

A 03

3M Specialty Materials

3M Center  
St. Paul, MN 55144-1000  
651 733 1100

MR43053

HIP  
~~1355~~ 1355 PL

AR226-0979

RECEIVED  
OPPT CBIC

2001 JAN 16 AM 11 06



VIA OVERNIGHT DELIVERY

ORIGINAL

FYI-0101-001378

December 22, 2000

Document Processing Center (7407)  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street SW  
Washington, D.C. 20460



FYI-00-001378

2001 JAN 18 AM 9:21

RECEIVED  
OPPT NCIC

Attention: For Your Information Docket - Docket No. AR-226

Re: Information on Perfluorooctane  
Sulfonates and Related Compounds

Dear Sir or Madam:

This continues 3M's voluntary submissions of data on perfluorooctane sulfonates and related compounds, as part of our ongoing dialog with EPA regarding fluorochemistry.

Contents of this Submission

In this submission, we are enclosing some new information and some older documents that have only recently been located. All of the information in this submission relates to health effects research. We expect to provide an environmental submission, along with additional new health effects research information, within the next few months.

Analytical Data for Previously Submitted Studies

- > This submission includes recent final analytical reports providing liver and serum values for the PFOS monkey study and the N-ethyl FOSE two-generation rat study. The PFOS monkey study report updates an interim analytical report submitted May 4, 2000. The analytical data for the N-ethyl FOSE two-generation study had not previously been submitted. As indicated in the draft Initial Assessment Report, the laboratory is continuing to update the serum and liver values for various studies. Additional revised values for other studies will be forthcoming in future submissions.



85010000008

Contain NO CBI

December 22, 2000  
Page 2

- This submission also includes analytical values on liver and serum from 1978/79 monkey studies with PFOS and N-ethyl FOSE. We have only recently located these old analytical reports.

#### **Additional Copies of Information Submitted to TSCA Section 8(e) Docket**

- For convenience, we have included in this package copies of three recent submissions under TSCA Section 8(e).
  - The first relates to an update of the mortality study at the Decatur plant where fluorochemicals are produced, showing a statistically significant excess of urinary bladder cancer (based on three cases). Based on all of the available toxicology information, we do not believe it is biologically plausible that the observed excess relates to fluorochemical exposure. We are continuing to investigate this finding, and as indicated in the Section 8(e) letter, the report is still in preparation.
  - The second Section 8(e) submission reproduced here for convenience pertains to findings from the 104-week bioassay using N-ethyl FOSE. That study resulted in a statistically significant excess of benign hepatocellular adenomas, and one hepatocellular carcinoma, in female rats only, and only at the high dose. The final report on that study will be delayed. Given the recent finding of an excess of bladder cancer in the Decatur mortality study, we have asked the laboratory to review urinary bladder tissue of all animals in the study. The laboratory had already examined the urinary bladders of high-dose and control animals, with no findings of note. However, we have asked for the additional work to ensure that there is no abnormal pathology present in the urinary bladders at any dose. We expect this work may delay issuance of the final report on the chronic N-ethyl FOSE study by several months. We have therefore included the Section 8(e) letter containing the significant information from the chronic bioassay in this package. (We have not yet received the results of the chronic PFOS study. We have made the same request regarding additional tissue examination in that study, and accordingly, also expect that study to be delayed pending completion of additional work.)
  - We have also included a report on a 13-week dietary study of N-Methyl Perfluorooctanesulfonamido Ethanol (N-Methyl FOSE) submitted to the TSCA docket by letter dated July 24, 2000.

### Mixtures

- Our series of data submissions have provided to you studies administering specified fluorochemicals (13 chemicals, counting PFOS acid and its various salts as one, were covered in the document submissions). We also provided a submission in June 2000 regarding mixtures in which PFOS is present as an intentional ingredient. That mixture submission essentially covered our AFFF product, known as Lightwater®, which contains small amounts of PFOS. As I discussed in a recent telephone conversation with Dr. Oscar Hernandez of EPA, the utility of these mixture studies for toxicological evaluation and risk assessment is limited. Accordingly, although 3M has located a few additional such studies, we agreed that these need not be submitted.
- Dermal or intravenous absorption studies that administered a covered fluorochemical to test animals have already been supplied in our previous submissions. We note that there also exist studies that administered a mixture to the test species, and then measured the presence of a covered chemical such as PFOS in the animal. The data from these studies is of limited utility, however. The studies were intended to be only a screening-level survey of exposure potential. They do not indicate the rate of absorption or whether the presence of any fluorochemical resulted from absorption versus metabolism. Many of the products administered to the animals were experimental reagents than commercial products, and in some cases only limited information is available as to the composition of the test material. As with the AFFF studies on mixtures, and particularly given the limited usefulness of these mixture studies, we are not providing copies of these studies.

### Compact Discs

We have previously provided you with a compact disc containing our April and early May submissions. We are now enclosing two additional CDs:

- Disc 2 covers the submissions dated May 23, 2000 through June 2000.
- Disc 3 covers the August submission and this submission.

Thus, you will now have all of the data submissions in electronic format. Note that each of the document submissions may include information on multiple chemicals.

As with the first CD, the enclosed CDs include a copy of the cover letter and index for each submission, followed by the data. Also as with the first CD, these electronic documents are for read-only purposes and are not to be

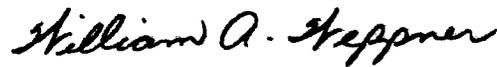
December 22, 2000  
Page 4

modified. 3M will maintain paper copies, and in the event of any discrepancy, the 3M paper copies will be considered controlling. The CDs include only the public version of any confidential information; EPA should refer to the paper copies for any information submitted under claim of confidentiality.

\*\*\*

We will continue to provide information on a regular basis as it becomes available. I will be retiring from 3M effective January 10, 2001. You may feel free to contact me until then, or after that, please contact my successor Michael Santoro (at the same phone number shown below) with any questions.

Very truly yours,



William A. Weppner, Ph.D.  
Director of Environmental, Health  
Safety and Regulatory Affairs  
Specialty Materials Markets  
3M  
Building 236-1B-10  
3M Center  
St. Paul, MN 55144  
651-733-6374 (phone)  
651-733-1958 (fax)  
E-mail: waweppner@mnim.com

cc: Dr. Charles Auer  
Dr. Oscar Hernandez  
(with index but without other enclosures)

ATTACHMENT TO LETTER OF DECEMBER 22, 2000  
SUPPLEMENTAL SUBMISSION

TOXICOLOGY AND MEDICAL SURVEILLANCE

PFOS	Perfluorooctane sulfonate
------	---------------------------

2001 JAN 16 AM 11:06

RECEIVED  
OPPT/CBIC

Medical Surveillance

- 180 1. Letter submitted under TSCA Section 8(e) regarding update of Decatur plant mortality study, December 15, 2000.

