SEARCHING FOR NEW ORGANOHALOGEN POLLUTANTS IN SAN FRANCISCO BAY HARBOR SEALS

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Introduction

Contaminant measurement in biological samples is almost always done in a targeted fashion using a set list of target compounds and methods specifically geared towards measurement or detection of those compounds. Consequently, the number of routinely targeted GC-able compounds with POP-like characteristics is probably <200 (1). Recent theoretical studies based on physical property and usage data suggest that the potential number of POP-like compounds in samples that can be determined by routine GC/MS methods may be several times this number (1, 2). Because of practical issues such as time and labor, environmental samples are rarely screened to look for these additional compounds.

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/MS allows for full electron impact ionization spectra to be acquired.

The goal of this study was to use GCxGC TOF/MS to screen harbor seal samples from San Francisco Bay, California for pollutants that may originate within the bay watershed. This paper is an update of work presented previously to now include the analysis of harbor seals from both inside and outside of San Francisco Bay. 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 in 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.

Prior to processing, blubber that was in contact with foil was trimmed away, and the sample was minced, mixed with sodium sulfate, and extracted using pressurized fluid extraction. Following extraction, lipid was removed from extracts by first passing through a 600 mm x 25 mm PlGel size exclusion column (Agilent Inc., Santa Clara, California) using dichloromethane (DCM) 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.

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

Serum samples (5 g) were fractioned into neutral and a phenolic phases using a method similar to given elsewhere (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) DCM:hexane by focused microwave extraction (CEM Discoverer, city, state). Extracts were concentrated, and phenolic compounds were isolated by back extraction with base. The phenolic fraction was then re-acidified and re-extracted with the DCM:hexane solution. Extract will be first cleaned up by size exclusion chromatography (SEC) as described above and then derivatized using MSTFA prior to analysis. Liver samples will be extracted by pressured fluid extraction using DCM, cleaned up by SEC, and then derivatized with MSTFA prior to analysis.

Samples were reduced to 150 μL, and 3 μL was introduced onto a Leco (St. Joseph, Michigan) 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, State College, Pennsylvania) 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. Samples were initially run using the NIST 2007 mass spectral database but were re-analyzed against the more recent NIST 2011 spectral library containing approximately 213,000 unique compounds. Spectra of non-legacy POPs or metabolites were noted and identified based on the library match, by fragmentation rules or by comparison to literature spectra.

Results and Discussion

To date, the fractionated blubber samples have been analyzed by the GC x GC TOF/MS. Numerous non-legacy POP compounds were identified in samples using the NIST library; however several compounds produced spectra that were not present in the NIST Mass Spectral Library (Figure 1). About 20 non-POP compounds were detected in each seal. The majority of compounds found in seals from different locations had good (>600) matches to spectra in the NIST Mass Spectral Library. The seal from Baker Beach, which is inside of San Francisco Bay, had the largest number of new compounds (32). Compounds detected included current use pesticides, halo acetate esters, C8 to C12 chloro and fluoroalkanes, as well as numerous chloro and fluoro ringed compounds. Work is currently underway to verify the presence of the compounds in the samples by acquiring the individual compounds and reanalyzing the samples by GC/MS. If the presence of compounds is confirmed, they will be quantified in the samples. Work is also in progress on identifying compounds that produced spectra that were not in the NIST library and analyzing seal blood and liver for polar compounds.

