# Analytical Report

# Analysis of PFOS in Test Organisms from the 96-Hour Static Acute Toxicity Test with the Freshwater Mussel

**Laboratory Report No. W2078**

**FACT Project No. FACT-TOX132**


***Testing Laboratory***

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***Requester***

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## 1 Introduction

This study was designed to quantitatively assess perfluorooctane sulfonate (PFOS, C8F17SO3-, CAS # 2795-39-3) levels in freshwater mussels after a 96-hour exposure. Details of exposure conditions, clinical observations and other toxicity data were generated by the in-life testing facility (Wildlife International) and are not included in this report. Analytical results of the test animals representing 5 dosage groups and 1 control group are presented here. In each dose group, there were 20 mussels exposed to PFOS. Within each group, 10 mussels were exposed in one of two chambers labeled A and B. Six of the twenty test animals per group (3 from chamber A and 3 from chamber B) were analyzed for PFOS.

PFOS was identified in all test animals analyzed, and most of the matrix mussels and control mussels. Matrix mussels were sacrificed upon arrival at the in-life testing facility, prior to the start of the study. Control mussels were housed near the test animals in water to which no PFOS was added.

PFOS levels were determined to increase with each increase in dose level. Refer to attachment D for results of individual mussel analysis.

Based on matrix spike analysis studies, data generated in support of this study should be considered to be semi-quantitative with a margin of error of +/-35%.

No data was collected for analytes other than PFOS.

Although rigorous quality control measures and 3M Environmental Laboratory Standard Operating Procedures were followed, the acquisition of this data was not necessarily collected according to applicable Good Laboratory Practices. Data presented here is the highest quality data available at this time.

In-phase testing was conducted according to applicable Good Laboratory Practices. The conduct of the in-life phase is the sole responsibility of Wildlife International Ltd. See attachment C for the in-life protocol.

## 2 Sample Receipt

Mussel specimens were collected on 06/01/99 and sent to the Environmental Laboratory on 06/07/99. The 3M Environmental Lab received, on 06/08/99, a total of 105 frozen mussel specimens. Twenty specimens were from group 1, 20 from group 2, 20 from group 3, 20 from group 4, 18 from group 5, 2 from group 6, and 5 matrix mussels. Upon specimen receipt, a chain of custody was initiated and a copy of this form can be found in Attachment C.

## 3 Holding Times

There are no holding time criteria associated with this analysis.

## 4 Methods - Analytical and Preparatory

**Preparatory and Analytical Methods**

FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry Spectrometry *with the following exception*: Due to the time constraints and lack of control material, standard curves were prepared in methanol instead of from mussel tissue. PFOS lot 171 was used as reference material.

ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry *with the following exception*:

Due to the time constraints and lack of control material, standard curves were prepared in methanol instead of from mussel tissue. PFOS lot 171 was used as reference material.

FACT-M-2.1, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

The characterization of the reference material (PFOS, lot #171), with respect to purity, stability, and homogeneity, was not complete on the date this report was issued. Once the characterization is complete, a copy of the report will on file in the 3M Environmental Lab archives.

**Sample preparation – ion-pairing extraction**
On July 01, 1999, 1.0 mL of each mussel homogenate (six specimens per group) were extracted following method FACT-M-1.1 which utilizes an ion pairing reagent [tetrabutyl ammonium hydrogen sulfate (TBA)] in a pH-controlled environment. The cationic reagent selectively targets anionic fluorochemicals. Once the anion-TBA pair is formed, the analyte is transferred into a non-polar organic solvent, dried, and reconstituted in 1.0 mL of methanol for MS analysis.

Samples of the test matrix from Group 4 animals were spiked with PFOS and extracted according to FACT-M-1.1 on July 01, 1999. Samples of test matrix from the control group were spiked and extracted following ETS-8-6.0 on July 22, 1999 and August 2, 1999.

Although two different extraction method numbers were used for these specimens, the extractions were performed as described in method ETS-8-6.0. For example, the July 01, 1999 extraction following method FACT-M-1.1 used methyl tert butyl ether solvent as stated in method ETS-8-6.0, instead of ethyl acetate as stated in FACT-M-1.1.

