SWAMP
Surface Water Ambient Monitoring Program

Statewide Pilot Monitoring Plan	2016

Constituents of Emerging Concern (CECs) Statewide Pilot Study Monitoring Plan

Office of Information Management and Analysis

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Background

In 2009, the State Water Board adopted a Recycled Water Policy (http://www.waterboards.ca.gov/board_decisions/adopted_orders/resolutions/2013/rs2013_0003_a.pdf) . Consequently, the State Water Board formed a Science Panel to recommend CEC monitoring framework for recycled water. For recycled water, the Science Panel recommended a risk based target compounds selection process for monitoring, and provided methodology to use when selecting compounds to monitor. In 2011, the State Water Board provided additional funding to expand the scope to include freshwater ecosystems. The Science Panel recommended the State have a phased monitoring approach that develops a list of CECs from a risk-based framework, performs an initial statewide monitoring study at appropriate spatial and temporal scales, analyzes and interprets initial monitoring data, and implements control actions. The final report is available at http://www.waterboards.ca.gov/water_issues/programs/swamp/cec_aquatic/docs/cec_ecosystems_rpt.pdf.

This statewide pilot study implements the second phase of the recommendation which is to gather data to determine the occurrence and biological impacts of CEC. The result of pilot study will help the State Water Board to develop a statewide CEC monitoring strategy and control action.

The State Water Resources Control Board contracted with Southern California Coastal Water Research Project (SCCWRP) to develop the statewide pilot CEC monitoring guidance document. SCCWRP developed the guidance document with input from stakeholders and a science advisory committee. The guidance document covers all potential tools for CEC monitoring, including methods that are still under development or need further studies to be fully implemented as a tool for routine monitoring. The guidance document did not consider budgetary constraints for monitoring, but budgetary constraints are important to be considered for successful implementation of the pilot study.

This statewide pilot study monitoring plan includes the proven methods to provide results and reasonable cost to conduct monitoring with undue burden to funding sources. The CEC program will incorporate new methods as they become fully developed. The Office of Information Management and Analysis (OIMA) will identify sources of support, such as discretionary funding, to develop or refine potential methods.

Objective

The objective of the CEC statewide pilot study monitoring plan is to generate statewide data to inform Water Board managers of the status and trends of CECs in water. The plan is designed to narrow the data gap among regions by producing comparable CEC data throughout the state.

The current regional CECs monitoring efforts produce uncoordinated and regionally focused monitoring data, making broad statewide synthesis, analysis and comparability difficult or impossible. In contrast, the Statewide CEC pilot study monitoring attempts to provide comprehensive CEC data to evaluate the overall condition of surface waters throughout the State. Thus providing the statewide information needed by Water Board Management to develop tier based management framework to manage CECs similar to the San Francisco Bay Regional Monitoring Program (SFRMP) model.

Brief Review of CEC Monitoring Programs

San Francisco Bay Regional Monitoring Program (SFRMP)

The SFRMP has investigated the occurrence and potential for impacts due to CECs since 2001. Much of the pioneering work on flame retardants (e.g. PBDEs) and more recently, perfluorinated compounds (PFCs) such as PFOS, have been conducted by the SFRMP as a result of recommendations made by the Emerging Contaminants Work Group (ECWG), a panel of stakeholders and internationally renowned scientists coordinated by the SFRMP. The role of the ECWG is to ensure the SFRMP is current with respect to CECs, and, as needed, to recommend, support and implement studies for consideration by the RMP Steering Committee. These studies have allowed for prioritization of these CECs using occurrence and toxicity data to determine the level of concern for individual contaminants in the Estuary.

The SFRMP recently synthesized the state of the science on occurrence of CECs in San Francisco Bay (Klosterhaus et al. 2013), including existing information on chemical usage, occurrence relative to other locations and toxicity.
The SFRMP then developed a three-element CEC monitoring strategy (Sutton et al. 2013), which combines:

a) traditional targeted monitoring guided by a risk-based framework, similar to that proposed by Anderson et al. (2012)
b) review of the scientific literature and other CEC monitoring programs as a means of targeting new CECs
c) non-targeted monitoring, including broad scan analyses of Bay biota samples and development of bioassays to identify estrogenic effects, both means of identifying previously unknown CECs present in the Bay. The major outcome of this effort is to provide updates on relevant information to the San Francisco Bay Regional Board and stakeholders including the ECWG, so that they may react and adapt to new information using a tiered risk-management action framework (Sutton et al. 2013).

Los Angeles Regional Water Quality Board

The Los Angeles Regional Board has conducted monitoring for CECs in the Los Angeles River and Santa Clara River watersheds in two separate studies. The first study was conducted in 2011 and focused on two effluent-dominated watersheds (Los Angeles River and San Gabriel River). The second study was conducted in 2013 and focused on a more natural watershed (Santa Clara River), although there is considerable influence from POTW discharges.

A suite of more than 60 CECs were analyzed in water samples collected during two low-flow events in 2011 from the Los Angeles River (as well as from the San Gabriel River). Samples were collected at 7 to 9 stations stretching from above the discharge of wastewater treatment plants to the river mouth to quantify the occurrence of the target CECs and characterize in-stream fate and transport. Concentrations of chlorinated phosphate flame retardants (TCEP, TCPP, TDCPP) were highest among the CECs tested. Maximum in-stream concentrations of pyrethroids (bifenthrin and permethrin), diclofenac, and galaxolide exceeded risk-based thresholds established for monitoring of CECs in effluent-dominated waters. Fipronil (a current-use pesticide) and its degradates were measured at levels that exceeded toxicity thresholds for estuarine, but not freshwater, invertebrate test species. In contrast, maximum concentrations of pharmaceuticals and personal care products commonly detected in treated wastewater (e.g., acetaminophen, N,N,diethyl-meta-toluamide [DEET], and gemfibrozil), were less than 10% of established thresholds. Attenuation of target CECs was not observed downstream of wastewater treatment plant discharges until dilution by seawater occurred in the tidal zone, partly because of the short hydraulic residence times in these highly channelized systems (<3 days). In addition to identifying CECs to target for future in-stream monitoring, these results suggest that conservative mass transport is an important boundary condition for assessment of the input, fate, and effects of CECs in estuaries at the bottom of these watersheds.

A similar suite of more than 60 CECs were analyzed in water samples collected during two low-flow events in 2013 from the Santa Clara River. Samples were collected at 10 stations stretching from the upper watershed to the estuary, to quantify the occurrence of the target CECs and characterize in-stream fate and transport. CECs were detectable at stations nearest to wastewater treatment plant discharges, but were rapidly attenuated downstream. Sucralose and the chlorinated phosphate flame retardants (TCEP, TCPP and TCDPP) were found at the highest concentrations in water among the CECs tested. Triclocarban, an antimicrobial agent that has been used for decades, was found in higher concentrations than triclosan or nonylphenol. Maximum concentrations of pyrethroids (bifenthrin and permethrin) and fipronil exceeded CEC-specific monitoring trigger levels recently established for freshwater and estuarine sediments by factors of 10 to 1000, respectively. Maximum fish tissue concentrations of polybrominated diphenyl ethers (PBDEs) for the Santa Clara River estuary (and other coastal embayments tested) exceeded the monitoring trigger level by up to a factor of 10.

In addition to stream and estuary monitoring, the Los Angeles Regional Board has required approximately two dozen POTWs to conduct special studies to evaluate effluent concentrations of target CECs in their discharges (including freshwater and ocean dischargers). Each facility is required to conduct annual monitoring once per year for a minimum of two years for a suite of approximately 34 CECs. This special study requirement has been incorporated into NPDES permits as they are renewed, so not all dischargers have completed the special studies as of September 2015. Regional Board staff plan to evaluate the overall data set upon completion of the special studies to determine which CECs merit continued monitoring in the future, which CECs pose

potential threats to water quality and beneficial uses throughout the Los Angeles Region, and whether there are significant differences in CEC loadings discharged by various POTWs.

