Monitoring of Constituents of Emerging Concern (CECs) in Aquatic Ecosystems – Pilot Study Requirements

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1 Introduction

In October 2009, the State of California Water Resources Control Board (SWRCB) provided support for a scientific advisory panel to review existing scientific literature on constituents of emerging concern (CECs) in aquatic ecosystems; determine the state of the current scientific knowledge regarding the risks that CECs in freshwater and marine water pose to human health and aquatic ecosystems; and provide recommendations on improving the understanding of CECs for the protection of public health and the environment. Seven experts were vetted and convened as the CEC Ecosystems Panel (Panel) to provide information and recommendations on CECs[1] in coastal and marine ecosystems, and was subsequently tasked to expand the scope to include freshwater ecosystems. The Panel collaborated with stakeholders, who provided their perspective of the water quality issues and additional information, during the development of their recommendations. In their final report, Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems: Recommendations of a Science Advisory Panel, SCCWRP Technical Report 692, Anderson et al. (2012) recommended a risk-based screening framework to identify CECs for monitoring, applied the framework using existing information to three representative receiving water scenarios to identify a list of appropriate CECs for initial monitoring, an adaptive phased monitoring approach and development of bioanalytical screening and predictive modeling tools to improve assessment of the presence of CECs and their potential risk to the environment.

Early in the process, the Panel was instructed by SWRCB staff to focus on ambient surface waters that receive discharge from sources regulated under the National Pollutant Discharge Elimination System (NPDES). As a result, permitted discharges from municipal wastewater treatment plants (WWTPs) and municipal separate storm sewer systems (MS4) were considered as the primary sources of CECs to receiving waters. Waterbodies that receive agricultural runoff were not considered.

1.1 Summary of Panel Recommendations

1.1.1 Adaptive Monitoring Strategy

The Panel recommended an adaptive monitoring approach with four sequential phases described below (**Fig. 1.1-1**) that is responsive to advances in assessment and monitoring technology.

PHASE 1 – DEVELOP INITIAL CEC LIST. The Panel met with scientists, managers and stakeholder groups representing local, regional and statewide interests, to learn about current CEC studies, regional and statewide monitoring programs, and NPDES permitted discharges that are relevant statewide. The Panel created a risk-based framework to identify high priority CECs based on available, peer-reviewed occurrence and toxicity information. In applying this framework, the Panel identified three exposure scenarios where WWTP and MS4 discharge could impact receiving water quality. These scenarios are (1) WWTP effluent dominated freshwater (rivers); (2) coastal embayments receiving both WWTP effluent and stormwater discharge; and (3) ocean discharge from large WWTP (> 100 million gallons per day) outfalls. The initial list of CECs was generated by comparing measured or predicted environmental concentrations (MECs or PECs) in aqueous, sediment and/or tissue to monitoring trigger levels (MTLs) based on biological effects thresholds that incorporated safety factors. CECs recommended for initial

[1] CECs may include a wide variety of substances including pharmaceuticals, flame retardants, newly registered contemporary use pesticides, commercial and industrial products, fragrances, hormones, antibiotics and nanoparticles that are not currently regulated in discharges to ambient waters across California.

monitoring exhibited a monitoring trigger quotient (MTQ = MEC/MTL) that exceeded unity and for which sufficiently robust analytical chemistry methods were available. The recommendations for Phase 1 was documented in the Panel's final report (Anderson et al. 2012).

PHASE 2 – IMPLEMENT MONITORING OF CECS. The objectives of this phase are to: 1) verify the occurrence of high priority CECs in aqueous, sediment and tissue samples; 2) initiate compilation of a data set that characterizes their occurrence in source and receiving waters, and in appropriate matrices (i.e., water, sediment and tissue); 3) evaluate improved/supplemental methods and surrogate measures (e.g., bioanalytical screening tools); and 4) utilize, modify and/or initiate development of environmental fate models where appropriate. Screening-level mass balance models synthesize knowledge of CEC loading, and predict environmental compartment transfer and loss rates, as well as temporal CEC concentration trends. Through insight gained from these models, prioritization efforts in Phases 3 and 4 can subsequently focus on issues with the greatest potential risk.

