RMP Study Proposal: Effects directed identification of estrogenic chemicals in sediment from San Francisco Bay

Estimated Cost: $25,000
Oversight Group: Exposure and Effect Workgroup (EEWG)
Proposed by: Alvine Mehinto and Keith Maruya (SCCWRP)

Proposed Deliverables and Timeline

Deliverable	Due Date
Confirmation of estrogenic responses in sediment samples from the Bay (Task 1)	CSD + 6 months
Concentrations of known estrogens in sediment samples (Task 2)	CSD + 9 months
Relative contribution of known estrogens to estrogenic equivalents (Task 3)	CSD + 12 months

Background

Current monitoring programs focus on individual contaminants based on chemistry and toxicity data that can be decades old. However, thousands of new chemicals are introduced each year, and some may find their way into the aquatic environment via treated wastewater effluents and urban/stormwater runoff, generating a complex mixture of contaminants that may impact aquatic and human health. Chemicals for which little information is known, aka contaminants of emerging concern or CECs, can thus missed by standard analytical and toxicity methods. To modernize our approach for monitoring of ambient environments, an effects-directed monitoring framework that features cell-based bioanalytical screening tools (**Fig. 1**) was recently endorsed by the State Water Board as part of their statewide CEC initiative for protecting beneficial uses of recycled and receiving waters. At the heart of this enhanced framework are cell bioassays, which provide an integrative measure of chemicals acting via a common mode of action and offer a rapid and efficient way to screen for both known and unknown chemicals that can be linked to a toxic effect. The response of a specific bioassay then "directs" what target analytes are likely causative candidates, and also informs what further biological testing endpoints would be most informative in determining and/or confirm a higher order effect. Similar frameworks have been embraced beyond California. For example, it is a guiding principle for efforts by an European Union consortium of water quality experts to deal with CECs (Altenburger et al. 2015). In California, initial results from pilot studies on freshwater and marine habitats have demonstrated the promise of bioanalytical tools to enhance and expand the scope of current monitoring practices (Crago et al. 2016, Mehinto et al. 2017, Maruya et al. 2018).

The proposed study will apply Tier I of the framework to 1) confirm the estrogenic bioactivity in samples from the Bay using the estrogen receptor (ER-α) cell assay, and 2) identify bioactive chemicals using targeted chemical analysis. Sample selection will be made in collaboration with Phase I-II ER-linkage PI Dr. Nancy Denslow and RMP personnel.

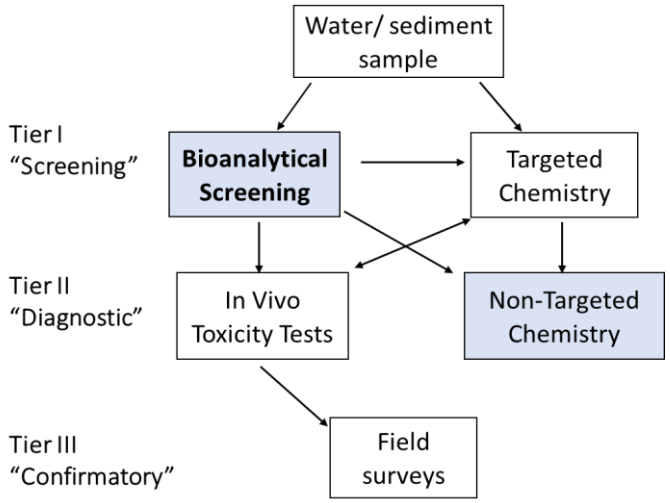

Figure 1. Effects directed monitoring framework that includes bioanalytical tools (cell assays) to screen for broader range of chemicals.

Study Objectives and Applicable RMP Management Questions

The goal of this study is to assess the contribution of known chemicals that exert estrogenic activity in sediment samples from San Francisco Bay. This study will further demonstrate the utility of the tiered framework in prioritizing sites for additional investigation, and what analyses are most appropriate based on the screening results. Specific objectives that address key management questions are shown in **Table 1**.

Table 1. Study objectives and questions relevant to RMP management questions.

