Special Study Proposal: Non-targeted Analysis of Water-soluble Compounds in Ambient Bay Water and Wastewater to Identify Emerging Contaminants

Summary: Non-targeted analysis, a key element of the RMP's CEC strategy and recent state CEC guidance, can help to provide a measure of assurance that the RMP is not missing unexpected yet potentially harmful contaminants simply because of failures to predict their occurrence based on use or exposure prioritization criteria. The RMP has completed non-targeted analysis of fat-soluble compounds in bivalve tissue and seal blubber, but another major class of chemicals, water-soluble (polar) organic contaminants, has not been evaluated. This proposed study will fill this data gap by conducting a broad screen of ambient Bay water (passive and grab samples) and wastewater (composite samples) for polar organic compounds such as: detergents and other surfactants, pesticide and pharmaceutical breakdown products, and plastic additives. This type of non-targeted study will lay the foundation for future targeted CEC monitoring by helping to identify new potential contaminants of concern without *a priori* knowledge of their occurrence.

Estimated Cost: $52,000

Oversight Group: ECWG

Proposed by: Rebecca Sutton (SFEI), Lee Ferguson (Duke University)

PROPOSED DELIVERABLES AND TIMELINE

Deliverable	***Due Date***
Task 1. Project Management (write and manage sub-contracts, track budgets)	Winter 2015 – Spring 2017
Task 2. Develop detailed sampling plan	Spring 2016
Task 3. Field Sampling	Summer 2016
Task 4. Lab analysis	Fall 2016
Task 5. QA/QC and contaminant risk review	Winter 2016
Task 6. Draft report and fact sheet	3/31/2017
Task 7. Final report and fact sheet	6/30/2017

Background

The RMP has developed a pro-active emerging contaminants program, and conducts policy-relevant monitoring via Special Studies to help identify and address problematic, unregulated contaminants before they cause significant harm to the Bay. The RMP has established a unified emerging contaminants strategy (Sutton et al. 2013) with three elements: 1) targeted chemical monitoring and relative risk evaluation using a tiered risk and management action framework; 2) review of the scientific literature and other aquatic monitoring programs as a means of identifying new emerging contaminants for which no Bay occurrence data yet exist; and 3) non-targeted analysis to create inventories of unanticipated contaminants in tissues, sediment, or water that can be used to direct targeted chemical monitoring or toxicity identification evaluations.

Recently completed state guidance on emerging contaminants in aquatic ecosystems echoes many aspects of the RMP strategy (Dodder et al. 2015). In particular, non-targeted analysis plays a key role in the comprehensive CEC management framework (see pg 40 Dodder et al. 2015). Non-targeted analysis is an essential means of assuring focus on the contaminants with greatest potential to impact an ecosystem, by seeking to remove a "knowledge bias" on previously identified problem chemicals. One form of non-targeted analysis specifically recommended by the state guidance document is development of bioanalytical tools; the RMP has commissioned one such study from scientists at the Southern California Coastal Water Resources Project (SCCWRP) and the University of Florida, which is nearing completion.

Other non-targeted methods highlighted by the state guidance are those "designed to screen for new or unexpected contaminants; i.e., unknown CECs" (pg 29, Dodder et al. 2015). The RMP, in collaboration with the National Institute of Standards and Technology (NIST), recently completed a non-targeted analysis of Bay harbor seal blubber and mussel tissues, which focused on persistent, fat-soluble (nonpolar), chlorine and bromine-rich chemicals (Sutton and Kucklick 2015). This investigation brought to light five contaminants not previously identified in Bay wildlife, and for which toxicity is largely unknown. However, most of the Bay chemical contamination was from high priority contaminants that the RMP already monitors, or closely related compounds. More polar, water-soluble organic compounds were not covered by this recent non-targeted tissue analysis. Polar organic contaminants are of significant concern to the water quality of the San Francisco Bay, as they may exhibit meso-range transport, be difficult to remove through treatment strategies, and cause effects on wildlife through endocrine disruption and other mechanisms. The following monitoring proposal would fill this important data gap. Detergents, plastics, and medications are examples of products that can contain such water-soluble, polar organic contaminants.

Study Objectives and Applicable RMP Management Questions

Given the increased burden on the RMP from multiple areas of interest to stakeholders, it is imperative that the RMP focus on those CECs that are the highest priority. Traditional, targeted contaminant monitoring focuses on specific lists of chemicals already identified as potentially problematic through either expert judgement, anticipation of high toxicity, use-based prioritization, or other *a priori* methods. Through non-targeted monitoring, we can provide a measure of assurance that the RMP is not missing unexpected, potentially harmful contaminants in the Bay water simply because of failures to predict their occurrence based on use or exposure prioritization criteria.

