AR226-0202

COMPOSITE ANALYTICAL LABORATORY REPORT

ON THE

Quantitative Analysis of Fluorochemicals in Environmental Samples

REPORT NO. FACT GEN-021, GEN-024, GEN-030, GEN-033
LRN—W2491, W2845, W3197, E00-1386

ANALYTICAL STUDY INITIATION

GEN021: 08/25/99
GEN024: 10/12/99
GEN030: 12/13/99
GEN033: 03/14/00

TABLE OF CONTENTS

LIST OF TABLES

ANALYTICAL STUDY PERSONNEL AND CONTRIBUTORS

Analytical Chemistry Laboratories

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

Kris Hansen, Ph.D., *PAI*
Harold Johnson, *Analytical Chemist*
Lisa A. Clemen, *Analytical Chemist*
Mark Ellefson, *Analytical Chemist*

Sponsor

3M ET&SS
Building 2-3E-09
St. Paul, MN 55133

Dale Bacon, *Sponsor Representative*

INTRODUCTION

Purpose

The purpose of this composite report is to provide a summary of the analytical data collected for 3M studies Gen-021, Gen-024, Gen-030, and Gen-033. All of the samples included in these studies are tissue samples collected from fish, birds, mammals, and amphibians; Dr. John Geisy of Michigan State University has supplied all samples to 3M. These analyses have been conducted to support studies designed by Dr. Geisy.

The target analytes for these four studies were perfluorooctane sulfonate (PFOS; CAS# 2795-39-3), perfluorooctanesulfonylamide (PFOSA; CAS# 754-91-6), perfluorooctanoate (PFOA or POAA; CAS# 3825-26-1), and perfluorohexane sulfonate (PFHS; no CAS# available).

Due to the variety of matrices analyzed (with respect to both species and tissues), and due to evolving analytical methods, some analytical data quality objectives, such as the limit of quantitation (LOQ) were quite variable. A summary of the achieved LOQ (by specie, tissue and study number) is presented in Table 2 of this report. The stated data quality is based on results of data collection quality controls, sample prep quality controls, and recovery of target analytes from prepared matrix spike samples. More specific data quality objectives and parameters for these analytical studies are outlined later in this report.

Test and Control Article

The test articles for each study consisted of various tissues from various species and are listed below, in Table 1. For all studies, the control article consisted of rabbit sera and rabbit liver, as appropriate. Rabbit tissues were chosen as the control articles because previous studies have indicated very low levels of endogenous fluorochemicals in these matrices. Samples of the control articles were provided by the 3M Environmental Laboratory.

This report does not include details for the collection of the test articles; these details should be obtained from Dr. Geisy.

Table 1. Description of Samples, by Study

STUDY NUMBER	SERA/PLASMA/BLOOD	LIVER	OTHER
Gen-021	Cormorant Blood, Caspian Seal Blood, Sea Otter Blood	California Sea Lion, Elephant Seal, Harbor Seal, Gozzi, Mink, River Otter, Sea Otter, Turtle	Sea Otter Brain, Sea Otter Kidney
Gen-024	Albatross sera, Albatross plasma, Cormorant plasma, Herring Gull Plasma, Bald Eagle plasma, Cormorant blood, Herring Gull blood	Loon, Brown Pelican, Albatross	Albatross kidney, Cormorant yolk, Gull yolk
Gen-030	Northern Fur Seal blood (juvenile, sub-adult, adult), Polar Bear blood, Stellar Sea Lion blood	Northern Fur Seal, Polar Bear, Mink, Map Turtle, Terrapin, Tuna, Green Frog, Chinook Salmon, Lake Whitefish, Brown Trout	Carp body, Frog muscle, Frog body, Green Frog eggs, Lake Whitefish eggs, Brown Trout eggs, Carp muscle, Chinook Salmon muscle, Lake Whitefish muscle, Brown Trout muscle
Gen-033	None Submitted	Mink, Baikal Seal, Ganges Dolphin, Cormorant (adult and juvenile), Bottlenose Dolphin, Striped Dolphin, Weddell Seal, Swordfish, Tuna, Blacktailed Gull	None Submitted

Following analysis, extracts generated from these samples have been retained in cold storage.

