

AR226-1837

## **Study Title**

ANALYTICAL PHASE REPORT FOR  
PFOS: A REPRODUCTION STUDY WITH THE MALLARD

## **Data Requirement**

FIFRA Subdivision E, Section 71-4  
OECD Guideline 206

## **Author**

Lisa A. Stevenson

## **Analytical Phase Initiation Date**

February 19, 2002

## **Analytical Phase Completion Date**

At Signing

## **Performing Laboratory**

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

## **Project Identification**

3M Environmental Laboratory Study # E01-1256  
Wildlife International Ltd: 454-109

## **Total Number of Pages**

216

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**GLP COMPLIANCE STATEMENT**

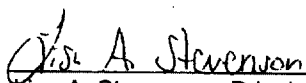
Study Title: Analytical Phase Report for PFOS: A Reproduction Study with the Mallard

Study Identification Number: E01-1256

This analytical phase was conducted in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards with the exceptions listed below:

Exceptions to GLP compliance:

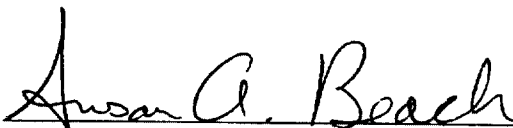
- Automated data collection systems are not validated. The hardcopy printouts are considered as the original raw data.
- The stability of the samples has not been determined.
- Not all data generated were recorded directly or promptly, or initiated and dated on date of entry.



Lisa A. Stevenson, Principal Analytical Investigator

01/22/03

Date



Susan A. Beach, Sponsor Representative

01/22/03

Date

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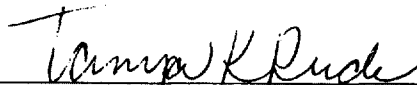
**QUALITY ASSURANCE STATEMENT**

Study Title: Analytical Phase Report for PFOS: A Reproduction Study with the Mallard

Study Identification Number: E01-1256

The analytical phase was audited by the 3M Environmental Laboratory Quality Assurance Unit (QAU), as indicated in the following table. The findings were reported to the study director and laboratory management.

| INSPECTION DATES            | PHASE        | DATE REPORTED TO   |                    |
|-----------------------------|--------------|--------------------|--------------------|
|                             |              | MANAGEMENT         | STUDY DIRECTOR     |
| 02/22/02                    | In-phase     | 02/22/02           | 02/22/02           |
| 04/03/02-04/08/02, 04/17/02 | Data         | 04/08/02, 05/21/02 | 04/08/02, 05/21/02 |
| 05/13/02-05/15/02           | Final Report | 05/16/02           | 05/16/02           |

  
Quality Assurance Representative

1/22/03  
Date

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**TABLE OF CONTENTS**

|                                                                                                                                                               |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| GLP Compliance Statement.....                                                                                                                                 | 2   |
| Quality Assurance Statement .....                                                                                                                             | 3   |
| List of Tables.....                                                                                                                                           | 5   |
| Summary.....                                                                                                                                                  | 7   |
| Purpose.....                                                                                                                                                  | 8   |
| Reference Substances.....                                                                                                                                     | 8   |
| Specimen Receipt and Storage .....                                                                                                                            | 9   |
| Method Summary.....                                                                                                                                           | 9   |
| Preparatory Method .....                                                                                                                                      | 9   |
| Sample Receipt, Preparation and Analysis.....                                                                                                                 | 10  |
| Analytical Method.....                                                                                                                                        | 11  |
| Analytical Results.....                                                                                                                                       | 12  |
| Data Summary .....                                                                                                                                            | 14  |
| Statement of Conclusion .....                                                                                                                                 | 21  |
| Statistical Methods .....                                                                                                                                     | 23  |
| Statement of Data Quality .....                                                                                                                               | 23  |
| References .....                                                                                                                                              | 24  |
| List of Attachments.....                                                                                                                                      | 24  |
| Signature Page .....                                                                                                                                          | 25  |
| Attachment A: Extraction and Analytical Method, ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices" ..... | 26  |
| Attachment B: Data Summary Tables.....                                                                                                                        | 46  |
| Attachment C: Sample Chromatograms, Calibration Information, and Instrument Information .....                                                                 | 55  |
| Attachment D: Sample Preparation Sheets .....                                                                                                                 | 144 |
| Attachment E: Study Protocol, Protocol Amendments, and Deviations .....                                                                                       | 178 |
| Attachment F: Example Calculations .....                                                                                                                      | 216 |