Central Valley Regional Water Quality Control Board

The Central Valley Regional Water Quality Control Board is not currently conducting any water quality monitoring of constituents of emerging concern (CEC) through its regional programs. Staff is also not aware of any ongoing CEC monitoring by other agencies or groups in the Central Valley. Historic CEC data in the region are quite limited. Below are a brief summary that pertain to past CEC-related projects in the region:

The U.S. Fish and Wildlife Service Environmental Contaminants Division has periodically deployed water sampling devices to assess potential contaminant effects on special status species in the Bay-Delta. This work was performed in collaboration with researchers from U.C. Davis monitoring Sacramento splittail and another team from the University of Florida who analyzed blood collected from splittail for the presence of vitellogenin (a precursor protein of egg yolk normally found only in females). This study found high levels of vitellogenin in 2 of 12 male splittail indicating the presence of endocrine disrupting chemicals.

A research team from U.C. Riverside and U.C. Berkeley found evidence for a relationship between mixes of toxic chemicals present at low levels and signs of endocrine disruption in fish. In their study, the U.C. team tested surface water samples collected throughout the Central Valley for signs of fish feminization and analyzed for more than 100 chemicals, including steroid hormones, pharmaceuticals, current use pesticides, and other emerging contaminants.

A researcher from the U.C. Davis Bodega marine laboratory examined the impact of endocrine disrupting compounds on the Mississippi silverside, an important forage fish in the Delta-Suisun food web. In 2009 and 2010, the team caught fish monthly from two beaches in Suisun Marsh: Suisun Slough and Denverton Slough. Suisun Slough receives urban runoff and wastewater effluent, and Denverton Slough receives runoff from a local ranch. A bioassay detected estrogenic EDCs at the ranch site and both estrogenic (compounds mimicking female sex hormones) and androgenic EDCs (compounds mimicking male sex hormones) at the urban site.

Scientists from the Southern California Metropolitan Water District (MWD) and the Orange County Water District assessed the occurrence of CECs in Delta water. Sampling took place from April 2008 to April 2009 on a quarterly basis at eleven sites representing source water for the State Water Project. The researchers evaluated the presence of endocrine disrupting compounds together with other pharmaceuticals and personal care products and organic contaminants typically found in wastewater. Detectable amounts of CECs were found at all but one site during one of the four sampling events.

U.C. Davis scientists deployed passive samplers to estimate concentration and applied an environmental model to estimate the load of pharmaceuticals from WWTPs and distribution in the

bay. The model was run for the 2006, 2007, and 2009 water years. Results indicate that it is feasible that WWTP discharges could result in chronic presence of these pharmaceuticals at low levels at all 45 model output locations and, therefore, aquatic organisms within the Delta may be continually exposed to these contaminants.

OIMA's Surface Water Ambient Monitoring Program (SWAMP) screened surface water samples collected in the Central Valley and northeastern area of California for estrogenic activity. The results showed that a majority of the surface water samples tested were below EEDC detection threshold concentration for the screening procedure utilized. To establish a more definitive assessment of EEDC occurrence, follow up screening at sites where statistically significant, but weak, estrogenic activity was observed, is recommended.

Overall Approach for a Statewide CEC Assessment

The State Water Board does not currently have a CEC management strategy. The current Recycle Water Policy is not comprehensive enough and did not require CEC monitoring in surface water. Statewide CEC data and information is scant and not organized in a comprehensive and consistent way to provide either baseline information for CECs or to develop management actions in the State. The monitoring goals are to verify the occurrence of target CECs in water, sediment, and tissue samples and to determine biological effects of these CECs. The chemical concentration and biological effects monitoring results will be combined in order to provide a comprehensive status and trends of CECs, and to determine if beneficial uses are impacted. An additional goal is to conduct non-targeted analysis to screen for unexpected chemicals, establish a baseline CEC inventory, and identify compounds with high concentrations missed by targeted monitoring. The monitoring plan covers waterbodies including freshwater, embayments, and the ocean.

The SFRMP has gathered CEC data in the bay since 2000. The SFRMP developed a risk based tiered monitoring approach with management actions associated with each tier (appendix IV). The SFRMP tiered scheme provides a good model for the statewide monitoring to follow. The results of statewide monitoring will finally be evaluated in SFRMP's risk based categories and management framework.

Statewide CECs Monitoring Coordination Team

A statewide coordination team will be established to

- review monitoring questions and designs
- develop management actions
- seek funding sources
- develop strategies to maintain a sustainable program
- organize workshops, and coordinate with other relevant entities

Monitoring Methods

A combination of chemistry, in vitro bioassay (IVB) and toxicity monitoring methods will be applied during monitoring. The monitoring methods are described below. The first section describes the chemistry approach, the monitoring design, locations, sampling frequency, monitoring questions, and analytical information. The second section describes the bioanalytical and toxicity testing methods, monitoring questions, toxicity, and sampling frequency.

The chemistry methods screen for and identify CECs and quantify their concentrations. This method helps to answer questions related to occurrences, temporal and spatial variability, sources, loading and attenuations of CECs. The concentration of the compounds will be measured in water, sediment, and tissue. Non-targeted analysis will be conducted on tissue samples to establish baseline contaminant inventories and identify high concentrated compounds missed by target monitoring.

The in vitro bioassays (IVB) and lab toxicity testing determine adverse effects to aquatic species. Bioanalytical methods will help answer: which concentrations trigger biological effects, what the biological responses are to mixture of chemicals (additive or antagonistic), which chemicals detected below Monitoring Trigger Levels (MTLs), and how it correlates to the chemistry data.

The targeted compounds for monitoring are selected by a risk based screening framework developed by Science Advisory Panel (http://www.waterboards.ca.gov/water_issues/programs/swamp/cec_aquatic/docs/cec_ecosystems_rpt.pdf 2012).
For consistent statewide monitoring maintain scientific integrity, the target CECs selected by Science Advisory Panel are recommended to be monitored in all Regions with the appropriate matrices and waterbodies.

Compound Selection Process

The Science Panel used a risk-based screening framework to select compounds for monitoring from thousands of CECs. The framework was developed by the CEC Recycled Water Expert Panel, also coordinated through SCCWRP. The framework utilizes maximum concentration and biological effects of each compound with safety factor.

The safety factor is used to extrapolate salt water effects from freshwater data, and accounts for specific mode of action and unknown mode of action. For biological effects no observed effect concentration (NOECs) were used and when NOECs were not available, LC50 values were used. To determine the most sensitive species and NOECs, two major databases were used: EPA EcoTox website and MistraWikiPharma database (http://cfpub.epa.gov/ecotox/ and http://www.wikipharma.org/welcome.asp). In addition, the Expert Panel used Pubmed, SciFinder Scholar, and Web of Science for journals that provided information about toxicity. For occurrences and concentration data, the Panel compiled data from various sources. The primary sources of data are results from the Recycled Water Panel, SCCWRP & SFEI (RMP & RB4 data are included), Water

Environmental Research Foundation, and published literature identified in Thomas Reuters Web of Knowledge (http://wokinfo.com).