Sample Collection and Analysis

Tissue samples were submitted to the Environmental Laboratory- Fluorine Analytical Chemistry Team by Kurunthachalan Kannan of Michigan State University. Details of the sample receipt are documented on the chain of custody forms located in appendices of this report.

Sample Receipt and Maintenance

Samples were received in the Environmental Lab cold or frozen on the following dates: Gen-021 (8/24/99), Gen-024 (10/11/99), Gen-030 (12/13/99), and Gen-033 (3/13/00). Sample receipt, identification, and chain of custody information are located in the study folder for each report; the folders are located in the 3M archives.

The sample extracts will be maintained in cold storage at the 3M Environmental Laboratory until the quality of preparation no longer affords preservation.

Chemical Characterization

The target analytes characterized in the samples include PFOS, PFOSA, PFOA, and PFHS. Procurement details of the reference standards used for analysis are summarized below.

Procurement

Table 2. Procurement Information for Reference Materials in the Analysis of Environmental Samples

Reference Material	Lot Number	Source
PFOS (potassium salt)	171	3M ICP/PCP Division
PFOSA	Gen-021: L-2353; all others: L-15709	3M Specialty Chemicals (R. Buckanin)
PFHS (potassium salt)	NB116638-16	3M Specialty Chemicals (G. Moore)
PFOA (ammonium salt)	Gen-024: 245; all others: commercial	Gen-024: 3M Specialty Chemicals; all others: Aldrich

Full chemical characterization studies, including purity and stability determination, have not been completed at this time. Upon completion of these studies, a report will be archived in the 3M Environmental Lab.

Method Summaries

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory. Copies of the actual methods used for these studies are located in attachment H.

PREPARATORY AND ANALYTICAL METHODS

- ETS-8-004.1, "Extraction of PFOS or Other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

  Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves. When sample size permitted, two matrix spikes were prepared in each tissue sample from each specie tested to provide some level of extraction efficiency determination.

  For some samples, less than 1 mL of sample was available. For these samples, the available volume was extracted according to the method with the exception that the final volume of extraction solvent was adjusted to match the volume of the initial sample.

  This method was used for the extraction of sera, plasma, and whole blood samples.

- ETS-8-005.1, "Analysis of PFOS or Other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

  Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves; as a result, all sample concentrations were adjusted by a factor of 1.25 to adjust for the removal of 4/5 of the MTBE from the extract. The factor is unnecessary when an extracted curve is used for evaluation.

- ETS-8-006, "Analysis of PFOS or Other Fluorochemical Compounds in Liver Extracts using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

  Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves. When sample size permitted, two matrix spikes were prepared in each tissue sample from each specie tested to provide some level of extraction efficiency determination.

  For some samples, less than 1 g of sample (as called for in the method) was available. For these samples, the available mass of tissue was extracted according to the method.

  Samples of kidney, brain, egg, and muscle were extracted by this method.

- ETS-8-007, "Extraction of PFOS or Other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

  Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves; as a result, all sample concentrations were adjusted by a factor of 1.25 to adjust for the removal of 4/5 of the MTBE from the extract. The factor is unnecessary when an extracted curve is used for evaluation.

  For Gen-030 and Gen-033 only: Due to the lack of excess test material for method development, all samples determined to contain greater than 0.015 µg/g of PFOS were subject to an additional PFOS verification process. Each sample was analyzed separately with respect to the 499 → 99 transition and the 499 → 80 transitions. The quantitative results

obtained from each transition analysis were compared. When these results agreed to with 30%, the identity of PFOS was confirmed (see Reference 1). Those samples where the identity of PFOS could not be confirmed are noted in the data table.

In Gen-021, no PFHS standard was available. In these samples, qualitative determination of PFHS was conducted based on reasonable retention time and a known PFHS transition (399 → 99).