Repeat Dose

- 981 1. Analytical Laboratory Report, September 19, 2000, "26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys, Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples," to accompany Covance Laboratories Inc., Study No. 6329-223, 3M Medical Department Study No. T-6295.7, Report No. FACT TOX-030 [unaudited draft of in-life report and interim report of preliminary analytical data were submitted May 4, 2000].

[Note: This report contains revised analytical data updating that in the October 2000 draft Initial Assessment Report. The draft Initial Assessment Report contained data available as of July 2000 and noted that revisions would be forthcoming.]

- 982 2. Technical Report Summary and Central Analytical Laboratory Report No. 6967, providing analytical data for Ninety-Day Subacute Rhesus Monkey Toxicity Study, with Fluorad® Fluorochemical Surfactant FC-95, International Research and Development Corporation, Project No. 137-092, 3M Reference No. T-3351, August 4, 1978 [final in-life report was submitted May 4, 2000].

Contain NO CBI

**ATTACHMENT TO LETTER OF DECEMBER 22, 2000  
SUPPLEMENTAL SUBMISSION**

N-EtFOSE alcohol	N-ethyl perfluorooctane sulfonamidoethanol
------------------	--

Genotoxicity

- 983
1. Final Report, L5178Y TK +/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with N-EtFOSE T-6316, Covance Laboratories, Inc., Study No. 20785-0-431 ICH, 3M Reference No. T-6316.12, September 5, 2000

[Note: This is a repeat of Final Report, Evaluation of the Mutagenic Activity of T-6906 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells, NOTOX Study No. 223458, 3M Reference No. FM-3923, December 22, 1998, submitted May 18, 2000, with a letter from Covance Laboratories (reviewing the NOTOX study and concluding it was technically inadequate and should be repeated)].

Repeat Dose

- 984
1. Letter submitted under TSCA Section 8(e) regarding results of 104-week chronic study, December 4, 2000.
  2. Technical Report Summary and Central Analytical Laboratory Report No. 6995, providing analytical data to accompany Ninety Day Subacute Rhesus Monkey Toxicity Study on FM-3422, International Research and Development Corporation, Study No. 137-088, August 16, 1978 [final in-life report submitted May 18, 2000].
- 985

Teratology & Reproductive

- 986
1. Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.5, Argus Protocol No. 418-009, FACT-TOX-013, June 30, 1999 ["two-generation study" previously reported to TSCA 8e docket]
    - a. Report Amendment 1, October 12, 2000, amending figure.
    - b. Analytical Laboratory Report, October 9, 2000, "Determination of the Presence and Concentration of PFOS, M556, PFOSAA, and PFOSA in the Liver and PFOS, M556, PFOSA, and EtFOSE-OH in the Sera of Crl:CD®BR VAF/Plus® Rats Exposed to Et-FOSE-OH"
- 987

**ATTACHMENT TO LETTER OF DECEMBER 22, 2000  
SUPPLEMENTAL SUBMISSION**

<b>N-MeFOSE alcohol</b>	<b>N-methyl perfluorooctane sulfonamido ethanol</b>
-------------------------	---

**Repeat Dose**

- 988  
990  
991
1. Covance Laboratory, 13-Week Dietary Study of N-Methyl Perfluorooctansulfonamide Ethanol (N-MeFOSE) in Rats, and 4-week Range-Finding Study, 3M Reference No. T-6314, along with letter dated July 24, 2000 submitting reports to TSCA Section 8(e) docket.

<b>PFOA</b>	<b>Perfluorooctanoic acid</b>
-------------	-------------------------------

**Genotoxicity**

- 989
1. Report to 3M Company, Reverse Mutation Studies with T-1485 in Five Salmonella Strains and One Saccharomyces Strain, Industrial Bio-Test Laboratories, Inc., IBT No. 8540-09238, August 10, 1976.

**Published Literature (Bibliography Only)**

1. Olsen, GW, Burris, JM, Burlaw, MM, and Mandel, JH, Plasma Cholecystokinin and Hepatic Enzymes, Cholesterol and Lipoproteins in Ammonium Perfluorooctanoate Production Workers, *Drug and Chemical Toxicology* 23(4): 603-620 (2000).
  2. Reo, NV, Adinehzadeh, NMR Spectroscopic Analyses of Liver Phosphatidylcholine and Phosphatidylethamine Biosynthesis in Rats Exposed to Peroxisome Proliferators - A Class of Nongenotoxic Hepatocarcinogens, *Toxicology and Applied Pharmacology* 2000; 164: 113-126.
- 7

**AR226-0980**

**has actually been processed  
as**

**8EHQ-0101-0373**

**but is included here in**

**FYI-0101-1378**

**as well as it was received as  
an attachment to this FYI.**

Dr. Charles Reich  
Executive Vice President

3M Specialty Material  
Markets

3M Center, Building 220  
St. Paul, MN 55144-1000  
651 733 0439  
651 575 8001 Fax

A 11

2P

8EHQ-0101  
~~1000~~-0373

December 15, 2000

AR 226-0980

3M

**CERTIFIED MAIL**

Document Processing Center (7407)  
ATTN: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
US Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

**COPY**

8EHQ-80-373

2001 JAN 16 AM 11:06

RECEIVED  
OPT HCIC

Re: TSCA 8(e) Notice for Perfluorooctane Sulfonyl Fluoride-based Production Processes

Dear Sir:

Pursuant to Section 8(e) of the Toxic Substances Control Act, 3M is reporting the draft results of an update of a retrospective cohort mortality study of workers at the Decatur, Alabama manufacturing facility. The study update was performed by the University of Minnesota's Division of Environmental and Occupational Health and included workers with at least one year of cumulative employment at the plant prior to December 31, 1997. The cohort of 2,083 employees was followed from 1961 through 1998.

This mortality study was originally initiated as part of an ongoing monitoring program of fluorochemical production workers that began in the late 1970's. Previous study results showed a healthy worker effect and no statistically significant elevations of standard mortality ratios for any cause among fluorochemical production workers at Decatur [Mandel, 1995]. In addition, testing done through regular medical monitoring has not associated abnormal clinical chemistry results with Decatur employees' serum levels for perfluorooctane sulfonate [Olsen, 1999].

