MONITORING ALTERNATIVE FLAME RETARDANTS IN SF BAY WATER, SEDIMENT, AND BIOTA

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ESTIMATED COST: $137,000
OVERSIGHT GROUP: Emerging Contaminants Work Group (ECWG)

PROPOSED DELIVERABLES AND TIMELINE

Deliverable	Due Date
Task 1. Collection of water samples	Jul – Aug 2013
Task 2. Project Management (write and manage sub-contracts, track budgets)	Jan – Dec 2014
Task 3. Collection of sediment, bivalve, and seal samples	Summer 2014
Task 4. Laboratory analysis	Fall 2014
Task 5. QA/QC and data management	Dec 2014
Task 6. Draft and final manuscript	Mar 2015

Background

Reduced use of polybrominated diphenyl ether (PBDE) flame retardants following management actions (bans and phase-outs) has already led to declines in PBDE contamination in Bay biota over the last decade. However, to meet California's strict flammability regulations, product manufacturers must substitute other flame retardant chemicals in place of PBDEs. Contamination with these alternative (non-PBDE) flame retardants may be on the rise in the San Francisco Bay ecosystem, and this potential increase in exposure could pose risks to aquatic life and humans.

Previous RMP studies have identified a number of alternative flame retardants in San Francisco Bay sediment and biota (Table 1). Non-PBDE flame retardants detected in Bay wildlife were hexabromocyclododecane (HBCD), Dechlorane Plus (DP), pentabromoethylbenzene (PBEB), bis(2,4,6 tribromophenoxy)ethane (BTBPE), tris(1-chloropropyl)phosphate (TCPP), tris(2-chloroethyl)phosphate (TCEP), tris(2-butoxyethyl)phosphate (TBEP), and triphenylphosphate (TPhP). Brominated flame retardants that were analyzed but not detected in Bay samples were EH-TBB and BEH-TEBP (the brominated components of the PentaBDE replacement commercial mixture, Firemaster 550, possibly not detected due to methodological issues), decabromodiphenylethane (DBDPE, a Deca-BDE replacement), and hexabromobenzene (HBB). The organophosphates TDCPP, TCPP, and TPhP have been detected in Bay sediments at estimated concentrations that are comparable to the PBDE and PCB concentrations in the same samples. A pilot study also detected TCEP, TCPP, and TBEP in cormorant eggs, with a total concentration of up to 200 ng/g lipid weight (Chen unpublished data). Several other organophosphate flame retardants were analyzed in cormorant eggs but were not detected. It is suspected that some of these organophosphate flame retardants (e.g. TDCPP) may have been metabolized into mono or di-esters (Chu et al. 2011).

Table 1. Summary of previous data and proposed measurements for alternative flame retardants in San Francisco Bay. + indicates previous detection; - indicates previous non-detection; indicates prioritized compound in current proposal.

	Previous RMP Analyses						Proposal				
Alternative Flame Retardants	Water*	Sediment	Mussels	Sport Fish	Bird Eggs	Seals	Water	Sediment	Mussels	Seals (2014)	Seals (archive)
HBCD		+	+	+	+	+	not prioritized for testing; Tier II (Low Concern)				
Dechlorane Plus (DP)		+	+	+	+	+					
PBEB		+	+	-	-	+					
DBDPE		-									
BTBPE		+	-	-	-	-					
HBB		-	-	-	-	-					
BEH-TBP**		-	-		-						
EH-TBB**		-	-	-	-	-					
TDCPP or Chlorinated Tris	+	+	-		-						
TCPP	+	+	-		+						
TPhP	+	+	+		-						
TCEP	+				+						
TBP	+				-						
TBEP	-				+						
TEHP	-				-						
TPrP					-						
Tris(2,3-dibromopropyl) phosphate, Tricresyl phosphate, 2-Ethylhexyl-diphenyl phosphate, Tris(2-bromo-4-methylphenyl) phosphate					-						
V6											
EBTEBPI											
DBE-DBCH or TBECH											
Dechlorane 602											
Organophosphate metabolites											

* Qualitative detections via passive water samplers (POCIS) indicating presence or absence in Bay waters; ** Possibly not detected due to methodological issues.