The panel further investigated the occurrence of the compounds which have documented potency to induce adverse biological effects in multiple environmental matrices. These matrices include WWTP & stormwater effluent, receiving waters (stream, estuaries, and coast-water), sediment, and tissue. The panel targeted compounds with NOEC <0.1 mg/L (100,000 ng/L) for aqueous exposure. The rationale for evaluating only CECs with <0.1 mg/L was based on the assumption that most compounds occur in concentrations within the ng-mg/L range. If the worst case safety factor of 1,000 was applied, then the compound with (NOEC/1000) in the ng/L range may exceed one and cause potential risk. For initial screening, 82 compounds were selected. In addition, they have investigated how many of the 82 compounds can be analyzed by commercial laboratories and dropped 17 compounds from the list because laboratories do not analyze them. Of the remaining compounds the risk-based model was performed and the ratio of maximum concentration and toxicity benchmark (NOECs, LOECs, or PNECs) of each compound were calculated to decide which compounds should be targeted for monitoring. If the ratio was > 1, the compound will be targeted for monitoring because it is assumed that the compound has a potential to cause a risk. If not, the compound was removed from the list.

1. Targeted CECs Monitoring—Chemistry Approach

The monitoring is primarily intended for targeted CECs in three waterbody scenarios and matrices:

1. Freshwater: effluent dominated water ways and stormwater (Scenario 1)
2. Bays and Embayments (Scenario 2)
3. Ocean (Scenario 3)

Sampling matrices are:

1. Water
2. Sediment
3. Tissue

1.1 Inland Freshwater (Scenario 1)

1.1.1 Waste Water Treatment Plant (WWTP) Effluent Dominated

Scenario 1 examines inland freshwater systems including rivers and lakes where the majority of the flow or volume during the dry season is WWTP effluent. Treated wastewater is expected to be the largest source of CECs during this time period.

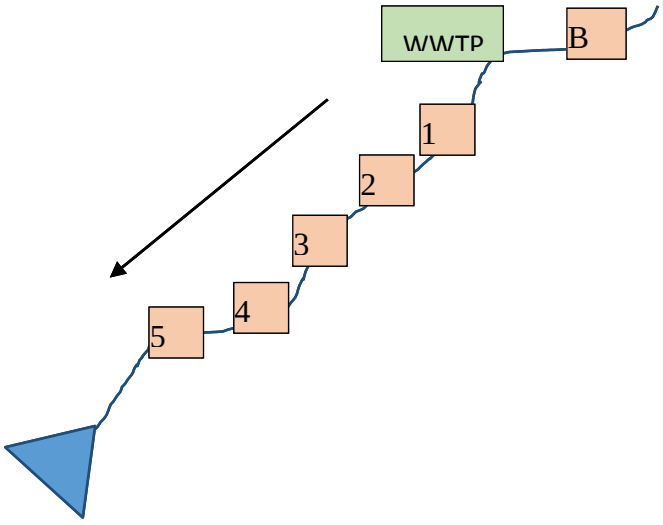
Monitoring Questions

1. Which CECs are detected in freshwaters and in which California watersheds are they detected?
2. Can the CECs be shown to originate from the inland WWTP, or are they present at background concentrations?
3. How quickly (i.e., at what distance) do the CECs attenuate once discharged?
4. What are the concentrations and loadings of target CECs in the dry vs. wet seasons?
5. Do the new occurrence data change the estimated monitoring trigger quotients (MTQs)?
6. Which detected CECs have been found to accumulate in sediments and fish tissue?

Design

The effluent of selected inland WWTPs and their corresponding waterways will be monitored. To determine the occurrence and attenuation of target CECs downstream of each identified WWTP (or series of upstream WWTPs), a minimum of 7 stations will be monitored: effluent sample in one station just downstream of the WWTP discharge location(s), five stations further downstream of the WWTP(s), and one background station located upstream of the WWTP(s). To assess repeatability, duplicate field samples will be collected at the WWTP and background stations. Both the wet and dry seasons will be monitored over a 2 year period (**Table 1.1.1-1).**

Figure 1. Design schematic for monitoring of CECs in Scenario 1.



Monitoring Locations:

- Southern California: **Los Angeles River and the Santa Clara River Watersheds**
- Delta and Central Valley: **Sacramento Regional WWTP, Alamo Creek downstream of the Vacaville Easterly WWTP and Pleasant Grove downstream of the City of Roseville Pleasant Grove WWTP**

Table 1.1.1-1 Aqueous sampling frequency for WWTP (Scenario 1)

Source	Receiving Water	Years	Waterways	Total Samples
WWTP effluent 1 station Wet and dry season 2 replicates Samples = 4/yr	Downstream 5 stations Wet and dry season Samples = 10/yr Background 1 station Wet and dry season 2 replicates Samples = 4/year 14 total samples/yr	2	5 (two in SoCal and Three in Delta/ Central Valley)	Effluent = 40 FW = 140

Table 1.1.1-1.2 Sediment sampling frequency for WWTP (Scenario 1)

Waterway Sediment	Years	Waterways	Total Samples
3 stations Dry season Samples = 3/yr	2	5 (two in SoCal and three Delta/CV)	Sediment = 30

1.1.2 Stormwater Discharge to Receiving Waters - MS4 (Scenario 1)

Unlike WWTP effluent, the vast majority of annual stormwater runoff and discharge occurs during the wet season (November through April) in all but the most arid regions of the State. It is critical to address both short term toxicity and long term loading, as well as to take into account the distribution and fate of CECs for monitoring in MS4 watersheds.

Monitoring Questions

1. Which CECs are detected in waterways dominated by stormwater?
2. What are their concentrations and loadings in the dry vs. wet seasons?
3. What is the relative contribution of CECs in WWTP effluent vs. stormwater?
4. What is the spatial and temporal variability in loadings and concentrations (e.g. between storm variability during the wet season; in stream attenuation rate during low flow, dry season conditions)?

Design

Wet Weather. Since annual loading is the main concern during wet weather, a design that focuses on detection of target CECs, and estimating total loads for those detected into MS4 receiving waters are the primary goals. Flow-weighted or time-interval sampling at fixed mass emission (FME) stations for two storms per year, per watershed will provide data to address monitoring questions 1-3 above (**Table 1.1.2-1**). Ideally, the storms sampled will include an early ("first flush") and late season event. A minimum of four watersheds statewide will be assessed over a 2-year pilot study period. Addressing question 4 will necessitate more intensive sampling during and/or between storm events, and, if warranted based on the results of the initial 2 year screening, should be planned during subsequent pilot study cycles. Non-filtered, whole water samples will be analyzed when addressing loading, and for effects/toxicity evaluation.

Dry Weather. Since short term maximum concentrations resulting in acute toxicity are the main concern, a strategy that focuses on capturing worst case exposure conditions for a relevant endpoint/receptor of interest is the primary goal. A design that targets receiving water near known or suspected incidental runoff sources (e.g. culverts or sections that drain parks or golf courses), is needed to include worst case exposure scenarios. Depositional area sediments (e.g. river mouths, oxbows, retention basins) will be sampled at the start and end of the dry season to examine (1) what has been washed in during the previous wet season and (2) degree of attenuation occurring during the dry season (**Table 1.1.2-1**).

In San Francisco Bay, the dry weather sampling sites will be paired with the wet weather sampling sites.

Table 1.1.2-1. Sampling matrix for MS4 watersheds. Monitoring of a minimum of 6 watersheds over a 2 year period is recommended.

Parameter	Sample Type	Stations	Frequency	Replication	Total Samples
Aqueous concentration, wet weather	Whole water (unfiltered)	1 (FME)	2 storms/yr	3	72
Aqueous concentration, dry weather	Whole water (unfiltered)	3 (source-related)	1/yr	1	36
Sediment concentration, dry	Whole (sieved) sediment	3 (depositional)	twice/yr	1	72

Candidate MS4 Watersheds

- San Francisco Bay: San Lorenzo Creek (Alameda County); Matadero Creek (Santa Clara County).
- Delta/Central Valley: Steelhead Creek, Morrison Creek, American River and the Sacramento River at the Hood integration site, Site 3 (Sacramento County);
- Southern California: Ballona Creek and Bouquet Canyon Creek (Los Angeles County); San Diego Creek and Salt Creek (Orange County); Chollas Creek and San Diego River

(San Diego County) including watersheds monitored by the SMC, SWAMP/SPoT and DPR.