PHASE 3 – UPDATE MONITORING AND RESPONSE PLANS. Using results from Phase 2, the list of CECs is re-evaluated and, if warranted, re-prioritized. Results of environmental fate modeling are evaluated to prioritize future monitoring and to conduct a preliminary review of the impacts of management actions.

PHASE 4 – ACTION PLAN TO MINIMIZE IMPACTS. If the assessment conducted during Phase 3 indicates certain CECs will persist and continue to present a concern, then during Phase 4 the Panel would develop guidance on the development and assessment of specific action plans for consideration by the SWRCB for implementation as part of their development of statewide policies, permits and/or guidance.

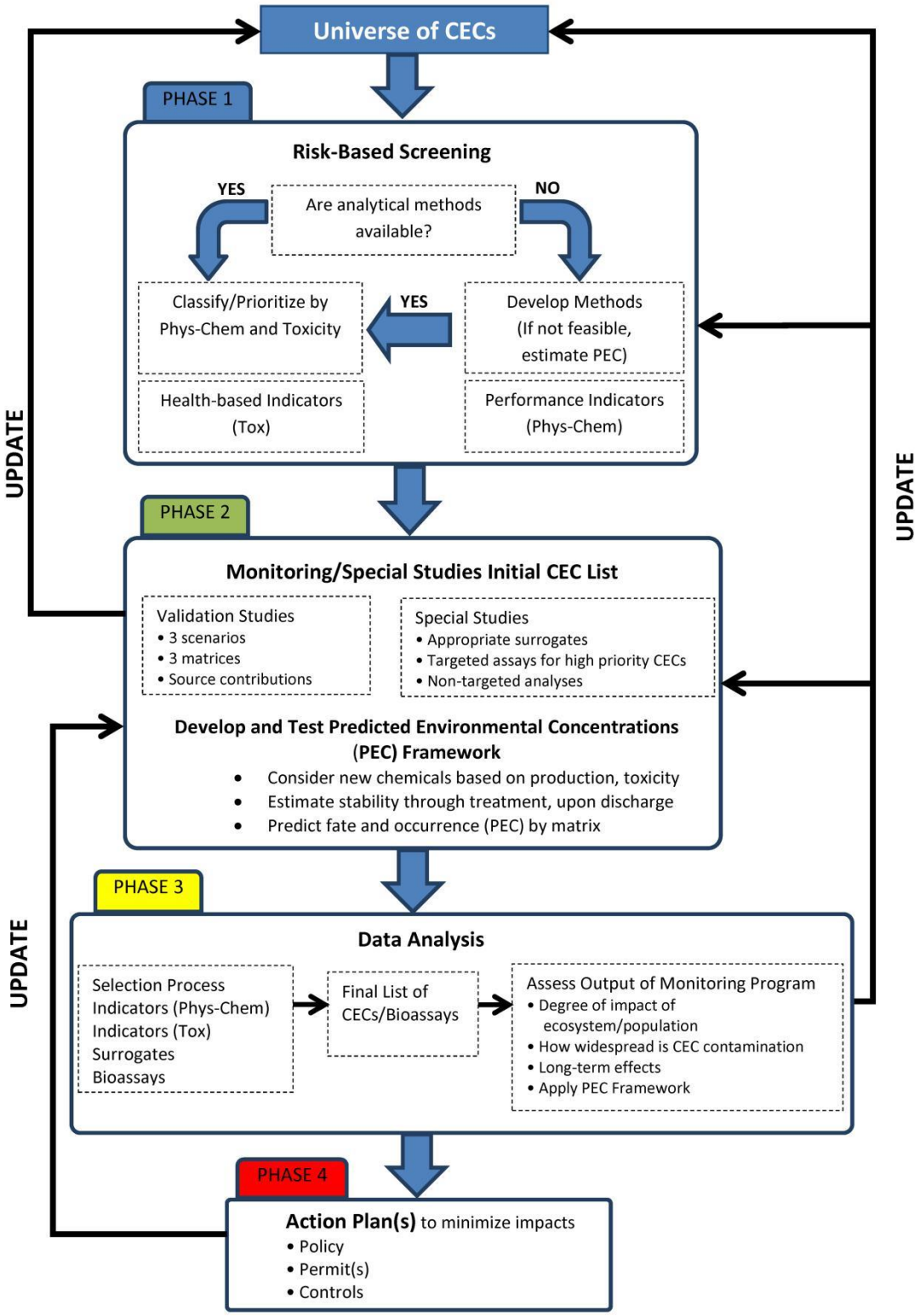

Figure 1.1-1. The adaptive monitoring strategy for constituents of emerging concern (CECs) developed by the Expert Panel convened to recommend CEC monitoring in California surface waters impacted by NPDES permitted discharges (i.e. treated wastewater effluent and stormwater runoff).

1.1.2 Discharge Scenarios

With guidance from the SWRCB and stakeholder community, the Panel identified three receiving water scenarios for which to provide CEC monitoring recommendations. These scenarios were selected based on the expected magnitude of CEC discharge from NPDES permitted sources and severity of exposure to both human and ecological receptors.

1. Inland freshwaters where flow is dominated by treated WWTP effluent discharge (dry season).
2. Coastal embayments receiving treated WWTP effluent and stormwater (MS4) discharge (dry and wet seasons).
3. Offshore marine waters receiving treated effluent from large (>100 mgd) WWTPs.

These scenarios were considered separately because they have distinct differences in spatial and temporal source characteristics, fate and transport processes, and receptors of interest that define beneficial uses of the resource. A detailed description of relative CEC source contributions and exposure conditions for each of the three scenarios is provided in the Panel's final report (Anderson et al. 2012).

1.1.3 Initial List of CECs by Discharge Scenario ("Targeted Monitoring")