In addition to RMP work, passive water samplers (POCIS) deployed by SFEI as part of the NOAA Mussel Watch Contaminants of Emerging Concern (CECs) Early Warning Network: California Pilot Project indicated the presence of several organophosphate flame retardants in San Francisco Bay waters: TCPP, TDCPP, TCEP, tributyl phosphate (TBP), and TPhP (Table 1). TBEP and tris(2-ethylhexyl)phosphate (TEHP) were not detected.

Few alternative flame retardants have been well characterized as to aquatic toxicity, and most are thus assigned to Tier I (Possible Concern) within the San Francisco Bay risk and management action framework, with the exception of HBCD (Tier II: Low Concern).

This proposal outlines a study to monitor five to fifteen alternative flame retardants in water, sediment, bivalves and seals (Table 1). The physical, chemical, and biological properties of each target analyte were used to determine the most appropriate matrix for monitoring. The result is a research plan optimized to detect those widely used flame retardant contaminants entering the Bay and potentially posing risks to wildlife. Measurements made as part of this study will be compared to previous measurements, where possible, to evaluate variation in contamination with time.

Applicable RMP Objectives and Management Questions

This study will address the following RMP Objectives and Management Questions:

MQ.1 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?

MQ.2 What are the concentrations and masses of contaminants in the Estuary and its segments?

- A: Do pollutant spatial patterns and long-term trends indicate particular regions of concern?

<u>Detailed Outline of Study Objectives</u>

1. Describe the distribution and trends of pollutants concentrations in the Estuary.

- This study will provide some of the first data to determine the distribution of concentrations of non-PBDE flame retardant compounds in the Estuary and to place these concentrations in context with concentrations observed in other locations.

2. Project future contaminant status and trends using current understanding of ecosystem processes and human activities.

- Comparison with earlier screens for alternative flame retardants can provide an initial indication as to whether levels of certain flame retardants are increasing or decreasing in Bay wildlife. Analysis of archived seal blubber samples can also be used to identify trends for a limited number of analytes.

3. Measure pollution exposure and effects on selected parts of the Estuary ecosystem (including humans).

- Flame retardants are considered a class of emerging contaminants. As such, it is important that we determine their concentrations in the Bay and biota to evaluate whether management actions are needed.
- Determining the concentrations of flame retardants in upper trophic level is important for assessing both ecological and human health risks.

4. Compare monitoring information to relevant benchmarks, such as TMDL targets, tissue screening levels, water quality objectives, and sediment quality objects

- The concentrations detected in this study will be compared to known threshold effect levels, where possible.

Relationship of the Study to the ECWG Priority Question and Current RMP List of Emerging Contaminants

The Emerging Contaminant Workgroup is focused on answering the following question: "What emerging contaminants have the greatest potential to adversely impact beneficial uses in the Bay?"

Following management actions to eliminate production and use of PBDEs, manufacturers must use alternative (non-PBDE) flame retardants in many products. Because use of these alternatives is expected to increase, it is essential to identify those flame retardants of highest concern for the Bay and conduct preliminary monitoring studies to assess contamination levels. Alternative flame retardants are included as a priority class of compounds in the ECWG five-year plan.

Approach

Flame retardants have diverse physical, chemical, and biological properties, such that the likelihood of detection in different Bay matrices varies widely by compound. Evaluation of flame retardant properties led to creation of prioritized lists of flame retardants appropriate to monitor in each Bay matrix: water, sediment, bivalves, and harbor seals. Emphasis was placed on re-examining those flame retardants examined during previous screenings (e.g., Klosterhaus et al. 2012) to allow for initial comparisons of measured levels over time. Additional flame retardants suggested for study include those identified by Howard and Muir as candidates for environmental monitoring due to persistence and bioaccumulative potential (Howard and Muir 2010), as well as those identified by USEPA as replacements for DecaBDE, which is being phased out in 2013 (USEPA 2012).