In the current update of this cohort mortality study, the cohort was divided into three exposure categories based on job titles in the employee's work history and knowledge of serum PFOS levels generally found in employees with that job title - no workplace exposure to perfluorooctanesulfonyl fluoride (POSF)-based chemistries; low potential workplace exposure to POSF-based chemistries; and ever had job with high potential workplace exposure to POSF-based chemistries. There were fewer than expected deaths from all causes (145 v. 230 expected) and from all cancers (39 v. 54 expected) in the overall cohort. In the highest exposed group, there were fewer than expected overall deaths (65 v. 93 expected) and deaths from cancer (18 v. 21 expected). The basis of this section 8(e) report is that the study did find a statistically significantly elevated standard mortality ratio (SMR) for bladder cancer (coded as malignant neoplasm of the bladder and other urinary organs) in the high exposure group. The SMR for this cause in the high potential exposure group was 16.1 (3 observed, 0.2 expected, 95% confidence interval 3.3-47.1). All three employees who died from bladder cancer worked in the facility beginning in the 1960's and the last retired by 1989. One died prior to 1992 and the other two died between 1992 and 1998.

2001 JAN 18 AM 9:21

RECEIVED  
OPT HCIC

Contain NO CBI

Although the study shows an association between workers ever employed in jobs with high exposure to FOSF-based fluorochemicals at Decatur and bladder cancer, existing toxicology data indicate it is unlikely that POSF chemistry exposure is related to these tumors. Perfluorooctane sulfonate and N-ethyl-perfluorooctanesulfonamidoethanol have both been extensively studied. Neither is genotoxic in multiple studies. Two-year bioassays on both compounds, in rats, are nearing completion. Preliminary information shows no tumors of the urinary bladder in the high dose group of either study. (Final reports pending.) No acute or subchronic study for either compound has shown any bladder or urinary tract toxicity. Other possible explanations for the study finding are chance, exposure to other chemicals in the work environment and personal risk factors such as smoking or recurrent urinary tract stones or infection. In general, the exposure stratification used in this study would apply to exposure to most chemicals used on the site, not just POSF-based chemistries. We are currently investigating whether there has been historical use of known or suspected bladder carcinogens at this location.

The liver has been the target organ in animal studies done with perfluorooctane sulfonate and N-ethyl-perfluorooctanesulfonamidoethanol. The two-year feeding study of N-ethyl-perfluorooctanesulfonamidoethanol resulted in a significant increase in benign liver adenomas in high dose female rats. (Supplemental 8(e) notice dated December 4, 2000) In this mortality study there were two cases of death from liver cancer - one in the low exposure group and one in the high exposure group.  $SMR = 3.1$ , observed = 2, expected = .65, 95% confidence interval = 0.4-11.1). In one of these cases, there is still uncertainty as to whether the death of this 85-year-old male was related to a primary liver cancer or a metastatic process to the liver. In the other case, the employment duration was brief (one year) and the worker was young (36 years), raising the possibility of another causative explanation (viral).

The final report will be forwarded to you when available from the University of Minnesota.

Please contact Dr. Larry Zobel, 3M Medical Director at 651-733-5181 if you have any questions concerning this filing.

Sincerely,



Charles Reich  
Executive Vice President

References:

Mandel J, Johnson R. Mortality study of employees at 3M plant in Decatur, Alabama. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, 1995.

Olsen GW, Burnis JM, Mandel JA, Zobel LR. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. Journal of Occupational and Environmental Medicine 1999; 41(9): 799-806.

**ORIGINAL**

**AR226-0981**

**231pp**

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**ANALYTICAL LABORATORY REPORT**

FROM THE

**26-Week Capsule Toxicity Study with  
Perfluorooctanesulfonic Acid Potassium Salt  
(T-6295) in Cynomolgus Monkeys**

ON THE

**Determination of the Presence and Concentration  
of Perfluorooctanesulfonate (PFOS) in  
Liver and Serum Samples**

***Project Identification***

3M Medical Department Study: T-6295.7  
Covance In-Life Study: #6329-223

Analytical Study: FACT TOX-030  
3M Laboratory Request No. U2279

***Study Completion Date***

At signing

***Total Number of Pages***

233

RECEIVED  
OPPT/KCIC  
2001 JAN 18 AM 9:23

Contain NO CBI

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**GLP COMPLIANCE STATEMENT**

Study Title: Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples

Study Identification Number: FACT TOX-030, T-6295.7, Covance #6329-223

This study was conducted in compliance with United States Environmental Protection Agency Good Laboratory Practice (GLP) Standards 40 CFR Part 792, with the exceptions in the bulleted list below. All raw data and samples for this study are retained in archives at the 3M Lab and will be retained for a period of at least ten years. The analytical phase completed at the 3M Lab was performed in accordance with 3M ET&SS Standard Operating Procedures.

Exceptions to GLP compliance:

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase study was considered to end at the generation and shipment of specimens. The analytical study was considered to start at the receipt of these specimens for analysis. This resulted in having two separate study directors, one for each phase of the same study. However, since the technical performance of each phase was entirely separate, no effect is expected from this exception.
- On a few occasions, data were not recorded or corrected exactly as required by the GLPs.
- The 3M TOX 030 protocol states in the Regulatory Compliance section that "This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practices Standards, 40 CFR 792, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor." Analyses were, however, completed on the concentration and homogeneity of the test material mixture, according to non-GLP validated methods, and are included in this report. As per the protocol, solubility and stability determinations were not conducted.

Andrew M. Seibert 9/19/00  
Study Director Date

John L. Butterloff 19 SEPT 2000  
Study Sponsor Date

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

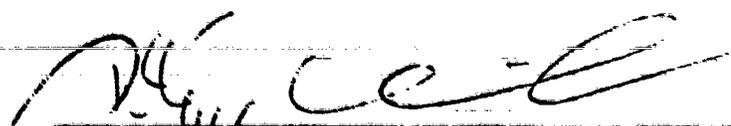
**GLP STUDY—QUALITY ASSURANCE STATEMENT**

**Study Title:** Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples

**Study Identification Number:** FACT TOX-030, T-6295.7, Covance #8329-223

The analytical phase of this study has been inspected by the 3M Lab Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and management.