**HPLC/ESMSMS: for detailed quanitative work**
On July 07, 1999 the mussel extracts were analyzed using tandem electrospray mass spectrometry (ESMSMS), analytical system "Soup020199" according to FACT-M-2.1, with the following exception. Because large amounts of blank matrix were not available, analyte levels were evaluated versus a solvent curve instead of versus a curve extracted from matrix. Extra matrix spike studies were conducted in order to validate this action. Results of these matrix spike studies were good (extraction efficiency greater than 65%), therefore, these sample results are considered to be acceptable.

In this analysis, a 10 µL aliquot of extract is injected and passed through a reverse phase liquid chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. For example, in a standard solution PFOS may elute at 5.0 minutes. Retention times between a standard PFOS solution and the analyte extracted from mussel in this analysis were matched on the HPLC system to within 2%.

Following HPLC separation, ESMSMS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray, an ionization technique used primarily for detection of molecular ions, is generally operated at relatively mild temperatures. Molecules are ionized, and a primary ion, characteristic of the analyte, is selected. This ion is bombarded with high-energy gas; subsequent collisions create smaller secondary ionic fragments unique to the primary ion, which are detected.

For example, for PFOS ($C_8F_{17}SO_3^-$) analysis, ion 499 is selected as the characteristic primary ion. This ion is fragmented into other ions such as 99 amu (corresponding to $FSO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected and can be used to differentiate PFOS from other compounds that

might have the same characteristic 499 amu primary ion, but different chemical compositions and secondary ion fragmentation patterns.

After analysis on July 07, 1999, it was determined that dilutions were required for extracts from group 2-6 test animals. Dilutions were prepared and analyzed on July 09, 1999 using analytical system "Madeline 040198" according to method FACT-M-2.1. The diluted samples were within the calibration range.

# 5 Analysis

## 5.1 Calibration

For quantitative determinations, a mid-level, unextracted methanol calibration curve verification (CCV) standard was analyzed every 5 to 10 samples to monitor instrumental drift. Calibration curve verification standards are compliant if instrumental response is within +/- 30% of the expected value. In this study, all CCV standards were compliant.

Quantitation of the target analytes is based on linear regression analysis (weighted 1/x) of two curves prepared in methanol, bracketing each group of samples. Quantitation of each analyte is based on the response of one or more specific daughter ions using the multiple response-monitoring mode of the instrument.

Analyses on 08/02/99 and 08/03/99 contained poor calibration curve response; these data were not reported.

## 5.2 Blanks

Four extraction blanks, utilizing water as surrogate matrix, were extracted with each batch of samples. Blanks are compliant if no target analyte is detected above the limit of quantitation for a specific analyte. In this study, all blanks were compliant.

## 5.3 Surrogates

Tetra-hydro perfluorooctane sulfonate is used as a surrogate in this analysis. Surrogate response is monitored to confirm gross instrumental failure, if necessary. Surrogate response results were acceptable.

## 5.4 Matrix Spikes

Four matrix spikes at 100 ppb and four matrix spikes at 1.0 ppm were extracted and analyzed utilizing all target analytes for which quantitative data is presented.

Samples of the test matrix from Group 4 animals were spiked with PFOS and extracted according to FACT-M-1.1 on July 01, 1999. Spike recoveries were erratic, probably due to exceptionally high endogenous levels of PFOS; the matrix spike studies were repeated on July 22, 1999 and August 02, 1999. Spike recoveries from the July 22, 1999 extraction at 100 ppb and 1.0 ppm were within analytical acceptance criteria of 65-130%. As the matrix spike data collected July 22, 1999 was acceptable, data collected from analyses of the August 2, 1999 extraction were not included in this report.

## 5.5 Laboratory Control Samples

Laboratory Control Samples are not a component of this study.

## 5.6 Sample Related Comments

The level of PFOS in samples of mussel tissue was determined by comparison with a standard curve prepared in solvent. Typically, PFOS levels are determined versus a curve extracted from tissue in order to more accurately assess any matrix effects. Extra matrix spike studies were conducted in order to validate this action. Results of these matrix spike studies were good (extraction efficiency greater than 65%), therefore these sample results are considered to be acceptable.