Flame Retardant Selection by Matrix

Water

Many widely used organophosphate flame retardants are relatively hydrophilic and have been detected in surface water samples. Passive water samplers (POCIS) deployed by SFEI as part of the NOAA Mussel Watch Contaminants of Emerging Concern (CECs) Early Warning Network: California Pilot Project indicated the presence of several organophosphate flame retardants in San Francisco Bay waters: TCPP, TDCPP, TCEP, TBP, and TPhP. TBEP and TEHP were not detected.

Aquatic toxicity thresholds, where available, are frequently reported as ambient water concentrations (e.g., LC_{50}s or EC_{50}s); thus, Bay water monitoring may indicate the relative level of risk posed by those organophosphate flame retardants for which aquatic toxicity data exist. A review of the literature indicates aquatic toxicity thresholds are available for TCPP, TDCPP, TCEP, TBP, and TPhP; the majority of data are derived for freshwater rather than marine conditions.

The RMP will collect water samples in July – August 2013 as part of the Annual Status and Trends monitoring effort. As part of this study proposal, water samples could be collected for organophosphate flame retardant analysis. Ten water samples could be collected, three in the Lower South Bay, three in the South Bay, two in the Central Bay (near Oakland and San Francisco, respectively), and one each in San Pablo and Suisun Bays. Sample locations will mimic those used by Klosterhaus et al. (2012) for previous screening of flame retardants in sediment samples (Figure 1). A replicate sample will be collected at a South or Lower South Bay site, for a total of eleven samples.

Analysis of water samples will be conducted by Dr. Da Chen of Southern Illinois University using a highly sensitive liquid chromatography–electrospray ionization(+)-triple quadrupole mass spectrometry (LC–ESI(+)-QQQ-MS/MS) based analysis method (Chen et al. 2012a; Chu et al. 2011). Limits of detection are typically in the range of 0.1 ppb.

While this method is capable of detecting a wide range of organophosphate flame retardants, those of particular interest to the RMP are: 1) TCPP; 2) TDCPP; 3) TCEP; 4) TBP; 5) TPhP; 6) V6 (a newly identified compound (Fang et al. 2013); and 7) tripropyl phosphate (TPrP, an organophosphate flame retardant with high water solubility). Analysis is expected to cost around $500 per sample.

These measurements would constitute the first ever quantitative testing for alternative flame retardants in San Francisco Bay water samples.

Sediment

Many flame retardants are halogenated and hydrophobic, and therefore tend to partition to sediment, making this a good matrix for determining which flame retardants can contaminate the Bay. A number of brominated flame retardants have already been detected in Bay sediment samples (Klosterhaus et al. 2012). In addition, organophosphate flame retardants TDCPP, TCPP, and TPhP have been detected in Bay sediment samples at levels comparable to PBDEs, suggesting periodic monitoring to assess trends in concentration with time would be appropriate. Finally, toxicity thresholds, where available, may be provided in units of concentration in sediment or sediment organic carbon, allowing evaluation of the level of risk associated with those flame retardant for which aquatic toxicity data are available. A review of the literature indicates aquatic toxicity thresholds are available for TDCPP and DBDPE; the majority of data are derived for freshwater rather than marine conditions.