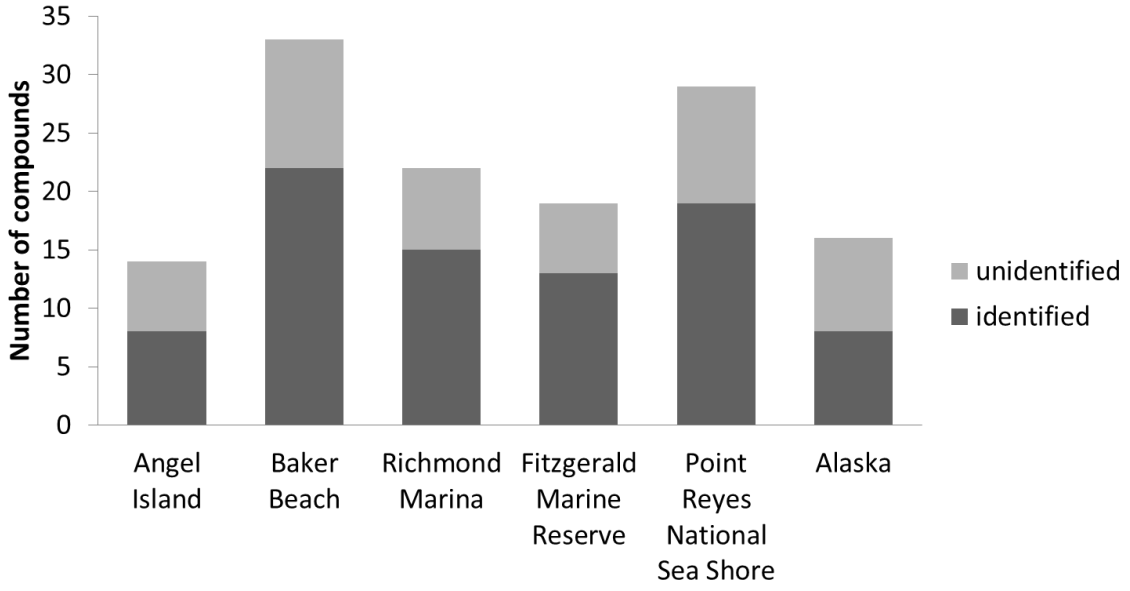

Figure 1: Number of non-POP spectra per site that were identified by the NIST Mass Spectral Library or were of compounds not in the library.

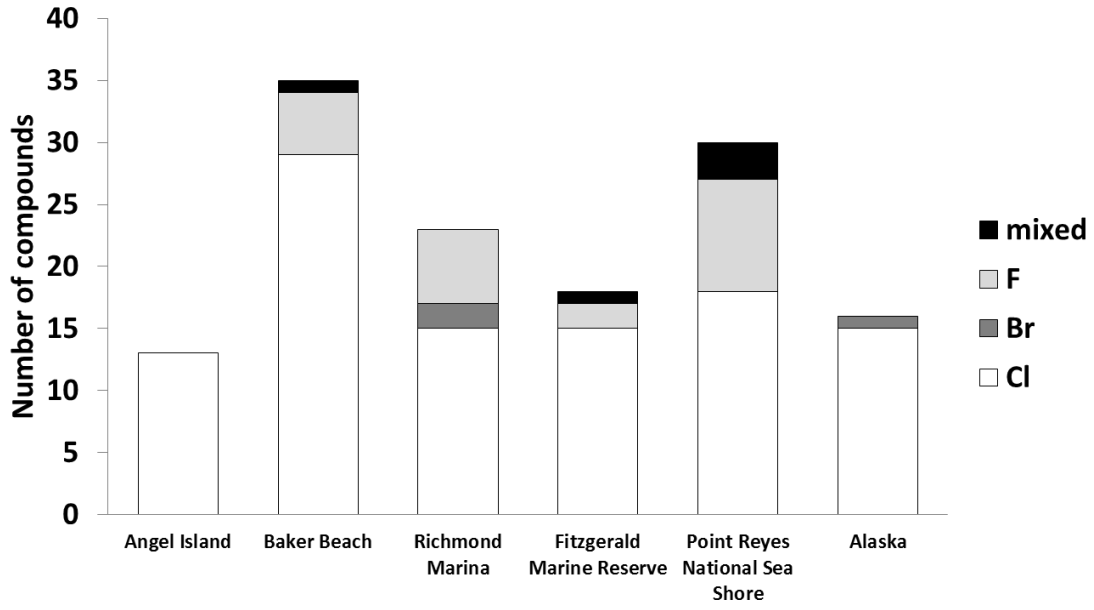

Figure 2: Number of non-POP spectra per site broken down by halogenation type.

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