## 6 Data Summary

PFOS was detected in the test animals analyzed. Refer to Table 1 for a summary of average PFOS levels in mussels by dose group. Please refer to the attached spreadsheets for individual test animal results.

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitiative to ± 35 %. The validity of this assumption has not been verified by other techniques.

The estimated limit of quantitation (LOQ) for PFOS is 25 ppb (ng/mL) for the July 09, 1999 analysis and 5.0 ppb (ng/mL) for the remaining analyses (July 07, 1999 and August 03, 1999).

The LOD and LOQ values are stated as ng/mL since the extracts were evaluated versus a solvent curve, not an extracted mussel curve.

This report reflects the most accurate data available at this time.

**Table 1 Analysis Results-Mussel**

**FRESHWATER MUSSEL**

| Group/ Dosage | Average Conc. PFOS in Mussel ug/g |
|---|---|
| Matrix | 0.0808 |
| Group 1<br>Control | A: <0.0174µg/g<br>B: 0.0502 |
| Group 2<br>5.3 ppm | A: 3.41<br>B: 3.96 |
| Group 3<br>12 ppm | A: 4.83<br>B: 5.61 |
| Group 4<br>20 ppm | A: 8.07<br>B: 6.58 |
| Group 5<br>41 ppm | A: 11.2<br>B: 12.5 |
| Group 6<br>79 ppm | A: 91.2<br>B: 86.4 |

**Matrix** = Mussel specimens which were sacrificed upon arrival of all mussel specimens at the in-life testing facility and not exposed to control or dosed water.
**A** = Specimens stored in Chamber A.
**B** = Specimens stored in Chamber B.

## 7 Data / Specimen Retention

Specimens and extracts will be retained for a minimum of one year for future evaluation, if necessary.

## 8 Attachments

**8.1** **Attachment A:** Analytical Report Addendum 1
**8.2** **Attachment B:** Wildlife International Protocol
**8.3** **Attachment C:** Extraction and Analytical Methods
**8.4** **Attachment D:** Chain of custody form(s)
**8.5** **Attachment E:** Complete Analytical Results
**8.6** **Attachment F:** Extraction and Analysis Information

## 9 Signatures

Lisa A Clemen - 06/12/00
Lisa Clemen, Advanced Chemist — Date

Kris Hansen 06/09/00
Kris Hansen, Ph.D., Specialist — Date

D RE LAC 06/12/00

# Analytical Report

# Analysis of PFOS in Test Organisms from the 96-Hour Static Acute Toxicity Test with the Freshwater Mussel

**Laboratory Report No. W2078**

**FACT Project No. FACT-TOX132**

***Attachment A***

Analytical Report Addendum 1

# Analytical Report Addendum 1

# Analysis of PFOS in Test Organisms from the 96-Hour Static Acute Toxicity Test with the Freshwater Mussel

**Laboratory Report No. W2078**

**FACT Project No. FACT-TOX132**

***Testing Laboratory***

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3M Environmental Laboratory
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FAX: (651) 778-6176

## 10 Introduction

This study addendum was designed to quantitatively assess perfluorooctane sulfonate (PFOS, C8F17SO3-, CAS # 2795-39-3) levels in unexposed, freshwater "matrix" mussels. Matrix mussels were sacrificed upon arrival at the in-life testing facility, prior to the start of the study.

In the original study, the samples of matrix mussels were determined to contain an average of 0.0808 ug/g of PFOS. This level of PFOS in unexposed mussels was unexpected, so further analyses were conducted. The results of the reanalysis, reported in this addendum indicate that PFOS levels in the samples of matrix mussels are less than 0.0174 µg/g. It is likely that the original samples of mussel matrix that were analyzed were contaminated during sample prep.

Although rigorous quality control measures and 3M Environmental Laboratory Standard Operating Procedures were followed, the acquisition of this data was not necessarily collected according to applicable Good Laboratory Practices. Data presented here is the highest quality data available at this time.