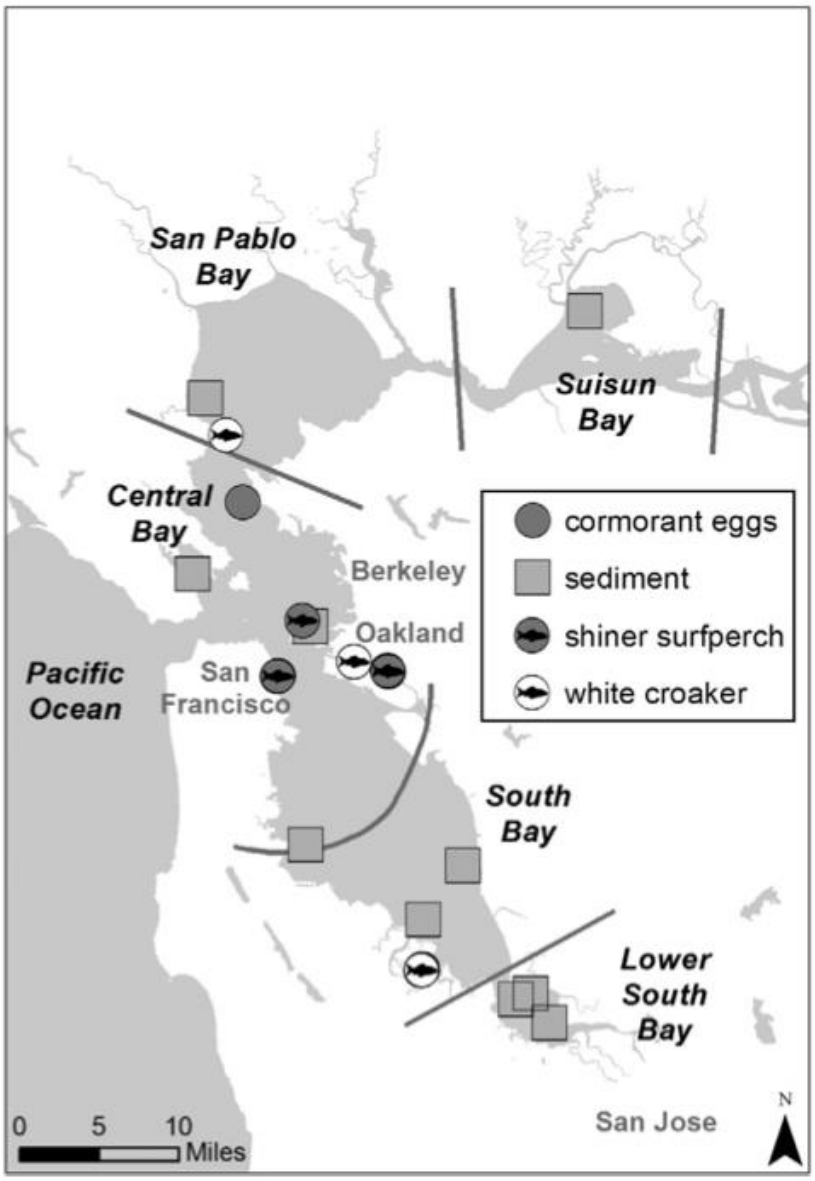

Figure 1. Sediment sites examined by Klosterhaus et al. (2012) will guide collection of water and sediment samples in the proposed study.

The RMP will collect sediment samples in the summer of 2014 as part of the Status and Trend monitoring. Additional samples could be collected for flame retardant analysis. Ten sediment samples will be collected, three in the Lower South Bay, three in the South Bay, two in the Central Bay (near Oakland and San Francisco, respectively), and one each in San Pablo and Suisun Bays. Sample locations will mimic those used by Klosterhaus et al. (2012) for previous screening of flame retardants in sediment samples (Figure 1). A replicate sample will be collected at a South or Lower South Bay site, for a total of eleven samples.

Analysis of sediment samples will be conducted by Dr. Da Chen of Southern Illinois University. Sediment will be examined for phosphates using a highly sensitive liquid chromatography–

electrospray ionization(+)-triple quadrupole mass spectrometry (LC–ESI(+)-QQQ-MS/MS) based analysis method (Chu et al. 2011; Chen et al. 2012a). Limits of detection are typically in the range of 0.1 ppb. Organophosphate flame retardants prioritized for analysis include those previously detected in sediment (TDCPP, TCPP, and TPhP) and a compound newly identified in consumer goods (V6).

Sediment will also be examined for halogenated, hydrophobic alternative flame retardants using GC-ECNI-MS (Chen et al. 2012b, c). Limits of detection vary with the compound, ranging from roughly 0.1 to 1 ppb. Hydrophobic flame retardants prioritized for quantification include: EH-TBB and BEH-TEBP (the brominated components of the PentaBDE replacement commercial mixture, Firemaster 550), DBDPE, BTBPE, PBEB, HBB, Dechlorane Plus, ethylene bis-tetrabromophthalimide (EBTEBPI), 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (DBE-DBCH or TBECH) and Dechlorane 602.