Specific instrumental parameters are available in appendix I-L of this report, stored in the 3M Environmental Lab archives.

ANALYTICAL EQUIPMENT

For HPLC-Electrospray Tandem Mass Spectrometry:
Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column:
1×30 mm C18 Betasil™
Column temperature: 30 degrees C
Cycle Time: 10 minutes

Mobile phase components:
Component A: 2mM ammonium acetate
Component B: Methyl alcohol
Flow rate: 300 µL/min

Injection volume: 10 µL
Solvent Gradient:

Time (min)	%B
0	10
1	10
5.5	95
7.5	95
8	10
10	10

For Detection:
Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro Ultima Triple Quadrapole system or Micromass® API/Mass Spectrometer Quattro II Triple Quadrapole system
Acquisition Mode: MRM (refer to Table 3)
Software: Mass Lynx™ 3.3
Mode: Electrospray Negative
Source Block Temperature: 125-150°C
Source: Z-spray

Table 3. Ions Monitored in the Analyses of Extracts of Groundwater

TARGET ANALYTE	PRIMARY ION (amu)	PRODUCT ION (amu)
PFOS	499.0	80, 99*
PFOSA	498	78
PFOA	413	169
PFHS	399	99

* Indicates the ion used for quantitation

Refer to the analytical methods and equipment logs found in the raw data for details on the actual analytical equipment settings used in the present study. These settings may have varied somewhat during actual data collection. However, slight variations in the instrument settings will not adversely affect the quality of the data. Exact settings during all phases of data collection are recorded and presented in the appendix of this report.

DATA SUMMARY, ANALYSES, AND RESULTS

Summary of Quality Control Analyses Results

- **Standard Curves:** The coefficient of determination (r^2) for all 1/X weighted curves bracketing useable data was $\geq$ 0.982. High or low curve points may have been excluded to provide a better fit over the linear range appropriate to the data. High or low curve points were deactivated if the calculated concentration varied from the theoretical concentration by more than 30%. Acceptable data was evaluated versus a standard curve containing at least 5 points. All actions are acceptable and are documented in specific data sets. All standard curves used to evaluate quantitative data were acceptable.
- **Continuing Calibration Verifications:** On average, one calibration check is analyzed for every five samples. Acceptable data is bracketed by calibration checks quantitated to be within 30% of the theoretical value, evaluated at least every ten samples. All quantitative data is bracketed by acceptable calibration checks, as required.
- **Blanks:** Extraction blanks were compliant if no target analyte was detected above the limit of quantitation (LOQ) for a specific analyte. In this study, extraction blanks were often higher than low curve points. Because analyte levels in the blank are used to determine the LOQ, by default, all blanks were determined to be below the limit of quantitation for the compounds of interest.
- **Internal Standards:** Internal standard response was monitored in Gen-030 and Gen-033 only. Internal standard response was required to be within ±50% of the theoretical value. If samples showed an internal standard response that deviated more than ±50%, the samples were reanalyzed. If the deviant IS response was confirmed, the analyte data was reported, but noted in the data table.

