UNTARGETED SCREENING OF SAN FRANCISCO BAY HARBOR SEALS FOR NEW ORGANOHALOGEN POLLUTANTS

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Introduction

The measurement of contamination in biological samples almost always uses a predetermined list of target compounds. This is done to address regulatory mandates or due to the availability of calibration standards for the compounds of interest. Recently, it has been shown that many compounds produced by society that are not routinely monitored have physical properties similar to well known bioaccumulative pollutants (1, 2). Therefore biological samples potentially harbor compounds other than routinely monitored pollutants such as PCBs, DDTs, organochlorine pesticides and many brominated flame retardants.

Recent advances in analytical instrumentation facilitate the identification of environmental contaminants in complex mixtures. In particular, commercial instruments combining two dimensional gas chromatography with time of flight mass spectrometry (GCxGC TOF/MS) have allowed for the successful identification of numerous non-routine analytes in environmental samples(3). The GCxGC technique allows for several thousand individual compounds to be resolved in a single run while the TOF portion allows for full electron impact ionization spectra to be acquired.

The goal of this study was to use the GCxGC TOF/MS instrument to screen harbor seal samples from San Francisco Bay, California for pollutants that may originate within the bay watershed. Results from this work will help to inform water managers in the San Francisco Bay region about the scope of contamination in the bay as reflected in organisms feeding at a trophic level that is similar to humans.

Materials and methods

Samples were collected from six dead stranded or euthanized harbor seals (*Phoca vitulina*) from a variety of locations around the San Francisco Bay (Table 1). Samples were collected during necropsy, wrapped in aluminum foil and then stored at -80 ^{o}C until analysis. Blood samples were collected into red-top serum tubes, allowed to coagulate and then centrifuged. Serum was transferred to 5 mL cryovials and frozen at -80 ^{o}C. In addition, liver and blubber samples were obtained from a harbor seal collected from Alaska to serve as a negative control for the study. This animal was harvested by subsistence hunters, sampled using established protocols developed by the NIST Marine Environmental Specimen Bank (ESB) with samples stored in the NIST ESB at -160 ^{o}C.

Table 1: Samples collected for this study.

ID	Sex	Age	Location	Tissues
HS-2118	M	Juvenile	Fitzgerald Marine Reserve	Blubber, Serum
CAS-RB-6050	F	Adult	Baker Beach	Blubber
HS-2120	M	Adult	Angel Island	Blubber, Serum
HS-2028	F	Adult	Point Reyes National Sea Shore	Blubber, Liver
HS-2122	M	Adult	Fitzgerald Marine Reserve	Serum
HS-2125	F	Adult	Richmond Marina	Blubber, Serum
HS-10	M	Adult	Kachamak Bay, Alaska	Blubber, Liver

Prior to processing, blubber that was in contact with foil was trimmed away and the sample was minced, mixed with sodium sulfate and extracted by pressurized fluid extraction. Following extraction, lipid was removed from extracts by first passing through a 600 mm x 25 mm PlGel size exclusion column (Varian Inc.) using dichloromethane as the mobile phase, concentrated again and then passed through a second size exclusion column (300 mm x 7.5 mm PlGel). Extracts were then fractionated into a non polar, intermediate polarity and a higher polarity fraction using a silica/alumina column. Fraction 1 contained mainly PCBs, fraction 2 contained primarily organochlorine pesticides and PBDEs, and fraction 3 contained dieldrin, and other more polar compounds.

Serum samples (5 g) were processed using a method similar to given elsewhere that allows for the separation of samples into a neutral and a phenolic fraction(4). Briefly, samples were acidified with 1.25 mL HCl, amended with 5 mL formic acid and then extracted twice with 5 mL 25% (volume fraction) dichloromethane:hexane by focused microwave extraction (CEM Discoverer). Extracts were concentrated and phenolic compounds isolated by back extraction with base. The phenolic fraction was then re-acidified and re-extracted with the dichloromethane:hexane solution. Extracts were concentrated and then derivatized using MSTFA.

Samples were reduced in volume to 150 μL and then 3 μL was introduced onto a Leco Pegasus GC x GC TOF/MS by splitless injection. The columns used were a 40 m x 180 μm x 0.20 μm Rtx-5 (Restek) in the first dimension and a 1 m x 100 μm x 0.10 μm Rxi-17 (Restek) in the second dimension. Acquired spectra were deconvoluted and compared to the NIST Mass Spectral Library using the LECO Chroma TOF software. Spectra of unknown compounds were noted and identified if possible using expert judgment and comparison to literature spectra.

Results and discussion

To date, the fractionated blubber samples have been analyzed by the GC x GC TOF/MS. Legacy POPs tend to dominate the contaminant profiles, however several unusual compounds have been tentatively identified in the samples. For example, identification has been made of several possible DDT-related byproducts or metabolites. These include DDMU which was a major compound found in the first fraction of HS-2125—a seal collected inside San Francisco Bay near the city of Richmond. Also tentatively identified were 1 chloro 2,2, bischlorophenyl ethane, dicofol and mitotane. Other compounds tentatively identified included chlorinated PAHs, current-use pesticides, PCB methyl sulfones, toxaphene congeners, as well as several brominated and fluorinated compounds. Work continues on these samples to determine the identity of compounds that did not match spectra in the NIST Mass Spectral Library.

The next steps in the sample analysis will include confirmation of compound identity by the analysis of pure reference compounds, determining the contribution of unusual compounds relative to more routinely determined POPs, and the analysis of serum samples. Results from this work will be compared to those currently being generated on cetacean samples from Southern California.

References:

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