December 01/98	Sample receipt	1/17/00	1/17/00
March 19,22,23/99	Analysis	3/25/99	3/25/99
October 14/99	Extraction	10/20/99	10/20/99
May 3,8-12,15-19,22-26,29-31/00, June 1,2,5,7,8/00	Data	6/14/00	6/14/00
June 1,5,7,12-16/00	Draft report	6/16/00	6/16/00
September 14/00	Draft report	9/14/00	9/14/00

  
QAU Representative

9/22/00  
Date

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**STUDY PERSONNEL AND CONTRIBUTORS**

**Study Director**

Andrew M. Seacat, Ph.D.  
3M Medical Department  
3M Center, Building 220-2E-02  
PO Box 33220  
St. Paul, MN 55133-3220  
(651) 575-3161

**Sponsor**

3M Toxicology Services - Medical Department  
3M Center, Building 220-2E-02  
St. Paul, MN 55133-3220

John L. Butenhoff, Ph.D., *Sponsor Representative*

**Analytical Chemistry Laboratory**

*Liver and Serum Analyses*

3M Environmental Technology and Safety Services (3M ET&SS)  
3M Environmental Laboratory (3M Lab)  
Fluorine Analytical Chemistry Team (FACT)  
2-3E-09  
935 Bush Avenue  
St. Paul, MN 55106

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator*

**Contributing Personnel**

David R. Barnidge  
Lisa A. Clemens  
Kelly J. Dorweiler  
Mark E. Ellefson  
Sara E. Estes  
Barb A. Gramenz  
Sarah A. Heimdal  
Cari S. Hewitt  
Marlene M. Heying

Harold O. Joki  
Kelly J. Kuehlwein  
Sally A. Linda  
Michael D. Livingston  
Joseph C. Pilon  
Scott R. Post  
Ian A. Smith  
Anh-Dao Vo  
Bob W. Wynne

**In-life Testing Laboratory**

Covance Laboratories, Inc.  
3301 Kinsman Boulevard  
Madison, WI 53704-2595

Peter J. Thomford, Ph.D., *In-Life Phase Study Director*

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**TABLE OF CONTENTS**

GLP Compliance Statement ..... 2

GLP Study—Quality Assurance Statement ..... 3

Study Personnel and Contributors ..... 4

Introduction and Purpose ..... 6

    Test System ..... 6

    Specimen Collection and Analysis ..... 7

Specimen Receipt ..... 7

    Dose Confirmation Analyses ..... 8

Materials and Methods ..... 8

    Chemical Characterization ..... 8

    Method Summaries ..... 8

    Analytical Equipment ..... 8

    Deviations ..... 10

Data Quality Objectives and Data Integrity ..... 10

Data Summary, Analyses, and Results ..... 11

    Summary of Quality Control Analyses Results ..... 11

    Summary of Sample Results ..... 12

Statistical Methods and Calculations ..... 12

Statement of Conclusion ..... 12

List of Attachments ..... 12

Attachment A: Control Matrix Characterization and Dose Confirmation Analyses ..... 13

Attachment B: Protocol and Deviation Summary ..... 15

Attachment C: Extraction and Analytical Methods ..... 43

Attachment D: Data Summary Tables ..... 179

Attachment E: Data Spreadsheets ..... 188

Attachment F: Example Calculations ..... 225

Attachment G: Interim Certificate of Analyses ..... 226

Attachment H: Report Signature Page ..... 233

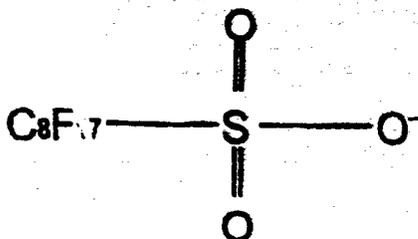
3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**INTRODUCTION AND PURPOSE**



**Perfluorooctanesulfonate (PFOS)**

CAS Number: 2758-39-3

Chemical Formula:  $\text{C}_8\text{F}_{17}\text{SO}_3^-$

Molecular Weight: 498.98

The purpose of the analytical phase of this study is to determine the presence and concentration of PFOS ( $\text{C}_8\text{F}_{17}\text{SO}_3^-$ ) in liver and serum specimens collected during the study of Cynomolgus monkeys orally dosed with perfluorooctane sulfonic acid potassium salt (T-6295).

**Test System**

The test system species and strain selected was the Cynomolgus monkey from Covance Research Products, Inc., identified using a collar tag. At the initiation of treatment, the Cynomolgus monkeys were young adult to adult, and weighed approximately 3-5 kg.

Twenty-two male and 22 female Cynomolgus monkeys were used as the test system in the present study. Four groups of test animals were established according to dosage levels. Group 1 consisted of control Cynomolgus monkeys that did not receive the test substance, but received the equivalent amount of lactose in gelatin capsules as that administered to the Group 4 animals. Groups 2, 3, and 4 were administered daily with 0.03 (low dose), 0.15 (mid dose), and 0.75 (high dose) mg respectively, of T-6295 per kg of body weight/day (mg/kg/day) triturated with lactose in gelatin capsules (see Table 1 for Dosage and Group Characteristics).

**Table 1. Dosage and Group Characteristics of Test System in Study T-6295.7**

STUDY GROUP	NUMBER OF ANIMALS	TOTAL	DOSAGE LEVEL (mg/kg/day)	DOSAGE RATIO (w:w)a
Total: Test System	22 males 22 females	44*	—	—
Group 1 (Control)	6 males 6 females	12	0	—
Group 2 (Low Dose)	4 males 4 females	8	0.03	1:499
Group 3 (Mid Dose)	6 males 6 females	12	0.15	1:39
Group 4 (High Dose)	6 males 6 females	12	0.75	1:39

a Test substance triturated with lactose

\* 48 animals were included in the baseline sera collection, but 44 animals were assigned for treatment

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

All treatment groups were dosed for a minimum period of 26 weeks. Sera specimens were collected from all test animals at various time points during the in-life phase of the 26-week study and sent to the 3M Lab for analysis (see Attachment D, Tables D-1a, D-1b).

Four animals each from Groups 1, 3 and 4 were designated as recovery group animals. Treatment was discontinued and the animals were monitored for elimination of compounds for one year post-treatment. The recovery groups were observed after the cessation of treatment until February 23, 2000 (Week 79) for Group 1 and Group 4 recovery animals, and until March 7, 2000 (Week 80) for Group 3 recovery animals.