Non-targeted analysis is an essential element of the RMP's CEC Strategy (Sutton et al. 2013). The RMP recently completed a non-targeted analysis focusing on fat-soluble (hydrophobic) compounds in tissue samples (Sutton and Kucklick 2015). This study identified a few unexpected contaminants, but the good news is that the majority of chemical contamination was from high priority contaminants that the RMP already monitors, or closely related compounds.

The current proposal is to use non-targeted analysis to scan for more water-soluble (polar) organic contaminants in the Bay (grab and passive samples) as well as in treated wastewater effluent, which is anticipated to be a major and important source of these compounds to the Bay. A special study on water-soluble contaminants would provide data on those contaminants that were not part of the study of fat-soluble compounds, essentially filling a major data gap in characterizing possible contaminant chemistries in the Bay. This would make the Bay the first ecosystem to be studied via non-targeted methods for both water- and fat-soluble contaminants.

Using the proposed non-targeted analytical strategies outlined below, Dr. Lee Ferguson at Duke University has tentatively identified 52 water-soluble compounds from seven functional classes including pharmaceuticals, flame retardants, pesticides, and consumer product chemicals in wastewater effluent discharged to surface waters in central North Carolina (Ferguson et al., in prep). Nine of these compounds have not been detected in the environment previously. Examples include ZPCA (a transformation product of the sleep-aide zolpidem [Ambien]), raltegravir (HIV treatment), and Atorvastatin lactone (transformation product of atorvastatin [Lipitor]).

Should a non-targeted study of the Bay identify unexpected water-soluble contaminants such as these, the information could indicate a need for a follow-up RMP Special Study designed to specifically assess the new "candidate" CECs on a quantitative basis. It could also point to ecotoxicity data gaps or suggest new management priorities. Thus, we anticipate that positive identifications resulting from the proposed study would be potentially very high in impact.

In contrast, because of the comprehensive nature of the non-targeted methods proposed herein, should few unexpected contaminants be identified, the RMP would then have considerable evidence that existing polar organic CEC monitoring is indeed already focusing on the highest priority contaminants for the Bay.

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?	Identify water-soluble contaminants not yet characterized by targeted monitoring efforts. Evaluate future monitoring needs and toxicity data gaps.	Have previous targeted monitoring efforts focused on contaminants with the highest relative risk to the Bay? Which newly identified contaminants merit further monitoring?
2) What are the concentrations and masses of contaminants in the Estuary and its segments? 2.1 Are there particular regions of concern?	Initial comparison of specific sites influenced by different pathways (agriculture-dominated river, stormwater, wastewater) with respect to detection.	Are there regional or pathway-related differences in the presence of newly identified contaminants?
3) What are the sources, pathways, loadings, and processes leading to contaminant-related impacts in the Estuary? 3.1. Which sources, pathways, etc. contribute most to impacts?	Gain an unbiased inventory of water-soluble (polar) organic contaminants in key, high-volume wastewater discharges. Allow an initial exploration of differences between secondary and advanced wastewater treatment with respect to contaminant removal. Investigate the influence of stormwater and river discharges on contaminants.	Are any newly identified contaminants in wastewater also detected in the Bay? Do differences in detection for wastewater and ambient Bay water suggest persistence, degradation, or additional pathways (e.g., stormwater) for specific contaminants? Do sites influenced by stormwater or agricultural discharges show different patterns of contamination?
4) Have the concentrations, masses, and associated impacts of contaminants in the Estuary increased or decreased? 4.1. What are the effects of management actions on concentrations and mass?	Establish a baseline for future studies.	
5) What are the projected concentrations, masses, and associated impacts of contaminants in the Estuary?	Identify sources of newly identified contaminants to evaluate effects of current management actions on potential discharges and project trends with likely changes in use and wastewater treatment technology.	Are relevant management actions having the intended effect? Will newly identified contaminants suggest the need for additional or different management actions?

This monitoring effort would most directly address questions 1, 2, and 3, identifying water-soluble contaminants not yet characterized by targeted monitoring efforts, and providing information useful to initial comparisons with respect to contaminants in sites influenced by different pathways (rural river, stormwater, wastewater) and discharged from secondary versus more advanced water treatment facilities. This proposal does not include an examination of potential sources of newly identified contaminants. Such a study could be completed in future years and would provide information useful in addressing questions 4 and 5, concerning likely past and future trends.