EBTEBPI was identified by Howard and Muir (2010) as a likely candidate for monitoring based on predictions of its persistence and bioaccumulative potential. It is a high production volume chemical and an alternative for DecaBDE, which is being phased out this year. DBE-DBCH was also identified by Howard and Muir (2010) as a likely candidate for monitoring based on predictions of its persistence and bioaccumulative potential. It has been detected in Arctic wildlife (Tomy et al. 2008) and causes reproductive toxicity in American kestrels (Marteinson et al. 2012b). DBE-DBCH has been identified as an androgen agonist (Larsson et al. 2006), and was found to modulate the thyroid axis in these fish at environmentally relevant concentrations (Park et al. 2011b). Finally, Dechlorane 602 has been detected in a number of aquatic species in other parts of the world (Peng et al. 2012; Shen et al. 2012).

Bivalves

Bivalves, being stationary filter feeders, can concentrate significant levels of contaminants in their tissues. They also often exhibit reduced rates of metabolism of CECs relative to organisms at higher trophic levels, which increases the likelihood of detecting certain flame retardants, like organophosphates, that are thought to be readily metabolized and excreted by higher trophic organisms. As such, they are an excellent matrix for investigating the presence of alternative flame retardants in Bay biota.

The RMP will deploy transplanted bivalves (*Mytilus californianus*) in 2014 at nine locations, with collection and analysis after 90 days of exposure. Deployment at six sites will be selected among those already in regular use by the RMP and will be distributed to allow characterization of each region of the Bay: one in the Lower South Bay, two in the South Bay, one in the Central Bay (near Oakland), and one each in San Pablo and Suisun Bays. A replicate sample of bivalves will also be deployed at a South or Lower South Bay site, for a total of seven samples.

Analysis of composite mussel tissue samples will be conducted by Dr. Da Chen of Southern Illinois University. Mussel tissue will be examined for phosphates and metabolites using a highly sensitive liquid chromatography–electrospray ionization(+)-triple quadrupole mass spectrometry (LC–ESI(+)-QQQ-MS/MS) based analysis method (Chen et al., 2012a; Chu et al. 2011). Limits of detection are typically in the range of 0.1 ppb. Organophosphate flame retardants prioritized

for analysis include one previously detected in Bay mussels (TPhP) and a compound newly identified in consumer goods (V6). Analysis for established metabolites will be included.

Mussel tissue will also be examined for halogenated, hydrophobic alternative flame retardants using GC-ECNI-MS (Chen et al. 2012b, c). Limits of detection vary with the compound, ranging from roughly 0.1 to 1 ppb. Hydrophobic flame retardants prioritized for quantification include those for which metabolism and excretion are expected or suspected, such that they might be less likely to be found in higher trophic level organisms: EH-TBB and BEH-TEBP (the brominated components of the PentaBDE replacement commercial mixture, Firemaster 550), DBDPE, and EBTEBPI.

As the two analytical methodologies will require different extractions, the total cost of analysis will be $1,000, double that of water samples.

Harbor seals
Seals are long-lived, apex predators that eat a diet consisting primarily of fish and tend to forage in areas that are frequently impacted by contamination (e.g., heavy marine traffic, urban and agricultural runoff, etc.). As a result, harbor seals are highly exposed to contaminants that can be bioaccumulative. A previous RMP investigation of alternative flame retardants generally found seal blubber to contain higher levels of hydrophobic compounds relative to other species studied in the Bay (Klosterhaus et al. 2012). For this reason, seal blubber is an important matrix to monitor to determine which alternative flame retardants may be accumulating in Bay biota, and is often preferable to other matrices representing higher trophic levels, such as sport fish or bird eggs.

The RMP proposes a sampling strategy that combines targeted sampling of adult female seals in the summer of 2014 (goal n=10) with use of archived samples to allow initial determination of contamination trends with time. One replicate sample will be collected in 2014 as well, for a total of eleven new samples.

A RMP seal capture and sampling campaign in 2014 may be considered a pilot investigation of the feasibility of incorporating regular characterizations of contaminants in these apex predators into RMP Status and Trends monitoring or other work. Additional samples could also be collected and archived for future RMP studies. Summer sampling of adult females is preferred because a) these subjects tend to have high levels of hydrophobic contaminants in blubber, particularly during the summer months when the animals are of lower body weight; b) capturing these subjects is easier in the summer both because they tend to be tired and because capture team logistics are easier; c) limiting characterization to female adults removes the additional variable of life stage that can radically affect contaminant concentrations (D. Greig, *personal communication*). Male adult blubber samples may be included as necessary.