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**LIST OF TABLES**

|                                                                                                                                                  |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. Summary Table, C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Concentration in Sera and Liver .....                        | 7  |
| Table 2. Summary Table, C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Concentration in Sera and Liver with Outliers Removed .....  | 8  |
| Table 3. Reference Substances .....                                                                                                              | 8  |
| Table 4. SPE Extraction Summary .....                                                                                                            | 9  |
| Table 5. Sample Analysis .....                                                                                                                   | 10 |
| Table 6. Serum QC Summary Table .....                                                                                                            | 13 |
| Table 7. Serum Matrix Spike Summary Table .....                                                                                                  | 13 |
| Table 8. Liver QC Summary Table .....                                                                                                            | 13 |
| Table 9. Liver Matrix Spike Summary Table .....                                                                                                  | 13 |
| Table 10. C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Data Summary, Female Mallard Sera (Adult and Offspring) .....              | 14 |
| Table 11. C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Data Summary, Male Mallard Sera (Adult and Offspring) .....                | 16 |
| Table 12. C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Data Summary, Female Mallard Liver (Adult and Offspring) .....             | 18 |
| Table 13. C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Data Summary, Male Mallard Liver (Adult and Offspring) .....               | 20 |
| Table 14. Summary Table, C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Concentration in Sera and Liver .....                       | 22 |
| Table 15. Summary Table, C <sub>8</sub> F <sub>17</sub> SO <sub>3</sub> <sup>-</sup> Concentration in Sera and Liver with Outliers Removed ..... | 23 |

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## STUDY INFORMATION

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Ognjenka Krupljanin, *Analytical Chemist*  
Marlene Heying, *Analytical Chemist*

**Experimental Dates**

Sample Analysis Initiation: 19 February 2002  
Sample Analysis Completion: 11 April 2002

**Archives**

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory according to 40 CFR Part 792. The analytical reference standard reserve samples, as well as the samples pertaining to the analytical phase of this study, are archived at the 3M Environmental Laboratory according to 40 CFR Part 792.

## SUMMARY

The concentration of the  $C_8F_{17}SO_3^-$  anion of perfluorooctane sulfonate, potassium salt (PFOS,  $C_8F_{17}SO_3^-K^+$ ) was determined in the liver and sera of adult mallards and their offspring in both a control group and a 10ppm dosed group from Wildlife feeding study #454-109. The following tables summarize the  $C_8F_{17}SO_3^-$  concentrations found in the liver and sera of study samples. Extracted-matrix PFOS standards are used to establish the HPLC-ES/MS/MS instrument calibration curve for  $C_8F_{17}SO_3^-$ . Concentrations of  $C_8F_{17}SO_3^-$  are reported after correction for the purity and the potassium contribution to the mass of the PFOS reference substance.

Table 1 summarizes all study sample data. Table 2 summarizes study data after removing certain results that have been statistically determined to be outliers utilizing the Dixon Q-Test.

**Table 1. Summary Table,  $C_8F_{17}SO_3^-$  Concentration in Sera and Liver**

| SAMPLING EVENT                 | NUMBER OF SERA SAMPLES | AVERAGE $C_8F_{17}SO_3^-$ CONC. SERA | Range of Results                                        | NUMBER OF LIVER SAMPLES | AVERAGE $C_8F_{17}SO_3^-$ CONC. LIVER | Range of Results  |
|--------------------------------|------------------------|--------------------------------------|---------------------------------------------------------|-------------------------|---------------------------------------|-------------------|
| Male Control Group             | 18                     | NA                                   | <100 ng/mL (14 of 18)<br>107-1400 ng/mL range (4 of 18) | 20                      | <50 ng/g                              | NA                |
| Female Control Group           | 20                     | NA                                   | <100 ng/mL (15 of 20)<br>166-2680 ng/mL range (5 of 20) | 20                      | <50 ng/g                              | NA                |
| Male 10ppm Group               | 20                     | 89700 ng/mL                          | 11300-210000 ng/mL                                      | 20                      | 60900 ng/g                            | 24200-125000 ng/g |
| Female 10ppm Group             | 20                     | 20300 ng/mL                          | 6350-91400 ng/mL                                        | 20                      | 10800 ng/g                            | 2480-30700 ng/g   |
| Male Offspring Control Group   | 3                      | NA                                   | <100 ng/mL (2 of 3)<br>103 ng/mL (1 of 3)               | 3                       | <50 ng/g                              | NA                |
| Female Offspring Control Group | 7                      | NA                                   | <100 ng/mL (7 of 7)                                     | 7                       | <50 ng/g                              | NA                |
| Male Offspring 10ppm Group     | 3                      | 4410 ng/mL                           | 3670 - 5450 ng/mL                                       | 3                       | 3170 ng/g                             | 2200-4640 ng/g    |
| Female Offspring 10ppm Group   | 7                      | 4960 ng/mL                           | 3420 - 6760 ng/mL                                       | 7                       | 3620 ng/g                             | 2240-5930 ng/g    |

For individual sample values see Tables 10 to 13 and Attachment B.