1.2. Coastal Embayment (Scenario 2)

Scenario 2 examines coastal embayments that receive CEC inputs at the land-ocean interface. These may originate from upstream WWTP discharge, direct WWTP discharge into the embayment, or stormwater runoff. As San Francisco Bay is by far the largest and most actively monitored coastal embayment in California, this scenario is based on monitoring in San Francisco Bay but may be extended to other coastal embayments across the State.

Monitoring Questions

1. Which CECs are detected in coastal embayment water?
2. Do CECs originate from the outfalls, or are embayment concentrations due to stormwater and other inputs?
3. Do the new occurrence data, change the estimated MTQs?
4. Which of the CECs conveyed through effluent and stormwater, accumulate in sediments in coastal lagoons and embayment?

Design

SFRMP is a multi-stakeholder collaboration among regulators, dischargers, and scientists with a well-established emerging contaminants monitoring program. The program regularly monitors for select compounds using a robust statistical design for site selection. It is recommended that monitoring for additional contaminants, identified by the state expert panel, take advantage of the existing SFRMP site selection and sampling activities. Collaboration between the state and the SFRMP will provide greater efficiency and allow for pooling of contaminant data.

In the Bay, there are multiple WWTP discharges with relatively close outfalls, tidal influences, and multi-directional currents that rapidly distribute contaminants. It is recommended that effluent samples of at least five representative WWTPs should be collected, as well as ambient bay water samples from up to 20 sites chosen through the SFRMP's statistical design (**Table 1.2-1**). At least two samples will be collected in each of the five Bay sub-embayments. Sampling should take place during the dry season, when dilution from runoff is lowest, and concentrations of WWTP-derived contaminants can be expected to be at their highest. Additional sediment monitoring is not required for San Francisco Bay, as the SFRMP already monitors those contaminants recommended for embayment sediment monitoring.

Table 1.2-1. WWTP effluent and ambient bay water sampling frequency for Scenario 2.

Effluent	Aqueous	Years	Total Samples
5 WWTPs Dry season Samples = 5/yr	Sample = 20	**2**	Effluent = 10 Ambient Bay water = 40

1.3 WWTP Effluent Discharge to the Ocean (Scenario 3)

Scenario 3 examines WWTP effluent discharged by outfalls at mid-continental shelf depths (50-100 m). Discharged CECs are diluted by the ambient water, transformed into breakdown products and/or are transported away from the outfall by currents. This scenario is monitored exclusively at marine outfalls within the Southern California Bight.

Monitoring Questions

1. Which CECs are detected in marine waters and sediments adjacent to WWTP outfalls, what are their concentrations, and how quickly do they attenuate?
2. Can the CECs be shown to originate from the outfalls, or are they present at background concentrations?
3. Is there a sub-annual change in discharged CECs?
4. Does the new occurrence data change the estimated MTQs?
5. What is the relative contribution of CECs in WWTP effluent vs. stormwater?

Design Considerations

The effluent and sediments at a minimum of two WWTP ocean outfalls will be monitored, with a grid of 8 sediment stations at each outfall (**Figure 2**). Observations of a stepwise decrease in concentrations away from the zone of initial dilution (ZID) will verify whether the compounds originate from the outfall and are not at background concentrations due to other inputs. The exact locations will consider the oceanic conditions and historic depositional patterns at each candidate outfall and may be changed based on the results of initial monitoring. Three stations will be located down current from the ZID. Three will be located cross current, and one background station will be located up current of the outfall. The frequency of analysis is semi-annual (wet and dry) for the effluent and annual for the sediment (**Table 1.3-1**). Exact station locations may be assigned, based on the results from the Bight '13 Special Study described in **Appendix C**.

Figure 2. Design schematic for sampling of CEC in Scenario 3

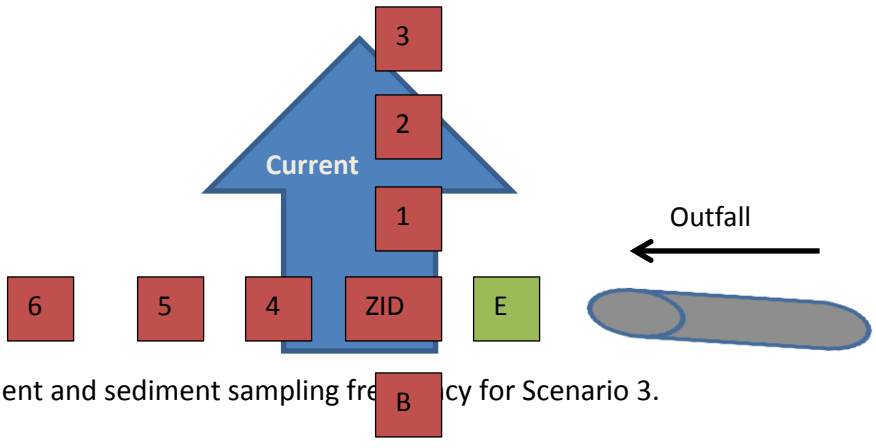


Table 1.3-1. Effluent and sediment sampling fre cy for Scenario 3.

Source	Sediment	Years	WWTPs	Total Samples
WWTP effluent 1 station Wet and dry seasons 2 replicates Samples = 4/yr	Grid 7 stations Samples = 7/yr Background 1 station 2 replicates Samples = 2/yr 9 total samples/yr	2	2	Effluent = 16 Sediment = 36

1.4. Tissue Monitoring

Chemicals that are hydrophobic (log *K*ow >3), remain un-ionized in either freshwater or saltwater environments, and they have the potential to bioaccumulate in aquatic biota. For CECs that biomagnify (e.g. PBDEs), an organism with a sub-critical body burden that comprises the majority of the diet of a higher level trophic receptor may pose an unacceptable risk to the predator organism if CEC concentrations exceed the predator-based critical body residue concentration. While several of the CECs considered have the potential to bioaccumulate, only two (PBDE and PFOS) have observable effect concentration (NOECs) from which body burden-based MTLs could be derived. The studies on birds used adult Mallard and Bobwhite Quail to set a PNEC of 1000 µg/kg for PFOS, and studies on the American Kestrel to set a NOEC of 289 µg/kg for the two PBDE congeners (47 and 99).

Monitoring Questions

1. What are the concentrations in tissues and do they exceed toxicity thresholds?
2. Do the new occurrence data change the recommendation to monitor?
3. Are concentrations of bioaccumulative CECs changing over time (annual to decadal time frames)?
4. Do bioaccumulative CECs occur in scenario-specific patterns?

Design

1.4.1 Toxicity Thresholds Based on Bird Eggs. Addressing changes in the MTQs requires analysis of bird eggs, since the thresholds for both PBDEs and PFOS were set using this matrix.

Table 1.4.1-1. Recommended sampling of bird eggs and marine mammals for the 2-year pilot study cycle. Additional tissue samples are to be analyzed through regional programs, as noted in the text.

Sample	Region	Number per 2 yr cycle	Total Samples
Bird eggs	Delta/Central Valley	10 egg composites	10
Marine Mammals Blubber (PBDEs) Blood	Southern California Bight	5 sea lion 5 bottlenose dolphin	Blubber = 10 Blood = 10

1.4.2 Fish and Bivalves. Compared with birds and marine mammals, some fish and all bivalves are more abundant and have higher site fidelity. These sentinels are therefore well suited to compare contaminants across scenarios, to assess temporal trends, to characterize exposure and to identify localized contamination sources. Candidate bivalve species are *Corbicula fluminea* (freshwater) and *Mytilus spp. (californianus or galloprovicialis)* for embayment and marine habitats.

