2.8	13.3 ± 2.5
Total Liveborn Pups per Group	N (%)	260 (98.1)	291 (97.0)	245 (99.2)	285 (99.3)	252 (99.6)	227 (98.3)	251 (99.6)
Mean Liveborn Pups per Litter	N	13.7 ± 2.7	14.6 ± 2.4	14.4 ± 2.4	15.0 ± 2.3	14.0 ± 2.9	13.4 ± 2.9	13.2 ± 2.5
Total Stillborn Pups per Group	N (%)	5 (1.9)	9 (3.0)	2 (0.8)	2 (0.7)	1 (0.4)	4 (1.7)	1 (0.4)
Mean Stillborn Pups per Litter	N	0.3 ± 0.6	0.4 ± 0.6	0.1 ± 0.3	0.1 ± 0.4	0.0 ± 0.2	0.2 ± 1.0	0.0 ± 0.2
Day 5 Viability Index	%	97.3	97.6	93.1	88.8	81.7	49.3**	17.1**
Percent Males day 1 postpartum	N	50.0 ± 17.4	46.4 ± 14.1	53.9 ± 11.3	46.9 ± 15.9	50.0 ± 14.1	45.8 ± 9.6	50.1 ± 14.0
Pup Weight per Litter day 1 postpartum	Grams	86.9 ± 15.4	85.2 ± 15.1	84.2 ± 10.4	85.8 ± 14.3	77.2 ± 16.2	67.0 ± 15.9**	58.5 ± 25.6**

* = significantly different from control at $p \leq 0.05$; ** $p \leq 0.01$

Table 16: Natural Delivery and Litter Observations -Rescue:

Parameter	Units	Tween 80 Control	MA Control	Chol. Control	1.6 mg/kg/day PFOS	1.6 mg/kg/day PFOS +MA	1.6 mg/kg/day PFOS +Chol.	2.0 mg/kg/day PFOS	2.0 mg/kg/day PFOS +MA	2.0 mg/kg/day PFOS +Chol.
F1 Natural Delivery Observations										
Pregnant	N (%)	19 (100.0)	18 (94.7)	17 (89.5)	17 (85.0)	18 (90.0)	20 (100.0)	19 (95.0)	19 (95.0)	19 (95.0)
Delivered Litters	N (%)	19 (100.0)	18 (100.0)	17 (100.0)	17 (100.0)	18 (100.0)	20 (100.0)	19 (100.0)	19 (95.0)	19 (100.0)
Maternal Body Weight at Delivery	Grams	410.6 ± 31.1	403.5 ± 24.8	428.0 ± 31.1^	398.3 ± 26.1	398.2 ± 34.1	392.0 ± 31.6**	397.5 ± 30.2	403.4 ± 35.1	394.5 ± 29.0**
Duration of Gestation	Days	22.9 ± 0.3	22.7 ± 0.5	22.8 ± 0.4	22.0 ± 0.0**	22.2 ± 0.4*	22.0 ± 0.4**	22.2 ± 0.4**	22.0 ± 0.4**	22.3 ± 0.4**
Dams with Stillborn Pups	N (%)	4 (21.0)	2 (11.1)	2 (11.8)	1 (5.9)*	4 (22.2)	2 (10.0)	1 (5.3)*	2 (10.5)	2 (10.5)
Dams with No Liveborn Pups	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
Dams with All Pups Dying During Days 1-5 Postpartum	N	0 (0.0)	0 (0.0)	0 (0.0)	4 (23.5)	6 (33.3)	8 (40.0)	14 (73.7)**	17 (89.5)**	14 (73.3)**
F2 Litter Observations										
Litters with ≥ 1 Liveborn Pup	N	19	18	17	17	18	20	19	19	18
Total Pups Delivered in Group	N	265	237	247	231	233	271	252	270	261
Mean Pups Delivered per Litter	N	13.9 ± 2.6	13.2 ± 2.2	14.5 ± 2.2	13.6 ± 2.8	12.9 ± 2.3	13.6 ± 2.1	13.3 ± 2.5	14.2 ± 1.4	14.5 ± 1.7
Total Liveborn Pups per Group	N (%)	260 (98.1)	234 (98.7)	245 (99.2)	227 (98.3)	227 (97.4)	269 (99.3)	251 (99.6)	268 (99.2)	259 (99.2)
Mean Liveborn Pups per Litter	N	13.7 ± 2.7	13.0 ± 2.2	14.4 ± 2.2	13.4 ± 2.9	12.6 ± 2.1	13.4 ± 2.3	13.2 ± 2.5	14.1 ± 1.4	14.4 ± 1.8
Total Stillborn Pups per Group	N (%)	5 (1.9)	3 (1.3)	2 (0.8)	4 (1.7)	6 (2.6)	2 (0.7)	1 (0.4)	2 (0.7)	2 (0.8)
Mean Stillborn Pups per Litter	N	0.3 ± 0.6	0.2 ± 0.5	0.1 ± 0.3	0.2 ± 1.0	0.3 ± 0.7	0.1 ± 0.3	0.0 ± 0.2	0.1 ± 0.3	0.1 ± 0.3
Day 5 Viability Index	%	97.3	98.7	98.0	49.3**	41.4 *	42.0*	17.1**	1.1**	14.3**
Percent Males day 1 postpartum	N	50.0 ± 17.4	56.2 ± 12.4	48.3 ± 14.5	45.8 ± 9.6	49.9 ± 10.8	47.3 ± 16.8	50.1 ± 14.0	48.0 ± 15.6	56.1 ± 11.9
Pup Weight per Litter day 1 postpartum	Grams	86.9 ± 15.4	83.0 ± 11.5	90.9 ± 12.5	67.0 ± 15.9**	63.1 ± 18.7**	67.7 ± 14.1**	58.5 ± 25.6**	57.1 ± 22.2**	68.6 ± 20.2**

* = significantly different from appropriate control at $p \leq 0.05$; ** $p \leq 0.01$

^ = significantly different from Tween 80 control group $p \leq 0.05$

Table 17 : Data Summary of PFOS Concentration—Rat Serum (µg/mL) – Dose Response

Timepoint	Female F0 Gestation Day 21	Pups F1 Gestation Day 21	Female F0 Lactation Day 5	Pups F1 Lactation Day 5
Group				
Tween 80 Control	0.007 ± 0.003 [a] (n=6)	<0.005 [b] (n=6)	0.018 ± 0.009 [a] (n=6)	0.030 ± 0.020 [a] (n=6)
0.4 mg/kg PFOS	N/A	N/A	27.2 ± 18.7** (n=6)	36.2 ± 3.84** (n=6)
0.8 mg/kg PFOS	N/A	N/A	42.6 ± 6.92** (n=6)	53.1 ± 27.7** (n=6)
1 mg/kg PFOS	N/A	N/A	52.3 ± 26.0** (n=6)	84.4 ± 17.5** (n=6)
1.2 mg/kg PFOS	N/A	N/A	86.0 ± 10.2** (n=6)	147 ± 162* (n=6)
1.6 mg/kg PFOS	142 ± 24.5** (n=6)	142 ± 33.2** (n=6)	169 ± 32.1** (n=6)	N/A
2 mg/kg PFOS	125 ± 19.8** (n=6)	170 ± 38.1** (n=6)	134 ± 26.6** (n=6)	138 (n=1)

N/A—Not applicable
NOTE: Results are expressed as group averages ± the standard deviation associated with the group.
NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, extracted calibration curves and multi-level continuing calibration verifications (CCVs), indicate that the sera data are quantitative to ±25%.
a: one or more values less than the limit of quantitation (LOQ) of 0.005 µg/mL; 0.005 used as the individual value for statistical equations
b: all values less than the LOQ; 0.005 used as the group average
* significantly different from control using students T-Test ($p \leq 0.05$); ** ($p \leq 0.01$)

Table 18: Data Summary of PFOS Concentration—Rat Serum (µg/mL) – Rescue Study

Timepoint	Female F0 Gestation Day 21	Pups F1 Gestation Day 21	Female F0 Lactation Day 5	Pups F1 Lactation Day 5
Group				
Tween 80 Control	0.007 ± 0.003 [a] (n=6)	<0.005 [b] (n=6)	0.018 ± 0.009 [a] (n=6)	0.030 ± 0.020 [a] (n=6)
MA control	0.018 ± 0.023 [a] (n=6)	0.078 ± 0.084 [a] ^ (n=6)	12.7 ± 27.4 (n=6)	3.33 ± 3.14 [a] ^ (n=6)
Chol. control	0.025 ± 0.004^^ (n=6)	0.083 ± 0.032^^ (n=6)	0.688 ± 1.62 (n=6)	0.188 ± 0.199 [a] (n=6)
1.6 mg/kg PFOS	142 ± 24.5** (n=6)	142 ± 33.2** (n=6)	169 ± 32.1** (n=6)	N/A
1.6 mg/kg PFOS + MA	55.8 ± 8.87** (n=6)	207 ± 25.6** (n=6)	127 ± 24.7** (n=6)	124 ± 18.7** (n=3)
1.6 mg/kg PFOS + Chol.	79.9 ± 15.5** (n=6)	154 ± 32.2** (n=6)	104 ± 14.6** (n=6)	109 ± 7.85** (n=2)
2 mg/kg PFOS	125 ± 19.8** (n=6)	170 ± 38.1** (n=6)	134 ± 26.6** (n=6)	138** (n=1)
2 mg/kg PFOS + MA	82.4 ± 22.6** (n=6)	251 ± 55.9** (n=6)	189 ± 48.2** (n=6)	N/A
2 mg/kg PFOS + Chol.	371 ± 214** (n=6)	166 ± 50.4** (n=6)	145 ± 7.59** (n=6)	155** (n=1)

N/A—Not applicable
Mevalonic acid lactone (MA); Cholesterol (Chol.)
NOTE: Results are expressed as group averages ± the standard deviation associated with the group.
NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, extracted calibration curves and multi-level continuing calibration verifications (CCVs), indicate that the sera data are quantitative to ±25%.
a: one or more values less than the limit of quantitation (LOQ) of 0.005 µg/mL; 0.005 used as the individual value for statistical equations
b: all values less than the LOQ; 0.005 used as the group average
* significantly different from appropriate control using students T-Test ($p \leq 0.05$); ** ($p \leq 0.01$)
^ significantly different from Tween 80 Control group using students T-Test ($p \leq 0.05$); ^^ ($p \leq 0.01$)

Table 19: Data Summary of PFOS Concentration—Rat Liver (µg/g) - Dose Response

Timepoint	Female F0 Lactation Day 5	Pups F1 Lactation Day 5
Group		
Tween 80 Control	<0.6 ± 0.9 [a] (6)	< 0.0 ± 0.0 [b] (6)
0.4 mg/kg PFOS	47.9 ± 5.1** (4)	73.4 ± 30.8** (6)
1.6 mg/kg PFOS	110.2 ± 13.4** (5)	247.8 ± 131.1** (6)
2 mg/kg PFOS	145.8 ± 20.2** (6)	245.2 ± 93.4** (4)

a: one or more values below the quantitation limit (BQL) of 12.5 ng/g; 12.5 ng/g used as the individual value(s) for statistical equations
b: all values BQL; 12.5 ng/g used for the individual values in the calculations
* significantly different from control using students T-Test ($p \leq 0.05$); ** ($p \leq 0.01$)

RS-III-43: *In Vitro* Microbiological Mutagenicity Assays of 3M Company Compounds T-2247 CoC [Perfluorooctyl sulfonate DEA salt] and T-2248 CoC.

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^{o} 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^{o} 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

RS-III-44: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with PFOS.

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*uvr*A

Metabolic activation: 0.1 ml S9 liver homogenate from Aroclor 1254 induced Sprague-Dawley rats

Concentrations tested: S. typhimurium: 33.3 µg/plate, 100 µg/plate, 333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000µg/plate µg/plate with activation and 0.333 µg/plate, 1.00 µg/plate, 3.33 µg/plate, 10.0 µg/plate, 33.3 µg/plate plate, 100 µg/plate, 3333 µg/plate, 1,000 µg/plate and 5,000 µg/plate without activation.

E. coli: 33.3 µg/plate, 100 µg/plate, 3333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000 µg/plate both with and without activation.

Statistical methods used: None

Remarks: There were no significant protocol deviations. (1) There were 3 plates per test concentration and control; the positive controls were strain and activation condition specific and included benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, ICR-191 and 4-nitroquinoline-N-oxide. The vehicle control was DMSO; (2) the solvent was DMSO; (3) the assay was not repeated. (4) For the test article to be considered positive in strains TA98, TA100 and WP2*uvr*A, 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

RS-III-45: *Salmonella Typhimurium* Spot Test on FC-95 [PFOS].

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

RS-III-46: Bacterial Reverse Mutation Test of ~~9~~-1.

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 WP2*uvr*A

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 WP2*uvr*A without activation: 39.1 µg/plate, 78.1µg/plate, 156µg/plate, 313 µg/plate, 625µg/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 *uvr*A and 5000 µg/plate with S. typhimurium TA98 without activation. 5000 µg/plate

with S. typhimurium TA100, TA1535, TA1537, and E. coli WP2 *uvr*A 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

RS-III-47: Mutagenicity Evaluation of T-2O14 CoC [PFOS] in the Ames *Salmonella*/Microsome Plate Test.

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: 0.1µg/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, nitroflourene, 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

RS-III-48: Chromosomal Aberrations in Human Whole Blood Lymphocytes with PFOS.

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 a 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

RS-III-49: Mutagenicity Test on T-6295 [PFOS] in an *In Vivo* Mouse Micronucleus Assay.

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; Crl: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 H_2O; 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

RS-III-50: Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS.

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 ^{3}H-thymidine added to the cultures for 30 minutes after which the cells were fixed, dried over night, coverslips were mounted on slides, dipped in emulsion and stored for 6 days at 2-8^{o} 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

RS-III-51: Eye Irritation Report on Sample T-1117.

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: 1 hr, 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.

RS-III-52: 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

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.

RS-III-53: One Generation Reproduction Study of PFOS – Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats

TEST SUBSTANCE

Identity: Potassium Perfluorooctanesulfonate (KPFOS), CAS No. 2795-39-3, 3M Company.

Remarks: The test article, FC-95 (lot 217) was received on August 25, 2000 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. Purity is 98.9 %. The report will contain a certificate of analysis. Potassium perfluorooctanesulfonate (KPFOS) was administered; however, for simplification, KPFOS and the dissociated anion, perfluorooctanesulfonate (PFOS), will both be referred to as PFOS throughout this document.

CONTROL ARTICLES

Identity: Mevalonic acid lactone, Lots 070K26603 and 080K2618, Sigma Chemical Company. Cholesterol, Lot 119H0218, Sigma Chemical Company. Tween® 80, J.T.Baker.

Remarks: Mevalonic acid lactone was received on August 1, 2000 and September 21, 2000 and stored frozen. Cholesterol was received on July 20, 2000 and stored at room temperature. Tween® 80 was received on March 7, 2000, and stored at room temperature.

METHOD

Method/Guideline followed: 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: Investigative, mechanistic one-generation reproduction study

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 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: 2000 - 2001

Species/Strain: Rat Crl:CD® (SD) IGS BR VAF/Plus® (Sprague-Dawley)

Sex (males/females/both): Both (males were not dosed and were only used for breeding)

Number of animals per dose: 20 - 29 females per group

Route of administration: Oral (gavage)

Dosing regimen: Female rats were given the test article, control article and/or vehicle daily beginning 42 days before cohabitation and continuing through gestation day 20 or lactation day 4, depending on whether rats were assigned to caesarean sectioning on gestation day 21 or sacrifice on lactation day 5, respectively. Rats in the process of delivering were not treated. The F1 generation pups were not given test article directly; however, exposure occurred during gestation (*in utero)* and via milk during the lactation period. Male rats were used for breeding

purposes only and were not treated.