**Table 2. Summary Table,  $C_8F_{17}SO_3^-$  Concentration in Sera and Liver with Outliers Removed**

| SAMPLING EVENT                 | NUMBER OF SERA SAMPLES | AVERAGE $C_8F_{17}SO_3^-$ CONC. SERA | Range of Results                                       | NUMBER OF LIVER SAMPLES | AVERAGE $C_8F_{17}SO_3^-$ CONC. LIVER | Range of Results |
|--------------------------------|------------------------|--------------------------------------|--------------------------------------------------------|-------------------------|---------------------------------------|------------------|
| Male Control Group             | 18*                    | *                                    | *                                                      | 20*                     | *                                     | *                |
| Female Control Group           | 17                     | NA                                   | <100 ng/mL (15 of 17)<br>166-172 ng/mL range (2 of 17) | 20*                     | *                                     | *                |
| Male 10ppm Group               | 18                     | 87300 ng/mL                          | 68700-108000 ng/mL                                     | 20*                     | *                                     | *                |
| Female 10ppm Group             | 19                     | 16600 ng/mL                          | 6350-42800 ng/mL                                       | 20*                     | *                                     | *                |
| Male Offspring Control Group   | 3*                     | *                                    | *                                                      | 3*                      | *                                     | *                |
| Female Offspring Control Group | 7*                     | *                                    | *                                                      | 7*                      | *                                     | *                |
| Male Offspring 10ppm Group     | 3*                     | *                                    | *                                                      | 3*                      | *                                     | *                |
| Female Offspring 10ppm Group   | 7*                     | *                                    | *                                                      | 7*                      | *                                     | *                |

\* No outliers removed from this data set. Refer to Table 1 for results. For individual sample values see Tables 10 to 13 and Attachment B.

## PURPOSE

The purpose of the analytical phase of this study is to quantify  $C_8F_{17}SO_3^-$  in the liver and sera of both control and dosed mallards and in their offspring.

## REFERENCE SUBSTANCES

**Table 3. Reference Substances**

| REFERENCE SUBSTANCE                 | PFOS                     | PFDA                   |
|-------------------------------------|--------------------------|------------------------|
| Source                              | 3M Internal              | Oakwood Products, Inc. |
| Expiration Date                     | 08/31/2006               | 12/01/2010             |
| Storage Conditions                  | Frozen -20°C +/- 10°C    | Frozen -20°C +/- 10°C  |
| 3M Laboratory Identification Number | SD-018, Lot # 217        | SD-036                 |
| Physical Description                | White Crystalline Powder | White Solid            |
| Purity                              | 86.9%                    | 98%                    |

PFDA is nonadecafluorodecanoic acid, or Perfluorodecanoic acid,  $C_{10}F_{19}O_2H$  and is used solely to monitor for gross instrument failure and not as an internal standard for quantitation.

## SPECIMEN RECEIPT AND STORAGE

Mallard serum and liver samples were obtained from the sacrifice of the test animals upon the conclusion of the in-life phase of the study (#454-109) conducted at Wildlife International, Ltd. and sent to 3M Environmental Laboratory for analysis. The specimens were received at 3M Environmental Laboratory, assigned a laboratory tracking (LIMS) number (E01-1256, E01-1326, or E01-1367), and were stored at -20°C +/- 10°C until prepared for analysis.

## METHOD SUMMARY

The determination of  $C_8F_{17}SO_3^-$  concentration in the liver and sera was performed according to 3M Environmental Laboratory method ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices".

The sera samples were prepared by measuring a volume of serum and diluting with water in a 1/20 ratio. The liver samples were prepared by measuring a weight of liver and diluting with water to a 1/10 ratio prior to homogenizing. Five mL of acetonitrile was added to 1.0mL of the diluted sera or 1.0mL of the homogenate of liver and was shaken for 20 minutes. These samples were then centrifuged for 10 minutes; the supernatant was decanted into 40 mL of water and passed through a conditioned solid phase extraction (SPE) column. After drying the column, 2.0 mL of methanol was passed through the column to elute the analyte. The samples were analyzed for  $C_8F_{17}SO_3^-$  using HPLC-ES/MS/MS.

## PREPARATORY METHOD

- **ETS-8-231.1 "Solid Phase Extraction and Analysis of Fluorochemical Components from Biological matrices."** This method describes the extraction of target analytes from biological matrices, such as sera and liver, using solid phase extraction (SPE). Sera samples were diluted with Kandiyohi (commercially bottled) water 1/20, before extraction. Liver samples were diluted 1/10, approximately 1g of liver and 9ml of Kandiyohi water, and then homogenized. A 1.0mL aliquot of the diluted sera or homogenate of liver was extracted by adding 5.0mL of acetonitrile, shaking for 20 minutes, and centrifuging for 10 minutes. The supernatant was transferred to a clean tube, diluted with 40 ml of Kandiyohi water, and filtered through a conditioned C18 (SPE) column. Analytes were eluted off the column, and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

**Table 4. SPE Extraction Summary**

| STEP | SAMPLE PREPARATION                                                                                                                            |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | Measure volume of sera or weigh portion of liver                                                                                              |
| 2    | Dilute sera (1/20) and liver (1/10) with water                                                                                                |
| 3    | Mix sera/water or homogenize liver/water                                                                                                      |
| 4    | Aliquot 1.0ml of diluted/homogenized sample and add 5.0ml of ACN. Spike samples accordingly. Shake for 20 minutes, centrifuge for 10 minutes. |
| 5    | Dilute supernatant with 40 ml of water                                                                                                        |
| 6    | Filter sample through conditioned SPE C18 column.                                                                                             |
| 7    | Elute target analytes from the column with 2.0mL methanol (MeOH).                                                                             |

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**SAMPLE RECEIPT, PREPARATION AND ANALYSIS**

All core and liver samples were received frozen and remained frozen until thawed prior to