1. For freshwater systems (e.g. Scenario 1 and MS4 monitoring), fish (PBDEs and PFOS) and bivalves (PBDEs) will be sampled in one system each in the San Francisco Bay watershed, southern California and the Delta/Central Valley region.

    a. For Scenario 1, bivalves and fish will be collected from a location in close proximity to the WWTP outfall, during the period of highest effluent loading.

    b. For MS4 watersheds, bivalves and fish will be collected in close proximity to FME/integrator stations (i.e. near the mouth of the watershed), where loadings are expected to be highest, during or near the end of the wet season.

2. For San Francisco Bay (Scenario 2), the SFRMP measures PBDEs in bivalves every 2 years, and PBDEs and PFCs in sport fish every 5 years. Recommended fish species are shiner surfperch, white croaker, topsmelt, and California halibut.

3. For marine outfall tissue monitoring (Scenario 3), fish will be monitored for PBDEs and PFOS at two outfalls that are also monitored for sediment concentrations (n = 10 fish, each outfall). Species that have high site fidelity will be selected. Recommended species include those collected in abundance historically at these outfalls (e.g. hornyhead turbot, Dover sole and scorpionfish).

Table 1.4.2-1. Fish and bivalve sampling frequency. Additional tissue samples are to be analyzed through regional programs, as noted in the text.

Sample	Scenario	Number per year	Locations	Years	Total Samples
Freshwater fish	Scenario 1 and MS4	5	14 Waterways each scenario	2	140
Marine fish	Scenario 3	5	2 WWTP outfalls	2	20
Bivalves	Scenario 1 and MS4	3	14 waterways each.	2	84

1.5 Non-Targeted Analysis.

The Panel recognized non-targeted analytical methods as of potential utility in periodically screening for unexpected contaminants, and in addition, as tool for toxicity identification evaluation (TIE) when responses and/or effects observed with in vitro, in vivo testing and/or in situ monitoring cannot be explained by targeted analytical chemistry. Non-targeted methods have recently been developed for analysis of bioaccumulative organic compounds in marine biota from the California coast (Hoh et al. 2012; Shaul et al. 2014). Application of non-targeted analysis to the tissue samples collected as part of this pilot study (this section), will establish baseline contaminant inventories and identify any high abundance compounds missed by targeted monitoring. In addition, the mass spectral libraries and retention time information, generated by such periodic monitoring, will allow for efficient identification of the contaminants in the future.

Table 2.2 Recommended non-targeted analysis of tissue samples collected for monitoring of PBDEs and PFOS.

Sample	Scenario/Region	Number per 2 yr cycle	Locations	Total Samples
Freshwater Fish	Scenario 1 and MS4	2	3 waterways ea. scenario	12
Marine mammal blubber	Scenario 2 (San Francisco Bay)	10	n/a	10
Marine fish	Scenario 3	5	2 WWTP outfalls	10
Marine mammal blubber (2 species)	Southern California Bight	5	n/a	10

2. Targeted CEC Monitoring ----Bioanalytical Approach

Tier	Method	Studies
I	*In Vitro* Bioassays - Evaluating CECs based on mode of action	Screening
II	*In Vivo* Animal Toxicity Assay - Fish reproduction assay for aqueous sample testing - Invertebrate toxicity assay for sediment samples testing	Diagnostic
III	*In Situ* Assessment of CECs Toxicity - Community/population analyses (e.g. species diversity/abundance) - Tissue analyses (e.g. histology, somatic indices) - Molecular analyses (e.g. gene or protein expression level)	Confirmatory

2.1 General Description of Bioanalytical Process

Bioanalytical studies will be conducted in three tiers: screening, diagnostic and confirmatory. In Tier I, high-throughput *in vitro* bioassays (IVBs) are conducted to screen for the occurrence of chemicals, including CECs, in environmental samples, based on their mode of action (MOA). *In vitro* assays are an efficient way to assess the ability of CECs to activate cellular receptors but stop short of predicting adverse outcomes at the organismal or population level. In Tier II, whole organism toxicity testing to determine if CECs present in aquatic ecosystems can have adverse effects at the organism level (e.g. impaired reproduction in fish exposed to model chemicals, receiving water samples and/or WWTP effluent). In the case that samples of interest demonstrate effects in Tier II analyses that warrant further investigation, Tier III analyses focuses on in situ evaluation which entails field collection of biological samples of sentinel organisms (e.g. invertebrates, fish, birds and/or mammals. The purpose is to investigate whether such MOAs identified using Tier I *in vitro* cell assays and adverse outcomes indicated by Tier II analyses are prevalent in the receiving water environment. Tier III tools endpoints would incorporate both advanced molecular tools such as quantitative polymerase chain reaction (qPCR) or gene microarrays as well as more conventional in situ biomonitoring and assessment parameters (e.g. histology, species abundance/diversity).

2.1.1 Tier I – Bioanalytical Screening Using High-Throughput *In Vitro* Assays

From the number of commercially available IVBs, CECs are proven to have a capacity to activate the endocrine-related receptors, Estrogen Receptor (ER) and Androgen Receptor (AR). At this initial stage, eight estrogenic compounds, and one androgenic will be evaluated (**Table 2.1.1-1**).

Table 2.1.1-1. In vitro bioassays that screen for endocrine disruption and general cell toxicity. *Table adapted from Anderson et al. (2012).*

Endpoint	Response	Mode of Action	Potential Adverse Outcome
Estrogen Receptor Alpha (ERa)	Activation and inhibition	Estrogen signaling	Feminization of males. Impaired reproduction, cancer
Androgen Receptor (AR)	Activation and inhibition	Male sexual phenotype	Andrgoen insensitivity, masculinization of female, impaired reproduction
Cytotoxicity	-	General cell toxicity	Tissue damage, death

Two types of investigations will be carried out. Evaluate ER and AR to determine their response to target CECs at exposure concentrations of monitoring relevance. Then, evaluate the ER and AR to determine the magnitude and range of response associated with real environmental samples and to assess the concordance with responses predicted using targeted analytical chemistry results. The output parameters, resulting from bioassays, are not directly comparable with individual chemical concentrations. We will then translate the bioassay into equivalent concentrations, or bioassay equivalents (BEQs) (**Table 2.1.1-2**). In vitro bioassay will not be applied for ibuprofen, diclofenac, and triclosan, because there is no developed method for these compounds. Similarly, no in vitro bioassay will be conducted for the PBDEs. Although PBDEs activate AhR, there is no known predicted adverse outcome.

Table 2.1.1-2. Output parameters of *in vitro* assays.

	Parameter
`Calibration	Dose response curve with reference toxicant
Concentration effect assessment	Relative Enrichment Factor (REF) (enrichment factor of extraction process and dilution of extract in the IVB)
Data analyses	Effect concentration (EC)
Output parameter	Bioassay equivalent concentration (BEQ)

2.2. *In Vitro* Screening of Targeted CECs

Questions to be addressed:

1. Which priority CECs are detectable at or below their respective monitoring trigger levels (MTLs) using the endocrine-related cell assays?
2. What are the responses (additive or antagonist) of priority CECs mixtures using the selected cell assays?

The objective of this study is to identify the concentration at which ER and AR assay are most responsive to screen for priority CECs at environmentally relevant levels (**Table 2.2-1**). For each chemical, four concentrations will be selected including the lowest at or below its MTL. A mixture of the selected CECs will also be tested with individual concentrations at and above MTLs to determine if additive or antagonist effects may occur.

Table 2.2-1. *In vitro* assays for screening of priority CECs.