Doses: 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 mg/kg/day (PFOS alone); 1.6 and 2.0 mg/kg/day (PFOS + 1000 mg/kg/d mevalonic acid lactone as two 500 mg/kg/day doses); 1.6 and 2.0 mg/kg/day (PFOS + 500 mg/kg/day cholesterol); 1000 mg/kg/day mevalonic acid lactone (given in two 500 mg/kg/day doses); 500 mg/kg/day cholesterol; 5 ml/kg 0.5 % Tween 80 (vehicle control). Dosage volumes were adjusted to 5 ml/kg.

Statistical methods used: Vehicle control data was first compared to mevalonic acid lactone and cholesterol control data. Then, vehicle control data was compared to PFOS-only dosed groups. Mevalonic acid lactone control data was compared to dose groups also supplemented with mevalonic acid lactone. Cholesterol control data was compared to dose groups also supplemented with cholesterol. 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 Ceasarean-sectioning and natural delivery involving discrete data (number of corpora lutea, number of pups per litter) were evaluated by the Kruskal-Wallis Test.

Remarks: Detail and discuss any significant protocol parameters:

F_0 Generation:

Female rats were used for this study. Due to the size and complexity of the study design, the rats were received in two shipments designated as replicate 1 and 2, respectively. The rats were randomly assigned to 13 dosage groups detailed below.

Dosage Group	**Identification**	**Number of Rats**			
		Total	**Replicate 1 C-sectioning**	**Replicate 1 Natural Delivery**	**Replicate 2 Natural Delivery**
1	Tween 80 Control	28	9	5	14
2	Mevalonic acid (MA) control	28	8	6	14
3	1.6 mg/kg PFOS + MA	29	8	6	15
4	2.0 mg/kg PFOS + MA	28	8	6	14
5	Cholesterol (Chol) Control	28	8	6	14
6	1.6 mg/kg PFOS + Chol.	28	8	6	14
7	2.0 mg/kg PFOS + Chol.	28	8	6	14

8	0.4 mg/kg PFOS	20	NA	10	10
9	0.8 mg/kg PFOS	20	NA	10	10
10	1.0 mg/kg PFOS	20	NA	10	10
11	1.2 mg/kg PFOS	20	NA	10	10
12	1.6 mg/kg PFOS	28	8	6	14
13	2.0 mg/kg PFOS	28	8	6	14

The first eight females per dosage group in Groups 1-7, 12, and 13 of replicate 1 with a confirmed date of mating were assigned to caesarean sectioning on day 21 of gestation. The remaining females were permitted to naturally deliver litters. Dosing began 42 days prior to cohabitation and continued until day 20 of gestation for rats assigned to caesarian sectioning or day 4 of lactation for rats assigned to natural delivery. Rats assigned to natural delivery and not producing a litter were dosed until gestation day 24.

Viability observations were made twice daily and rats were observed for clinical signs of treatment effect daily prior to dosage administration, approximately one-hour after the second dosage and at the end of the working day. Body weights were taken weekly to cohabitation and daily through presumed gestation, and on days 1 and 5 of lactation. Mating was confirmed by vaginal smear or observation of a vaginal plug.

Rats assigned to natural delivery were observed for adverse clinical signs during parturition. Other indices examined were duration of gestation, pup viability at birth, fertility index, gestation index, number of offspring per litter, number of implantation sites, general condition of dam and litter during the post-partum period, and lactation day 5 viability index.

Eight rats from groups 1-7, 12, and 13 of replicate 1 were sacrificed on gestation day 21 and caesarian sectioned. Number of corpora lutea in each ovary was recorded. The uterus was examined for pregnancy, number of implantation sites, live and dead fetuses, and early and late resorptions. Placentae were examined for size, color, and shape. Blood samples were collected, the liver was excised, and liver weight was recorded. A sample of liver (right lateral lobe) was flash frozen in liquid nitrogen and remained frozen. The median liver lobe was frozen. A section of the remaining portion of liver was fixed in glutaraldehyde.

Clinical chemistry parameters observed were cholesterol, glucose, LDL-direct, HDL-direct, triglycerides, total and free thyroxin, total and free triiodothyronine, and thyroid stimulating hormone. Milk cholesterol was determined in those dams allowed to litter. In animals supplemented with mevalonic acid lactone, plasma mevalonate was also determined.

Liver and serum PFOS concentrations were determined in selected groups. Individual samples were used.

F_1 Generation:

Litter observations were made for number and sex of pups, stillborn, live births, and gross alterations. Viability of pups was observed twice daily. Pups were counted once daily. Pooled litter weights were taken through day 5 of lactation.

Fetuses taken by caesarian section on day 21 of gestation were pooled by litter and litter body

weights were recorded. A blood sample and liver were collected from each fetus. One fetal liver from each litter was fixed in glutaraldehyde. The remaining fetal livers in each litter were divided into three samples of equal number. Two of these were frozen. The third was flash frozen in liquid nitrogen and remained frozen.

Pups were sacrificed on day 5 of lactation and examined for gross lesions. Necropsy included a single cross section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. Blood samples were collected from each pup. In replicate 1, blood samples were pooled per litter. In replicate 2, two litters per sample from each dose group were pooled. Liver was collected from each pup, pooled by sex per litter, and the pooled liver weight was recorded. One liver per litter pool was fixed in glutaraldehyde. The remaining fetal livers in each litter were divided into three samples of equal number. Two of these were frozen. The third was flash frozen in liquid nitrogen and remained frozen. The hearts were collected from the first two male and two female pups from each litter and fixed in glutaraldehyde. Thyroids were excised from each pup, pooled by sex per litter and retained.

Clinical chemistry parameters observed were cholesterol, glucose, LDL-direct, HDL-direct, triglycerides, total and free thyroxin, total and free triiodothyronine, and thyroid stimulating hormone. In those animals supplemented with mevalonic acid lactone, plasma mevalonate was also determined.

Liver and serum PFOS concentration was determined in selected groups. Pooled samples were used.

Selected thyroid and heart tissues were examined by light microscopy.

RESULTS

Toxic response/effects by dose level and generation:

Mortality, morbidity, and necropsy observations

No deaths among dams were attributed to administration of the test article. With the exception of aversion to the taste of mevalonic acid lactone, which occurred equally among all mevalonic acid lactone treated groups, there were no untoward symptoms attributed to treatment. Necropsy observations were considered unrelated to administration of test or control article.

Maternal body weight and feed consumption

Significant reductions in maternal body weight as compared to control were observed in all groups receiving 1.6 and 2.0 mg/kg/day PFOS. Absolute and relative feed consumption was significantly less in dams receiving 2.0 mg/kg PFOS only, 1.6 and 2.0 mg/kg PFOS plus mevalonic acid lactone, and 1.6 and 2.0 mg/kg PFOS plus cholesterol on premating days 1-42. In the 0.8 mg/kg/day and higher dose groups, significant reductions in absolute and relative feed consumption were observed during the first week of gestation and during days 1-5 of lactation with the exception of relative feed consumption on days 1-5 of lactation in the 2.0 mg/kg/day PFOS alone dose group, which was apparently lower but did not reach statistical significance. Significant decreases in absolute and relative feed consumption were seen at other weeks of gestation and lactation in the 1.6 and 2.0 mg/kg/day dose groups.

Clinical Chemistry

On day 21 of gestation, no significant changes in the selected clinical chemistry parameters were

seen in dams dosed with PFOS alone or with PFOS plus cholesterol as compared to appropriate control. In the mevalonic acid lactone supplemented dams, a significant increase in LDL was observed as compared to the mevalonic acid control. Significant decreases in cholesterol and increases in LDL were observed in all fetuses from the 1.6 and 2.0 mg/kg PFOS dose groups, with or without supplementation, on day 21 of gestation. A significant increase in HDL was observed in fetuses from the 1.6 and 2.0 mg/kg PFOS plus cholesterol dose groups and a significant decrease in triglycerides was observed in the 2.0 mg/kg PFOS plus mevalonic acid lactone fetuses on day 21 of gestation as compared to appropriate controls. On day 5 of lactation, all dams receiving PFOS, with or without supplementation, had significantly decreased cholesterol levels as compared to appropriate control. Milk cholesterol was significantly increased in the 2.0 mg/kg PFOS plus mevalonic acid lactone dams on lactation day 5 as compared to the mevalonic acid lactone control. Serum glucose was significantly increased in dams receiving 2.0 mg/kg PFOS alone or plus mevalonic acid lactone on gestation day 5. Serum triglycerides were significantly decreased in dams receiving 1.6 and 2.0 mg/kg PFOS alone or with cholesterol as compared to appropriate control on day 5 of lactation. Plasma mevalonic acid levels were significantly increased in the 1.6 and 2.0 mg/kg PFOS plus mevalonic acid lactone dose groups as compared to the mevalonic acid lactone control. In pups on lactation day 5, a significant decrease in cholesterol was observed in the 1.6 mg/kg PFOS plus mevalonic acid lactone dose group. Significant increases in LDL were seen in the 1.6 mg/kg PFOS plus mevalonic acid lactone, 1.6 mg/kg PFOS plus cholesterol, and 2.0 mg/kg PFOS plus cholesterol dose groups as compared to appropriate control. HDL was significantly increased in the 1.6 and 2.0 mg/kg PFOS plus cholesterol dose groups as compared to the cholesterol control. Triglycerides were significantly increased in pups from the 1.6 mg/kg PFOS plus mevalonic acid lactone and 2.0 mg/kg PFOS plus cholesterol dose groups as compared to appropriate control. Plasma mevalonic acid levels in all mevalonic acid control groups were significantly increased over the Tween 80 control groups.

Thyroid hormone determination

Dams in all groups receiving PFOS had lower free and total T4 and T3 at gestation day 21 when compared to control with no significant change in TSH. Significant decreases in free T4 were observed in fetuses in the PFOS dose groups on gestation day 21. Measurement of total T4, free T3, and TSH was complicated by low sample number and/or control values. On lactation day 5, total T4 was significantly reduced in dams receiving 0.8 mg/kg and higher doses of PFOS. Total and free T3 were reduced in dams in the 1.2mg/kg PFOS and higher dose groups. Free T4 was significantly reduced in all dams receiving PFOS. Free and total T4 were significantly reduced in pups from all dose groups on lactation day 5. TSH values in pups were elevated in the 1.0 and 1.6 mg/kg group. (NOTE: Further investigation of thyroid hormone levels using equilibrium dialysis is currently underway at the Mayo Thyroid Lab, Rochester, MN. Preliminary results using this method indicate there is no difference in free T4 levels between treated and control animals.)

Natural delivery and litter observations

Day five viability indices for the 0.0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/day PFOS groups were 97.7, 97.6, 93.5, 89.8, and 82.5 percent, respectively. The no observed effect level for pup viability and growth was determined to be 1.0 mg/kg/day. Day five viability indices for the 1.6 mg/kg/day PFOS plus mevalonic acid lactone, 1.6mg/kg/day PFOS plus cholesterol, 2.0 mg/kg/day PFOS plus mevalonic acid lactone, and 2.0 mg/kg/day PFOS + cholesterol were 41.4,

42.0, 1.1, and 14.3, respectively. Supplementation with mevalonic acid lactone or cholesterol had no positive effect on perinatal survival through lactation day 5. Day one postpartum pup weight per litter was significantly decreased in all 1.6 and 2.0 mg/kg/day dose groups. The number of dams with all pups dying was significantly increased in all 2.0 mg/kg/day dose groups. The number of dams with stillborn pups was significantly increased in the 0.4 mg/kg PFOS only dose group; however, this parameter was significantly decreased in the 1.0, 1.2, 1.6, and 2.0 mg/kg/day PFOS only dose groups. Duration of gestation was slightly yet significantly decreased in the 0.8 mg/kg/day and higher dose groups, including those supplemented with mevalonic acid lactone and cholesterol.

Serum and Liver PFOS

Serum PFOS concentrations on gestation day 21 equaled 0.007 ± 0.003 ug/ml in control dams and were below the limit of quantitation (0.005 ug/ml) in the control fetuses. Dams and fetuses in the 1.6 mg/kg dose group had serum levels of approximately 142 ug/ml. Serum PFOS levels in the 2.0 mg/kg dose group equaled 125 ± 19.8 ug/ml in the dams and 170 ± 38.1 ug/ml in the fetuses. Serum PFOS levels were approximately equal in dams and pups at lactation day 5 and increased in a dose response manner. Liver PFOS levels in dams at lactation day 5 were approximately equal to the lactation day 5 serum levels. Liver PFOS levels in the pups at lactation day 5, however, were approximately twice as high as the pup serum levels and the dam liver levels. Serum PFOS levels in the supplemented dose groups were approximately equal in dams and pups and increased in a dose response manner at both time points. Liver PFOS was not measured in the supplemented dose groups.

Histology

Light microscopy was performed on hearts and thyroids from lactation day 5 pups in the 0.0, 0.4, 1.6, and 2.0 mg/kg/day PFOS only dose groups. No abnormalities were observed in any of the tissues.

CONCLUSIONS

The final report is not available at the time of this review. Based on the data currently available from an audited draft final report of May 7, 2001, co-administration of mevalonic acid lactone or cholesterol was unable to prevent or mitigate adverse effects experienced by the dam, fetus, or pup in prior reproduction studies. Perinatal mortality is not a result of cholesterol deficiency in the perinatal period. The relationship of apparent reductions in thyroxin and triiodothyronine in treated rats and their offspring to the observed effects is unclear and the subject of further investigation.

NOEL/NOAEL: The NOEL for the F_0 generation females = 0.4 mg/kg/day, the lowest dose tested. For dams, the 0.8 mg/kg/day dosage and higher dosages caused an increase in relative liver weight, decreased body weight gain during gestation and lactation and decreased relative and absolute feed consumption during gestation and lactation. The duration of gestation was also decreased at 0.8 mg/kg/day. The NOEL for viability and growth in the F1 pups is 1.0 mg/kg/day [NOTE: Current draft of report gives 1.2 mg/kg/day as NOEL. After discussion with Dr. York, we have revised this to 1.0 mg/kg/day].

REFERENCE

Hoberman, A.M., and York, R.G.. Argus Research Laboratories, Inc. Argus Study Number: 418-018, Sponsor Study Number: 6295.25.

APPENDIX IV

BENCHMARK DOSE CALCULATIONS FOR PFOS

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BENCHMARK DOSE CALCULATIONS FOR PFOS:

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Benchmark Dose Calculations for PFOS: 14-Week Study

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Introduction

Perfluorooctanesulfonate (PFOS) was administered as the potassium salt in the diet at 0, 0.5, 2.0, 5.0, and 20 ppm to male and female Sprague Dawley rats for 14 weeks. Benchmark doses estimated to produce a 10% excess risk of abnormal levels are calculated for liver weight, liver to body weight ratio, and clinical chemistry measurements related to PFOS exposure reported by Seacat *et al.* (*Toxicology*, 2002). Benchmark doses for PFOS are calculated in terms of the administered concentration (ppm) in the diet, mg/kg body weight per day, serum concentration, and liver concentration.

If measurements below the 1.5^{th} percentile or above the 98.5^{th} percentile of a normal distribution are considered abnormal, then a shift of the mean of the distribution equal to the size of the standard deviation results in an excess of 10% of measurements in the abnormal range. Hence, the procedure for calculating a benchmark dose is to determine the mean and standard deviation among the control animals for a selected endpoint and to establish the dose response relationship of that endpoint to PFOS exposure.
A polynomial dose response curve often provided a good fit to the bioassay data. In some cases a linear relationship was sufficient. When there was extreme curvature due to an effect only at the highest dose tested, a power model provided a better fit to the data. The estimated PFOS dose corresponding to a change of the mean of the endpoint equal to the size of the standard deviation is the benchmark dose (BMD_{10}) where an additional 10% of the animals are in the abnormal range. Further, a lower 95% confidence limit ($LBMD_{10}$) is estimated that can be divided by uncertainty (safety) factors to establish a reference dose associated with a negligible risk.