A total of 16 individual CEC analytes were recommended for chemical-specific (or "targeted") Phase 2 monitoring; however not all 16 CECs were selected for all scenarios (**Table 1.1.3-1**). Due primarily to the limited degree of attenuation (e.g. by dilution), the number of CEC analytes recommended for monitoring was greatest for the WWTP effluent dominated inland freshwater (Scenario I). In contrast, the smallest number of CECs recommended were for sediment and tissue, due in large part to the paucity of MECs and MTLs available for these matrices compared with water (aqueous phase).

The Panel was also charged to provide guidance on implementation of targeted CEC monitoring. Guidance on the number of waterbodies and discharges, spatial coverage and temporal (frequency of monitoring) considerations from the Panel was given to address the highest priority questions identified by the Panel (**Table 1.1.3-2**), e.g. what is the occurrence (magnitude, pervasiveness) of target CECs in waterbodies representing each scenario? What is the spatial and temporal variation in CEC occurrence in these scenarios?

1.1.4 Special Studies to Improve CEC Monitoring

One of the key limitations to the risk-based framework utilized by the Panel to identify CECs for targeted monitoring is the lack of robust monitoring/occurrence/toxicity data (i.e. MECs and MTLs) for the vast array of possible environmental contaminants. In recognition of this limitation, the Panel recommended a number of special studies using emerging technologies and/or methods that if successful, will provide a more comprehensive and efficient monitoring program for receiving waters (Anderson et al. 2012). These studies will complement and/or direct traditional targeted analytical methods while providing additional information on the occurrence of unknown CECs, and based on biological responses of aquatic organisms at the cellular (bioanalytical screening) and organism (in vivo testing) level (**Table 1.1.4-1**).

Table 1.1.3-1. Constituents of emerging concern (CECs) recommended for pilot (Phase 2) monitoring by the CEC Ecosystems Panel. Each column lists exposure scenarios (E = coastal embayment; F = inland freshwater, O = ocean) and matrices of interest (i.e., aqueous, sediment, tissue). M = monitor; NA = not applicable. WWTP – municipal wastewater treatment plant.