Compound	**estrogenic**	**androgenic**	**AhR**
Estrone	x		
Ibuprofen	No IVBs, more research needed		
Bisphenol A	x		
17-beta-estradiol	X		
Galaxolide (HHCB)		X	
Diclofenac	No IVB, more research needed		
Triclosan	No IVB, more research needed		
Bis(2-ethylhexyl) phthalate (BEHP)	x		
Butylbenzyl phthalate (BBP)	x		
p-nonylphenol	x		
PBDE-47			X (no biological response)
PBDE-99			X (no known biological response)
PFOS	x		

2.3 *In Vitro* Screening of Environmental Extracts

Questions to be addressed:

1. How efficient is ER and AR *in vitro* bioassay in detecting known and unknown CECs present in complex environmental mixtures (e.g. WWTP effluent and receiving water)?
2. How do cell assay responses correlate with analytical chemistry data?

It is important to evaluate the correlation between ER and AR in vitro assay responses and chemistry data to understand the contribution of known (i.e. measurable) CECs. This study will be conducted over a three-year period. Water samples will be collected, extracted and split on an annual schedule for targeted monitoring and testing (Table 2.3-1).

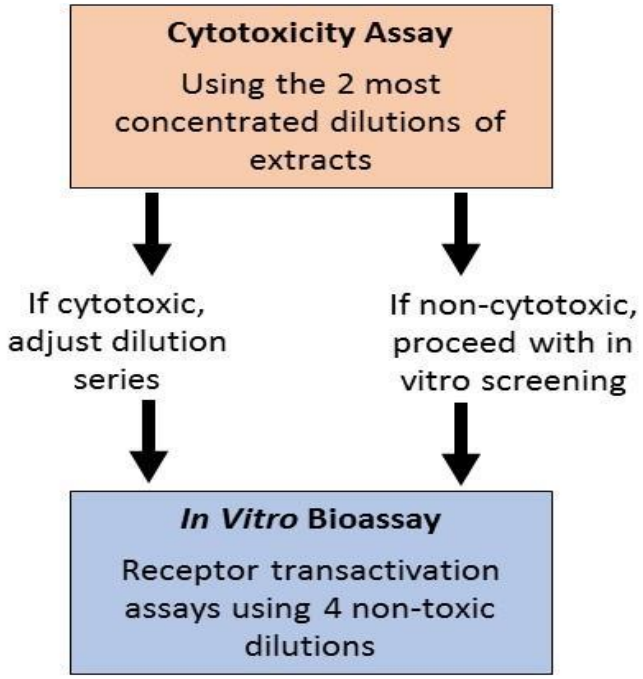
Table 2.3-1. Sampling locations and frequency for *in vitro* screening.

	Sample Type	**Location**	**Sampling Frequency**	**Waterways**
Scenario 1 Freshwater	WWTP effluent	Outfall	2/year (wet & dry season)	2
	River water	Stations # B, 1, 3 and 5 (Section 2.2.1)	2/year (wet & dry season)	
Scenario 2 Embayment	WWTP effluent Receiving water	Outfall	1/year 1/year	1
Scenario 3 Ocean	WWTP effluent Receiving water	Outfall Stations # B, ZID, 3 and 6	1/year 1/year	2
Scenario 4 MS4	Watershed	1 FME 3 source-related	2 storms/year dry weather 1/year	3

The General Process of IVBs

Prior to *in vitro* screening, the environmental water extracts will be solvent exchanged to dimethylsulfoxide (DMSO). Screening of sample extracts for cytotoxicity is performed prior to screening of the remaining candidate endpoints (or MOAs) (**Fig. 2.1-1**).

Figure 2.3-1. In vitro bioassay endpoints are sequenced to screen for cytotoxicity prior to testing for specific modes of action.



2.4 Tier II – Toxicity Testing Using Whole Organisms

In vivo tests should be conducted to evaluate the effects of environmental CECs on key biological processes such as development, reproduction, and behavior in whole organisms. Toxicity testing using whole organisms will be implemented to (1) determine the levels of exposure to CECs and complex mixtures affecting sensitive organisms; and (2) to establish linkage between *in vitro* screening results and *in vivo* apical endpoints.

2.4.1 Linkage of In Vitro Responses with Effects on Fish Reproduction

Questions to be addressed:

1. What are the NOECs and LOECs of model compounds *in vivo*?
2. What is the relationship between ER *in vitro* assay responses and adverse effects on fish reproduction?

These studies will provide quantitative linkage between effects measured *in vitro* (i.e. induction/ suppression of receptor activity) and *in vivo* (i.e. reproductive output, sexual characteristics). The

21-day fathead minnow (*Pimephales promelas*) reproductive assay should be performed in accordance with USEPA (2007) and OECD (2012) guidelines. The toxicity of model compounds known to affect ER receptors will be investigated. Water samples should be collected directly from the exposure tanks and extracted and analyzed using the appropriate cell receptor assay and targeted chemistry.

Table 2.4-1. Key test parameters for linkage study of *in vitro* and *in vivo* responses to model compounds.

	Test parameters - ER agonist
Chemicals	17-beta estradiol Solvent control (TEG or ethanol, less than 0.05%) Water control (no solvent)
In vitro endpoint	ER receptor transactivation
Fish assay endpoints	- % survival and changes in behavior relative to controls - No eggs laid and fertilized - Levels of plasma steroids and vitellogenin (males) relative to controls - Reduction of the number of nuptial tubercles in males - Gonadosomatic index - Gonad histopathology (possible testis-ova in males) - qPCR (e.g. vtg, aromatase) and/or microarrays

2.4.2 Effects of CECs in Complex Environmental Matrices on Fish Reproduction

Questions to be addressed:
1. Do CECs, present in complex mixtures, affect fish physiology, behavior and reproduction?
2. What is the relationship between results of *in vitro* and *in vivo* assays?

The fish reproduction assay will be conducted using water samples from locations previously monitored by targeted chemical analyses and Tier I *in vitro* analyses. The specific fish reproduction parameters to be measured in this study are described in **Table 2.4-1** with samples in **Table 2.5-1**.

Table 2.5-1. Aqueous test samples for fish reproduction assay.

Scenario	Sample	Dilutions
Scenario 1 Freshwater	2 WWTP effluents Receiving river water Station #1 & 5 (Section 2.3.1)	1x – undiluted effluent 1x – undiluted samples
Scenario 2 Embayment*	2 WWTP effluents	1x – undiluted effluent 10x – worst case 100x – best case
Scenario 3 Oceans*	2 WWTP effluents	1x – undiluted effluent 50x – worst case > 1000x – best case

2.6 Tier III – In Situ Toxicity Assessment

In situ analyses will be conducted using fish species residing in the waterways previously monitored using targeted chemical analyses, Tier I (*in vitro* screening) and Tier II (*in vivo* laboratory exposures) assays.

The State Water Resources Control Board (SWRCB) has developed guidelines to sample and measure environmental chemicals (e.g. metals, PCBs, alkylphenols) in fish and invertebrates (Davis et al. 2014, SWAMP 2014). Tier III analyses will be conducted using the same fish species collected for tissue monitoring (Section 1.4.2). Recommended species include common carp, channel catfish, Sacramento sucker and largemouth bass for freshwater environments (scenario 1); topsmelt, white croaker, shiner surfperch and California halibut for coastal environments (scenario 2); white croaker, Dover sole, English sole, scorpion fish and hornyhead turbot (scenario 3). For in situ monitoring in the Delta, largemouth bass can serve as a sentinel fish species. For each waterway, a minimum of 2 species and 5 fish per species (n = 10 fish minimum) will be collected. Liver-somatic (LSI) and gonadosomatic (GSI) indexes will be evaluated. Gonads and liver will then be preserved for histopathological analyses.

3. Cost of the Pilot Study

Table 3.1 Summary two-year cost.