Average experimental levels of PFOS from 14-week exposure to the potassium salt of PFOS in the diet are listed in Table 1.

Table 1. Average experimental exposures to PFOS.

Dietary concentration (ppm)	0	0.5	2.0	5.0	20
Males					
Dose (mg/kg body wt. per day)	0	0.03	0.13	0.34	1.33
Serum concentration (μg/mL)	<LOQ[a]	4.04	17.1	43.9	148
Liver concentration (μg/g)	0.46	23.8	74.0	358	568
Females					
Dose (mg/kg body wt. per day)	0	0.04	0.15	0.40	1.56
Serum concentration (μg/mL)	2.67	6.96	27.3	64.4	223
Liver concentration (μg/g)	12.0	19.2	69.2	370	635

[a] Limit of quantification = 0.046 μg/mL. One-half of this value (0.023 μg/mL) was used in fitting the dose response curve.

Male Liver Weight

The average liver weights in males after 14 weeks of exposure to PFOS were 15.5, 15.5, 14.0, 18.8, and 20.3 grams at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm of PFOS, respectively.

Fitting a polynomial model to PFOS concentration in the diet (C_D) resulted in a linear relationship

Liver weight (grams) = 15.37 + (0.263 x dietary concentration)

with a standard deviation of 2.35 grams. The BMD_{10} is the dose where the liver weight is estimated to be (15.37 + 2.35) = 17.72 grams, i.e., changes by 2.35 grams, giving BMD_{10} = 2.35 / 0.263 = 8.9 ppm. The lower 95 % confidence limit is $LBMD_{10}$ = 6.1 ppm of PFOS in the diet.

Fitting a polynomial model to liver weight as a function of PFOS dose expressed as mg/kg body weight per day gives a linear relationship

Liver weight (grams) = 15.37 + (3.97 x dose)

with a standard deviation of 2.34 grams, giving BMD_{10} = 0.59 and $LBMD_{10}$ = 0.40 mg/kg body weight per day of PFOS.

Fitting a polynomial model to liver weight as a function of PFOS concentration in the serum (C_S) gives a linear relationship

Liver weight (grams) = 15.27 + (0.0364 x C_S)

with a standard deviation of 2.32 grams, giving BMD_{10} = 64 and $LBMD_{10}$ = 44 μg/mL of PFOS in the serum after 14 weeks of exposure.

Fitting a polynomial model to liver weight as a function of the PFOS concentration in the liver (C_L) gives a linear relationship

Liver weight (grams) = 14.79 + (0.010 x C_L)

with a standard deviation of 2.13 grams giving BMD_{10} = 215 and $LBMD_{10}$ = 154 μg/g of PFOS in the liver after 14 weeks of exposure.

The results for liver weights in males are summarized below:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	8.9	0.59	64	215
$LBMD_{10}$	6.1	0.40	44	154

Male Liver /(Body Weight)

The average liver to body weight ratios, expressed as a percent of body weight, were 3.2, 3.2, 3.2, 3.6, and 4.3 % after a 14-week exposure to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm, respectively, for males.

Fitting a polynomial model to these data as a function of concentration in the diet (C_D) of PFOS gives

$$\text{Liver/body weight (\%)} = 3.15 + (0.087 \times C_D) - (0.00147 \times C_D^2)$$

with a standard deviation of 0.27 %. The BMD_{10} = 3.2 ppm and LBMD = 1.8 ppm of PFOS in the diet.

Fitting a polynomial model as a function of dose (mg/kg body weight per day) gives

$$\text{Liver/body weight (\%)} = 3.15 + (1.29 \times \text{dose}) - (0.315 \times \text{dose}^2)$$

with a standard deviation of 0.27 %. The BMD_{10} = 0.22 mg/kg body weight per day and $LBMD_{10}$ = 0.12 mg/kg/d of PFOS.

The polynomial for concentration of PFOS in the serum (C_S) is

$$\text{Liver/body weight (\%)} = 3.15 + (0.0096 \times C_S) - (0.000012 \times C_S^2)$$

with a standard deviation of 0.27%. The BMD_{10} = 29 μg/mL and $LBMD_{10}$ = 16 μg/mL of PFOS in the serum following 14 weeks of exposure.

For concentration of PFOS in the liver (C_L),

$$\text{Liver/body weight (\%)} = 3.20 - (0.00029 \times C_L) + (0.0000039 \times C_L^2)$$

with a standard deviation of 0.26 %. The BMD_{10} = 297 μg/g and $LBMD_{10}$ = 163 μg/g of PFOS in the liver after a 14-week exposure.

A summary of the results for liver weight to body weight ratio for males are:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	3.2	0.22	29	297
$LBMD_{10}$	1.8	0.12	16	163

Male Segmented Neutrophils

The average concentrations of segmented neutrophils (N-Seg) for males were 1.1, 1.3, 1.2, 1.2, and 1.6 ($10^3/\mu L$) after 14 weeks of exposure to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm, respectively. The fitted models versus dietary concentration (C_D), dose, serum concentration (C_S), and liver concentration (C_L) of PFOS:

N-Seg ($10^3/\mu L$) = $1.19 - (0.0012 \times C_D) + (0.00107 \times C_D^2)$; std. dev = 0.35

N-Seg ($10^3/\mu L$) = $1.20 - (0.032 \times \text{dose}) + (0.252 \times \text{dose}^2)$; std.dev. = 0.35

N-Seg ($10^3/\mu L$) = $1.20 - (0.0005 \times C_S) + (0.000022 \times C_S^2)$; std.dev. = 0.35

N-Seg ($10^3/\mu L$) = $1.16 + (0.0006 \times C_L)$ std. dev. = 0.36

Estimates of the BMD_{10} and $LBMD_{10}$ for 14-week exposures to PFOS for males were:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	19	1.2	139	605
$LBMD_{10}$	7.5	0.52	66	366

Male Cholesterol

Average levels of cholesterol in males were 63, 53, 51, 57, and 37 (mg/dL) after 14-week exposures to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm, respectively. The fitted models as a function of dietary concentration, dose, serum concentration, and liver concentration of PFOS were, respectively:

Cholesterol (mg/dL) = $57.0 - (0.44 \times C_D) - (0.0278 \times C_D^2)$ std. dev. = 13.4

Cholesterol (mg/dL) = $56.9 - (5.37 \times \text{dose}) - (7.12 \times \text{dose}^2)$ std. dev. = 13.4

Cholesterol (mg/dL) = $56.9 - (0.0375 \times C_S) - (0.000648 \times C_S^2)$ std. dev. = 13.4

Cholesterol (mg/dL) = $58.0 - (0.0283 \times C_L)$ std. dev. = 14.0

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	15	1.0	118	497
$LBMD_{10}$	5.8	0.40	51	322

Male Alanine Aminotransferase

Average values of alanine aminotransferase (ALT) in males were 36, 41, 41, 44, and 65 (IU/L) following 14 weeks exposure of PFOS in the diet at 0, 0.5, 2.0, 5.0, and 20 ppm, respectively. Fitted models as a function of dietary concentration, dose, serum concentration, and liver concentration of PFOS were, respectively:

$ALT\ (IU/L) = 35.7 + (2.79 \times C_D)$ std. dev. = 6.1

$ALT\ (IU/L) = 35.0 + (34.1 \times dose)$ std. dev. = 6.7

$ALT\ (IU/L) = 34.5 + (0.401 \times C_S) - (0.001073 \times C_S^2)$ std. dev. = 5.9

$ALT\ (IU/L) = 35.2 + (0.072 \times C_L)$ std. dev. = 8.6

The BMD_{10} and $LBMD_{10}$ for 14-week exposures of males to PFOS were:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	2.2	0.20	15	119
$LBMD_{10}$	1.6	0.11	9	34

Male Urea Nitrogen

Average values of urea nitrogen (UN) for males were 13, 14, 13, 14, and 16 (mg/dL) after 14-week exposure to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm. Models fitted to these data as a function of dietary concentration, dose, serum concentration, and liver concentration of PFOS in males were, respectively:

$UN\ (mg/dL) = 13.3 + (0.10 \times C_D) + (0.0018 \times C_D^2)$ std. dev. = 1.8

$UN\ (mg/dL) = 13.3 + (1.44 \times dose) + (0.445 \times dose^2)$ std. dev. = 1.8

$UN\ (mg/dL) = 13.3 + (0.0096 \times C_S) + (0.000059 \times C_S^2)$ std. dev. = 1.8

$UN\ (mg/dL) = 13.3 + [(1.1 \times 10^{-8}) \times C_L^{3.0}]$ std. dev. = 1.8

Estimates of the BMD_{10} and $LBMD_{10}$ for 14-week exposures of males to PFOS were:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	14	0.96	111	498
$LBMD_{10}$	5.1	0.35	46	359

Female (Liver Weight) / (Body Weight)

The average liver to body weight ratio, expressed as percent, were 3.3, 3.1, 3.2, 3.5, and 4.3% for females after 14-week exposures to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm, respectively. Models fit to these data as a function of dietary concentration, dose, serum concentration, and liver concentration of PFOS were:

$$\text{Liver/Body Weight (\%)} = 3.17 + (0.056 \times C_D) \qquad \text{std. dev.} = 0.22$$

$$\text{Liver/Body Weight (\%)} = 3.17 + (0.723 \times \text{dose}) \qquad \text{std. dev.} = 0.22$$

$$\text{Liver/Body Weight (\%)} = 3.15 + (0.0051 \times C_S) \qquad \text{std. dev.} = 0.21$$

$$\text{Liver/Body Weight (\%)} = 3.21 - (0.00045 \times C_L) + [(3.4 \times 10^{-6} \times C_L^2)] \qquad \text{sd.} = 0.21$$

Estimates of the BMD_{10} and $LBMD_{10}$ for 14-week exposures of PFOS to females were:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	3.8	0.30	42	325
$LBMD_{10}$	2.6	0.20	28	156

Female Urea Nitrogen

Average values of urea nitrogen (UN) in females were 12, 13, 13, 14, and 17 mg/dL after 14-week exposures to PFOS at dietary concentrations of 0, 0.5, 2.0, 5.0, and 20 ppm. Models fit to these data as a function of dietary concentration, dose, serum concentration, and liver concentration of PFOS were, respectively:

$$\text{UN (mg/dL)} = 12.4 + (0.366 \times C_D) - (0.0067 \times C_D^2) \qquad \text{std. dev.} = 2.1$$

$$\text{UN (mg/dL)} = 12.4 + (4.59 \times \text{dose}) - (1.04 \times \text{dose}^2) \qquad \text{std. dev.} = 2.1$$

$$\text{UN (mg/dL)} = 12.3 + (0.0283 \times C_S) - (0.0000329 \times C_S^2) \qquad \text{std. dev.} = 2.1$$

$$\text{UN (mg/dL)} = 12.6 + (0.00026 \times C_L) + (0.0000104 \times C_L^2) \qquad \text{std. dev.} = 2.2$$

Estimates of the BMD_{10} and $LBMD_{10}$ for 14-week exposures of PFOS for females were:

	Dietary conc. (ppm)	Dose (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
BMD_{10}	6.6	0.53	84	443
$LBMD_{10}$	3.2	0.25	40	255

Summary

Estimates of the BMD_{10} and $LBMD_{10}$ based on various effects in male and female rats are listed in Table 2 for 14-week exposures to PFOS in the diet. The benchmark dose approach utilizes the results from the dose response curve and therefore has more sensitivity to detect and estimate biological effects than simple comparisons of the results from individual dose groups with the control animals. The most sensitive endpoints to PFOS exposure were liver weight to body weight ratio and analine aminotransferase in male rats.

Table 2. Estimates of the BMD_{10} and $LBMD_{10}$ for 14-week exposures of rats to PFOS in the diet.

Effect	Dietary conc. (ppm)	Dose conc. (mg/kg/d)	Serum conc. (μg/mL)	Liver conc. (μg/g)
		BMD_{10}		
Males				
Liver weight	8.9	0.59	64	215
(Liver wt.) / (Body wt.)	3.2	0.22	29	297
Segmented neutrophils	19	1.2	139	605
Cholesterol	15	1.0	118	497
Alanine aminotransferase	2.2	0.20	15	119
Urea nitrogen	14	0.96	111	498
Females				
(Liver wt.) / (Body wt.)	3.8	0.30	42	325
Urea nitrogen	6.6	0.53	84	443
		$LBMD_{10}$		
Males				
Liver weight	6.1	0.40	44	154
(Liver wt.) / (Body wt.)	1.8	0.12	16	163
Segmented neutrophils	7.5	0.52	66	366
Cholesterol	5.8	0.40	51	322
Alanine aminotransferase	1.6	0.11	9	34
Urea nitrogen	5.1	0.35	46	359
Females				
(Liver wt.) / (Body wt.)	2.6	0.20	28	156
Urea nitrogen	3.2	0.25	40	255

Benchmark Doses for Liver Tumors in Sprague Dawley Rats fed Perfluorooctane Sulfonic Acid Potassium Salt (PFOS)

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January 24, 2002

Introduction

The carcinogen risk assessment guidelines proposed by the U.S. Environmental Protection Agency (1999) recommend the use of a benchmark dose (BMD) approach for low dose cancer risk assessment. Unless stipulated otherwise, the BMD is the dose at which the excess lifetime tumor incidence is 10%, denoted by BMD_{10}. A value of 10% was selected as this is about the lowest incidence that can be estimated with adequate precision from typical chronic bioassays in rodents. Further, a lower 95% confidence limit is calculated for the benchmark dose ($BMDL_{10}$) to account for the experimental variation of the bioassay. The $BMDL_{10}$ is then used as a point-of-departure for low dose cancer risk assessment. When a nonlinear dose response curve is expected in the low dose range, a margin of exposure between the $BMDL_{10}$ and anticipated human exposure levels is considered. Otherwise, linear extrapolation from the $BMDL_{10}$ to zero is used for low dose cancer risk estimation. In either case, the $BMDL_{10}$ serves as the point-of-departure.

Bioassay Data

The data used for calculation of the $BMDL_{10}$ were collected in the 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats. The BMDL is calculated for hepatocellular adenomas and carcinomas combined for males and females. All tumors were adenomas except for one carcinoma in the high dose females.

In order to calculate lifetime incidence rates for each dose group, it is necessary to calculate the number of animals at risk. Clearly, animals that were removed from the study for interim sacrifices or that died before the terminal sacrifice were not at risk for a lifetime. The Poly-3 approach developed by the National Toxicology Program (Bailer and Portier, 1988) is used here to calculate the effective number of animals at risk. Obviously, an animal that survives for the lifetime of the study until the terminal sacrifice counts as a whole lifetime exposure. Also, any animal that is removed from the study with a hepatocellular adenoma/carcinoma prior to the terminal sacrifice lived long enough to develop the tumor is counted as a lifetime exposure. All other animals are given a weight of $(t/T)^3$, where t is the week that an animal was removed from the study without a hepatocellular adenoma/carcinoma and terminal sacrifices began at week T=105.. Relatively little weight is given to an animal removed early in a study. For example, the animals removed at an interim sacrifice halfway through the study

at 53 weeks receive a weight of $(53/105)^3 = 0.13$ of a lifetime, whereas an animal that died on week 96 receives a weight of $(96^3/105) = 0.76$ of a lifetime. The weights are summed for each dose group to obtain the effective number of animals at risk for each group.

The number of animals with hepatocellular adenoma/carcinoma, effective number of animals at risk, and average serum levels of PFOS at 14 weeks for each dose group are displayed in Table 1.

Table 1. Results from the 104-week carcinogenicity study in SD rats fed PFOS.

