Screening of San Francisco Bay mussels and harbor seals for anthropogenic pollutants: Year 2

Estimated Cost: $70,000 for 2011 (Total project cost is $125,000 including $55,000 from RMP for 2010)
Oversight Group: Emerging Contaminants Work Group
Proposed by: John Kucklick, National Institute of Standards and Technology (NIST), and Susan Klosterhaus, SFEI

Background

Though monitoring efforts by the RMP have generally targeted chemicals with the highest known potential to impact beneficial uses of the Bay, there are thousands of current-use and phased-out chemicals which have not yet been monitored due to limited resources and analytical method availability, as well as limited knowledge of the chemicals used in consumer products, their potential for release to the environment, and their fate in aquatic systems. An alternative to the traditional analytical approach, which targets a specific chemical or chemical class in a sample, is to take advantage of recent advancements in analytical methods and instrumentation that screen samples using a non target approach and then work to identify the compounds detected. In 2002 the RMP conducted a similar exercise in which full scan chromatograms from Bay water, sediment, and bivalve samples were screened for previously unmonitored chemical contaminants (Oros 2003). This work was extremely valuable to the RMP because it identified several chemicals of concern present in San Francisco Bay. More advanced analytical approaches are now available, however, that provide an improved ability to identify chemicals in very complex mixtures. An example of this new approach is the use of GC x GC/time of flight (TOF) mass spectrometry, which has been used successfully by NIST to screen human samples for many different types of anthropogenic compounds. Though more labor intensive, this type of screening approach would provide a more thorough method for assessing chemicals which have the greatest potential to adversely affect the Bay foodweb.

Applicable RMP Management Question (MQ) and Study Objective

MQ1. Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?

A: Which chemicals have the potential to impact humans and aquatic life and should be monitored?

B: What potential for impacts on humans and aquatic life exists due to contaminants in the Estuary ecosystem?

The objective of this study is to screen Bay tissue samples for chemicals that are not on a target list but are predicted to be in the environment due to a set of intrinsic chemical properties that would likely lead to a high degree of environmental persistence and the potential to accumulate in organisms (MQ1A). If possible, a second objective is to quantify the newly identified

compounds in tissue samples and to provide exposure data to complement existing toxicological information (MQ1B).

Study Outcomes

The primary outcome of this work will be a list of previously unmonitored chemicals present in Bay tissue samples. Where possible, and with resources permitting, chemicals concentrations in the tissue samples will be provided using commercial chemical standards.

Approach: Year 1 (2010)

The project is divided into a two year effort. The first year is aimed at (1) developing protocols for the collection of samples from dead, stranded harbor seals and mussels for analysis; (2) developing analytical methods for the screening of harbor seal liver and blubber for non targeted compounds; (3) applying the methods to the screening of harbor seal blubber and liver from dead, stranded harbor seals for chemical contaminants.

Tissue samples from mussels and harbor seals will be used to characterize chemical exposure to the entire Bay foodweb. Samples from harbor seals, which represent exposure to the entire Bay foodweb, will be collected by personnel from The Marine Mammal Center (MMC) using protocols developed by NIST. Because tissues may accumulate different types of pollutants, samples of blubber, liver will be collected. Only samples collected from dead stranded seals or seals that died at the MMC will be used because of the large amount of sample required. **No vertebrates will be killed or harassed for this work.** Samples of mussels will be analyzed to characterize exposure to chemicals that are metabolized by higher trophic level species. Mussels that are deployed as part of the 2010 RMP Status and Trends monitoring will be used. Rather than analyzing samples from several individual seals or mussel deployment sites, analyses of harbor seal tissues and mussels will be conducted on a limited number of pooled samples so that a sufficient amount of material is available for a variety of analytical approaches. Samples of mussels and seals collected from reference locations will also be analyzed so that chemical exposure specific to Bay processes can be determined. Reference site mussel samples will be collected from Bodega Bay, CA, coinciding with RMP monitoring in 2010. Seal samples archived in the NIST Marine Mammal Tissue Bank collected from remote regions will be used as control samples.

The primary method for sample analysis will be two dimensional gas chromatography (GCxGC) time-of-flight (TOF) mass spectrometry (GCxGC TOF/MS). The instrument being used for this work, the LECO Pegasus 4D GCxGC TOF/MS (LECO St. Joseph, MI), has a unique capability to separate chemicals of interest from a very complex mixture and identify the chemicals using comprehensive mass spectral libraries. Both fat soluble (non-polar) and more polar chemical contaminants will be screened for in the samples. More polar compounds will be first derivatized to products that are amenable to GC analysis.