**Specimen Collection and Analysis**

In the analytical phase reported here, liver and sera specimens collected from all test animals were sent to the 3M Lab and analyzed for the presence of PFOS (some samples were analyzed to determine the presence of EFOSE, PFOSA, POAA, PFOSEA, M556, PFOSAA, and the monoester; however, these data were collected for informational purposes only, and are not reported). Specimens other than serum and liver tissues were collected and received from Covance Laboratories (6329-223), but were not part of the current scope of analysis determined by the study director and sponsor. Additional analyses of feces are being completed and will be issued as an amendment to this final report.

Blood specimens were centrifuged within one hour of collection. The serum was then harvested and stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Liver specimens collected from each animal were flash frozen in liquid nitrogen and then stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Liver and sera specimens were shipped to the 3M Lab frozen and on dry ice. Liver specimens from Group 3 (9/1/00) and Group 4 (9/22/99) recovery animals were collected via biopsy.

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

**Specimens Collected from Study Groups 1 through 4 (through 2/25/99):**

- Serum Specimens—550 specimens: 9–14 specimens/animal**
- Liver Specimens—30 specimens**

**Specimens Collected from the Recovery Group from 2/27/99 to 3/07/00:**

- Serum Specimens—224 specimens: 18–20 specimens/animal**
- Liver Specimens—12 specimens from Group 3 and Group 4 animals (8 via biopsy)**

**SPECIMEN RECEIPT**

Specimens were received from Covance Laboratories periodically, during the in-life phase of this study, from August 1998 through March 2000. Specimens received were frozen and on dry ice. Specimens were logged in with the 3M Lab and transferred to freezers for storage at either -55°C ±10–20°C or -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-030 were obtained from commercial sources and are presented in Attachment A (see Table A-1). Samples analyzed at the 3M Lab will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: 295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279**Dose Confirmation Analyses**

Dose confirmation analyses were performed on lactose dose samples (1:39 and 1:499) collected on 8/11/98 during the in-life phase of the study; the results are presented in Attachment A (see Table A-2, A-3). The dose confirmation data were collected according to a method that was not fully validated.

Dose confirmation was performed by diluting the lactose dose samples (1:39 - 1,000x and 1:499 - 1,000x) with Milli-Q water, then extracted using the ion-pair procedure, diluted 1:50 and 1:5 respectively into the linear range of the instrument. For each sample (top, middle, bottom), a matrix spike was prepared (approximately 5000 µg/g and 400 µg/g) by spiking the dose solution and then diluting and extracting as described above. In all cases, samples were analyzed versus an unextracted curve using HPLC-ES/MS/MS. The instrumental parameters and analytical conditions described in ETS-8-5.1 were used for dose solution analyses. The average dose level measured was confirmed to be 99 ±27% of the target concentration. Matrix spikes were recovered at >60%.

**MATERIALS AND METHODS****Chemical Characterization**

Table 2 presents information regarding characterization of the test substance used in the in-life phase of this study, and the analytical reference substance used in the analytical phase of this study.

Table 2. Characterization of Test and Analytical Reference Substances in Study FACT TOX-030

	TEST SUBSTANCE	ANALYTICAL REFERENCE SUBSTANCES		
CHEMICAL NAME	KPFOS Potassium Perfluorooctanesulfonate	KPFOS Potassium Perfluorooctanesulfonate	THPFOS-1H,1H,2H,2H-perfluorooctanesulfonic acid	
SOURCE	3M Specialty Chemicals Div.	3M Specialty Chemicals Div.	ICN Biomedics, Inc.	
EXPIRATION DATE	8/31/2001	8/31/2001	10/1/2020	
STORAGE CONDITIONS	Frozen ≤-10°C	Frozen ≤-10°C	Ambient temperature	
CHEMICAL LOT NUMBER	217	171	59909	53406
PHYSICAL DESCRIPTION	FC-95, White crystalline powder	White crystalline powder	Brown powder	Brown waxy solid
PURITY	86.9%	86.4%	N/A	N/A

Reserve samples of the analytical reference substance will be stored at the 3M Lab for a period of 10 years, as will any reserve samples of test substance returned from the in-life phase of the study.

**Method Summaries**

Following is a brief description of the latest methods used during the analytical phase of this study by the 3M Lab. Detailed descriptions of the methods used in this analytical phase are located in Attachment C. As the present analytical phase of this study progressed, more advanced methods evolved and earlier methods were used with deviations until amendments to the protocol were written. Changes to the methods included the use of methyl-*tert*-butyl ether (MtBE) instead of ethyl acetate, curves plotted by linear regression weighted 1/x instead of unweighted curves, a reduction in the size of the analytical column from 100mm to 50mm, gradient changes, and faster HPLC cycle times. A summary of protocol and method deviations is presented in Attachment B (see Table B-1) of this report.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

#### Preparatory Methods:

- **ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"**

Liver samples were homogenized in water. An aliquot of each homogenate was spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MIBK. The extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter, using a 3 cm<sup>3</sup> disposable plastic syringe into glass autosampler vials.

- **ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"**

Sera samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MIBK. The MIBK extract was removed and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter, using a 3 cm<sup>3</sup> disposable plastic syringe into glass autosampler vials.

#### Analytical Methods:

- **ETS-8-7.0, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"**
- **ETS-8-5.1, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"**

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC/ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS (C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup>) analysis, was fragmented to produce ion 99 (FSO<sub>3</sub><sup>-</sup>). The characteristic ion 99 was monitored for quantitative analysis.

#### Analytical Equipment

The actual analytical equipment settings used in the present analytical phase of this study varied slightly during actual data collection. The following is representative of the settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system

Analytical column: Keystone® Betasil™ C<sub>18</sub> 2x50 mm (5 µm)

Column temperature: Ambient

Mobile phase components:

Component A: 2mM ammonium acetate in water

Component B: methanol

Flow rate: 300 µL/min

Injection volume: 10 µL

Solvent Gradient: 10 minutes

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

Start at 10%B  
 Hold at 10%B for 1.0 minute  
 Increase to 95%B over 4.5 minutes  
 Hold at 95%B for 2.0 minutes  
 Return to 10%B over 0.5 minutes  
 Hold at 10%B for 2.0 minutes

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system  
 Software: Mass Lynx™ 3.2  
 Cone Voltage: 60V  
 Collision Gas Energy: 40–60eV  
 Mode: Electrospray Negative  
 Source Block Temperature: 150°C ±10°C  
 Electrode: Z spray  
 Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored in FACT TOX-030

TARGET ANALYTE	PRIMARY ION (amu)	PRODUCT ION (amu)
PFOS	499.0	99.0
THPFOS	427.0	80.0

### Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Earlier methods were used with deviations until amendments to the protocol were written. Deviations from the original protocol and methods are documented in the Attachment B (see Table B-1).