Dose (ppm)	14-wk Serum (ug/ml)	Number of animals with liver tumors[a]	Effective number of animals
		Males	
0	0.05	0	33
0.5	4.04	3	32
2.0	17.1	3	37
5.0	43.9	1	38
20.0	148	7	38
		Females	
0	2.67	0	39
0.5	6.96	1	35
2.0	27.3	1	29
5.0	64.4	1	37
20.0	223	6	41

[a] Hepatocellular adenomas except one hepatocellular carcinoma in the high dose females.

As noted before, the effective numbers of animals at risk reflect the lower survival in the controls and low dose males and the females fed 2 ppm and the higher survival in the high dose females.

Benchmark Dose Calculations

The numbers of animals with hepatocellular adenoma/carcinoma and the effective number of animals at risk were entered into the U.S. Environmental Protection Agency benchmark dose software program (BMDS). Estimates of the benchmark dose were obtained using the multistage model

$$P = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + q_3 d^3 + q_4 d^4)]$$

where P represents the proportion of animals with tumors, d is the dietary dose or serum level, and the q's are estimated from the experimental dose response data. Goodness-of-fit p-values for the multistage model are above 0.1 indicating an adequate fit of the model. Small p-values would indicate a statistically significant deviance from the multistage model. The goodness-of-fit p-values, $BMD_{10,}$ and $BMDL_{10}$ in terms of dietary concentration and 14-week serum levels of PFOS for males and females are displayed in Table 2.

Table 2. Goodness-of-fit p-values for the multistage model, BMD_{10}, and $BMDL_{10}$ values obtained using the US EPA benchmark dose software program (BMDS).

Sex	p-value	BMD_{10}	$BMDL_{10}$
	Dietary Concentration		
Male	0.24	18.2 ppm	7.9 ppm
Female	0.54	16.7 ppm	8.0 ppm
	14-Week Serum Level		
Male	0.23	135 ug/ml	62 ug/ml
Female	0.54	193 ug/ml	92 ug/ml

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Benchmark Dose Calculations for PFOS: Combined Data from the Two-Generation and One-Generation Studies

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Introduction

Benchmark dose calculations for PFOS were reported previously in
"Benchmark Dose Calculations for PFOS: Two-Generation Study", 6/26/02, and
"Benchmark Dose Calculations for PFOS: One-Generation Study", 7/22/02.
F_0 female body weight gains over the 42-day exposure to PFOS prior to cohabitation with males and F_1 litter size four days after birth exhibited statistically significant dose response relationships with PFOS exposure in both studies. The purpose of the following analysis is to demonstrate the gain in information obtained by combining the data from the two studies in order to obtain better estimates of the benchmark doses for these two effects.

F_0 Female Day-42 Body Weight Gains

In the Two-Generation Study, average body weight gains were: 37.1, 36.0, 34.5, 25.0, and 5.4 grams at PFOS doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg body weight per day, respectively. In the One-Generation Study, average body weight gains were: 50.2, 61.0, 50.8, 55.0, 49.8, 44.5, and 39.2 grams at PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively. The body weight gains in the One-Generation Study were higher, but body weight gains compared to the body weight gains for their respective study controls were similar in the two studies over the PFOS dose range. Fitting a polynomial model to these data gave

$$\text{Body Wt. Change from the Controls (grams)} = 0.64 + (1.825 \times \text{dose}) - (3.8043 \times \text{dose}^2)$$

with an average standard deviation of 14.2 grams. For example, at a PFOS dose of 2 mg/kg body weight per day, the body weight gain difference from the controls is estimated to be

$$\text{Body Wt. Change from the Controls} = 0.64 + (1.825 \times 2) - (3.8043 \times 2^2) = -10.9 \text{ grams.}$$

That is, at a PFOS dose of 2 mg/kg body weight per day, the average Day-42 body weight of F_0 dams is estimated to be 10.9 grams less than the average body weight gain of the control animals.

As discussed previously, the benchmark dose associated with an excess risk of 10% of the animals with abnormally low body weight gains (BMD_{10}) occurs at the dose corresponding to a decrease in average body weight gains equal to the standard deviation. The combined data result in a BMD_{10} = 2.18 mg/kg body weight per day of PFOS with a lower 95% confidence limit of LBMD10 = 1.93 mg/kg per body weight per day.

The $LBMD_{10}$'s were 1.20 and 1.78 mg/kg body weight per day for the Two-Generation and One-Generation study, respectively. Because of the additional information resulting from combining the data from the two studies, a higher (less conservative) $LBMD_{10}$ = 1.93 mg/kg per body weight per day of PFOS is obtained.

As reported in the 7/22/02 Draft, "Benchmark Dose Calculations for PFOS: One-Generation Study", the following relationship between gestation Day-0 serum concentration (S_0) and PFOS dose was obtained

$$S_0\,(\mu g/ml) = 0.027 + (99.51 \times dose).$$

Hence, the BMD_{10} can be expressed in terms of gestation Day-0 serum concentration as

$$S_0\,BMD_{10} = 0.027 + (99.51 \times 2.18) = 217\ \mu g/ml$$

and $S_0\,LBMD_{10} = 192\ \mu g/ml$.

In summary, the BMD_{10} and $LBMD_{10}$ for the PFOS dose and serum GD0 concentration based on the pooled data are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)
BMD_{10}	2.18	217
$LBMD_{10}$	1.93	192

F_1 Litter size (Day-4)

Average litter sizes on Day-4 in the Two-Generation Study were: 13.4, 14.2, 13.3, 8.4, and 0 for PFOS doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg body weight per day, respectively. Average litter sizes in the One-Generation Study were: 13.3, 14.2, 13.4, 13.3, 11.4, 6.6, and 2.3 at PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively. Fitting a power model to these pooled data gave

$$\text{Litter Size} = 13.93 - (1.738 \times \text{dose}^{2.59})$$

with a standard deviation of 2.80 for the control animals. The average litter size, at a change from the controls equal to one standard deviation, is estimated to be (13.93 – 2.80) = 11.13 at the BMD_{10} = 1.20 mg/kg body weight per day of PFOS and $LBMD_{10}$ = 1.05 mg/kg body weight per day.

The $LBMD_{10}$'s for the Two-Generation and One-Generation Study were 0.59 and 1.01 mg/kg body weight per day, respectively. In this case, combining the data from the two studies resulted in only a slightly higher (less conservative) $LBMD_{10}$ = 1.05 mg/kg body weight per day than that obtained from the One-Generation alone of 1.01 mg/kg body weight per day. That is, the One-Generation Study was an efficient experiment for estimating the BMD_{10} and little information was gained by adding the Two-Generation Study data.

Converting the PFOS doses to GD0 serum levels gives: $S_0\,BMD_{10}$ = 119 μg/ml and $S_0\,LBMD_{10}$ = 105 μg/ml.

As reported in "Benchmark Dose Calculations for PFOS:One-Generation Study", 7/22/02, conversion of PFOS doses to gestation Day-21 serum (S_{21}) and liver (L_{21}) concentrations can be calculated from the following relationships

$$S_{21} = (99.65 \times \text{dose}) - (15.4302 \times \text{dose}^2)$$

$$L_{21} = (270.23 \times \text{dose}) - (35.9443 \times \text{dose}^2).$$

Hence,

$$S_{21}\,BMD_{10} = (99.65 \times 1.20) - (15.4302 \times 1.20^2) = 97\ \mu g/ml$$

$$S_{21}\,LBMD_{10} = (99.65 \times 1.05) - (15.4302 \times 1.05^2) = 88\ \mu g/ml$$

$$L_{21}\,BMD_{10} = (270.23 \times 1.20) - (35.9443 \times 1.20^2) = 273\ \mu g/g$$

$$L_{21}\,LBMD_{10} = (270.23 \times 1.05) - (35.9443 \times 1.05^2) = 244\ \mu g/g.$$

In summary, the BMD_{10} and $LBMD_{10}$ for the PFOS dose, serum, and liver concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	1.20	119	97	273
$LBMD_{10}$	1.05	105	88	244

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Benchmark Dose Calculations for PFOS: Two-Generation Study

Gaylor and Associates, LLC

Introduction

In a two-generation study conducted by the Argus Research Laboratories (ARL), Inc. (Combined Oral Gavage Fertility, Developmental, and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, 10 June 1999) for the 3M Company, male and female Sprague-Dawley rats were administered doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day of PFOS. Only the 0.1 and 0.4 mg/kg/day dosage groups were continued into the second generation due to excessive toxicity observed in the 1.6 and 3.2 mg/kg/day F_1 pups.

Serum and liver concentrations of PFOS for the F_0 females were measured by the 3M Environmental Laboratory. Results were listed in the Analytical Report No. FACT Tox-110; LRN-U2849. Serum concentrations measured on gestation day 0 (GD0) and GD21 and liver concentrations for GD21 were used for benchmark concentration calculations. These data were used to establish dose response relationships between effects and serum and liver concentrations in the F_0 generation female rats. Hence, benchmark doses also can be expressed in terms of F_0 female serum and liver concentrations of PFOS, as well as in terms of the administered doses of PFOS. The average serum and liver concentrations are listed in Table 1.

Table 1. Average serum concentrations for GD0 and GD21 and average liver concentrations on GD21 from Report No. FACT Tox-110; LRN-U2849.

PFOS dose (mg/kg/day)	Serum on GD0: S_0 (μg/ml)	Serum on GD21: S_{21} (μg/ml)	Liver on GD21: L_{21} (μg/g)
0	0.06	0.08	0.23
0.1	8.9	6.7	22.8
0.4	40.7	29.9	87.6
1.6	160	124	348
3.2	318	160	495

Benchmark Doses for Continuous Data

For quantal incidence data such as pup survival, the benchmark dose corresponding to a 10% increase in mortality (BMD_{10}) is clearly defined. For continuous data such as body weight gain and litter size, the dose associated with a 10% risk requires a somewhat more complicated procedure. In such cases, there generally is not a specific weight gain or litter size that is universally considered adverse. Animals or litters that are below the one to two percentile values of the controls can certainly be considered to be somewhat abnormal. For effects that are approximately normally distributed, such as body weight gain, the dose that causes a shift in the average effect that is equivalent to one standard in magnitude results in approximately an extra 10% of the animals or litters with abnormal levels. Hence, the procedure used to estimate the BMD_{10} for continuous data is to fit a dose response curve (polynomial or power model) and determine that dose where the mean has shifted by an amount equivalent to the standard deviation of the controls. A lower 95% confidence limit ($LBMD_{10}$) also is calculated to use as a point of departure for setting a reference dose (RfD). The $LBMD_{10}$ can be divided by uncertainty factors for extrapolation from an effect level, animal to human extrapolation, and inter-individual sensitivities to obtain an RfD that is likely to have, at most, a negligible risk.

Of course, levels other than the one to two percentile range can be used to define the abnormal range. Then, the size of the shift in the mean relative to the value of the standard deviation of the controls would need to be determined that corresponds to an additional 10% of animals or litters in the abnormal range.

In the following sections, benchmark doses are calculated for the effects related to PFOS exposure that were identified in the ARL Two-Generation Report, except for terminal body weight gains in the F_1 pups where sufficient data did not exist to estimate the BMD.

F_0 Male Terminal Body Weight Gain

Average terminal body weight gains for F_0 males of 154, 149, 133, 122, and 91 grams were reported for doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day of PFOS, respectively (Table B3, ARL Two-Generation Report). Fitting a polynomial model to these data as a function of the PFOS dose gave

$$\text{Terminal Body Weight Gain (grams)} = 149.6 - (20.92 \times \text{dose}) + (0.8914 \times \text{dose}^2)$$

with an average standard deviation of 34.1 grams. The BMD_{10} corresponds to the dose of PFOS where the average terminal weight gain equals the average weight gain of the controls (149.6 grams) minus 34.1 grams, i.e., (149.6 – 34.1) = 115.5 grams. This occurs at a dose of BMD_{10} = 1.76 mg/kg/day. The lower 95% confidence limit is $LBMD_{10}$ = 1.16 mg/kg/day of PFOS.

F_0 Female Day 42 Body Weight Gain

Average body weight gains for F_0 females on Day 42 (the last day before co-habitation with males) were 37.1, 36.0, 34.5, 25.0, and 5.4 grams for doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day of PFOS, respectively (Table C3, ARL Two-Generation Report). Fitting a polynomial model to these data as a function of the PFOS dose gave

$$\text{Day 42 Body Weight Gain (grams)} = 36.8 - (5.02 \times \text{dose}) - (1.4964 \times \text{dose}^2)$$

with an average standard deviation of 12.3 grams. The BMD_{10} corresponds to the PFOS dose where the average weight gain is one standard deviation below the weight gain estimated for the controls, i.e., (36.8 – 12.3) = 24.5 grams. This occurs at a dose of BMD_{10} = 1.64 mg/kg/day, with $LBMD_{10}$ = 1.20 mg/kg/day of PFOS.

Fitting a polynomial model to the F_0 female day 42 body weight gain as a function of the GD0 serum concentration (S_0) gave

$$\text{Day 42 Body Weight Gain (grams)} = 36.8 - (0.049 \times S_0) - (0.000156 \times S_0^2)$$

again with an average standard deviation of 12.3. The BMD_{10} corresponds to the GD0 serum concentration where the average weight gain is (36.8 – 12.3) = 24.5 grams. This occurs at a GD0 serum concentration of 164 μg/ml in the F_0 female rats and LBMD = 121 μg/ml.

Note that the serum BMD, expressed as μg/ml, is very close to 100 times the BMD for the PFOS dose, expressed as mg/kg/day, because there is a nearly linear relationship between GD0 serum concentrations (S_0) and PFOS dose, with S_0 very close to 100 times the dose (Table 1).

In summary, body weight gains in the F_0 female are slightly more sensitive to PFOS (lower BMD) than the F_0 males. The BMD_{10} and $LBMD_{10}$ for the Day 42 body weight gains for F_0 females for doses of PFOS and the GD0 serum concentrations of PFOS are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)
BMD_{10}	1.64	164

$LBMD_{10}$	1.20	121

F_1 Preimplantation Loss (Average Number of Implants per Litter)

The average numbers of implants per litter were 14.9, 15.4, 14.7, 14.8, and 12.5 for PFOS doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day, respectively (Table C34, ARL Two-Generation Report). With an effect only at the highest dose, the power model provided a better fit than the polynomial model to these data giving

$$\text{Average Number of Implants per Litter} = 15.02 - (0.0444 \times \text{dose}^{3.47})$$

with an average standard deviation of 2.23. The BMD_{10} corresponds to the PFOS dose where the estimated average number of implants per litter is one standard deviation below the estimated number for the controls (15.02 – 2.23) = 12.79 implants. This occurs at a dose of 3.09 mg/kg/day of PFOS, with $LBMD_{10}$ = 2.50 mg/kg/day.

Fitting the power model to the implant data as a function of the GD0 serum concentration (S_0) gave

$$\text{Average Number of Implants per Litter} = 15.02 - (4.329 \times 10^{-9} \times S_0^{3.50})$$

again with an average standard deviation of 2.23. The BMD_{10} corresponds to the GD0 serum concentration where the average number of implants per litter is (15.02 – 2.23) = 12.79 implants. This occurs at a GD0 serum concentration of BMD_{10} = 307 μg/ml and $LBMD_{10}$ = 249 μg/ml.