Region	Chemistry	Tissue	Non Target	Total
Delta/Central Valley	$492,000	$122,000	$36,400	$650,400
San Francisco Bay	$296,000	$42,000	$36,400	$380,800
Southern California	$240,000	$64,000	$20,800	$324,800
Ocean	$68,000	$40,000	$52,000	$160,000
Bioanalytical (all regions)				$566,250
Total	$1,096,000	$268,000	$145,600	$2,075,850

The total cost for the pilot plan is $2,075,850. However, the Regional Boards recommended adding additional compounds for monitoring in their respective Regions which raised the total cost by $517,200. When the amount is added to the total pilot study, the total cost will be $2,593,050. The detail of this additional cost is shown in Tables 3.1.a, 3.1.b and 3.1.c. below. The San Francisco Bay region has added a total of $66,000 and Los Angeles region has added a total of $451,200.

3.1. a Summary of additional cost for RB 2				
	Matrix	**Total Samples**	**Total Cost/Sample**	**Total Coast (two years)**
SF Bay WWTPs & MS4				
sulfamethoxazole, erythromycin	WWTP- Water (effluent)	10	$600	$6,000
	Ambient Bay Water	40	$600	$24,000
	MS4- Water (wet weather)	24	$600	$14,400
	MS4- Water (dry weather)	12	$600	$7,200
	MS$- Sediment	24	$600	$14,400
			Total cost	$66,000

3.1.b Summary of additional cost for RB 4 WWTP				
	Matrix	Total Cost/Sample	Total Samples	Total cost (two years
Bifenthrin, Permethrin, Fipronil & 3 degradates (desulfinyl, sulfide, sulfone)	Water (receiving)	$1,000	56	$56,000
	Water (effluent)	$1,000	16	$16,000
	Sediment (receiving)	$1,000	56	$56,000
Fipronil	Tissue (fish) Fipronil	$600	20	$12,000
TCEP, TCPP, TDCPP	Water (receiving)	$600	56	$33,600
	Water (effluent)	$600	16	$9,600
	Sediment (receiving)	$1,000	56	$56,000
Sucralose	Water (receiving)	$500	56	$28,000
	Water (effluent)	$500	16	$8,000
	Sediment (receiving)	$500	56	$28,000
			Total cost	**$303,200**

3.1.c Summary of additional cost for RB 4 MS4				
	Matrix	Total Cost/Sample	**Total Samples**	Total cost
Bifenthrin, Permethrin, Fipronil & 3 degradates (desulfinyl, sulfide, sulfone)	Water (wet weather)	$1,000	24	$24,000
	Water (dry weather)	$1,000	12	$12,000
	Sediment	$1,000	24	$24,000
Fipronil	Tissue (fish) Fipronil	$1,100	20	$22,000
TCEP, TCPP, TDCPP,	Water (wet weather)	$600	24	$14,400
	Water (dry weather)	$600	12	$7,200
	Sediment	$600	24	$14,400
Sucralose	Water (wet weather)	$500	24	$12,000
	Water (dry weather)	$500	12	$6,000
	Sediment	$500	24	$12,000
			Total cost	**$148,000**
			Grand T	**$451,200**

Data and Deliverables

Data will be submitted to CEDEN database and will be formatted to submit in electronic submittal system of the database. The deliverables are:

1. Submit data to CEDEN
2. Annual technical report and factsheet
3. QAPP of the project

Appendix I

Target CECs and Matrices by Scenarios

Table A. Bay Delta Freshwater (Scenario 1)					
Compound	Matrices				Rationale
	Effluent	Ambient water	Sediment	Tissue	
Estrone	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
Ibuprofen	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
Bisphenol A	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
17-beta-estradiol	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
Galaxolide (HHCB)	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
Diclofenac	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data
Triclosan	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	No data

Table B. Southern California - Fresh Water (Scenario 1)					
Compound	Matrices				Rationale
	Effluent	Ambient Water	Sediment	Tissue	
Estrone	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	Sediment no data
Ibuprofen	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	
Bisphenol A	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	
17-beta-estradiol	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	
Galaxolide (HHCB)	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	
Diclofenac	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	
Triclosan	Monitor	Monitor	Monitor	Fish, Bivalve, Bird egg	

Table C. Southern California – Ocean (Scenario 3)					
Compound	Matrices				Rationale
	Effluent	Ambient Water	Sediment	Tissue	
Estrone	Monitor	Monitor	Monitor	Not Applicable	
Bis(2-ethylhexyl) phthalate (BEHP)	Monitor	Monitor	Monitor	Not Applicable	
Butylbenzyl phthalate (BBP)	Monitor	Monitor	Monitor	Not Applicable	
p-nonylphenol	Monitor	Monitor	Monitor	Bivalve	
PBDE-47	Monitor	Monitor	Monitor	Fish, Bivalve, Blubber	
PBDE-99	Monitor	Monitor	Monitor	Fish, Bivalve, Blubber	
PFOS	Monitor	Monitor	Monitor	Fish, Bivalve, Blood	

Table D. San Francisco Bay - Fresh Water (Scenario 1)					
Compound	Matrices				Rationale
	Effluent	Receiving	sediment	Tissue	
Estrone	Monitor	Monitor	??	Not applicable	No data
Bisphonel A	Monitor	Monitor	??	Not applicable	No data
17-beta-estradiol	Monitor	Monitor	yes	Not applicable	No data
Galaxolide (HHCB	Monitor	Monitor	Monitor	Not applicable	only one year data (available
Diclofenac	Monitor	Monitor	Monitor	Not applicable	no data
Triphenyl phosphate	Monitor	Monitor	Monitor	Not applicable	Recommended by the Region
Sulfamethoxazole	Monitor	Monitor	Monitor	Not applicable	Recommended by the Region
Erythromycin	Monitor	Monitor	Monitor	Not applicable	Recommended by the Region

Table E. San Francisco Bay – Embayment (Scenario 2)					
Compound	Matrices				Rationale
	Effluent	Receiving	sediment	Tissue	
Estrone	Monitor	Monitor	Not applicable	Not applicable	No data
Bisphonel A	Monitor	Monitor	Not applicable	Not applicable	No data
17-beta-estradiol	Monitor	Monitor	Not applicable	Not applicable	No data
Galaxolide (HHCB	Monitor	Monitor	Not applicable	Not applicable	only one year data (available
Diclofenac	no	no	Not applicable	Not applicable	no data

Appendix II

List of Compounds Added and Removed from the Original Target List

Table 1. Compounds added in the target list by Regional Boards.

Compound	Recommended by	Matrices
Bifenthrin	Regional Board 4	Water & sediment
Perimethrin	Regional Board 4	Water & sediment
fipronil	Regional Board 4	Water, sediment & tissue
Fipronil desulfinyl	Regional Board 4	Water & sediment
Fipronil sulfide	Regional Board 4	Water & sediment
Fipronil sulfone	Regional Board 4	Water & sediment
TCEP	Regional Board 4	Water & sediment
TCPP	Regional Board 4	Water, sediment
TDCPP	Regional Board 4	Water, sediment
Sucralose	Regional Board 4	Water, sediment
Triphenyl phosphate	Regional Board 2	Bay Water; in River-- Water & sediment
Sulfamethoxazole	Regional Board 2	Bay Water; in River Water & sediment
Erythromycin	Regional Board 2	Bay Water; in River Water & sediment

*****For fipronil, sampling and chemistry cost are added with the rest of the pesticides. The cost indicated in its line item is for tissue analysis.
**Total cost includes both sampling and analytical cost for two years.

Table 2. Compounds recommended by Science Panel but removed by Stakeholders.