## 11 Sample Receipt

Mussel specimens were collected on 06/01/99 and sent to the Environmental Laboratory on 06/07/99. The 3M Environmental Lab received, on 06/08/99, a total of 105 frozen mussel specimens. One hundred and five specimens were received; 5 of these were matrix mussels. Upon specimen receipt, a chain of custody was initiated and a copy of this form can be found in Attachment C of the final analytical report (TOX-132).

## 12 Holding Times

There are no holding time criteria associated with this analysis.

## 13 Methods - Analytical and Preparatory

**Preparatory and Analytical Methods**

Please refer to the analogous section in the full analytical report.

## 14 Analysis

### 14.1 Calibration

For quantitative determinations, a mid-level, unextracted methanol calibration curve verification (CCV) standard was analyzed every 5 to 10 samples to monitor instrumental drift. Calibration curve verification standards are compliant if instrumental response is within +/- 30% of the expected value. In this study, all CCV standards were compliant.

Quantitation of the target analytes is based on linear regression analysis (weighted 1/x) of two curves prepared in methanol, bracketing each group of samples. Quantitation of each analyte is based on the

response of one or more specific daughter ions using the multiple response-monitoring mode of the instrument.

### 14.2 Blanks

Two extraction blanks, utilizing water as surrogate matrix, were extracted with each batch of samples. Blanks are compliant if no target analyte is detected above the limit of quantitation for a specific analyte. In this study, all blanks were compliant.

### 14.3 Surrogates

Tetra-hydro perfluorooctane sulfonate is used as a surrogate in this analysis. Surrogate response is monitored to confirm gross instrumental failure, if necessary. Surrogate response results were acceptable.

### 14.4 Matrix Spikes

Two matrix spikes at 50 ppb were extracted and analyzed for PFOS. The matrix spikes showed recoveries of 119% and 75%, within the acceptable range of +/-30%.

### 14.5 Laboratory Control Samples

Laboratory Control Samples are not a component of this study.

### 14.6 Sample Related Comments

The level of PFOS in samples of mussel tissue was determined by comparison with a standard curve prepared in solvent. Typically, PFOS levels are determined versus a curve extracted from tissue in order to more accurately assess any matrix effects. Results of matrix spike studies conducted in the original study and described in the final analytical report validate the choice of using an unextracted curve.

## 15 Data Summary

The results of the reanalysis, reported in this addendum indicate that PFOS levels in the samples of matrix mussels are less than 0.0174 µg/g. It is likely that the original samples of mussel matrix that were analyzed were contaminated during sample prep.

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitative to ± 35 %. The validity of this assumption has not been verified by other techniques.

## 16 Data / Specimen Retention

Specimens and extracts will be retained for a minimum of one year for future evaluation, if necessary.

## 17 Attachments

**17.1** None.

## 18 Signatures

Lisa A Clemen — 06/12/00
Lisa Clemen, Advanced Chemist — Date

Kris Hansen — 06/09/00
Kris Hansen, Ph.D., Principal Analytical Investigator — Date

# FACT-TOX-132

| | | | |
|---|---|---|---|
| Study: | 96 Hour Static Acute Toxicity Study with the Freshwater Mussel | | |
| Product Number(Test Substance): | PFOS | | |
| Matrix: | Freshwater Mussel | Filename: | See Attachments |
| Method/Revision: | ETS-8-6.0, ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.2 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 09/14/99 RWW | | |
| Date of Analysis/Analyst: | 09/19/99 MEE | | |
| Date of Data Reduction/Analyst: | 09/20/99 MEE | | |

Sample Data

## MUSSEL

| Group Dose | Sample # | Concentration of PFOS ug/g | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|
| Method Blk | H2O Blk-1 | <LOQ (0.0347 ug/g) | | NA |
| | H2O Blk-2 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| QC - 100 ppb, 500 ppb 36417 | 1A-10-100 ppb-1 | 105% | | |
| | 1A-10-100 ppb-2 | 110% | 108% | 5% |
| | 1A-10-500ppb-1 | 99% | | |
| | 1A-10-500ppb-2 | 90% | 95% | 10% |
| Matrix clean mussels | MATRIX-1-1 | <LOQ (0.0347 ug/g) | | |
| | MATRIX-1-2 | <LOQ (0.0347 ug/g) | | |
| | MATRIX-1-3 | <LOQ (0.0347 ug/g) | | |
| | MATRIX-1-4 | <LOQ (0.0347 ug/g) | | NA |
| | MATRIX-1-5 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| Group 1 Control | 1A-6 | <LOQ (0.0347 ug/g) | | NA |
| | 1A-10 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| | 1B-3 | <LOQ (0.0347 ug/g) | | |
| | 1B-6 | 0.0663 | NA | NA |