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and GD0 serum concentrations are:

	PFOS dose (mg/kg/day)	Serum conc. (μg/ml)
BMD_{10}	3.09	307
$LBMD_{10}$	2.50	249

F_1 Litter Size (Day 1)

Average litter sizes on Day 1 were: 13.6, 14.4, 13.5, 12.7, and 7.8 for PFOS doses of 0, 0.1, 0.4, 1.6, and 3.3 mg/kg/day, respectively (Table C18, ARL Two-Generation Rpt.). Fitting a power model to these results gave

$$\text{Litter Size (Day 1)} = 13.86 - (0.376 \times \text{dose}^{2.39})$$

with a pooled estimate of the standard deviation for the controls of 2.61. The BMD_{10} occurs at a dose where the estimated average number of pups per litter is (13.86 – 2.61) = 11.25. This occurs at a PFOS dose of $BMD_{10} = 2.25$ mg/kg/day and $LBMD_{10} = 1.63$ mg/kg/day.

Fitting the power model to the litter size data as a function of the GD0 serum concentration (S_0) gave

$$\text{Litter Size (Day 1)} = 13.86 - (5.66 \times 10^{-6} \times S_0^{2.41})$$

giving the $BMD_{10} = 224$ μg/ml and $LBMD_{10} = 162$ μg/ml for S_0.

Fitting the power model to litter size as a function of the GD21 serum concentration (S_{21}) gave

$$\text{Litter Size (Day 1)} = 13.83 - (6.24 \times 10^{-15} \times S_{21}^{6.80})$$

giving the $BMD_{10} = 141$ μg/ml and $LBMD_{10} = 128$ μg/ml.

Fitting the power model to litter size as a function of the GD21 concentration of PFOS in the liver (L_{21}) gave

$$\text{Litter Size (Day 1)} = 13.83 - (3.612 \times 10^{-13} \times L_{21}^{4.91})$$

giving the $BMD_{10} = 417$ μg/g and $LBMD_{10} = 363$ μg/g of PFOS in the liver at GD21.

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and serum GD0 and GD21 concentrations of PFOS and liver GD21 concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	2.25	224	141	417
$LBMD_{10}$	1.63	162	128	363

F_1 Litter Size (Day 4)

Average litter sizes on Day 4 were: 13.4, 14.2, 13.3, 8.4, and 0 for PFOS doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day, respectively (Table C18, ARL Two-Generation Rpt.). Fitting the power model to these results gave

$$\text{Litter Size (Day 4)} = 13.89 - (2.70 \times \text{dose}^{1.48})$$

with an estimate of the standard deviation for controls of 2.52. The BMD_{10} occurs at the dose where the estimated average number of pups per litter on Day 4 is one standard deviation less than the controls, i.e., (13.89 – 2.52) = 11.37 pups per litter. This occurs at a PFOS dose of BMD_{10} = 0.95 mg/kg/day and $LBMD_{10}$ = 0.59 mg/kg/day.

Fitting the power model to the Day 4 litter size data as a function of the GD0 serum concentration (S_0) gave

$$\text{Litter Size (Day 4)} = 13.89 - (0.00304 \times S_0^{1.47})$$

giving the BMD_{10} = 95 μg/ml and $LBMD_{10}$ = 59 μg/ml for S_0.

Fitting the power model as a function of the GD21 serum concentration of PFOS (S_{21}) gave

$$\text{Litter Size (Day 4)} = 13.89 - (0.00546 \times S_{21}^{1.43})$$

giving the BMD_{10} = 73 μg/ml and $LBMD_{10}$ = 45 μg/ml for S_{21}.

Fitting the power model as a function of the GD21 concentration of PFOS in the liver (L_{21}) gave

$$\text{Litter Size (Day 4)} = 13.89 - (0.000856 \times L_{21}^{1.50})$$

giving the BMD_{10} = 209 μg/g and $LBMD_{10}$ = 128 μg/g for L_{21}.

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and the serum GD0 and GD21 concentrations of PFOS and GD21 liver concentrations are :

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	0.95	95	73	209
$LBMD_{10}$	0.59	59	45	128

F_1 Pup Growth (Body Weight Gain Day 1-21)

Average body weight gains for pups from Day 1 through Day 21 were: 43.2, 42.1, 41.8, and 34.6 grams for PFOS doses of 0, 0.1, 0.4, and 1.6 mg/kg/day (Table C18, ARL Two-Generation Rpt.). There were no Day 21 survivors in the 3.2 mg/kg/day dose group. Fitting the polynomial model to these data gave

Pup Body Wt. Gain for Day 1-21 (grams) = $42.8 - (2.31 \times \text{dose}) - (1.7714 \times \text{dose}^2)$

with an average standard deviation of 4.3 grams. The BMD_{10} is the dose where the estimated body weight gain is (42.8 – 4.3) = 38.5 grams. This occurs at a PFOS dose of BMD_{10} = 1.04 mg/kg/day and $LBMD_{10}$ = 0.55 mg/kg/day.

Fitting the polynomial model to the pup body weight gains as a function of the GD0 serum concentration (S_0) gave

Pup Body Wt.Gain for Day 1-21 (grams) = $42.8 - (0.0208 \times S_0) - (0.00019 \times S_0^2)$

giving the BMD_{10} = 106 μg/ml and $LBMD_{10}$ = 57 μg/ml for S_0.

Fitting the polynomial model as a function of the GD21 serum PFOS concentration (S_{21}) gave

Pup Body Wt. Gain for Day 1-21 (grams) = $42.8 - (0.0305 \times S_{21}) - (0.000288 \times S_{21}^2)$

giving the BMD_{10} = 81 μg/ml and $LBMD_{10}$ = 42 μg/ml for S_{21}.

Fitting the polynomial model to the pup body weight gain for Day 1-21 as a function of the GD21 concentrations of PFOS in the liver (L_{21}) gave

Pup Body Wt.Gain Day 1-21 (grams) = $42.8 - (0.0107 \times L_{21}) - (3.738 \times 10^{-5} \times L_{21}^2)$

giving the BMD_{10} = 227 μg/g and $LBMD_{10}$ = 119 μg/g for L_{21}.

In summary, The BMD_{10} and $LBMD_{10}$ for the doses of PFOS and serum GD0 and GD21 concentrations of PFOS and liver GD21 concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	1.04	106	81	227
$LBMD_{10}$	0.55	57	42	119

F_1 Pup Viability (Day 1-4 Mortality)

Mortality of pups for Day 1-4 was obtained for each litter containing live births from Table C37, ARL Two-Generation Report. The logistic model was fit to these data giving the risk of pup mortality for Day 1-4 of

$$\text{Probabilty of Pup Death Day (1-4)} = 1 / [1 + \exp(5.702 - 3.021 \times \text{dose})].$$

The PFOS dose corresponding to an extra risk of 10% is BMD_{10} = 1.17 mg/kg/day and the $LBMD_{10}$ = 1.09 mg/kg/day.

Similarly, fitting the logistic model to the Day 1-4 mortality data as a function of the PFOS concentration on GD0 in the serum (S_0) gave

$$\text{Probability of Pup Death Day (1-4)} = 1/ [1 + \exp(5.714\text{-}0.0303 \times S_0)].$$

The GD0 serum concentration of PFOS in F_0 females corresponding to an extra risk of 10% for Day 1-4 mortality is BMD_{10} = 117 μg/ml and $LBMD_{10}$ = 109 μg/ml.

Fitting the logistic model to the mortality data as a function of the PFOS concentration on GD21 in the serum (S_{21}) gave

$$\text{Probability of Pup Death Day (1-4)} = 1 / [1 + \exp(7.440 - 0.05666 \times S_{21})].$$

The GD21 serum concentration of PFOS corresponding to an extra risk of 10% for Day 1-4 mortality is BMD_{10} = 93 μg/ml and $LBMD_{10}$ = 86 μg/ml for S_{21}.

Fitting the logistic model to the early pup mortality data as a function of the PFOS concentration on GD21 in the liver (L_{21}) gave

$$\text{Probability of Pup Death Day (1-4)} = 1 / [1 + \exp(7.056 - 0.0186 \times L_{21})].$$

The GD21 liver concentration of PFOS corresponding to an extra risk of 10% for Day 1-4 mortality is BMD_{10} = 261 μg/g and $LBMD_{10}$ = 243 μg/g for L_{21}.

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and GD21 concentrations of PFOS for serum and liver are listed below.

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	1.17	117	93	261
$LBMD_{10}$	1.09	109	86	243

F_1 Pup Survival (Day 4-21 Mortality)

Pups were culled on Day 4. The mortality of culled pups for Day 4-21 were obtained for each litter with Day 4 survivors from Table C37, ARL Two-Generation Report. The logistic model was fit to these mortality data giving the risk of pup death for Day 4-21 of

Probability of Pup Death (Day 4-21) = 1 / [1 + exp(5.648 – 1.576 x dose)].

The PFOS dose corresponding to an extra risk of 10% is the BMD_{10} = 2.21 mg/kg/day and the $LBMD_{10}$ = 1.75 mg/kg/day.

Fitting the logistic model to the Day 4-21 mortality data as a function of the GD0 concentration of PFOS in the serum (S_0) gave

Probability of Pup Death (Day 4-21) = 1/ [1 + exp(5.654-0.0158 x S_0)].

The GD0 serum concentration of PFOS in F_0 females corresponding to an extra risk of 10% for Day 4-21 mortality is BMD_{10} = 221 μg/ml and $LBMD_{10}$ = 174 μg/ml.

Fitting the logistic model to the mortality data as a function of the GD21 concentration in the serum (S_{21}) gave

Probability of Pup Death (Day 4-21) = 1 / [1 + exp(5.626 – 0.0201 x S_{21})].

The GD21 serum concentration of PFOS corresponding to an extra risk of 10% for Day 4-21 mortality is BMD_{10} = 172 μg/ml and the $LBMD_{10}$ = 135 μg/ml for S_{21}.

Fitting the logistic model to the pup mortality for Day 4-21 as a function of the PFOS concentration of GD21 in the liver of the F_0 females (L_{21}) gave

Probability of Pup Death (Day 4-21) = 1 / [1 + exp(5.654 – 0.00726 x L_{21})].

The GD21 liver concentration of PFOS in the F_0 females corresponding to an extra pup mortality risk of 10% for Day 4-21 is BMD_{10} = 481 μg/g and the $LBMD_{10}$ = 380 μg/g for L_{21}.

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and GD21 concentrations of PFOS for serum and liver in the F_0 females are listed below.

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	2.21	221	172	481
$LBMD_{10}$	1.75	174	135	380

Discussion

The most sensitive endpoints (lowest BMD_{10}'s and $LBMD_{10}$'s) occurred for effects during lactation: F_1 litter size on day 4 and F_1 pup mortality days 1-4, that are highly correlated with each other; and F_1 pup body weight gain during lactation for days 1-21 (Table 2).

Summary of Results

The estimates for the BMD_{10} and $LBMD_{10}$ are listed in Table 2 for the various effects in rats related to PFOS exposure in the Two-Generation study.

Table 2. Estimates of the BMD_{10} and $LBMD_{10}$ for effects in rats related to PFOS exposure in the Two-Generation study.

	PFOS Dose (mg/kg/d)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
		BMD_{10}		
F_0 Male Terminal Body Wt Gain	1.76	no data [a]	no data	no data
F_0 Female Day 42 Body Wt Gain	1.64	164	na [b]	na
Number of Implants per Litter	3.09	307	na	na
Litter Size (Day 1)	2.25	224	141	417
Litter Size (Day 4)	0.95	95	73	209
F_1 Pup Body Wt Gain (Day 1-21)	1.04	106	81	227
F_1 Pup Mortality (Day 1-4)	1.17	117	93	261
F_1 Pup Mortality (Day 4-21)	2.21	221	172	481
		$LBMD_{10}$		
F_0 Male Terminal Body Wt Gain	1.16	no data	no data	no data
F_0 Female Day 42 Body Wt Gain	1.20	121	na	na
Number of Implants per Litter	2.50	249	na	na
Litter Size (Day 1)	1.63	162	128	363
Litter Size (Day 4)	0.59	59	45	128
F_1 Pup Body Wt Gain (Day 1-21)	0.55	57	42	119
F_1 Pup Mortality (Day 1-4)	1.09	109	86	243
F_1 Pup Mortality (Day 4-21)	1.75	174	135	380

[a] No data for serum or liver concentrations in males.
[b] Not applicable time.

Benchmark Dose Calculations for PFOS: One-Generation Study

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Introduction

In a one-generation study conducted for the 3M Company by the Argus Research Laboratories (ARL), Inc., "One Generation Reproduction Study of PFOS-Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats", female Sprague-Dawley were administered perfluorooctane sulfonic acid potassium salt (PFOS) in the diet. Exposure of dams to PFOS at dosages of 0, 0.4, 0.8, 1.0, 1.2, 1.6 ,and 2.0 mg/kg body weight per day began 42 days before cohabitation and continued through gestation and Day 4 of lactation.

Serum and liver concentrations of PFOS for F_0 females measured at the 3M Environmental Laboratory were reported in the Analytical Report No. FACT Tox-110; LRN-U2849. From these results it was possible to obtain regression equations relating gestation Day 0 serum concentration (S_0), gestation Day 21 serum concentration (S_{21}), and gestation Day 21 liver concentration (L_{21}) to the PFOS dose:

$$S_0 = 0.027 + (99.51 \times dose)$$

$$S_{21} = (99.65 \times dose) - (15.4302 \times dose^2)$$

$$L_{21} = (270.23 \times dose) - (35.9443 \times dose^2)$$

Hence, a benchmark dose (BMD) or lower confidence limit (LBMD) for PFOS can be converted to serum and liver concentrations by these equations.

Benchmark Doses for Continuous Data

For continuous data, the BMD_{10} was calculated for doses associated with an extra risk of 10% for dams or pups displaying abnormal effects. The BMD_{10} was obtained by determining the dose of PFOS that caused a change in the mean response equal to one standard deviation of the effect in control (unexposed) animals. For effects that are approximately normally distributed, such as body weight gain, the abnormal range is defined by effects below approximately the 1.5^{th} percentile or above the 98.5^{th} percentile of the distribution of effects for the control animals. These abnormal levels are observed in approximately 1.5% of control animals that may or may not possess adverse health effects, but these values are extreme enough that they may be associated with elevated risk of adverse health effects. Hence, the procedure used to estimate the BMD_{10} was to

fit a dose response curve to the bioassay data and determine the dose where the mean response had shifted by an amount equivalent to the standard deviation for the controls. A lower 95% confidence limit ($LBMD_{10}$) also was calculated to use as a point of departure for setting a reference dose (RfD). The $LBMD_{10}$ can be divided by uncertainty factors for extrapolation from an effect level, animal to human extrapolation, and inter-individual sensitivity to PFOS in order to obtain an RfD that is likely not to produce adverse health effects, or at most, produce only negligible levels of risk.

Benchmark doses are calculated for the biological effects listed in the Conclusions of the ARL Report that were related to PFOS exposure, except for decreased body weight gain early in gestation that was transient and had disappeared by gestation Day 21. Further, no increase in stillbirths due to PFOS was observed (Table 20, page B-66, ARL One-Generaration Report), thus a BMD was not calculated for this endpoint.

Pre-mating Body Weight Gain of Dams

Female rats were administered PFOS in the diet for a period of 42 days prior to cohabitation. Average body weight gains during this period of 50.2, 61.0, 50.8, 55.0, 49.8, 44.5, and 39.2 grams were reported for doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg per day of PFOS, respectively (Table 5, page B-18, ARL One-Generation Report).

Fitting a polynomial model to these data as a function of PFOS dose gave

$$\text{Pre-mating Body Weight Gain (grams)} = 52.4 + (8.60 \times \text{dose}) - (7.8890 \times \text{dose}^2)$$

with an average standard deviation of 15.3 grams. The BMD_{10} is the dose of PFOS where the average body weight gain decreased by 15.3 grams from the controls, i.e., where the body weight gain is (52.4 – 15.3) = 37.1 grams. This occurs at a PFOS dose of BMD_{10} = 2.04 mg/kg per day. The lower 95% confidence limit is $LBMD_{10}$ = 1.78 mg/kg per day.