Management Question	Study Objective	Example Information Application
1) Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?	Confirm estrogenic activity in sediment samples from the Bay.	Bioanalytical data will help prioritize sites where further analytical and toxicity testing is needed.
	Identify known estrogenic chemicals and estimate their contribution to the observed cell assay response	Comparison of chemical and bioanalytical data will provide an understanding of the contribution of known estrogenic chemicals in samples showing elevated estrogenicity.

Approach

Task 1 – Bioscreening analyses of sediment samples. Archived sediment samples or ASE extracts of sediment samples (10 g sample equivalents, n=5 - 6 sites) that are suspected of elevated estrogenicity will be shipped to SCCWRP, where they will be processed for bioanalytical screening

using previously published protocols (Maruya et al. 2018). Splits of sample extracts (5 g sediment sample equivalent) will be solvent exchanged to DMSO and analyzed using the GeneBLAzer ER-α assay standardized by the PIs (Mehinto et al. 2015). The collected data will be validated against criteria for calibration, blank, DMSO control, cytotoxicity (cell viability) and sample dose response. Bioscreening responses will be expressed as bioassay equivalent concentration (BEQ, in ng/g) relative to the reference chemical 17β-estradiol. Estimated limits of detection will be ~0.05 ng/g dry weight.

Task 2 – Contribution of measured chemicals to the ER bioscreening responses. The remaining split of sample extracts will be exchanged to methanol or hexane and analyzed using mass spectrometry coupled to liquid chromatography (LC-MS) and/or gas chromatography (GC-MS). The list of candidate analytes is shown in **Table 2** below. Instrumental methods for analysis of individual chemicals will be selected and/or optimized to meet minimum reporting limits (RLs) recommended by the State's Expert Panel (Anderson et al. 2012). Analytical data will be validated against criteria for instrument calibration, analysis of blanks, and matrix spikes.

Table 2. List of candidate estrogenic compounds

Name	Estimated relative potency	Chemical class
17β-estradiol (E2)	1.0	hormone
Estrone (E1)	0.1	hormone
Bisphenol A	10e-2	industrial surfactant
4-Nonylphenol	10e-3	industrial surfactant
PBDE-47, -99	10e-4	flame retardant
DDTs	10e-4	organochlorine pesticide
Bifenthrin	?	pesticide
Oxybenzone	?	sunscreen

Task 3 – Comparative analyses of bioanalytical and analytical chemistry equivalents. For each sample, the chemical equivalent concentration (CEQ, ng/g) will be calculated as the sum of the product of individual analyte concentration and their relative estrogenic potency (**Table 2**). The CEQs will then be directly comparable to BEQs determined in Task 1. The percent contribution of each known estrogenic compound, as well as their total summed contribution, will then be estimated by comparing CEQ to BEQ.

Reporting

At the end of the proposed one-year study (CSD + 12 months), a final report will be produced to synthesize our findings regarding the levels of ER bioactivity and the relative contribution of known estrogenic compounds to the measured estrogenicity.

Budget

The following budget represents estimated costs for this proposed special study (**Table 3**).

Table 3. Proposed Budget.

Expense	Estimated Hours	Estimated Cost
Labor Project staff	256	$22,000
Direct Costs Supplies		$3,000
Grand Total	256	$25,000

Budget Justification

Labor. PI Mehinto will oversee all aspects of the project (including reporting) as the cell assay analyses. Co-PI Maruya will oversee the chemical analysis and will be assisted by Dr. Bowen Du of SCCWRP. The estimated cost for ER bioscreening and targeted analyses is $4,000 per sample.

Direct costs. Supplies include cell assay kits and reagents, high purity solvents, spiked chemicals, glassware, and other GC-MS and LC-MS consumables.

Field Costs are not included in this budget. Instead, we propose to use frozen archived sediment (sample extracts preferred) from previous RMP sampling efforts.

References

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Crago J, Xu EG, Kupsco A, Jia F, Mehinto AC, Lao W, Maruya KA, Gan J, Schlenk D. 2016. Trophic transfer and effects of DDT in male hornyhead turbot (Pleuronichthys verticalis) from Palos Verdes Superfund site, CA (USA) and comparisons to field monitoring. Environmental Pollution 213: 940-948.

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