Scenario	**Source: WWTP Effluent**	**Source: Storm Water (MS4)**	**Scenario 1 Effluent Dominated Inland Freshwater**	**Scenario 2 Embayment**		**Scenario 3 Ocean**	**All Scenarios**
Matrix	**Aqueous**	**Aqueous, Sediment**	**Aqueous**	**Aqueous**	**Sediment**	**Sediment**	**Tissue**
Additional Information in Panel Report			Tables 6.1 & 6.6	Table 6.2	Table 6.3	Table 6.4	Table 6.5
Bis(2-ethylhexyl) phthalate (BEHP)	O	NA	NA	NA	NA	M	NA
Butylbenzyl phthalate (BBP)	O	NA	NA	NA	NA	M	NA
p-Nonylphenol	O	NA	NA	NA	NA	M	NA
Bifenthrin	E F	M	M	M	M	NA	NA
Permethrin	E F	M	M	M	M	NA	NA
Chlorpyrifos	E F	M	M	M	NA	NA	NA
Estrone	E F	M	M	M	NA	NA	NA
17-beta estradiol	E F	M	M	M	NA	NA	NA
Galaxolide (HHCB)	E F	M	M	M	NA	NA	NA
Bisphenol A	E F	M	M	M	NA	NA	NA
Ibuprofen	F	M	M	NA	NA	NA	NA
Diclofenac	F	M	M	NA	NA	NA	NA
Triclosan	F	M	M	NA	NA	NA	NA
PBDE -47 and -99	E F O	M	NA	NA	M	M	M
PFOS	E F O	M	NA	NA	M	M	M

Table 1.1.3-2. Preliminary design guidance for pilot monitoring of CECs (Phase 2) in each of the three receiving water scenarios and for stormwater (MS4) discharge. F = freshwater; M = monitor; NA = not applicable; RW = receiving water.

	Source	Scenario 1	Scenario 2	Scenario 3
General Monitoring Design Parameters	**Stormwater (MS4) Discharging to Receiving Water[a]**	**WWTP Discharging to Inland Freshwater[b]**	**WWTP Discharging to Coastal Embayment[c]**	**WWTP Discharging to Ocean[d]**
Spatial coverage – Receiving Water (RW)	1-D gradient (up to 6 sites for each location)	1-D (up to 6 sites for each location)	2-D gradient (up to 7 sites in estuary)	2-D grid (up to 7 sites each location)
Number of POTW and/or FW Locations	Two large FW streams and the Delta	Two POTWs and RW	Five POTWs in one estuary/embayment	Two POTWs and corresponding RWs
Frequency	Wet and Dry Season over three years	Wet and Dry Season over three years	Semi-annual (aqueous) or annual (sediment, tissue) over three years	Semi-annual (aqueous) or annual (sediment, tissue) over three years
Background	M	M	M	M
Aqueous (non-filtered)	M	M	M	NA
Sediment (top 5 cm)	M	M	M	M
Tissue [e]	M	M	M	M

a - Potentially conduct pilot investigation for one stream in the San Francisco Bay Area; one stream in Southern California, and one stream in the Sacramento-San Joaquin Delta.

b - Potentially conduct pilot investigation in Southern California.

c - Daily discharge <100 mgd; potentially conduct pilot investigation in San Francisco Bay.

d - Daily discharge $\geq$100 mgd; potentially conduct pilot investigation in southern California.

e - Identify appropriate species and tissues (e.g., bivalve and fish tissue for PBDEs; bird eggs for PFOS).

Table 1.1.4-1. Special studies recommended for pilot evaluation (Phase 2) to improve CEC monitoring in aquatic ecosystems. WWTP – municipal wastewater treatment plant.