Converting these PFOS doses to the Day 0 serum concentration gives

$S_0\ BMD_{10} = 0.027 + (99.51 \times 2.04) = 203\ \mu g/ml$

$S_0\ LBMD_{10} = 0.027 + (99.51 \times 1.78) = 177\ \mu g/ml.$

In summary, the BMD_{10} and $LBMD_{10}$ for the dose of PFOS and serum GD0 concentration of PFOS are:

PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)

BMD_{10}	2.04	203
$LBMD_{10}$	1.78	177

Body Weight Gain of Dams during Lactation

Average body weight gains of dams during Days 1-5 of lactation were 17.4, 15.7, 4.3, 4.0, 7.5, 1.1, and –3.5 grams at 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day of PFOS, respectively (Table 9, page B-33, ARL One-Generation Report). Fitting a polynomial model to these data gave

$$\text{Lactation Body Wt. Gain of Dams} = 18.0 - (13.59 \times \text{dose}) + (1.6086 \times \text{dose}^2)$$

with an average standard deviation of 15.5 grams. The BMD_{10} is the PFOS dose where the weight gain is estimated to be (18.0 – 15.5) = 2.5 grams. This occurs at a PFOS dose of BMD_{10} = 1.36 mg/kg body weight per day and $LBMD_{10}$ = 0.78 mg/kg per day.

The concentration of PFOS in the serum on gestation Day 0 at the BMD_{10} and $LBMD_{10}$ are estimated to be

$S_0\ BMD_{10} = 0.027 + (99.51 \times 1.36) = 135\ \mu g/ml$

$S_0\ LBMD_{10} = 0.027 + (99.51 \times 0.78) = 77\ \mu g/ml.$

The concentration of PFOS in the serum on gestation Day 21 at the BMD_{10} and $LBMD_{10}$ are estimated to be

$S_{21}\ BMD_{10} = (99.65 \times 1.36) - (15.4302 \times 1.36^2) = 107\ \mu g/ml$

$S_{21}\ LBMD_{10} = (99.65 \times 0.78) - (15.4302 \times 0.78^2) = 68\ \mu g/ml.$

The concentration of PFOS in the liver on gestation Day 21 at the BMD_{10} and $LBMD_{10}$ are estimated to be

$L_{21}\ BMD_{10} = (270.23 \times 1.36) - (35.9443 \times 1.36^2) = 301\ \mu g/g$

$L_{21}\ LBMD_{10} = (270.23 \times 0.78) - (35.9443 \times 0.78^2) = 189\ \mu g/g.$

In summary, the BMD_{10} and $LBMD_{10}$ for body weight gains of dams during lactation Days 1-5 are:

PFOS dose	Serum GD0	Serum GD21	Liver GD21

	(mg/kg/day)	(μg/ml)	(μg/ml)	(μg/g)
BMD_{10}	1.36	135	107	301
$LBMD_{10}$	0.78	77	68	189

Ratio of Liver to Body Weight of Dams

The average ratio of liver to body weight of dams on Day 5 of lactation were 0.041, 0.042, 0.045, 0.044, 0.048, 0.044, and 0.046 at PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively (Table 3, page B-12, ARL One-Generation Report). Using a polynomial model for these data resulted in a linear fit

Liver to Body Weight Ratio for Dams = 0.0416 + (0.00289 x dose)

with an average standard deviation of 0.0043. An average ratio of (0.0416 + 0.0043) = 0.0459 gives the BMD_{10} = 1.48 mg/kg body weight per day of PFOS and $LBMD_{10}$ = 1.04 mg/kg per day.

The conversion of these doses to serum and liver concentrations of PFOS are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	1.48	147	114	321
$LBMD_{10}$	1.04	104	87	243

Duration of Gestation

Average duration of gestation was 22.9, 22.6, 22.5, 22.4, 22.3, 22.0, and 22.2 days for PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively (Table 19, page B-63, ARL One-Generation Report). Fitting a polynomial model to these data gave

Duration of Gestation (days) = 22.91 – (0.72 x dose) + (0.1647 x $dose^2$)

with an average standard deviation of 0.44 days. The average duration of gestation is estimated to be (22.91 – 0.44) = 22.5 days at the BMD_{10} = 0.73 mg/kg body weight per

day of PFOS and $LBMD_{10}$ = 0.49 mg/kg per day. Estimates of the BMD_{10} and $LBMD_{10}$ for serum and liver concentrations of PFOS are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	0.73	72	64	177
$LBMD_{10}$	0.49	48	45	123

Viability (Surviving Pups per Litter)

The average number of live pups per litter on Day 5 of lactation were 13.3, 14.2, 13.4, 13.3, 11.4, 6.6, and 2.3 at PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively (Table 20, page B-67, ARL One-Generation Report). A power model provided a good fit to the data

$$\text{Surviving Pups per Litter (Day 5 of Lactation)} = 14.20 - (1.871 \times \text{dose}^{2.63})$$

with an estimated standard deviation of 3.05 pups for the controls. The estimated average number of live pups per litter on Day 5 of lactation is (14.20 – 3.05) = 11.15 at the BMD_{10} = 1.20 mg/kg body weight per day of PFOS with $LBMD_{10}$ = 1.01 mg/kg per day. The conversion of these doses to serum and liver concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	1.20	120	98	273
$LBMD_{10}$	1.01	101	85	237

Pup Body Weight (Lactation Day 1)

Average body weights of pups on lactation Day 1 were 6.42, 5.94, 5.93, 5.85, 5.68, 5.35, and 5.30 grams for PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively. Fitting a polynomial model to these data gave

$$\text{Pup Body Weight (grams)} = 6.35 - (0.65 \times \text{dose}) + (0.0549 \times \text{dose}^2)$$

with an average standard deviation of 0.58 grams. An average pup weight of

(6.35 – 0.58) = 5.77 grams is estimated at the BMD_{10} = 0.96 mg/kg body weight per day of PFOS with the $LBMD_{10}$ = 0.61 mg/kg per day. Conversion of these doses to serum and liver concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	0.96	96	82	227
$LBMD_{10}$	0.61	61	55	153

Pup Body Weight Gain (Lactation Days 1-5)

Average pup body weight gains during the first five days of lactation were 3.42, 2.67, 2.53, 2.21, 1.69, 1.69, and 1.57 grams at PFOS doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg body weight per day, respectively. Fitting a polynomial model to these data gave

$$\text{Pup Body Weight Gain (grams)} = 3.40 - (1.635 \times \text{dose}) + (0.3445 \times \text{dose}^2)$$

with an average standard deviation of 0.92 grams. The average pup body weight is estimated to be (3.40 – 0.92) = 2.48 grams at the BMD_{10} = 0.65 mg/kg body weight per day of PFOS with $LBMD_{10}$ = 0.44 mg/kg per day. The corresponding estimated serum and liver concentrations are:

	PFOS dose (mg/kg/day)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)
BMD_{10}	0.65	65	58	161
$LBMD_{10}$	0.44	44	41	111

Summary of Results

The estimates for the BMD_{10} and $LBMD_{10}$ for the effects related to PFOS exposure in the One-Generation Study are listed below.

BMD_{10}	PFOS Dose (mg/kg/d)	Serum GD0 (μg/ml)	Serum GD21 (μg/ml)	Liver GD21 (μg/g)

Dam Body Wt Gain (Days 1-42)	2.04	203	not applic.	not applic.
Dam Wt Gain (Lac. Days 1-5)	1.36	135	107	301
Dam Liver/Body Wt (Lac. Day 5)	1.48	147	114	321
Duration of Gestation	0.73	72	64	177
Pup Viability (Lactation Day 5)	1.20	120	98	273
Pup Body Wt (Lactation Day 1)	0.96	96	82	227
Pup Body Wt Gain (Lac. Days 1-5)	0.65	65	58	161

$\underline{LBMD_{10}}$

Dam Body Wt Gain (Days 1-42)	1.78	177	not applic.	not applic.
Dam Wt Gain (Lac. Days 1-5)	0.78	77	68	189
Dam Liver/Body Wt (Lac. Day 5)	1.04	104	87	243
Duration of Gestation	0.49	48	45	123
Pup Viability (Lactation Day 5)	1.01	101	85	237
Pup Body Wt (Lactation Day 1)	0.61	61	55	153
Pup Body Wt Gain (Lac. Days 1-5)	0.44	44	41	111

Discussion

For the One-Generation study, the most sensitive endpoints (lowest BMD_{10}'s and $LBMD_{10}$'s) to PFOS exposure were pup body weight gain during days 1-5 of lactation and duration of gestation. In the Two-Generation Study, pup body weight gain also was a sensitive endpoint.

12/18/02

Benchmark Dose Calculations for Subchronic Exposure of PFOS to Monkeys

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Introduction

Perfluorooctanesulfonate (PFOS) potassium salt was administered to both sexes of cynomolgus monkeys for six months. This report contains calculations of benchmark doses and benchmark serum and liver concentrations of PFOS and their lower 95% confidence limits for effects on total cholesterol, high-density lipoprotein (HDL) cholesterol, and liver weight after 6-month exposures to PFOS. Monkeys were administered PFOS by intragastric intubation at doses of 0, 0.03, 0.15, or 0.75 mg/kg body weight per day. Average concentrations of PFOS in serum and the liver after 183 days of administration are displayed in Table 1.

Table 1. Average concentrations of PFOS in serum and liver after 183 days of oral administration.

PFOS Dose (mg/kg/d)	Males		Females	
	Serum Conc. (ppm)	Liver Conc. (ppm)	Serum Conc. (ppm)	Liver Conc. (ppm)
0	0.05	0.12	0.05	0.11
0.03	15.8	17.3	13.2	22.8
0.15	82.6	58.8	66.8	69.5
0.75	173	395	171	273

For quantal incidence data such as the proportion of animals with tumors, the benchmark dose corresponding to a 10% increase (BMD_{10}) in the incidence of tumors is clearly defined. For continuous data such as cholesterol concentrations and liver weight, there generally is not a specific value that divides levels into acceptable and adverse. In the absence of a sharp demarcation between normal an adverse levels, consider animals with measurements below the 1.5^{th} percentile or above the 98.5^{th} percentile as abnormal. If the measurements are approximately normally distributed, under these conditions Crump (!995) shows that a shift of the mean response from the controls equivalent to the size of the standard deviation results in approximately an additional 10% of animals with abnormal measurements. This process provides the BMD_{10} for continuous data.

Male Total Cholesterol at Day 182

The averages for total cholesterol concentrations at Day 182 in males were 152, 110, 147, and 48 mg/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg per day of PFOS, respectively. None of the models (linear, polynomial, or power) utilized by the Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS) program provided a good fit to these data, due in part to the somewhat erratic results at the two low doses. The shape of the dose response for total cholesterol in males is uncertain between 0.15 and 0.75 mg/kg per day of PFOS.

The estimated linear model as a function of the PFOS dose (mg/kg per day)

Total Cholesterol (mg/dl) = 146 – (125 x dose)

likely provides a conservative underestimate of the benchmark dose. This is considered a conservative underestimate because the linear model indicates a decrease in total cholesterol over the dose range whereas the data are also consistent with no change in the total cholesterol at low doses. The average standard deviation was estimated to be 28 mg/dl. The BMD_{10} corresponding to a change in the total cholesterol of 28 mg/dl to (146 – 28) = 118 mg/dl estimated from the linear model is

BMD_{10} = (146 – 118) / 125 = 0.22 mg/kg per day

with $LBMD_{10}$ = 0.16 mg/kg per day of PFOS.

Fitting a linear model as a function of serum concentration (ppm) of PFOS

Total Cholesterol (mg/dl) = 150 – (0.464 x serum conc.)

gives an average estimated average standard deviation of 33.5 mg/dl and likely provides a conservative underestimate of BMD_{10} = 72 and $LBMD_{10}$ = 48 ppm of PFOS in the serum.

Fitting a linear model as a function of liver concentration (ppm) of PFOS

Total Cholesterol (mg/dl) = 145 – (0.241 x liver conc.)

gives an estimated average standard deviation of 27 mg/dl and likely provides a conservative underestimate of the BMD_{10} = 111 and $LBMD_{10}$ = 80 ppm of PFOS in the liver.

These decreases in total cholesterol at the BMD_{10} apparently would not be considered adverse and may be beneficial. These BMDs and LBMDs are listed in Table 2.

Male Change in Total Cholesterol

Since there are differences among animals in the baseline levels of total cholesterol prior to the administration of PFOS, it is informative to study the change in total cholesterol from baseline to Day 182 for each animal as a function of the PFOS dose. Average changes in total cholesterol were 14, -0.2, -4.2, and –97 mg/dl at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg per day, respectively.

A linear model provided an adequate fit of the data as a function PFOS dose (mg/kg/d)

Total Cholesterol Change (mg/dl) = 9.9 – (135 x dose)

with an estimated standard deviation of 15 mg/dl for the controls and BMD_{10} = 0.11 and $LBMD_{10}$ = 0.08 mg/kg per day of PFOS.

None of the available models provided a good fit to the data as a function of serum concentration (ppm). Fitting a linear model

Total Cholesterol Change (mg/dl) = 8.0 – (0.192 x serum conc.)

gives an estimate of the standard deviation for the controls of 16 mg/dl and likely provides a conservative underestimate of the BMD_{10} = 86 and $LBMD_{10}$ = 25 ppm of PFOS in the serum.

The linear model provided an adequate fit of the data as a function of liver concentration (ppm)

Total Cholesterol Change (mg/dl) = 4.4 – (0.256 x liver conc.)

with an estimate of the standard deviation of 17 for the controls and BMD_{10} = 68 and $LBMD_{10}$ = 39 ppm of PFOS in the liver.

Since the change in total cholesterol is negative, it apparently is not adverse at these levels and may be beneficial. The BMDs and LBMDs are listed in Table 2.

Male HDL

The average values of HDL at Day 182 were 63, 42, 48, and 13 mg/dl at 0, 0.03, 0.15, and 0.75 mg/kg per day of PFOS, respectively. None of the available models provided a good fit to the data. The linear model likely provides a conservative underestimate of the BMD_{10}.

Fitting a linear model as a function of the PFOS dose (mg/kg/d)

HDL (mg/dl) = 56 – (57.5 x dose)

gives an estimated average standard deviation of 11 mg/dl and likely provides a conservative underestimate of BMD_{10} = 0.20 and $LBMD_{10}$ = 0.14 mg/kg per day of PFOS.

Fitting a linear model as a function of the serum concentration (ppm) of PFOS

HDL (mg/dl) = 59 – (0.239 x serum conc.)

with an estimated average standard deviation of 12 mg/dl and likely provides a conservative underestimate of the BMD_{10} = 52 and $LBMD_{10}$ = 37 ppm of PFOS in the serum.

Fitting a linear model as a function of the liver concentration (ppm) of PFOS

HDL (mg/dl) = 55 – (0.108 x liver conc.)

with an estimated average standard deviation of 11 mg/dl and likely provides a conservative underestimate of the BMD_{10} = 105 and $LBMD_{10}$ = 77 ppm of PFOS in the liver. The BMDs and LBMDs are listed in Table 2.

Male HDL/(Total Cholesterol)

The ratio of HDL to Total Cholesterol at Day 182 was calculated for each monkey. The average ratio was 0.42, 0.38, 0.32, and 0.25 at doses of 0, 0.03, 0.15, and 0.75 mg/kg per day of PFOS, respectively.

The linear model provided an adequate fit to the data as a function of PFOS dose (mg/kg/d)

HDL/(Total Cholesterol) = 0.39 – (0.194 x dose)

giving an estimated average standard deviation of 0.06 and BMD_{10} = 0.30 and $LBMD_{10}$ = 0.21 mg/kg per day of PFOS.

