

**3M Environmental Laboratory- Fluorine Analytical Chemistry Team**

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**Screening of PFOS levels in Eagles and Albatross**

**Summary:**

Ten samples of bird sera of bird sera, five from juvenile bald eagle and five from Layson albatross, we received at the Environmental Lab. The eagle sera was collected from birds in Northern Minnesota (1 sample), the Upper Peninsula of Michigan (2 samples), and the Lower Peninsula of Michigan (2 samples). All five albatross samples were obtained from birds found at the Officer Club plot on Sand Island within the Midway Atoll in the north central Pacific Ocean.

The sera samples were extracted; extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high-pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a five-point solvent curve.

The samples of eagle sera were determined to contain 30-77 ppb perfluorooctane sulfonate (PFOS); no PFOS was detected in the albatross above the limit of quantitation (10 ppb). Specific results and analytical parameters are attached to this report.

The presence of PFOS in samples of eagle sera was verified using HPLC-ESMSMS. This technique provides an additional degree of certainty to the analyte identification by specifically monitoring fragments daughter ions ( $m/z=80, 99, 130, 180, 230$ ) characteristic of the PFOS primary ion ( $m/z = 499$ ).

The presence of low levels of PFOSA, perfluorooctanesulfonylamide, was also confirmed in each of the samples of eagle sera.

**Experimental summary:**

*Sample preparation: Ion-pairing extraction*

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for HPLC-ESMS analysis.

*HPLC: Characteristic retention times for PFOS*

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

*ESMS: Detection and monitoring of the molecular ion*

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is  $m/z = 499$  amu, corresponding to the mass of the anionic surfactant ( $C_8F_{17}SO_3^-$ ). This ion was monitored selectively to maximize sensitivity. A scan of  $m/z=100$  to 1210 (negative only) was also collected.

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Exhibit  
1504

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

*ES/MSMS: Confirmation of analyte identification*

Several samples in this set were analyzed by ES/MSMS to verify the identity of the PFOS analyte ion. ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a compound specific ion is selected. After selection, the selected ion is characterized further by smashing it apart with high-energy gas. As a result of the smashing, ionic fragments, characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the compound specific primary ion. This ion is smashed into other ions such as 80 amu (corresponding to  $\text{SO}_3^-$ ), 99 amu (corresponding to  $\text{FSO}_3^-$ ), 130 amu (corresponding to  $\text{CF}_2\text{SO}_3^-$ ), 180 amu ( $\text{C}_2\text{F}_4\text{SO}_3^-$ ), and 230 amu ( $\text{C}_3\text{F}_6\text{SO}_3^-$ ). If PFOS is present in the samples, each of these secondary fragments is detected at the detector.

**Quality control summary:**

Due to sample size limitations, duplicate extraction and matrix spike analysis were not carried out on these samples. Quantitation of PFOS levels was determined relative to a standard solvent curve instead of an extracted matrix curve. As the extraction efficiency of PFOS is unknown, these results should be considered semi-quantitative and of screening quality. The standard curve was analyzed twice, before and after the samples. One 250-ppb calibration check was analyzed after five samples; recovery was acceptable at 87%. Minimal quality control was associated with this analysis. If more precision is required, the extracts may be re-analyzed.

*Limit of Quantitation*

The low standard analyzed with these samples was 10 ppb; this is the limit of quantitation associated with the analysis.

**Instrumental specifics:**

*HPLC system*

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil  $\text{C}_{18}$

2 x 100 mm

5  $\mu\text{m}$  particle size

Flow rate: 300  $\mu\text{L}/\text{min}$ .

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:

45 to 90 %B in 9.50 mins.

Hold at 90 %B for 3.50 mins.

Return to 45 %B in 1.50 mins.

Hold at 45 %B for 3.5 mins.

Injection volume: 10  $\mu\text{L}$

Injections / sample: 1

*Electrospray mass spectrometer*

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software

Cone voltages: -60v

Mode: electrospray negative

Source temperature: 115  $^{\circ}\text{C}$ .

Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar

Ions: 499, 413, 369

Electrode: cross-flow

*Electrospray MSMS*

Micromass Quattro II API Mass Spectrometer, "Madeline"

MassLynx 3.1 Software

Cone voltages: -60v

Collision gas energy: 45 V

Mode: electrospray negative

Source temperature: 115 °C.

Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar

Primary Ion: 499; 498

Daughter ions: 80, 99, 130, 180, 230; 78

Electrode: Z-spray

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# Fluorine Analytical Chemistry Team

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Samples Received: prior to 1/98  
Samples Extracted: 4/8/98  
Samples Analyzed: 4/9/98

Analysis by: ESMS (-)  
Calibration Curve Rang 10-250 ppb  
r<sup>2</sup> of Calibration Curve: 0.995  
LOQ/LOD<sup>1</sup>: 10 ppb

## Analysis of PFOS in sera of eagles and albatross

Sample ID	Age and Gender	Location	[PFOS] ppb	Sample ID	Age	[PFOS] ppb
Eagle 1: 629-39339	163 days, F	LP, MI	30	Alb 1: 1137-90880	6 yrs	b.d.l.
Eagle 2: 629-39332	228 days, F	LP, MI	34	Alb 2: 1247-60080	0 yrs	b.l.q.
Eagle 3: 629-31740	unkn, unkn	UP, MI	77	Alb 3: 1367-32659	8 yrs	b.d.l.
Eagle 4: 629-31748	unkn, unkn	UP, MI	31	Alb 4: 1067-19156	15 yrs	b.d.l.
Eagle 5: 629-14593	82 day, M	Voyagers, MN	34	Alb 5: 1247-60095	0 yrs	b.l.q.

## Analysis of PFOSA in sera of eagles and albatross

PFOSA was identified, but not quantitated, in all samples of eagle sera.  
PFOSA was not identified in the albatross sera.

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1 - LOD/LOQ = Limit of Detection/Limit of Quantitation

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