Screening of PFOS Levels in Eagles
And Albatross
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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.

ES/MSMS: Confirmation of analyte identification

Several samples in this set were analyzed by ESMSMS 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 SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). 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 C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/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 µL

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 °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 voltage: -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

LOCATIONS AND DATA FROM AVIAN BLOOD PLASMA SAMPLES

TO:

3M Corporation
Minneapolis, Minnesota

BY:

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July 18, 1994

Avian blood plasma samples collected from two species, the bald eagle (*Haliaeetus leucocephalus*) and the Laysan albatross (*Diomedea immutabilis*) were sent to 3M for analysis for $C_8F_{17}SO_2$ and $C_8F_{17}CO_2$. Additional data for these blood samples follows:

Laysan albatrosses

All samples of Laysan albatrosses were collected from the Officers Club plot on Sand Island within the Midway Atoll in the north-central Pacific Ocean. Table 1 provides additional data on the birds sampled:

Table 1. Band number, year bird was banded, age at collection of blood sample, blood sample number, and date blood was collected for 5 Laysan albatrosses from Midway Atoll, north-central Pacific Ocean.

Band Number	Year Banded	Collection Age	Sample Number	Date Collected
1247-60080	1993	0	LAC 08093	18 May 1993
1247-60095	1993	0	LAC 09593	18 May 1993
1367-32659	1985	8	LA 616	13 Dec 1992
1137-90880	1987	6	LA 607	13 Dec 1992
1067-19156	1978	15	LA 617	13 Dec 1992

No other data on organochlorine pesticides from these birds is currently available, however, a seperate split sample of each has been archived.

Bald eagles

All samples of bald eagles were collected within the Midwest, with one from Minnesota, and two each from the upper and lower peninsulas of Michigan (Figure 1). Table 2 provides additonal data on the birds sampled:

Table 2. Band number, location, county, region, date of blood sample collection, sex, weight, and age in days of 5 nestling bald eagles from Minnesota and Michigan.

Band No.	Location	County	Region	Date Collected	Sex	Weight	Age in Days
629-31740	Devil's Lake	Menominee	U. Peninsula, MI	1989	Unknown	Unknown	Unknown
629-31748	Slate River Falls	Baraga	U. Peninsula, MI	1989	Unknown	Unknown	Unknown
629-14593	Steve's Island	St. Louis	Voyageurs NP, MN	17 June 1992	Male	2.7 kg	82
629-39332	Caulkins Creek	Otsego	L. Peninsula, MI	3 June 1993	Female	4.85 kg	228
629-39339	Pickerel Channel	Emmet	L. Peninsula, MI	5 June 1993	Female	4.25 kg	163