In addition, the study will directly and explicitly address the emerging contaminants priority question: What emerging contaminants have the potential to adversely impact beneficial uses of the Bay?

Approach

Ambient Bay Water Sampling

Bay water sampling will be conducted using both grab samples and passive sampling devices called Polar Organic Chemical Integrative Sampler (POCIS, see Figure 1; Environmental Sampling Technologies, St. Joseph, MO). Grab samples have the advantage of providing analytical data for polar organic contaminants that is less convoluted by sampling bias and more representative of actual water conditions, but also has the disadvantage of providing only a snapshot of the pollutants in a particular location at a particular time, rather than more broadly integrated information. Passive samplers, while semi-quantitative at best, can be used to provide an integrated assessment of the pollutants present (or absent) in a location over a longer time span (e.g., 28 days). The lengthy time of deployment also means contaminants at trace levels are more likely to be detected, provided they have favorable uptake dynamics into the sampler.

Three POCIS canisters will be deployed, one at each of three sites: 1) a site at or near the mouth of the Napa River, probing potential agricultural and pesticide influences (spring or summer 2016 deployment, timed to coincide with pesticide applications); 2) a site influenced by stormwater discharges, San Leandro Bay for example (winter or spring 2016 wet season deployment); and 3) a site in the Lower South Bay influenced by WWTP discharges (summer 2016, when WWTP-derived contaminant levels are often highest due to low river inflow and POTW-system infiltration/inflow). Site selection and deployment will be conducted in collaboration with nutrients researchers at SFEI and elsewhere, as they have deployed and are monitoring and servicing a number of moored nutrient sensors throughout the Bay. Each POCIS holder will be deployed for a maximum of 28 days. The POCIS samplers contain a solid phase sorbent (Waters Oasis HLB) that is widely used for sampling a large range of water-soluble organic chemicals from water.

Each POCIS canister will contain three POCIS samplers to provide triplicate measurements at each location; however, only two of the three will be analyzed using RMP funds. The third POCIS from each site will be kept in reserve and would be analyzed at no additional cost to the RMP if unusual variability is observed in the first two POCIS. A total of seven POCIS samples will be analyzed using RMP funds, two from each of three sites and a single blank.

Grab samples (4 L glass) will be collected in the same locations on deployment and retrieval of the POCIS, to provide a snapshot, non-integrated picture of polar organic contaminant loadings in water at each location. A total of eight grab samples will be analyzed, two from each of three sites, along with a field duplicate and a blank. Each grab sample will be shipped (on ice) to Dr. Ferguson's laboratory at Duke University (NC) after collection for immediate extraction and analysis as described below.

Figure 1. Deployment holder featuring one POCIS holder containing three POCIS. Dimensions 15 cm high x 16 cm wide. Environmental Sampling Technologies, est-lab.com

Effluent Sampling

Effluent samples provide essential information on a major pathway for polar organic contaminants to enter the Bay. The state guidance on CECs directs agencies to include sampling wastewater treatment plant (WWTP) effluent when screening for emerging contaminants (Dodder et al. 2015). Compounds that persist in treated effluent at significant levels are likely to be polar and water-soluble rather than fat-soluble, making the focus of this proposed study particularly useful to the wastewater community.

24-hour composite samples of WWTP effluent (4 L glass) voluntarily provided by two to four high volume Bay Area dischargers will be characterized. Participants will include a WWTP employing secondary treatment, as well as one using more advanced measures. Sampling will occur in the summer of 2016, when inflow and infiltration are insignificant. A total of five samples will be analyzed, up to four effluent samples and a blank. As with water samples described above, these will be shipped (on ice) to Dr. Ferguson's laboratory at Duke University (NC) immediately after collection for extraction and analysis as described below.

One local discharger has agreed to participate and contribute in-kind services for sample collection but is not specifically named here, as dischargers will have the option to keep their identities confidential in subsequent reporting of the data. Measurements for each discharger will be reported individually.

Analytical Methods

Non-targeted analysis of 20 samples will be conducted by Dr. Ferguson's Lab (Duke University) using cutting-edge Orbitrap liquid chromatography high resolution mass spectrometry (LC-HRMS). POCIS samples (shipped directly from SFEI to Duke University) will be processed as recommended by the vendor (e.g., elution with methanol/MTBE prior to evaporation and reconstitution in HPLC-MS mobile phase). Water samples will be immediately filtered (< 0.45μm GF/F) for particle removal and processed for solid-phase extraction using an automated SPE system (Dionex Autotrace 280) fitted with custom layered-bed extraction cartridges (containing cation exchange, anion exchange, hydrophobic, and amphiphilic resins) and eluted with sequential basic and acidic methanol/MTBE solvent systems prior to combination and concentration of the extracts.