The linear model provided a good fit to the data as a function of serum concentration (ppm) of PFOS

HDL/(Total Cholesterol) = 0.41 – (0.000927 x serum conc.)

giving an estimated average standard deviation of 0.054 and BMD_{10} = 58 and $LBMD_{10}$ = 41 ppm of PFOS in the serum.

The linear model provided an adequate fit to the data as a function of the liver concentration (ppm) of PFOS

HDL/(Total Cholesterol) = 0.39 – (0.000355 x liver conc.)

giving an estimated average standard deviation of 0.06 and BMD_{10} = 170 and $LBMD_{10}$ = 114 ppm of PFOS in the liver.

The BMDs and LBMDs are listed in Table 2.

Female Total Cholesterol at Day 182

The average concentrations of total cholesterol at Day 182 in female monkeys were 160, 122, 129, and 82 mg/dl at 0, 0.03, 0.15, and 0.75 mg/kg per day of PFOS, respectively.

The linear model provides an adequate fit to the data as a function of PFOS dose (gm/kg/d)

Total Cholesterol (mg/dl) = 144 – (82.9 x dose)

with an estimated standard deviation of 35 mg/dl for the controls and BMD_{10} = 0.42 and $LBMD_{10}$ = 0.29 mg/kg per day of PFOS.

The linear model provides an adequate fit to the data as a function of serum concentration (ppm) of PFOS

Total Cholesterol (mg/dl) = 150 –(0.395 x serum conc.)

giving an estimated standard deviation of 37 mg/dl for controls and BMD_{10} = 93 and $LBMD_{10}$ = 64 ppm of PFOS in the serum.

The linear model provides an adequate fit to the data as a function of the PFOS concentration (ppm) in the liver

Total Cholesterol (mg/dl) = 146 – (0.235 x liver conc.)

giving an estimated standard deviation of 35 mg/dl for the controls and BMD_{10} = 150 and $LBMD_{10}$ = 103 ppm of PFOS in the liver.

These BMDs and LBMDs are listed in Table 2.

Female Change in Total Cholesterol

Baseline concentrations of total cholesterol prior to the administration of PFOS differ among the female monkeys. The change in total cholesterol from baseline to Day 182 was calculated for each animal. The average changes in total cholesterol for female monkeys were 13, -32, -14, and –72 mg/dl at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, resectively.

The linear model provided an adequate fit to the data as a function of PFOS dose (mg/kg/d)

Change in Total Cholesterol (mg/dl) = 0 – (96.8 x dose)

giving an estimated average standard deviation of 31 mg/dl and BMD_{10} = 0.32 and $LBMD_{10}$ = 0.24 mg/kg/d of PFOS.

The linear model provided an adequate fit to the data as a function of serum concentration (ppm) of PFOS

Change in Total Cholesterol (mg/dl) = 2 – (0.416 x serum conc.)

giving an estimated average standard deviation of 32 mg/dl and BMD_{10} =76 and $LBMD_{10}$ = 52 ppm of PFOS in the serum.

The linear model provided an adequate fit to the data as a function of the concentration (ppm) of PFOS in the liver

Change in Total Cholesterol (mg/dl) = 0 – (0.265 x liver conc.)

giving an estimated average standard deviation of 31 mg/dl and BMD_{10} = 117 and $LBMD_{10}$ = 81 ppm of PFOS in the liver.

These BMDs and LBMDs are listed in Table 2.

Female HDL

The average concentrations of HDL in female monkeys at Day 182 were
56, 42, 36, and 21 mg/dl at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided an adequate fit to the data as a function
of PFOS dose (mg/kg/d)

HDL (mg/dl) = 48 – (37.7 x dose)

giving an estimated average standard deviation of 12 mg/dl and BMD_{10} = 0.32
and $LBMD_{10}$ = 0.22 mg/kg/d of PFOS.

The linear model provided an adequate fit of HDL concentration for female monkeys as a function of the serum concentration (ppm) of PFOS

HDL (mg/dl) = 51 – (0.181 x serum conc.)

giving an estimate of the average standard deviation of 11 mg/dl and BMD_{10} = 63
and $LBMD_{10}$ = 45 ppm of PFOS in serum.

The linear model provided an adequate fit of HDL concentration as a function of PFOS concentration (ppm) in the liver

HDL (mg/dl) = 49 –(0.109 x liver conc.)

giving an estimate of the average standard deviation of 12 mg/dl and BMD_{10}= 108
and $LBMD_{10}$ = 76 ppm of PFOS in the liver.

These BMDs and LBMDs are displayed in Table 2.

<u>Female HDL/(Total Cholesterol)</u>

The ratio of the HDL to Total Cholesterol at Day 182 was calculated for each of the female monkeys. The average ratios were 0.36, 0.34, 0.29, and 0.26 at 0, 0.03, 0.15, and 0.75 mg/kg per day of PFOS, respectively.

The linear model provided a good fit of the average HDL/(Total Cholesterol) ratios as a function of PFOS dose (mg/kg/d)

HDL/(Total Cholesterol) = 0.34 – (0.115 x dose)

giving an estimate of the average standard deviation of 0.078 and BMD_{10} = 0.68
and $LBMD_{10}$ = 0.38 mg/kg/d of PFOS.

The linear model provided a good fit of the ratio as a function of serum concentration (ppm) of PFOS

HDL/(Total Cholesterol) = 0.35 – (0.000575 x serum conc.)

giving an estimated average standard deviation of 0.076 and BMD_{10} = 133
and $LBMD_{10}$ = 77 ppm of PFOS.

The linear model provided a good fit of the ratio as a function of the PFOS concentration (ppm) in the liver

HDL/(Total Cholesterol) = 0.34 – (0.000334 x liver conc.)

giving an estimated average standard deviation of 0.078 and BMD_{10} = 232 and $LBMD_{10}$ = 131 ppm of PFOS in the liver.

These values of the BMDs and LBMDs are listed in Table 2.

Discussion of Cholesterol Endpoints

Total cholesterol decreased in both male and female monkeys with exposure to PFOS. The most sensitive endpoint (lowest LBMD) for an adverse effect related to PFOS exposure was the decrease of HDL in male monkeys obtained at $LBMD_{10}$ = 0.14 mg/kg per day of PFOS. None of the available models in the EPA Benchmark Dose Software provided adequate fits of male HDL as a function PFOS dose. The linear model used to obtain the BMD_{10} possibly provides a conservative underestimate. Thus, the $LBMD_{10}$ = 0.14 mg/kg per day for use in risk assessments possibly is conservatively low. The total cholesterol also decreased and the $LBMD_{10}$ = 0.21 mg/kg per day of PFOS for the ratio of HDL to Total Cholesterol in the male monkeys.

Table 2. Summary of the BMD_{10} and $LBMD_{10}$ for Total Cholesterol at Day 182, Cholesterol Change from Baseline, HDL at Day 182, and HDL/(Total Cholesterol) at Day 182 in Monkeys Exposed to PFOS.

BMD_{10}	PFOS Dose (mg/kg/d)	Serum Conc. (ppm)	Liver Conc. (ppm)
Male Total Cholesterol at Day 182	0.22[a]	72[a]	111[a]
Male Change in Total Cholesterol	0.11	86[a]	68
Male HDL	0.20[a]	52[a]	105[a]
Male [HDL/(Total Cholesterol)]	0.30	58	170
Female Total Cholesterol at Day 182	0.42	93	150
Female Change in Total Cholesterol	0.32	76	117
Female HDL	0.32	63	108
Female [HDL/(Total Cholesterol)]	0.68	133	232
$LBMD_{10}$			
Male Total Cholesterol at Day 182	0.16[a]	48[a]	80[a]
Male Change in Total Cholesterol	0.08	25[a]	39
Male HDL	0.14[a]	37[a]	77[a]
Male [HDL/(Total Cholesterol)]	0.21	41	114
Female Total Cholesterol at Day 182	0.29	64	103
Female Change in Total Cholesterol	0.24	52	81
Female HDL	0.22	45	76
Female [HDL/(Total Cholesterol)]	0.38	77	131

[a] Poor fit of data to models. Linear model likely to provide conservative underestimates of the BMD_{10}.

Male Liver Weight at Day 184

Average liver weights for male monkeys at Day 184 were 54.9, 62.1, 57.3, and 85.3 g at 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively. Due in part to the somewhat erratic results at the low doses, dose response models provided extremely poor fits to the data. The shape of the dose response between 0.15 and 0.75 mg/kg/d of PFOS is uncertain. Hence, a BMD_{10} was not obtained for male liver weights.

Male (Liver Wt.)/(Body Wt.)

Ratios of liver weights to the total body weight (expressed as percent) at Day 184 were calculated for each animal. The average percent of the total body weight for the liver of male monkeys were 1.6, 1.7, 1.8, and 2.7% at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

A linear model provided a good fit of liver weight relative to body weight as a function of PFOS dose (mg/kg/d)

Relative Liver Wt. (% of body wt.) = 1.6 + (1.45 x dose)

with an estimated average standard deviation of 0.20% and BMD_{10} = 0.14 and $LBMD_{10}$ = 0.10 mg/kg/d of PFOS.

A polynomial model provided a good fit of liver weight relative to body weight as a function of the serum concentration (ppm) of PFOS

Rel. Liver Wt.(% of bw) = 1.6 – (0.0014 x serum conc.) + 0.0000436 x (serum conc.)^2

with an estimated average standard deviation of 0.20% and BMD_{10} = 86 and $LBMD_{10}$ = 53 ppm of PFOS in the serum.

The linear model provided a good fit of liver weight relative to the body weight as a function of the liver concentration (ppm) of PFOS

Relative Liver Wt. (% of body weight) = 1.6 + (0.00273 x liver conc.)

with an estimated average standard deviation of 0.20% and BMD_{10} = 73 and LBMD = 55 ppm of PFOS in the liver.
The BMDs and LBMDs are listed in Table 3.

Female Liver Weight at Day 184

The average liver weights at Day 184 for female monkeys were 51.1, 56.8, 57.0, and 75.3 grams at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided a good fit of liver weight for female monkeys as a function of the PFOS dose (mg/kg/d)

Liver Wt. (grams) = 52.8 + (30.0 x dose)

with an estimated average standard deviation of 9.3g and BMD_{10} = 0.31 and $LBMD_{10}$ = 0.22 mg/kg/d of PFOS.

The linear model provided a good fit of liver weight as a function of the concentration (ppm) of PFOS in the serum

Liver Wt. (grams) = 51.4 + (0.134 x serum conc.)

with an estimated average standard deviation of 9.5 g and BMD_{10} = 71 and $LBMD_{10}$ = 49 ppm of PFOS in the serum.

The linear model provided a good fit of liver weight as a function of the liver concentration (ppm) of PFOS

Liver Wt. (grams) = 52.1 + (0.0847 x liver conc.)

with an estimated average standard deviation of 9.3 grams and BMD_{10} = 109 and 77 ppm of PFOS in the liver.

The BMDs and LBMDs are listed in Table 3.

Female (Liver Wt.)/(Body Wt.)

The liver weight expressed as a percent of the body weight at Day 184 was calculated for each animal. The average relative liver weights for female monkeys were 1.8, 1.9, 2.1, and 2.9% at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided a good fit of relative liver weights as a function of the PFOS dose (mg/kg/d)

Relative Liver Wt. (% of body wt.) = 1.8 + (1.42 x dose)

with an estimated average standard deviation of 0.20 and BMD_{10} = 0.14 and $LBMD_{10}$ = 0.11 mg/kg/d of PFOS.

The linear model provided a good fit of the relative liver weights as a function of the serum concentration (ppm) of PFOS

Relative Liver Wt. (% of body wt.) = 1.8 + (0.0064 x serum conc.)

with an estimated average standard deviation of 0.21 and BMD_{10} = 32 and $LBMD_{10}$ = 25 ppm of PFOS in the serum.

The linear model provided a good fit of the relative liver weight as a function of the PFOS concentration (ppm) in the liver

Relative Liver Wt. (% of body wt.) = 1.8 + (0.0040 x liver conc.)

with an estimated average standard deviation of 0.20 and BMD_{10} = 49 and LBMD = 38 ppm of PFOS in the liver.

These BMDs and LBMDs are listed in Table 3.

Discussion of Liver Endpoints

The most sensitive endpoint (lowest BMD_{10}) at Day 184 was the ratio of liver weight to body weight. The $LBMD_{10}$ for the PFOS dose was similar for males (0.10 mg/kg/d) and females (0.11 mg/kg/d).

Table 3. Summary of the BMD_{10} and $LBMD_{10}$ for Liver Weight and (Liver Weight/Total Body Weight) after 183 Days Exposure of Monkeys to PFOS.

BMD_{10}	PFOS Dose (mg/kg/d)	Serum Conc. (ppm)	Liver Conc. (ppm)
Male Liver Weight at Day 184	NC[a]	NC	NC
Male (Liver Wt.)/(Body Wt.)	0.14	86	73
Female Liver Weight at Day 184	0.31	71	109
Female (Liver Wt.)/(Body Wt.)	0.14	32	49
$LBMD_{10}$			
Male Liver Weight at Day 184	NC[a]	NC	NC
Male (Liver Wt.)/(Body Wt.)	0.10	53	55
Female Liver Weight at Day 184	0.22	49	77
Female (Liver Wt.)/(Body Wt.)	0.11	25	38

[a] Not calculated. Extremely poor fit of dose response models. Dose response shape highly uncertain.

Estradiol at Day 182

The standard deviations among animals for estradiol concentrations were large relative to the mean values. Only the males exhibited a dose response for estradiol concentrations at Day 182, but not for changes from the baseline concentrations.

The average concentrations of estradiol in males at Day 182 were: 23.0, 24.1, 23.2, and 0.80 (pg/ml) at 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively. Fitting a power model to describe the flat dose response at low doses followed by a sharp decline at 0.75 mg/kg/d gave a good fit to the data as a function of the PFOS dose (mg/kg/d)

Estradiol (pg/ml) = $24.2 - (35.76 \times \text{dose}^{1.47})$

with an estimate of the standard deviation for controls of 10.0 pg/ml, $BMD_{10} = 0.42$, and $LBMD_{10} = 0.17$ mg/kg/d of PFOS.

Fitting the power model as a function of serum concentration (ppm) of PFOS provided a good fit to the data

Estradiol (pg/ml) = $24.2 - [2.4613 \times 10^{-6} \times (\text{serum conc.})^{3.12}]$

with an estimate of the standard deviation for controls of 10.0 pg/ml, $BMD_{10} = 132$, and $LBMD_{10} = 89$ ppm of PFOS in the serum.

Fitting the power model as a function of the concentration (ppm) of PFOS in the liver gave a good fit to the data

Estradiol (pg/ml) = $23.4 - [7.7903 \times 10^{-21} \times (\text{liver conc.})^{8.265}]$

with the estimated standard deviation for the controls of 9.8 pg/ml, $BMD_{10} = 357$, and $LBMD_{10} = 124$ ppm of PFOS in the liver.

The results for the BMD_{10} and $LBMD_{10}$ are listed in Table 4 for the hormones.

Total Triiodothyronine (T_3)

Benchmark dose calculations were considered for concentrations of T_3 at Day 182, Day 184, and Day 184 confirmatory analyses conducted at the Mayo Clinical Laboratories and changes from baseline for both males and females. None of the models provided adequate fits to the Day 182 data. Hence, only the Day 184 and confirmatory Mayo results were used. Further, no dose response relationships were observed for changes of T_3 from baseline concentrations for the females and benchmark doses were not calculable in that case.

Male T_3 at Day 184

The average values of total T_3 in males at Day 184 were: 115, 110, 94, and 43 ng/dl at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively. Fitting a linear model gave a good fit to the data as a function of the PFOS dose

T_3 concentration (ng/dl) = 112.1 – (93.24 x dose)

with an estimated standard deviation of 13.7 ng/dl, BMD_{10} = 0.15, and $LBMD_{10}$ = 0.11 mg/kg/d of PFOS.