PFOS = Perfluorooctanesulfonate NA: not applicable

Limit of Quantitation (LOQ): PFOS = 0.0347 ug/g

| | |
|---|---|
| Date Entered/By: | 09/21/99 LAC |
| Date Verified/ By: | 12/13/99 GML |

BACK TO MAIN

| | | | |
|---|---|---|---|
| Study: | 96 Hour Static Acute Toxicity Study with the Freshwater Mussel | | |
| Product Number(Test Substance): | PFOS | | |
| Matrix: | Freshwater Mussel | Filename: | See Attachments |
| Method/Revision: | ETS-8-6.0, ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.2 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 9/22/99 MCH | | |
| Date of Analysis/Analyst: | 09/24/99 IAS | | |
| Date of Data Reduction/Analyst: | 09/27/99 IAS | | |

**Sample Data**

**MUSSEL**

| Group Dose | Sample # | Concentration of PFOS ug/g | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|
| Method Blk | H2O Blk-9 | <LOQ (0.0174 ug/g) | | |
| | H2O Blk-10 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| QC - 50 ppb | MATRIX-1-3-MS-9 | 119% | | |
| ext. 9/22/99 | MATRIX-1-3-MSD-10 | 75% | 97% | 45% |
| Matrix | MATRIX-1-1 | <LOQ (0.0174 ug/g) | | |
| clean mussels | MATRIX-1-2 | <LOQ (0.0174 ug/g) | | |
| | MATRIX-1-3 | <LOQ (0.0174 ug/g) | | |
| | MATRIX-1-4 | <LOQ (0.0174 ug/g) | | NA |
| | MATRIX-1-5 | <LOQ (0.0174 ug/g) | <LOQ | NA |

PFOS = Perfluorooctanesulfonate NA: not applicable

Limit of Quantitation (LOQ): PFOS = 0.0174 ug/g

| | |
|---|---|
| Date Entered/By: | 10/06/99 LAC |
| Date Verified/ By: | 12/13/99 GML |

# Analytical Report

# Analysis of PFOS in Test Organisms from the 96-Hour Static Acute Toxicity Test with the Freshwater Mussel

**Laboratory Report No. W2078**

**FACT Project No. FACT-TOX132**

***Attachment E***

Complete Analytical Results

# FACT-TOX-132

| | | | |
|---|---|---|---|
| Study: | 96 Hour Static Acute Toxicity Study with the Freshwater Mussel | | |
| Product Number(Test Substance): | PFOS | | |
| Matrix: | Freshwater Mussel | Filename: | See Attachments |
| Method/Revision: | FACT-M-1.1, FACT-M-2.1, ETS-8-6.0, ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.2 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 07/01/99, 07/22/99, 08/02/99 SRP/RWW | | |
| Date of Analysis/Analyst: | 07/07/99, 07/09/99, 08/02/99, 08/03/99 DRB/GML/SAH | | |
| Date of Data Reduction/Analyst: | 07/09/99, 07/30/99, 08/04/99 DRB/GML | | |