Summary of Sample Results

- **GEN-021:**
  - PFOS was detected in at least one sample from the following matrices: California Sea Lion liver, Harbor Seal liver, Gozzi liver, Mink liver, River Otter liver, Turtle liver, Cormorant blood, Otter blood, and Caspian Seal blood.
  - PFOSA was tentatively identified in at least one sample from the following matrices: California Sea Lion liver, River Otter liver, Sea Otter liver, Sea Otter brain, and Otter blood.
  - PFOA was tentatively identified in at least one sample from the following matrices: California Sea Lion liver and Caspian Seal blood.
  - PFHS was tentatively identified in at least one sample from the following matrices: California Sea Lion liver, Gozzi liver, Mink liver, River Otter liver, Sea Otter liver, Turtle liver, Cormorant blood, Caspian Seal blood, and Otter blood.
- **GEN-024:**
  - PFOS was detected identified in at least one sample from the following matrices: Albatross plasma, Albatross sera, Cormorant plasma, Cormorant blood, Herring Gull plasma, Herring Gull blood, Bald Eagle plasma, Loon liver, Albatross liver, Brown Pelican liver, Albatross kidney, Cormorant yolk, and Gull yolk.
  - PFOSA was tentatively identified in at least one sample from the following matrices: Cormorant blood, Bald Eagle plasma, Loon liver, Brown Pelican liver, and Albatross liver.
  - PFOA was tentatively identified in at least one sample from the following matrices: Cormorant blood, Albatross liver, Cormorant yolk, and Gull yolk.
  - PFHS was tentatively identified in at least one sample from the following matrices: Herring Gull plasma and Bald Eagle plasma, Loon liver, Albatross liver, Brown Pelican liver, Albatross kidney, Cormorant yolk, and Gull yolk.
- **GEN-030:**
  - PFOS was detected in at least one sample from the following matrices: Polar Bear blood, Polar Bear liver, Mink liver, Northern Fur Seal liver, Map Turtle liver, Tuna liver, Green Frog liver, Chinook Salmon liver, Lake Whitefish liver, Brown Trout liver, Whole Carp, Frog muscle, Lake Whitefish eggs, Brown Trout eggs, Carp muscle, Chinook Salmon muscle, Lake Whitefish muscle, and Brown Trout muscle.
  - PFOSA was tentatively identified in at least one sample from the following matrices: Mink liver.
  - PFOA was not tentatively identified in any sample analyzed.
  - PFHS was not tentatively identified in any sample analyzed.
- **GEN-033:**
  - PFOS was detected in at least one sample from the following matrices: Mink liver, Baikal Seal liver, Cormorant liver, Bottle Nosed Dolphin liver, Ganges Dolphin liver, Striped Dolphin liver, Swordfish Liver, Tuna liver, and Black Tailed Gull liver.
  - PFOSA was tentatively identified in at least one sample from the following matrices: Mink liver, Cormorant liver, and Bottle Nosed Dolphin liver.

- PFOA was tentatively identified in at least one sample from the following matrices: Cormorant liver.
- PFHS was tentatively identified in at least one sample from the following matrices: Mink liver, Striped Dolphin liver, and Swordfish Liver.

Appendices contain data summary tables.

DATA QUALITY OBJECTIVES

No circumstances existed during the present study that would have affected the quality or integrity of the data. The data quality objectives (DQOs) followed during the present are indicated below.

- **Linearity:** The coefficient of determination (r^2) of the standard curve was equal to or greater than 0.985 with at least 5 active points using a linear regression curve with 1/x weighting.
- **Instrument Quantitation Limit (IQL):** The IQL is equal to the lowest acceptable standard in the calibration curve (acceptable standard is defined as a standard within 30% of the theoretical value). As this value is not useful in consideration of the sample data, the IQL was not specifically determined or stated for every study.
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within 30% of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks. The LOQ may vary due to the amount of sample available for analysis (particularly for samples extracted according to ETS-8-006) or to day-to-day variations in the analytical system. The ranges of LOQs for various tissues are listed in Table 4 (sera, plasma, and blood) and Table 5 (liver, kidney, muscle, egg, and brain).

Table 4. Range of LOQs for Sera, by Study

ANALYTE	GEN-021	GEN024	GEN-030	GEN-033
PFOS	0.0116 µg/mL	0.00116 µg/mL	0.0029-0.0579 µg/mL	NA
PFOSA	0.00625µg/mL	0.00626 µg/mL	0.000625 µg/mL	NA
PFHS	NA	0.00114 µg/mL	0.00114 µg/mL	NA
PFOA	0.00599 µg/mL	0.0299 µg/mL	0.00240-0.00958 µg/mL	NA