Additional analysis of archived samples will allow characterization of contaminant levels at earlier time points, allowing an exploration of contaminant trends with time similar to that provided by She et al. (2002) for PBDEs in seal blubber. Archived samples will be analyzed by AXYS Analytical for flame retardants already examined in an earlier RMP screening, including DBDPE, BTBPE, HBB, PBEB, PBDEs, and Dechlorane Plus. The first five of these compounds

are measured using a single HR-MS method ($850/sample), while the method associated with the final compound is still under development (estimated cost $400-600/sample, may include other compounds as well). Only PBDEs and Dechlorane Plus were detected in the previous RMP screening of seal blubber samples (Klosterhaus et al. 2012), but the method used was less sensitive.

Samples collected in 2014 will be analyzed by both the AXYS and Chen labs, allowing for a broader screen of potential alternative flame retardants while maximizing the potential for comparison of levels with earlier work. The Chen lab analyses will permit a broader investigation of potential contaminants, including organophosphate metabolites, Firemaster 550 components, DBE-DBCH or TBECH, and Dechlorane 602. The Chen lab will also be able to screen for and determine any potential degradation products of the priority flame retardants. The degradation products may exhibit different environmental behavior compared to their parent compounds, which may bring additional environmental and human health concerns.

Reporting

Results of these proposed study elements will be reported (together) as an RMP Technical Report and/or manuscript in early 2015. Comparisons will be made to past screening efforts in the Bay and in the literature from other locations, as well as to relevant toxicological information on these emerging contaminants available at that time.

Proposed Budget

The budget is presented as separate tasks that can be performed as separate elements or combined. The analysis of archived seal blubber could be deferred without greatly affecting the goal of a general screening of relevant Bay matrices for alternative flame retardants. However, without the analysis of archived samples, we will greatly limit our ability to observe variance over time in Bay contamination levels.

Task	**Estimated Cost**
Analysis of archived seal blubber (n=10+1 replicate), data management and reporting (AXYS)	$27,000
Collection of 2014 seal blubber samples (n=10+1 replicate)	$12,000
Analysis of 2014 seal blubber (n=10+1 replicate), data management and reporting; Option 1 = Chen lab & AXYS, Option 2 = Chen lab only, limiting comparison to archived samples	*Option 1:* $48,000 *or Option 2:* $21,000
Analysis of 2014 bivalves (n=6+1 replicate), data management and reporting (Chen lab)	$18,000
Analysis of 2014 sediment (n=10+1 replicate), data management, and reporting (Chen lab)	$21,000
Analysis of 2013 water (n=10+1 replicate), data management and reporting (Chen lab)	$11,000
Total (Option 1 for 2014 seal samples)	**$137,000**

For the full proposal outlined above, AXYS analysis of the 2014 seal samples serves two purposes: a) providing high quality measurements that can be more reliably compared to measurements of archived seal samples, and to previous years' PBDE measurements; and b) enabling comparison with the Chen lab seal blubber measurements, which will be obtained using methodologies that evaluate a broader variety of flame retardants, but in some cases provide less sensitivity and accuracy. Should the archived seal sample component of the study be eliminated, for cost-cutting purposes it may be preferable to analyze 2014 seal samples by the Chen lab only (Option 2 in the table above), rather than by both the Chen lab and AXYS (Option 1). In this case, a reduced budget eliminating all AXYS analyses is as follows:

Task	**Estimated Cost**
Collection of 2014 seal blubber samples (n=10+1 replicate)	$12,000
Analysis of 2014 seal blubber (n=10+1 replicate), data management and reporting; Option 2 = Chen lab only	*Option 2:* $21,000
Analysis of 2014 bivalves (n=6+1 replicate), data management and reporting	$18,000
Analysis of 2014 sediment (n=10+1 replicate), data management, and reporting	$21,000
Analysis of 2013 water (n=10+1 replicate), data management and reporting	$11,000
Total	**$83,000**

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