**Sample Data**

**MUSSEL**

| Group<br>Dose | Sample # | Concentration of PFOS ug/g | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|
| Method Blk | H2O Blk-1 | <LOQ (0.0174 ug/g) | | |
| | H2O Blk-2 | <LOQ (0.0174 ug/g) | | |
| | H2O Blk-3 | <LOQ (0.0174 ug/g) | | |
| | H2O Blk-4 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| Precision Check | 4B Blk-1-1 | 7.89 | | |
| | 4B Blk-1-2 | 7.68 | | |
| | 4B Blk-1-3 | 8.76 | | 7.38 |
| | 4B Blk-1-4 | 7.40 | 7.93 | 0.585 |
| Matrix Blk<br>07/22/99 | 1A-10-Blk-1 | <LOQ (0.0174 ug/g) | | |
| | 1A-10-Blk-2 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| QC - 100 ppb, 1.0 ppm<br>07/22/99 | 1A-10-100 ppb-1 | 102% | | |
| | 1A-10-100 ppb-2 | 91% | | |
| | 1A-10-100 ppb-3 | 121% | | 8% |
| | 1A-10-100 ppb-4 | 103% | 104% | 12% |
| | 1A-10-1.0 ppm-1 | 89% | | |
| | 1A-10-1.0 ppm-2 | 83% | | |
| | 1A-10-1.0 ppm-3 | 92% | | 4% |
| | 1A-10-1.0 ppm-4 | 96% | 90% | 5% |
| Matrix Blk<br>08/02/99 | 1A-10-Blk-1 | <LOQ (0.0174 ug/g) | | |
| | 1A-10-Blk-2 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| QC - 1.0 ppm, 10 ppm<br>08/02/99 | 1A-10-1.0 ppm-MS-1 | 83% | | |
| | 1A-10-1.0 ppm-MSD-1 | 74% | | |
| | 1A-10-1.0 ppm-MS-2 | 80% | | 4% |
| | 1A-10-1.0 ppm-MSD-2 | 85% | 80% | 5% |
| | 1A-10-10 ppm-MS-1 | 89% | | |
| | 1A-10-10 ppm-MSD-1 | 89% | | |
| | 1A-10-10 ppm-MS-2 | 77% | | 5% |
| | 1A-10-10 ppm-MSD-2 | 87% | 86% | 6% |
| Group1<br>Control | 1A-6 | <LOQ (0.0174 ug/g) | | |
| | 1A-9 | <LOQ (0.0174 ug/g) | | |
| | 1A-10 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| | 1B-1 | 0.0227 | | |
| | 1B-3 | <LOQ (0.0174 ug/g) | | 77.5 |
| | 1B-6 | 0.0778 | 0.0502 | 0.0389 |
| Group2<br>5.3 ppm | 2A-4 | 3.87 | | |
| | 2A-5 | 3.70 | | 19.1 |
| | 2A-7 | 2.67 | 3.41 | 0.654 |
| | 2B-6 | 3.25 | | |
| | 2B-7 | 3.15 | | 33.2 |
| | 2B-10 | 5.47 | 3.96 | 1.31 |
| Group3<br>12 ppm | 3A-7 | 4.85 | | |
| | 3A-8 | 5.76 | | 19.5 |
| | 3A-9 | 3.88 | 4.83 | 0.940 |
| | 3B-5 | 5.11 | | |
| | 3B-7 | 5.75 | | 8.00 |
| | 3B-10 | 5.97 | 5.61 | 0.449 |
| Group4<br>20 ppm | 4A-5 | 6.91 | | |
| | 4A-8 | 10.2 | | 23.2 |
| | 4A-10 | 7.07 | 8.07 | 1.87 |
| | 4B-1 | 8.06 | | |
| | 4B-2 | 4.79 | | 25.2 |
| | 4B-6 | 6.88 | 6.58 | 1.66 |
| Group5<br>41 ppm | 5A-7 | 13.1 | | |
| | 5A-8 | 10.9 | | 16.1 |
| | 5A-9 | 9.55 | 11.2 | 1.79 |
| | 5B-4 | 14.0 | | |
| | 5B-7 | 14.5 | | 24.8 |
| | 5B-9 | 8.92 | 12.5 | 3.10 |
| Group6<br>79 ppm | 6A-1 | 91.2 | | NA |
| | 6B-1 | 86.4 | 88.8 | 0.0537 |
| Matrix<br>clean mussels | MATRIX-1-1 | 0.213 | | |
| | MATRIX-1-4 | 0.0434 | | 110 |
| | MATRIX-1-5 | 0.0251 | 0.0937 | 0.103 |

PFOS = Perfluorooctanesulfonate NA = Not applicable

Limit of Quantitation (LOQ): 0.0867 ug/g (070999Files)