The linear model provided a good fit of T_3 as a function of the concentration (ppm) of PFOS in the serum

T_3 concentration (ng/dl) = 118.6 – (0.412 x serum conc.)

with an estimated standard deviation of 14.6 ng/dl, BMD_{10} = 35, and $LBMD_{10}$ = 27 ppm of PFOS in the serum.

The linear model provided a fair fit to the data as a function of the concentration of PFOS in the liver

T_3 concentration (ng/dl) = 110.8 – (0.174 x liver conc.)

with an estimated standard deviation of 14.0 ng/dl, BMD_{10} = 81, and $LBMD_{10}$ = 62 ppm of PFOS in the liver.

These values of the BMD_{10} and $LBMD_{10}$ are listed in Table 4 that summarizes the results for hormones.

Male T_3 at Day 184 (Mayo Confirmation)

The average values of total T_3 concentrations at Day 184 from the Mayo Clinical Laboratories results were: 146, 145, 129, and 76 ng/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

The linear model provided an excellent fit of T_3 as a function of PFOS dose

T_3 concentration (ng/dl) = 146.0 – (93.8 x dose)

with an estimated standard deviation of 15.3 ng/dl, BMD_{10} = 0.16, and $LBMD_{10}$ = 0.12 mg/kg/d of PFOS.

The linear model provided a fair fit of T_3 concentration as a function of the concentration (ppm) of PFOS in the serum

T_3 concentration (ng/dl) = 151.8 – (0.414 x serum conc.)

with an estimated standard deviation of 16.5 ng/dl, BMD_{10} = 40, and $LBMD_{10}$ = 28 ppm of PFOS in the serum.

The linear model provided an excellent fit of T_3 as a function of the concentration (ppm) of PFOS in the liver

T_3 concentration (ng/dl) = 145.0 – (0.176 x liver conc.)

with an estimated standard deviation of 15.6 ng/dl, BMD_{10} = 88, and $LBMD_{10}$ = 63 ppm of PFOS in the liver.

These results are listed in the Table 4 summary for hormones.

Male Change from Baseline in T_3 by Day 184

The change in the total T_3 concentration from the baseline value to the concentration by Day 184 was calculated for each animal for which this information was available. The average changes in T_3 were: +13.0, -2.0, -18.8, and –89.3 ng/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

The linear model provided a good fit of the change in the concentration of T_3 by Day 184 as a function of the PFOS dose (mg/kg/d)

Change in T_3 concentration (ng/dl) = 4.7 – (127.0 x dose)

with an estimated standard deviation of 19.3 ng/dl, BMD_{10} = 0.15, and $LBMD_{10}$ = 0.11 mg/kg/d of PFOS.

The linear model provided a fair fit of the change from baseline in T_3 as a function of the concentration (ppm) of PFOS in the serum

Change in T_3 concentration (ng/dl) = 13.7 – (0.552 x serum conc.)

with an estimated standard deviation of 20.6 ng/dl, BMD_{10} = 37, and $LBMD_{10}$ = 26 ppm of PFOS in the serum.

The linear model provided a fair fit of changes from baseline in T_3 as a function of the concentration (ppm) of PFOS in the liver

Change in T_3 concentration (ng/dl) = 3.0 –(0.238 x liver conc.)

with an estimated standard deviation of 19.7 ng/dl, BMD_{10} = 83, and $LBMD_{10}$ = 59 ppm of PFOS in the liver.

These results are summarized in Table 4.

Male Change from Baseline in T_3 by Day 184 (Mayo Confirmation)

The change from the baseline concentration of total T_3 based on the Mayo Clinical Laboratory measurement for Day 184 was calculated for each animal for which this information was available. The average changes in T_3 concentrations were: 49.0, 32.8, 27.7, and –56.0 ng/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

The linear model provided a good fit for the T_3 change from baseline as a function of the dose of PFOS (mg/kg/d)

Change in T_3 concentration (ng/dl) = 42.8 – (131.0 x dose)

with an estimated standard deviation of 17.6 ng/dl, BMD_{10} = 0.13, and $LBMD_{10}$ = 0.10 mg/kg/d of PFOS.

The linear model provided a marginal fit for the change in T_3 from baseline as a function of the concentration of PFOS in the serum

Change in T_3 concentration (ng/dl) = 50.7 – (0.559 x serum conc.)

with an estimated standard deviation of 21.8 ng/dl, BMD_{10} = 39, and $LBMD_{10}$ = 27 ppm of PFOS in the serum.

The linear model provided a good fit of the change in T_3 from baseline as a function of the concentration (ppm) of PFOS in the liver

Change in T_3 concentration (ng/dl) = 41.5 – (0.247 x liver conc.)

with an estimated standard deviation of 17.4 ng/dl, BMD_{10} = 70, and $LBMD_{10}$ = 50 ppm of PFOS in the liver.

These results are listed in Table 4.

Female T_3 at Day 184

The average concentrations of total T_3 at Day 184 in females were: 106, 92, 80, and 58 ng/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

None of the available models provided an adequate fit of T_3 concentration as a function of the dose of PFOS (mg/kg/d). The goodness-of-fit was P=0.03 for the linear model

T_3 concentration (ng/dl) = 96.5 – (52.42 x dose)

with an estimate of the standard deviation for the controls of 15.9 ng/dl, BMD_{10} = 0.30, and $LBMD_{10}$ = 0.23 mg/kg/d of PFOS, which is questionable.

The polynomial model provided a fair fit of T_3 as a function of the concentration (ppm) of PFOS in the serum

T_3 concentration (ng/dl) = 103.7 – (0.431 x serum conc.) + (0.000941 x {serum conc.}^{2})

with an estimated standard deviation for the controls of 15.6 ng/dl, BMD_{10} = 40 , and $LBMD_{10}$ = 26 ppm of PFOS in the serum.

The linear model provided a barely adequate fit, with a goodness-of-fit test with P=0.06, for the concentration of T_3 as function of the concentration of PFOS in the liver

T_3 concentration (ng/dl) = 98.0 – (0.150 x liver conc.)

with an estimated standard deviation in the controls of 15.7 ng/dl, BMD_{10} = 105, and $LBMD_{10}$ = 80 ppm of PFOS in the liver.

These results are listed in Table 4.

Female T_3 at Day 184 (Mayo Confirmation)

The average concentrations of total T_3 in females at Day 184 measured by the Mayo Clinical Laboratories were: 148, 139, 116, and 99 ng/dl at doses of 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

The linear model provided an adequate fit of T_3 as function of PFOS dose (mg/kg/d)

T_3 concentration (ng/dl) = 138.4 – (56.21 x dose)

with an estimate of the standard deviation of 17.4 ng/dl, BMD_{10} = 0.31, and $LBMD_{10}$ = 0.20 mg/kg/d of PFOS.

Neither serum nor liver concentrations could be adequately modeled for benchmark dose calculations.

Thyroid Stimulating Hormone (TSH)

Since no dose response or poor fits of models were obtained with the measurements of TSH from the Mayo Clinical Laboratories, no calculations of benchmark doses were performed for these data. Since the baseline levels of TSH were zero or nearly zero for males, the concentrations at Day 182 and Day 184 also represent the change from the baseline level.

Male TSH at Day 182

Average concentrations of TSH in males at Day 182 were: 0.43, 0.34, 0.74, and 0.93 μU/ml at 0, 0.03, 0.15, and 0.75 mg/kg/d of PFOS, respectively.

The linear model provided a good fit for TSH concentration as a function of PFOS dose (Mg/kg/d)

TSH (μU/ml) = 0.470 + (0.6736 x dose)

with an estimated standard deviation of 0.53 μU/ml, BMD_{10} = 0.79, and $LBMD_{10}$ = 0.38 mg/kg/d of PFOS.

The linear model provided a good fit for TSH as a function of the concentration (ppm) of PFOS in the serum

TSH (μU/ml) = 0.399 + (0.003298 x serum conc.)

with an estimated standard deviation of 0.52 μU/ml, BMD_{10} = 159, and $LBMD_{10}$ = 82 ppm of PFOS in the serum.

The linear model provided a good fit of TSH as a function of the concentration (ppm) in the liver

TSH (μU/ml) = 0.483 + (0.001218 x liver conc.)

with an estimated standard deviation of 0.54 μU/ml, BMD_{10} = 440, and $LBMD_{10}$ = 208 ppm of PFOS in the liver.

These results are listed in Table 4.

Male TSH at Day 184

The average concentrations of TSH in males at Day 184 were: 0.37, 0.56, 0.70, and 0.93 μU/ml at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d.

The linear model provided a fair fit of TSH as a function of PFOS dose (mg/kg/d)
TSH (μU/ml) = 0.492 + (0.632 x dose)
with an estimated standard deviation of 0.26 μU/ml, BMD_{10} = 0.41, and $LBMD_{10}$ = 0.26 mg/kg/d of PFOS.

The linear model provided a good fit of TSH as a function of the concentration (ppm) of PFOS in the serum
TSH (μU/ml) = 0.430 + (0.00302 x serum conc.)
with an estimated standard deviation of 0.25 μU/ml, BMD_{10} = 82, and $LBMD_{10}$ = 53 ppm of PFOS in the serum.

The linear model provided a fair fit of TSH as a function of the concentration (ppm) of PFOS in the liver
TSH (μU/ml) = 0.503 + (0.00116 x liver conc.)
with an estimated standard deviation of 0.26 μU/ml, BMD_{10} = 227, and $LBMD_{10}$ = 140 ppm of PFOS in the liver.

These results are summarized in Table 4.

Female TSH at Day 182

The lack of a dose response relationship precluded the calculation of benchmark doses.

Female TSH at Day 184

The average concentrations of TSH in females at Day 184 were: 0.53, 0.43, 0.47, and 1.03 μU/ml at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided a good fit of TSH concentration as a function of the PFOS dose (mg/kg/d)
TSH (μU/ml) = 0.441 + (0.762 x dose)
with an estimated standard deviation of 0.305 μU/ml, BMD_{10} = 0.40, and

$LBMD_{10}$ = 0.26 mg/kg/d of PFOS.

The linear model provided a fair fit of TSH as a function of the concentration (ppm) of PFOS in serum
TSH (μU/ml) = 0.415 + (0.00322 x serum conc.)
with an estimated standard deviation of 0.32 μU/ml, BMD_{10} = 99, and $LBMD_{10}$ = 63 ppm of PFOS in serum.

The linear model provided a good fit of TSH as a function of the concentration (ppm) of PFOS in the liver
TSH (μU/ml) = 0.426 + (0.00211 x liver conc.)
with an estimated standard deviation of 0.31, BMD_{10} = 146, and $LBMD_{10}$ = 96 ppm of PFOS in the liver.

These results are summarized in Table 4.

Female Change from Baseline for TSH at Day 182

Unlike males, the females exhibited some low concentrations of TSH prior to the administration of PFOS. Hence, the change from these baseline levels was calculated for each animal where this information was available. The average changes in TSH for females at Day 182 were: 0.52, 0.68, 0.93, and 0.84 at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided a good fit of the change from baseline of TSH as a function of the PFOS dose (mg/kg/d)
TSH change from baseline (μU/ml) = 0.681 + (0.267 x dose)
with an estimated standard deviation of 0.70 μU/ml, BMD_{10} = 2.62, and $LBMD_{10}$ = 0.66 mg/kg/d of PFOS.

The linear model provided a good fit of the THS change from baseline as a function of the concentration (ppm) of PFOS in the serum
TSH change from baseline (μU/ml) = 0.642 + (0.00158 x serum conc.)
with an estimated standard deviation of 0.70, BMD_{10} = 440, and $LBMD_{10}$ = 135 ppm of PFOS in the serum.

The linear model provided a good fit of the change from baseline for TSH at Day 182 in females as a function of the concentration of PFOS in the liver
TSH change from baseline (μU/ml) = 0.668 + (0.000818 x liver conc.)
with an estimated standard deviation of 0.70, BMD_{10} = 854, and $LBMD_{10}$ = 229 ppm of PFOS in the liver.

These results are listed in Table 4.

Female TSH Change from Baseline at Day 184

The average changes from the baseline values of TSH for females at Day 184 were: 0.32, 0.43, 0.13, and 1.02 μU/ml at PFOS doses of 0, 0.03, 0.15, and 0.75 mg/kg/d, respectively.

The linear model provided a good fit of the changes from baseline of TSH as a function of the PFOS dose (mg/kg/d)

TSH change from baseline (μU/ml) = 0.230 + (0.993 x dose)

with an estimated standard deviation of 0.56 μU/ml, BMD_{10} = 0.57, and $LBMD_{10}$ = 0.34 mg/kg/d of PFOS.

The linear model provided a fair fit of the change from baseline for TSH as a function of the concentration (ppm) of PFOS in serum

THS change from baseline (μU/ml) = 0.211 + (0.00399 x serum conc.)

with an estimated standard deviation of 0.58 μU/ml, BMD_{10} = 146, and $LBMD_{10}$ = 82 ppm of PFOS in the serum.

The linear model provided a fair fit of the change in TSH from baseline as a function of the concentration (ppm) of PFOS in the liver

TSH change from baseline (μU/ml) = 0.212 + (0.00274 x liver conc.)

with an estimated standard deviation of 0.57 μU/ml, BMD_{10} = 207, and $LBMD_{10}$ = 122 ppm of PFOS in the liver.

These results are listed in Table 4.

Discussion of Hormonal Endpoints

Among the hormonal endpoints (total T_3, estradiol, and TSH), the most sensitive endpoint was T_3 in males at Day 184. The $LBMD_{10}$ consistently ranged from 0.10 to 0.12 mg/kg/d of PFOS for both the concentration at Day 184 and the change from the baseline concentration, including the confirmatory analyses performed by the Mayo Clinical Laboratories.

Reference

Crump, K.S. Calculation of benchmark doses from continuous data. *Risk Analysis* 15: 79-89 (1995).

Table 4. Summary of the BMD_{10} and $LBMD_{10}$ for Hormonal Effects in Monkeys Exposed to PFOS.

BMD_{10}	PFOS Dose (mg/kg/d)	Serum Conc. (ppm)	Liver Conc. (ppm)
Male Estradiol at Day 182	0.42	132	357
Male T_3 at Day 184	0.15	35	81
Male T_3 at Day 184 (Mayo)	0.16	40	88
Male T_3 change from baseline at Day 184	0.15	37	83
Male T_3 change from baseline at Day 184 (Mayo)	0.13	39	70
Female T_3 at Day 184	NA [a]	40	105
Female T_3 at Day 184 (Mayo)	0.31	NA	NA
Male TSH at Day 182	0.79	159	440
Male TSH at Day 184	0.41	82	227
Female TSH at Day 184	0.40	99	146
Female TSH change from baseline at Day 182	2.62	440	854
Female TSH change from baseline at Day 184	0.57	146	207

Table 4. (Continued)

$LBMD_{10}$	PFOS Dose (mg/kg/d)	Serum Conc. (ppm)	Liver Conc. (ppm)
Male Estradiol at Day 182	0.17	89	124
Male T_3 at Day 184	0.11	27	62
Male T_3 at Day 184 (Mayo)	0.12	28	63
Male T_3 change from baseline at Day 184	0.11	26	59
Male T_3 change from baseline at Day 184 (Mayo)	0.10	27	50
Female T_3 at Day 184	NA	26	80
Female T_3 at Day 184 (Mayo)	0.20	NA	NA
Male TSH at Day 182	0.38	82	208
Male TSH at Day 184	0.26	53	140
Female TSH at Day 184	0.26	63	96
Female TSH change from baseline at Day 182	0.66	135	229
Female TSH change from baseline at Day 184	0.34	82	122

[a] Not available, lack of an adequate fit of a model to the data precluded the calculation of benchmark doses.