Table 5. Range of LOQs for Liver and Other Tissues, by Study

ANALYTE	GEN-021	GEN024	GEN-030	GEN-033
PFOS	0.0348 µg/g	0.0348 µg/g	0.00696-0.0696 µg/g	0.00696- 0.0694µg/g
PFOSA	0.0375 µg/g	0.00750 µg/g	0.0188 µg/g	0.0376 µg/g
PFHS	NA	0.00683 µg/g	0.00683-0.0342 µg/g	0.00683 µg/g
PFOA	0.0359 µg/g	0.180 µg/g	0.0180-0.0719 µg/g	0.00719-0.0718 µg/g

NA = not applicable

- **Duplicate/acceptable precision (extraction):** Spikes conducted on samples of control tissues were reproducible to within 15%
- **Quality Control Response:** A continuing calibration verification (CCV) was analyzed every 5–10 samples. Acceptable CCV response was within ±30% of the theoretical value. No more than 10 samples were analyzed between acceptable CCVs.
- **Spike/acceptable recoveries:** Due to the number of different matrices analyzed, there was great variability in spike recoveries. For any given matrix (specie and tissue), spike recoveries within 70–130% of the expected concentration indicate quantitative data (good to ±30%); spike recoveries between 50–150% indicate semi-quantitative data for that matrix (good to ±50%). Spike recoveries outside of this range indicate that sample data should be used for qualitative purposes only. Due to sample limitations, matrix spike studies were not conducted for all matrices. For PFOS analyses, sample data that is not supported by matrix spike studies should be considered for qualitative purposes only. Since no identity verification experiments were performed for PFOA, PFHS, and PFOSA, for these analytes, all analyses that are not supported by matrix spike studies should be considered to provide unconfirmed qualitative data only.
- **Use of Internal Standards:** Tetrahydro-perfluorooctane sulfonate (THPFOS) was spiked into the extracts post-extraction and used as an internal standard for samples in Gen-030 and Gen-033. For all samples in these studies, THPFOS levels were monitored to verify the analytical soundness of the data. THPFOS levels that were determined to be deviant from expected values by more than ±50% were reanalyzed. If the deviant THPFOS levels were confirmed, analyte levels were reported but are noted in the results table.
- **Use of confirmatory methods:** Given the selectivity of the analytical tool used (HPLC-ESMSMS) and lack of a viable alternative for analysis, no confirmatory methods were used.
- **Demonstration of specificity:** Specificity was demonstrated by chromatographic retention time (matched to standards to within 3%) and the response of at least one characteristic product ion arising from collisions of an analyte-specific parent ion.

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, matrix spike studies have been used as an indicator of data quality (see above). The validity of this assumption has not been verified by other techniques.

STATEMENT OF CONCLUSION

Under the conditions of the present studies, the presence of fluorochemicals was observed in the quantitative analysis of a selection of environmental matrices.

REFERENCES

1) "Acceptance Criteria for Ultratrace HPLC-Tandem Mass Spectrometry: Quantitative and Qualitative Determination of Sulfonylurea Herbicides in Soil"; Li, L.Y.; Campbell, D.A.; Bennet, P.K.; Henion, J.; *Anal. Chem.*, **68** (19), 3397-3404, 1996

ATTACHMENTS

- Attachment A: Gen-021 Sera/Plasma/Blood Results
- Attachment B: Gen-021 Liver/Miscellaneous Results
- Attachment C: Gen-024 Sera/Plasma/Blood Results
- Attachment D: Gen-024 Liver/Miscellaneous Results
- Attachment E: Gen-030 Sera/Plasma/Blood Results
- Attachment F: Gen-030 Liver/Miscellaneous Results
- Attachment G: Gen-033 Liver Results
- Attachment H: Analytical Methods
- Attachment I – L (additional bound document available in the 3M Environmental Lab archives): Analytical Details for Gen-021, Gen-024, Gen-030, and Gen-033

REPORT SIGNATURE

Kristen Hansen 5/10/00

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

Dale L. Bacon 5/10/2000

Dale L. Bacon, Sponsor Representative — *Date*