Extracts will be separated using UHPLC (Thermo Hypersil Gold column, 1.9 μm particle size, 2.1 x 100 cm) over a 70 minute gradient prior to introduction into the mass spectrometer. The LTQ-Orbitrap MS/MS will be operated at 100,000 resolution to achieve < 2 ppm mass accuracy across the mass range of interest. Sample extracts will be spiked with internal mass calibration/quantitation standards (chosen from a set of stable-isotope labeled compounds available in the PI's laboratory) immediately prior to injection. Ionization will be performed by either electrospray in either positive or negative polarity mode, depending on the analyte. High resolution detection of analytes in MS mode will be performed by the Orbitrap analyzer, while simultaneous data-dependent MS/MS will be performed in the LTQ Velos module before the Orbitrap. Ions for MS/MS analysis (10 per Orbitrap scan) will be dynamically chosen on a per-scan basis, with priority given to accurate mass values corresponding to compounds in compiled "suspect" lists (already compiled based on production volume, toxicity, and/or literature reports), with secondary priority given to "non-target" analytes in order of decreasing intensity. These MS/MS data will provide important information to aid in identification of non-target analytes.

Data generated through these approaches will be applied to both commercially-available (ThermoFisher Scientific TraceFinder, Compound Discoverer, and MassFrontier) and custom-written processing software designed to aid in identifying polar organic compounds based on HRMS/MS data. Final validation of tentative identities will be made based on authentic standard match wherever possible.

The Ferguson laboratory has extensive experience in use of accurate mass MS and MS/MS for identifying non-target compounds in complex mixtures (Benotti et al. 2003; Eichhorn et al. 2005; Cui et al. 2009; Stapleton et al. 2011), and this strategy has proved successful for identifying emerging contaminants in wastewater (preliminary work as described above), as well as in coastal surface waters impacted by water reuse activities (e.g., on Kiawah Island, SC). These new identifications include several micropollutants that have not, to our knowledge, been previously reported to occur in environmental media such as wastewater or surface water. Dr. Ferguson's laboratory was chosen for this work because it is uniquely qualified and experienced to undertake the experiments described. The Ferguson Lab has also agreed to contribute up to $10,000 of in-kind services to the project (e.g., technician and PI effort) because of the high priority and potential for high-impact results to be generated from the work.

Budget

The following budget represents estimated costs for this proposal. Efforts and costs can be adjusted by changing the number of matrices explored or the number of samples evaluated.

Table 2. Budget summary.

Expense	Estimated Hours	Estimated Cost ($)
Labor		
Project Staff	135	19000
Senior Management Review	21	4200
Project Management	0*	
Contract Management	0*	
Data Technical Services	0	
GIS Services	8	650
Creative Services	25	2000
IT Services	0	0
Communications	0	0
Operations	0	0
Subtotal		
Subcontracts		
Name of contractor		
Lee Ferguson		20000
Linda W.		3000
Direct Costs		
Equipment		2000
Travel		400
Printing		250
Shipping		500
Other		
		52000

*Not needed because core RMP funding provides this service.

Budget Justification

Field Costs

Details concerning passive sampling equipment:

POCIS: $65/each x 3/site x 3 sites + 1 blank = $260

POCIS holder (rental): $220 x 3 sites = $660

Total POCIS equipment costs ~$1,000

Reporting Costs

Preparation of a draft manuscript for publication in a peer-reviewed journal would be the responsibility of the analytical partner, and will require relatively little RMP staff time. RMP staff will produce a 2-page fact sheet to describe the results and their implications for RMP stakeholders and the general public. This fact sheet would be a companion to one recently completed for non-targeted analysis of fat-soluble compounds (Sutton and Kucklick 2015).

Laboratory Costs

The RMP can benefit from a significant discount in laboratory costs currently available due to outside funding of the Ferguson Lab. This discount will *not* be available in the future. For non-targeted analyses conducted in 2016, the estimated cost is $1,000/sample; in the future, the cost will be at least $1,500/sample.

Data Management Costs

No data management is needed for this proposed project, as it is not targeted, analyte-specific analysis.

Reporting

Deliverables will include: a) a draft manuscript[1] that serves as an RMP technical report due by 3/31/2017; b) a plain language RMP fact sheet describing the results and their implications due by 3/31/2017; and c) additions to other RMP publications such as the Pulse.

[1] The draft manuscript will be distributed by email, not published on the website, so as to not jeopardize publication of the manuscript in a peer-reviewed journal.

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