### DATA QUALITY OBJECTIVES AND DATA INTEGRITY

The following data quality objectives (DQOs) were indicated in the protocol for this study:

- **Linearity:** The coefficient of determination ( $r^2$ ) equal to or greater than 0.98
- **Limits of Quantitation (LOQ):** The Method Detection Limit (MDL) for PFOS is 12 ppb for serum and 15 ppb for liver. The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Duplicate/Acceptable Precision:** Precision was reproducible to within 30%
- **Spike/Acceptable Recoveries:** 70–130%
- **Confirmatory Methods:** Indeterminate samples may be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol should be written.
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and mass spectral daughter ion characterization.

**DATA SUMMARY, ANALYSES, AND RESULTS**

Data quality objectives for the analytical phase of this study outlined in the 3M Lab protocol for FACT TOX-030 (see Attachment B) were met with the exceptions noted in this report.

**Summary of Quality Control Analyses Results**

- **Linearity:** The coefficient of determination ( $r^2$ ) of the standard curve was  $\geq 0.985$ .
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis (unweighted – prior to 3/5/99, unweighted or 1/x weighted–3/5/99 to 3/19/99, and 1/x weighted–3/19/99 to end of the study) of two extracted matrix curves bracketing each group of samples, except as noted in the deviation summary. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier was deactivated. Quantitation of each analyte was based on the response of one specific product ion using the multiple response-monitoring mode of the instrument (see Attachment C).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within  $\pm 30\%$  of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks. This value does not exceed the validated LOQ of the method for data that is accepted (see Attachment D, Table D-6).

Table 4. Determination of PFOS LOQ in TOX-030 Analyses

ANALYTE-MATRIX	LOQ
PFOS-Sera	4.39–15.2 ng/mL
PFOS-Liver	26.9–60.1 ng/g

- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed monkey sera, rabbit sera was selected as a suitable surrogate matrix.
- **Duplicate/Acceptable Precision:** Precision was determined by analysis of MS/MSD and was reproducible to within 30%.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs at the 3M Lab. All sera matrix spikes were within  $\pm 30\%$  of the theoretical concentration. Matrix spikes prepared in liver were compliant within  $\pm 30\%$ , with the exception of one spike that was prepared with Day-93 samples and had a low recovery. The matrix spike was reextracted and the recovery was within  $\pm 30\%$  of the theoretical concentration.
- **Spike/Acceptable Recoveries:** Spike recoveries of  $\pm 30\%$  of expected values were achieved for all matrix spikes prepared in sera. With one exception (noted earlier), matrix spikes prepared in liver were within  $\pm 30\%$ .
- **Use of Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. After 11/04/99, the surrogate response of each analytical run was verified to determine that it did not vary more than  $\pm 50\%$  from the mean within each analytical run. No problems were observed with these data.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitative to  $\pm 30\%$ . The validity of this assumption has not been verified by other techniques.

#### Summary of Sample Results

- **Samples from Control Animals:** Low levels of PFOS were often detected in the sera and liver of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, PFOS levels found in the sera and liver of the test animals increased with dose group. PFOS levels increased as dosage increased; significant differences between male and female PFOS levels were not observed in sera. However, Group 4 males had notably higher PFOS levels in liver samples than Group 4 females. Detailed sample data is presented in Attachments D and E.

---

### STATISTICAL METHODS AND CALCULATIONS

Statistical methods were limited to the calculation of means and standard deviations. See Attachment F for example calculations used to generate the liver and serum sample data in TOX-030.

---

### STATEMENT OF CONCLUSION

Under the conditions of the present analytical phase of this study, PFOS was detected in the sera and liver samples of Groups 2, 3, and 4 animals. The Control Group 1 animals showed minimal amounts of PFOS. PFOS levels increased as dosage increased; significant differences between male and female PFOS levels were not observed in sera. However, Group 4 (high dose) males had notably higher PFOS levels in liver samples than Group 4 females.

Data quality objectives for the analytical phase of this study outlined in the 3M Lab protocol for FACT TOX-030 (see Attachment B) were met with the exceptions noted in this report.

---

### LIST OF ATTACHMENTS

- **Attachment A:** Control Matrix Characterization and Dose Confirmation Analyses
- **Attachment B:** Protocol and Deviation Summary
- **Attachment C:** Extraction and Analytical Methods
- **Attachment D:** Data Summary Tables
- **Attachment E:** Data Spreadsheets
- **Attachment F:** Example Calculations
- **Attachment G:** Interim Certificate of Analyses
- **Attachment H:** Report Signature Page

3m Medical Department Study: T-6295.7

Report No. FACT TOX-030  
laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

**ATTACHMENT A**  
**CONTROL MATRIX CHARACTERIZATION AND DOSE CONFIRMATION ANALYSES**

Table A-1. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT TOX-030

CONTROL MATRIX	RABBIT SERUM	RABBIT SERUM	MONKEY SERUM	MONKEY SERUM	MONKEY SERUM
Source	Sigma Chemicals	Sigma Chemicals	Lampire Biological	Sierra Biomedical	N/R
Expiration Date	01/01/2010	01/01/2010	N/R	01/01/2010	01/01/2010
Storage Conditions	Frozen -20°C	Frozen -20°C	Frozen -50°C	Frozen -20°C	Frozen -20°C
Chemical Lot #	118H8418	47H4841	111022515	#LY2N0	N/R
Physical Description	Rabbit Serum	Rabbit Serum	Monkey Serum	Monkey Serum	Monkey Serum
CONTROL MATRIX	RABBIT LIVER	RABBIT LIVER	RABBIT LIVER	RABBIT LIVER	MONKEY LIVER
Source	Coming Hazleton	N/R	Coming Hazleton	Coming Hazleton	Sierra Biomedical
Expiration Date	12/01/1999	12/01/1999	01/01/2010	01/01/2010	01/01/2010
Storage Conditions	Frozen -20°C	Frozen -20°C	Frozen -20°C	Frozen -20°C	Frozen -20°C
Chemical Lot #	F00007	N/R	F00005	F00009	N/R
Physical Description	Rabbit Liver	Rabbit Liver	Rabbit Liver	Rabbit Liver	Monkey Liver