Special Study	WWTP Discharging to Inland Freshwater (Scenario 1)	WWTP Discharging to Coastal Embayment (Scenario 2)	WWTP Discharging to Ocean (Scenario 3)	Stormwater (MS4) Discharging to Receiving Water
Bioanalytical Screening Assays[a]	yes	yes	yes	yes
Toxicity[b]	yes	yes	yes	no
Antibiotic Resistance[c]	yes	yes	no	no
Passive Sampling Devices (PSDs)[d]	yes	no	yes	no

a – Conduct evaluation and validation of bioanalytical screening methods in combination with targeted and non-targeted chemical analyses to identify bioactive substances using a toxicity identification evaluation (TIE) process.

b – e.g. 21 d fathead minnow recrudescence assay for freshwater matrices. Implement periodic reproduction assessments using appropriate fish and invertebrate species. Coordinate efforts with NPDES WET and bioassessment monitoring. This assay should be used for investigative purposes.

c -- Conduct a pilot investigation using a bioassay to screen for antibiotic resistance in effluent, water and/or sediment.

d – Conduct a pilot investigation using PSDs that provide adequate capacity to concentrate the CECs in the priority list. These devices should have demonstrated acceptable performance in laboratory or field validation studies, and published guidance on translation of results.

1.2 Pilot Monitoring (Phase 2) Design Requirements

The objective of this document is to generate requirements for pilot monitoring and special studies for CECs that address elements described in Phase 2 of the Panel's adaptive monitoring strategy (Fig. 1.1-1). These elements are broadly classified into targeted (chemical-specific) monitoring and special studies. ***The intent of this effort is to translate the Panel's guidance into requirements at a sufficient level of specificity and detail that can direct and be incorporated into local, regional and/or statewide workplans for future monitoring***.

To ensure relevance to the management decision making process, the Panel emphasized the need for a purposive (i.e. question or hypothesis driven) approach to monitoring, offering several questions to be answered by the proposed pilot monitoring and special studies monitoring:

1. Which CECs are detected in freshwaters and depositional stream sediments, and in which large California watersheds are they detected?
2. Which CECs are detected in marine waters and sediments adjacent to WWTP and significant stormwater outfalls and how quickly do they attenuate?
3. Which CECs are detected in coastal embayment/estuarine water and sediments?
4. What is the relative contribution of CECs in WWTP effluent vs. stormwater?

5. What is the extent and magnitude of PBDE and PFOS contamination in tissues of aquatic wildlife across the State? Does tissue occurrence correspond with sediment occurrence?
6. What is the direction and magnitude of change in CEC concentrations (in water, sediment and tissues) over a multi-year (3 to 5 year) time period?
7. How does the Panel's assumed relationships, based on the new CEC data (e.g., MEC or PEC, NOEC and MTL), change the estimated MTQs?
8. Does the new information (Question 7 above) modify the Panel's assumption regarding CEC potential risk and if so, does it trigger the need to evaluate CEC control efforts?
9. Which bioanalytical screening assays are effective to screen for target CECs in environmental samples?
10. How efficient are bioanalytical screening tools to detect unknown CECs?
11. What is the relationship between effects of CECs in vitro and toxicity observed in vivo?
12. What are the toxic effects of CECs of aquatic organisms?
13. How do CECs affect microbial antibiotic resistance?
14. Can passive samplers be used as a robust monitoring tool for CECs?

1.2.1 Targeted Monitoring

The design requirements to be specified for targeted monitoring for the CECs, scenarios and matrices listed in Tables 1.1.3-1 and 1.1.3-2, and as described in project agreement, are:

1. List of target CEC analytes, preferred methods and desired reporting limits
2. List of candidate waterbodies that represent exposure scenarios identified by the Science Advisory Panel
3. List of target media (e.g. water, sediment, biological tissue), and candidate target species
4. Frequency, number, and location of sampling stations with each candidate waterbody
5. QA/QC goals for measurement of CECs for incorporation into the Project Supplemental Guidance for Quality Assurance/Quality Control document (see Task 5 in Contract)
6. List of appropriate monitoring questions for each exposure scenario
7. Data analysis and assessment methods for each exposure scenario
8. Data management plan
9. Strategy to coordinate with existing monitoring programs

The development of targeted monitoring requirements is addressed in Section 2 of this document.

1.2.2 Special Studies

The design requirements to be specified for special studies monitoring for the elements in Table 1.1.4-1, and as described in project agreement, are:

1. List of target parameters, preferred methods and desired measurement goals
2. List of candidate waterbody(ies) for each special study
3. List of target media (e.g. water, sediment, biological tissue), and candidate target species
4. Frequency, number and location of sampling stations to be evaluated within each candidate waterbody

5. Quality assurance/quality control (QA/QC) goals for measurement of specific parameters
6. Rationale for exclusion/inclusion of studies that differ from the Panel's final recommendations

The development of special studies requirements is addressed in Section 3 of this document.