Limit of Quantitation (LOQ): 0.0174 ug/g (070799 and 080399 Files)

Date Entered/By: 07/09/99, 07/30/99, 08/04/99 LAC

Date Verified/ By: 08/05/99 GML

# FACT-TOX-132

| | | | |
|---|---|---|---|
| Study: | 96 Hour Static Acute Toxicity Study with the Freshwater Mussel | | |
| Product Number(Test Substance): | PFOS | | |
| Matrix: | Freshwater Mussel | Filename: | See Attachments |
| Method/Revision: | ETS-8-6.0, ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.2 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 09/14/99 RWW | | |
| Date of Analysis/Analyst: | 09/19/99 MEE | | |
| Date of Data Reduction/Analyst: | 09/20/99 MEE | | |

Sample Data

**MUSSEL**

| Group Dose | Sample # | Concentration of PFOS ug/g | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|
| Method Blk | H2O Blk-1 | <LOQ (0.0347 ug/g) | | NA |
| | H2O Blk-2 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| QC - 100 ppb, 500 ppb | 1A-10-100 ppb-1 | 105% | | |
| 36417 | 1A-10-100 ppb-2 | 110% | 108% | 5% |
| | 1A-10-500ppb-1 | 99% | | |
| | 1A-10-500ppb-2 | 90% | 95% | 10% |
| Matrix | MATRIX-1-1 | <LOQ (0.0347 ug/g) | | |
| clean mussels | MATRIX-1-2 | <LOQ (0.0347 ug/g) | | |
| | MATRIX-1-3 | <LOQ (0.0347 ug/g) | | |
| | MATRIX-1-4 | <LOQ (0.0347 ug/g) | | NA |
| | MATRIX-1-5 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| Group 1 | 1A-6 | <LOQ (0.0347 ug/g) | | NA |
| Control | 1A-10 | <LOQ (0.0347 ug/g) | <LOQ | NA |
| | 1B-3 | <LOQ (0.0347 ug/g) | | |
| | 1B-6 | 0.0663 | NA | NA |

PFOS = Perfluorooctanesulfonate NA: not applicable

Limit of Quantitation (LOQ): PFOS = 0.0347 ug/g

Date Entered/By: 09/21/99 LAC

Date Verified/ By: 12/13/99 GML

# FACT-TOX-132

| | | | |
|---|---|---|---|
| Study: | 96 Hour Static Acute Toxicity Study with the Freshwater Mussel | | |
| Product Number(Test Substance): | PFOS | | |
| Matrix: | Freshwater Mussel | Filename: | See Attachments |
| Method/Revision: | ETS-8-6.0, ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.2 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 9/22/99 MCH | | |
| Date of Analysis/Analyst: | 09/24/99 IAS | | |
| Date of Data Reduction/Analyst: | 09/27/99 IAS | | |

**Sample Data**

**MUSSEL**

| Group Dose | Sample # | Concentration of PFOS ug/g | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|
| Method Blk | H2O Blk-9 | <LOQ (0.0174 ug/g) | | |
| | H2O Blk-10 | <LOQ (0.0174 ug/g) | <LOQ | NA |
| QC - 50 ppb | MATRIX-1-3-MS-9 | 119% | | |
| ext. 9/22/99 | MATRIX-1-3-MSD-10 | 75% | 97% | 45% |
| Matrix | MATRIX-1-1 | <LOQ (0.0174 ug/g) | | |
| clean mussels | MATRIX-1-2 | <LOQ (0.0174 ug/g) | | |
| | MATRIX-1-3 | <LOQ (0.0174 ug/g) | | |
| | MATRIX-1-4 | <LOQ (0.0174 ug/g) | | NA |
| | MATRIX-1-5 | <LOQ (0.0174 ug/g) | <LOQ | NA |

PFOS = Perfluorooctanesulfonate NA: not applicable

Limit of Quantitation (LOQ): PFOS = 0.0174 ug/g

| | |
|---|---|
| Date Entered/By: | 10/06/99 LAC |
| Date Verified/ By: | 12/13/99 GML |