Compound	Recommended by	Reason
Bifenthrin	Science Panel	monitored by other program
Perimethrin	Science Panel	monitored by other program
Chlorpyrifos	Science Panel	monitored by other program
fipronil	Science Panel	monitored by other program

Appendix III

Reporting Limits

Table 1. Target CECs: Monitoring trigger levels (MTLs) and reporting limits (RLs). Recommended RLs are derived from MTLs as reported by the CEC Ecosystems Panel. WWTP and MS4

Compound	Panel Freshwater MTL	Recommended RL *	Achievable RL *
Aqueous Phase - Effluent dominated inland waterways (Scenario 1) (ng/L)			
Estrone	6.0	3.0	
Ibuprofen	100	50	
Bisphenol A	60	30	
17-beta-estradiol	2.0	1.0	
Galaxolide (HHCB)	700	350	
Diclofenac	100	50	
Triclosan	250	125	
Bis(2-ethylhexyl) phthalate (BEHP)			3.0
Butylbenzyl phthalate (BBP)			3.0
p-nonylphenol			22
PBDE-47			0.10
PBDE-99			0.10
PFOS			1.0

***** Minimum RL reported by commercial services laboratories. Missing values indicate the achievable value is at or below the recommended RL.

Table 2. Reporting Limit for target CECs: Coastal embayments (Scenario 2).

Aqueous Phase - (ng/L)			
Compound	**Panel Bay/Estuarine MTL**	**Recommended RL ***	**Achievable RL ***
Bisphenol A	6.0	3.0	
Estrone	0.60	0.30	2.0
17-beta-estradiol	0.20	0.10	0.4
Galaxolide (HHCB)	70	35	

***** Minimum RL reported by commercial services laboratories. Missing values indicate the achievable value is at or below the recommended RL.

Table 3. Target CECs: Reporting limits for Coastal embayment (Scenario 2).

Sediment - (ng/g dw)			
Compound	**Panel Bay/Estuarine MTL**	**Recommended RL ***	**Achievable RL ***
PBDE-47	0.030	0.015	
PBDE-99	0.030	0.015	
PFOS4	NA	0.1	

***** Minimum RL reported by commercial services laboratories. Missing values indicate the achievable value is at or below the recommended RL.

Table 4. Target CECs for *All Scenarios.*

Tissues (ng/g dw)		
Compound	**Panel MTL**	**Recommended RL**
PBDE-47	*28.9*	*14.5*
PBDE-99	*28.9*	*14.5*
PFOS	*1000*	*500*

Appendix IV

Tier Assignments Framework

Tier Assignments of Targeted CECs by Region
Based on RB 2 Tier Framework

Categories	San Francisco Bay	Southern California	Delta/Central Valley	Monitoring	Management
Tier IV High Concern				Studies to support cleanup plan	303(d) listing, Cleanup Plan (TMDL), Aggressive Control
Tier III Moderate Concern				Status and trends monitoring; and/or Studies of fate, effects, and sources and pathways	Action plan or strategy; Aggressive pollution prevention; Low-cost control
Tier II Low Concern				Reduced frequency screening in water, sediment, or biota. Periodic screening in pathways, track trends	Low-cost source ID and control; Low-level pollution prevention; Track use trends
Tier I Possible Concern				Screening in water, sediment, biota, wastewater, urban runoff	Prioritize contaminants of potential concern, track other efforts; Develop analytical methods

CECs will be assigned in each category when results are available

Definition of Tiers

Tier IV High Concern	**Moderate or High Impact.** High probability of moderate or high level effect on wildlife.
Tier III Moderate Concern	**Low Impact** High probability of low level effect on wildlife
Tier II Low Concern	**No Impact** High probability of no effect on wildlife
Tier I Possible Concern	**Unclear** uncertainty in toxic thresholds.

Monitoring Strategy

Tier IV High Concern	Studies to support Total Maximum Daily Load (TMDL) or alternatives
Tier III Moderate Concern	Trends monitoring and/or fate, effects and sources and loadings studies
Tier II Low Concern	Periodic ambient and/source trend screening
Tier I Possible Concern	Ambient and source screening

Appendix V

Detail Cost of Pilot Study

5. Detailed two-year monitoring cost

5.1 Cost for Southern California WWTPs (Scenario 1).

Southern California WWTP			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (receiving)	$2,000	56	$ 112,000
Water (effluent)	$2,000	16	$ 32,000
Sediment (receiving)	$1,000	56	$56,000
Tissue (fish and bivalves)	$1,000	20	$ 20,000
Tissue (bivalves)	$1,000	12	$ 12,000
Tissue (non-targeted, fish)	$2,600	4	$ 10,400
		Subtotal	$ 242,400

5.2 Cost for Southern California MS4 (Scenario 1)

Southern California Stormwater			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (wet weather)	$2,000	24	$ 48,000
Water (dry weather)	$2,000	12	$ 24,000
Sediment	$1,000	24	$ 24,000
Tissue (fish)	$1,000	20	$ 20,000
Tissue (bivalve)	$1,000	12	$ 12,000
Tissue (non-targeted, fish)	$2,600	4	$ 10,400
		Subtotal	$ 138,400

5.3 Coast for Southern California Ocean (Scenario 3).

Southern California Ocean			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (effluent only)	$2,000	16	$32,000
Sediment	$1,000	36	$36,000
Tissue (fish)	$1,000	20	$20,000
Tissue (mammals)	$1,000	20	$20,000
Tissue (non-targeted, fish and mammal)	$2,600	20	$52,000
		Ocean Subtotal	$160,000
		Subtotal	$540,800

5.4 Cost for San Francisco Bay Embayment (Scenario 2).

SF Bay WWTP Embayment			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Coast (Two Years)**
Water (effluent)	$2,000	10	$20,000
Ambient Bay Water	$2,000	40	$80,000
Tissue Non targeted - mammal	$2,600	10	$26,000
		Subtotal	$126,000

5.5 Cost San Francisco Bay MS4 (Scenario 1).

SF Bay Stormwater			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (wet weather)	$2,000	24	$48,000
Water (dry weather)	$2,000	12	$24,000
Sediment	$1,000	24	$24,000
Tissue (eggs)	$1,000	10	$10,000
Tissue (fish)	$1,000	20	$20,000
Tissue (bivalve)	$1,000	12	$12,000
Tissue (non-targeted, fish)	$2,600	4	$10,400
		Subtotal	$148,400
		Total	$274,400

5.6 Cost of Delta/Central Valley WWTP (Scenario 1).

Delta/Central Valley WWTP			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (receiving)	$2,000	84	$ 168,000
Water (effluent)	$2,000	24	$ 48,000
Sediment (receiving)	$1,000	84	$ 84,000
Tissue (fish bivalves)	$1,000	30	$ 30,000
Tissue (bivalves)	$1,000	18	$ 18,000
Tissue (non-targeted, fish)	$2,600	6	$ 15,600
		Subtotal	$ 363,600

5.7 Cost for Bay Delta MS 4 (Scenario 1).

Bay Delta Stormwater			
Matrix	**Total Cost/Sample**	**Total Samples**	**Total Cost (Two Years)**
Water (wet weather)	$2,000	48	$ 96,000
Water (dry weather)	$2,000	24	$ 48,000
Sediment	$1,000	48	$ 48,000
Tissue (eggs)	$1,000	10	$ 10,000
Tissue (fish)	$1,000	40	$ 40,000
Tissue (bivalve)	$1,000	24	$ 24,000
Tissue (non-targeted, fish)	$2,600	8	$ 20,800
		Subtotal	$ 286,800
		Total	$ 650,400

5.8 Cost for bioanalytical monitoring

	ER-a	AR	Anti-Ar	AhR	Total
In vitro targeted CEC	$36,000	-------	$18,000	$18,000	$72,000
In vitro Environmental Extracts	$48,750	----	$48,750	$48,750	$146,250
				Sub total (in vitro)	$218,250
In vitro with fish reproduction	$50,000	$50,000	$50,000		$150,000
Effects of CECs in receiving water sample on fish reproduction					$150,000
				Sub total (in vivo)	$300,000
In situ assessment					$48,000
				Total	$566,250