N/R—not recorded

3m Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

Table A-2. Lactose Dose Verification (PFOS) for Study 6329-223—8/21/99

	EXPECTED CONC. (ng/mL)	MEASURED CONC. (ng/mL)	%REC. FOR ng/mL	EXPECTED CALC. CONC. (µg/g)	MEASURED CALC. CONC. (µg/g)	AVERAGE	STD DEVIATION	%REC. FOR µg/g
<b>1:39 DOSE (25000 PPM PFOS)</b>								
Top	580	479	83%	25000	20647			83%
Middle	500	650	130%	25000	32537			130%
Bottom	500	422	84%	25000	21108	24764	6736 ±27%	84%
average / std. deviation=								99% ±27%
<b>1:499 DOSE (2000 PPM PFOS)</b>								
Top	410	305	74%	2000	1490			74%
Middle	404	287	71%	2000	1425			71%
Bottom	400	272	68%	2000	1361	1425	64.5 ±5%	68%
average / std. deviation=								71% ±3%

Actual MS Concentration—Actual background concentration, divided by expected, times 100  
(Spiked too low which accounts for the wide differences in recovery)

Table A-3. Lactose Dose Verification (PFOS—Matrix Spikes) for Study 6329-223—8/21/99

	EXPECTED CONC. (ng/mL)	ACTUAL CONC. (ng/mL)	%REC. FOR ng/mL	CALCULATED CONC. (µg/g)	EXPECTED CONC. (µg/g)	ACTUAL CALC. CONC. (µg/g)	AVERAGE	STD DEVIATION	%REC. FOR µg/g	AVERAGE
<b>1:39 DOSE (25000 PPM PFOS) MS</b>										
Top	604	507	84%	21858	10.8	12.1			112%	
Middle	524	485	92%	24252	12.5	-82.8			-664*	
Bottom	524	438	83%	21889	12.5	7.82	22666	1374 ±6%	63%	87%
<b>1:499 DOSE (2000 PPM PFOS) MS</b>										
Top	606	475	78%	2320	9.77	8.30			85%	
Middle	600	447	75%	2217	9.92	7.93			80%	
Bottom	596	440	74%	2202	10.0	8.41	2246	64.5 ±3%	84%	83%

\* This value is an outlier and was not used in any calculations

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279**ATTACHMENT B**  
**PROTOCOL AND DEVIATION SUMMARY**

Table B-1. Deviation Summary for FACT TOX-030

DEVIATION	DATES OF OCCURRENCE	IMPACT ON STUDY
MIBE was used as an extraction solvent instead of ethyl acetate.	2/5/99, 2/9/99, 5/18/99, 6/11/99	No negative impact on the study—MIBE improved the absolute recoveries and shortened extraction time.
Pipetta was used instead of Oxford dispenser.	10/14/99	No negative impact on the study.
Curves plotted by linear regression weighted 1/x rather than by linear regression as specified in the protocol.	2/13/99, 3/5/99, 3/12/99, 3/19/99, 3/20/99, 3/21/99, 3/23/99, 3/24/99, 3/25/99, 4/7/99, 4/11/99, 4/12/99, 4/17/99, 5/19/99, 5/22/99, 6/9/99, 6/14/99	No negative impact on the study—1/x weighted curves improved the precision and accuracy of analysis.
A second extracted matrix curve was not used to bracket samples.	3/5/99, 3/9/99, 3/15/99, 3/16/99, 5/19/99, 5/22/99, 10/26/99, 1/21/00, 3/24/00, 4/27/00	No negative impact on the study—The accuracy of calibration checks analyzed every five to ten samples was monitored to ensure continued accuracy of the analysis. The QC provided by the calibration checks is sufficient and the data quality will not be adversely affected.
Recorded extraction method FACT-M-1.0 rather than FACT-M-1.1.	8/1/99	No negative impact on the study—New method was followed, even though old method was recorded.
Followed extraction method ETS-8-6.0 rather than FACT-M-1.1.	10/14/99, 10/25/99, 1/19/00, 3/22/00	No negative impact on the study—New validated method provides improvements in precision and extraction time.
Followed analytical method ETS-8-7.0 rather than FACT-M-2.1.	7/29/99, 10/20/99, 10/22/99, 10/26/99, 10/27/99, 1/28/00, 3/24/00, 3/28/00	No negative impact on the study—New validated method provides improvements in precision, accuracy and analysis time.
Followed analytical method ETS-8-5.0 rather than FACT-M-4.1.	3/05/99, 3/08/99	No negative impact on the study—New validated method provides improvements in precision, accuracy and analysis time.
Samples extracted using 0.5 mL rather than 1.0 mL due to insufficient sample.	10/25/99	No negative impact on the study—Studies indicate that data quality is not jeopardized using 0.5 mL of sera.
Followed extraction method ETS-8-4.0 rather than FACT-M-3.1.	3/02/99, 3/03/99	No negative impact on the study—New validated method provides improvements in precision and extraction time.
Matrix spikes were not spiked with standard (Used as blanks).	11/3/99	Adequate QC was prepared with the sample set; unspiked samples pose no negative impact on the study.
Continuing calibration standards were not spiked with standard due to analyst error.	11/3/99	Mid-level curve standards were substituted as QC for the non-spiked calibration check standards; the unspiked calibration standards pose no negative impact to the study.
Samples extracted using <0.5 mL due to insufficient initial sample volume.	2/5/99, 2/9/99, 3/2/99, 3/3/99, 3/10/99, 3/12/99, 3/15/99, 3/18/99, 4/6/99, 4/8/99, 8/25/99, 11/3/99, 4/21/00	Studies indicate that data accuracy and precision may be affected when sera samples less than 0.5 mL were extracted. Data reported from extraction of samples less than 0.5 mL is noted in the data tables.

3M Medical Department Study: T-6295.7  
3M Environmental Technology  
and Services

PO Box 33331  
St. Paul, MN 55133-3331  
612 778 6442

Report No. FACT TOX-030  
Laboratory Request Number-U2279  
Protocol #FACT-TOX-030



**Study Title**

**26-Week Capsule Toxicity Study with  
Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in  
Cynomolgus Monkeys**

**PROTOCOL**

**Author**

Lisa Clemen

**Date:**

January 25, 1999

**Performing Laboratory**

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**

FACT-TOX-030

**Study Identification**

**26-Week Capsule Toxicity Study with  
Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in  
Cynomolgus Monkeys**

**Test Material**

Perfluorooctane sulfonic acid potassium salt  
(T-6295)

**Sponsor**

3M Toxicology Services - Medical Department  
3M Center, Building 220-2E-02  
St. Paul, MN 55144-1000

**Sponsor Representative**

Andrew Seacat, Ph.D.  
3M Toxicology Services  
Telephone: 612-575-3161  
Facsimile: 612-733-1773

**Study Director**

Kristen Hansen, Ph.D.  
3M Environmental Technology and Safety  
Services  
Building 2-3E-09  
651-778-6018

**Study Location(s)  
In vivo Testing Facility**

Covance Laboratories, Inc.  
3301 Kinsman Boulevard  
Madison, Wisconsin 53704

**Analytical Testing Laboratory**

3M Environmental Laboratory  
Building 2-3E-09  
935 Bush Avenue  
St. Paul, MN 55106

**Proposed Study Timetable**

**Study Initiation Date**

January 25, 1999

**Study Completion Date**

January 25, 2000

## 1. STUDY

Twenty-six week capsule toxicity study with potassium perfluorooctane sulfonic acid (T-6295) in cynomolgus monkeys.