Approach: Year 2 (2011)

The effort in the second year will build on the year 1 effort. The major tasks for year 2 are as follows:

1. **Develop methodology for the analysis of mussel tissues by GCxGC TOF/MS.** A modification of the methods developed in year 1 will be required for the analysis of mussel tissue. Chemical contaminants are typically present in mussel tissue in lower concentrations than marine mammal tissue hence a larger mass of tissue (several times that of harbor seal tissue) will be needed for analysis. A new cleanup scheme and possibly a fractionation scheme will need to be developed.

2. **Apply methods to the screening of deployed mussel samples from San Francisco Bay.** Samples from at least two sites will be analyzed and compared to pre-deployed mussels. To compare sites, sample peaks will be classified and the results compared using either LECO software (Statistical Compare) or third party statistical software to allow for factor analysis to be conducted.

3. **Build library of new chemicals to augment existing NIST/EPA/NIH Mass Spectral Library.** The list of non target chemicals put forth by Howard and Muir (2010) will be used as guide for the production of an electron impact spectra library for his project. This will be a "user library" and will be generated by comparing the Howard and Muir list to the NIST library to determine which compounds are not present in the NIST library. Missing chemicals will be purchased if a commercial source is available. These compounds will be acquired, put into solution and evaluated to see if they are amenable for GC analysis. If so, they will be analyzed by GC/MS on a 5% phenyl dimethyl polysiloxane column and a retention index will be calculated relative to a solution of alkanes. Furthermore, a spectrum for the compound will be derived either by GC-MS quadrupole or GC-MS-TOF. The spectrum will be added to the "user library." Once developed, previously run samples (harbor seals, mussel tissues) will be screened against the library to look for compounds in the "user library."

4. **Quantify non target compounds.** If non target compounds are found in samples, we will attempt to acquire the compound from a commercial source and produce a method for estimating the concentration of the compound in the sample. The analysis will likely be by quadrupole MS and not GC-MS-TOF, as the former is more sensitive and selective.

   Note: During the first year of the project we discussed the possibility of using liquid chromatography quadrupole TOF/MS as an analytical platform for the analysis of polar samples. After further consideration, the use of this technique is outside the scope of the project unless a partner can be identified to assist with method development. The technique will require intensive work with a Q-TOF instrument using different sources and optimizing conditions for analysis. Focusing on this element in year 2 may jeopardize the completion of the above tasks hence this task will not be done in year 2.

Proposed Budget

	2010 Estimated Costs	2011 Estimated Costs
Sampling supplies	$2,500	$2,500
Analytical standards	$5,000	$5,000
Other laboratory supplies	$2,500	$2,500
Analytical costs, data management, analysis, and reporting	$40,000	$40,000
SFEI project management	$5,000	$5,000
SFEI data management		$7,000
SFEI reporting		$8,000
Total Funded in 2010 (Year 1)	**$55,000**	
Total Requested for 2011 (Year 2)		**$70,000***

***SCCWRP will be contributing ~$50,000 in additional funding and resources**

NIST Assistance in Kind

1. John Kucklick will need to travel to NIST Gaithersburg to perform analysis of samples on the LECO Pegasus instrument. There will be approximately 4-6 of these trips during each year with each trip lasting roughly three days. The cost of this travel is will be covered by NIST as these trips will also include other duties. These trips typically cost about $1500 each.

2. The actual time spent on the project may exceed the funds provided by SFEI for salaries. The salaries provided by SFEI ($40K/year) will cover about 2 months of John Kucklick's salary and NIST overhead per year. We anticipate that NIST will spend more on salaries than is currently budgeted. NIST justifies this in that if new compounds are found, they will attempt to assign concentration values to these compounds in selected Standard Reference Materials.

3. Travel to scientific meetings to present results will be covered by NIST. Typically, John Kucklick attends 2 to 3 scientific meetings per year. The average trip cost is approximately $2500. The information on the project presented at the meetings will be in addition to information presented on other NIST measurement activities.

4. John Kucklick, Michele Schantz, and Elizabeth MaGaw were sent to LECO training to learn the software. The cost of the training with travel was about $5000/person. The training of John Kucklick on the LECO instrument was necessary for the completion of this project.

References

Howard, PH and Muir DCG. 2010. Identifying new persistent and bioaccumulative organics among chemicals in commerce. Environ Sci Technol. 44:2277–2285.

Oros et al. 2003. Surveillance for previously unmonitored organic contaminants in the San Francisco Estuary. Marine Poll Bull. 46:1102-1110.