## 2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in the liver and serum of cynomolgus monkeys. Additional tissues or fluids may be analyzed. The in-life portion of this study was conducted at Covance Laboratories, study #6329-223.

## 3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practices Standards, 40 CFR 792, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

## 4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

## 5. TEST MATERIAL

5.1 Refer to Covance Laboratory protocol for study #6329-223.

## 6. CONTROL MATRICES

6.1 **Identification** Monkey liver and serum and/or rabbit liver and serum, traceability numbers will be recorded in the raw data and included in the final report

6.2 **Source** Covance Research and/or Sigma Chemical

6.3 **Physical Description** Monkey liver and serum and/or rabbit liver and serum

6.4 **Purity and Stability** Not applicable

6.5 **Storage Conditions** Frozen at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$  or  $-55\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$

6.6 **Reserve Matrix** A portion of the control matrix will be retained in the archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 **Disposition** Matrices will be retained per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
Laboratory Request Number-U2279  
Protocol #FACT-TOX-030

**6.8 Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

## 7. REFERENCE MATERIAL

**7.1 Identification** Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

**7.2 Source** 3M Specialty Chemicals

**7.3 Physical Description** White powder

**7.4 Purity and Stability** Responsibility of the Sponsor

**7.5 Storage Conditions** Room temperature

**7.6 Reserve Material** A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

**7.7 Disposition** Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

**7.8 Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

## 8. TEST SYSTEM

Cynomolgus monkeys were used as the test system, and were maintained and dosed as described in Covance protocol #6329-223. Group 1 control animals did not receive the test substance. Groups 2, 3, and 4 received the test substance daily for 25 weeks, at concentrations of 0.02, 0.5, and 2.0 mg/kg/day, respectively. Refer to Covance protocol #6329-223 for tabular presentation of data. Two animals each from Groups 1, 3, and 4 were designated as recovery animals and were allowed at least a 13 week, which may be extended, recovery period after cessation of treatment.

## 9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory will receive homogeneity samples for dose analysis and specimens of the following body tissues and fluids from the indicated points in the study. All specimens will be packed on dry ice for shipping.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
 Laboratory Request Number-U2279  
 Protocol #FACT-TOX-030

Body tissue/fluid	Collected	Expected # of specimens
Serum - all animals	7 days prior to treatment 7 days post treatment every two weeks during treatment and recovery	616 from main study 24 additional from recovery
Urine and feces - recovery animals	Day zero of recovery 6, 30, and 90 (with a potential 180) days of recovery	24 urine 24 feces
Liver - all animals	After termination of the study	44

Total number of test animals: 32

Total number of control animals: 12

Specimens sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

#### 10. PREPARATORY METHODS

- 10.1** FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.2** FACT-M-3.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.3** If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

#### 11. ANALYTICAL METHODS

- 11.1** FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.2** FACT-M-4.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.3** If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

## 12. DATA QUALITY OBJECTIVES

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators are included in the analytical methods. In addition, the following criteria will be met:

**12.1 Linearity**  $r^2 \geq 0.98$

**12.2 Limits of detection / quantitation**

**12.2.1 Method Detection Limit (MDL) for PFOS**

a) Serum: 12 ppb

b) Liver: 15 ppb

**12.2.2 Practical Quantitation Limit (PQL) – Equal to the lowest standard in the calibration curve**

**12.3 Duplicate acceptable precision** < 30% for the method

**12.4 Spike acceptable recoveries** 70% - 130%

**12.5 Use of confirmatory methods** Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

**12.6 Demonstration of specificity** Chromatographic retention time, mass spectral daughter ion characterization.

## 13. SUB-CONTRACTED ANALYSIS

**13.1** All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

**13.2** An amendment to this protocol will be written if analyses are performed at laboratories other than the 3M Environmental Laboratory.

## 14. STATISTICAL ANALYSIS

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

**14.1 Data transformations and analysis** Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

**14.2 Statistical analysis** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

**15. REPORT**

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

- 15.1** Name and address of the facility performing the study
- 15.2** Dates upon which the study was initiated and completed
- 15.3** A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards
- 15.4** Objectives and procedures as stated in the approved protocol, including any changes in the original protocol
- 15.5** The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor
- 15.6** Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor
- 15.7** A description of the methods used to conduct the test(s)
- 15.8** A description of the test system
- 15.9** A description of any circumstances that may have affected the quality or the integrity of the data
- 15.10** The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study
- 15.11** A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses
- 15.12** Statistical methods used to evaluate the data, if applicable
- 15.13** The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable
- 15.14** The location where raw data and the final report are to be stored
- 15.15** A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

### **16. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT**

Original data, or copies thereof, will be available at 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

**16.1** The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

- 16.1.1** Approved protocol and amendments
- 16.1.2** Study correspondence
- 16.1.3** Shipping records
- 16.1.4** Raw data
- 16.1.5** Approved final report (original signed copy)
- 16.1.6** Electronic copies of data

**16.2** The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

- 16.2.1** Training records
- 16.2.2** Calibration records
- 16.2.3** Instrument maintenance logs
- 16.2.4** Standard Operating Procedures, Equipment Procedures, and Methods

### **17. SPECIMEN RETENTION**

Specimens will be maintained in the laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

### **18. PROTOCOL AMENDMENTS AND DEVIATIONS**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030  
laboratory Request Number-U2279  
Protocol #FACT-TOX-030

**19. ATTACHMENTS**

**19.1 Attachment A** Preparatory and analytical methods

**20. SIGNATURES**

Andrew M. Seacat  
Andrew Seacat, Ph.D., Sponsor Representative

1/28/98  
Date

Kristen Hansen  
Kristen Hansen, Ph.D., 3M Environmental Laboratory Study Director

1/29/98  
Date