1.2.3 Supporting/Related Documentation

In addition to the design requirements specified herein, guidance for QA/QC will be generated as a supplement to this document. This supplemental guidance document will provide criteria and guidelines to ensure that robust measurement of targeted monitoring and special study parameters is achieved.

1.3 Relevant Water Quality Monitoring Programs in California

1.3.1 SWAMP

The Surface Water Ambient Monitoring Program (SWAMP, (http://www.waterboards.ca.gov/water_issues/programs/swamp/about.shtml) was created to unify and coordinate all water quality monitoring conducted by the State and Regional Water Boards. The SWAMP mission is to provide resource managers, decision makers, and the public with timely, high-quality information to evaluate the condition of all waters across the State. SWAMP accomplishes this through the design and external review of monitoring programs, and by assisting others in generating comparable data for integrated assessments that provide answers to current management questions. SWAMP monitoring programs are each designed to address one or more of the following assessment questions:

- Status: What is the overall quality of California's surface waters?
- Trends: What is the pace and direction of change in surface water quality over time?
- Problem Identification: Which water bodies have water quality problems and are at risk?
- Diagnostic: What are the causes and sources of water quality problems?
- Evaluation: How effective are clean water projects and programs?

Current SWAMP efforts focus on two critical assessment needs: fish consumption safety in fishable waters (Bioaccumulation Monitoring Program) and aquatic ecosystem health in streams and rivers (Bioassessment Monitoring Program and the Stream Pollution Trends Monitoring Program [SPoT]).

The Bioaccumulation Monitoring Program addresses whether fish found in California's streams, lakes and coastal areas are safe to eat by measuring contaminant concentrations in fish tissue. The Bioaccumulation Oversight Group (BOG) guides the implementation of the Bioaccumulation Monitoring Program. Previous investigations have focused on legacy organochlorine pollutants (DDTs, PCBs, chlordanes) in bivalves and sport fish from freshwater systems (lakes, rivers and streams) and coastal marine waters. The most recent results for sportfish from California rivers and streams and taken along the coast (Davis et al. 2013, 2014) suggest levels of methylmercury exceeding State guidelines for safe human consumption were widespread, in contrast to levels of legacy organochlorine pollutants (PCBs, DDT and chlordanes) which did not regularly exceed such guidelines. The BOG next plans to focus on California lakes, asking why some lakes have higher methylmercury levels in sportfish than others (SWAMP 2014).

Initiated in 2008, SPoT measures contaminant concentrations and toxicity in sediments that accumulate in the lower reaches of large watersheds throughout California and relates contaminant concentrations to watershed land uses. Sediment samples are collected annually when streams return to base flow conditions after pollutant mobilization in runoff and during the wet season has abated. Each sample is analyzed for industrial compounds, pesticides, and metals, and is tested for toxicity to a resident aquatic crustacean, the amphipod *Hyalella azteca*. Results are compared across watersheds statewide, and pollutant concentrations are compared to land use and other human activities. In 2012, samples were collected from 100 of the nearly 200 major hydrologic units in California.

The most current SPoT summary report for the period 2008-12 provides evidence that pesticides are associated with ambient toxicity in California waters (Phillips et al. 2014). As a result, certain emerging pesticides are being prioritized for future SPoT monitoring. In 2013, fipronil was added as a SPoT analyte due to increasing use and the potential for surface water toxicity. Also, SPoT began collaborating with the California Department of Pesticide Regulation (DPR) to evaluate the effectiveness of new restrictions on the use of pyrethroid pesticides in urban applications. Four "intensive" monitoring sites were jointly sampled by SPoT and DPR to determine whether new regulations result in reduced pyrethroid concentrations and associated effects.

SPoT has plans to continue its monitoring focus on emerging pesticides. In 2015, SPoT will add the additional indicator organism *Chironomus dilutus* to assess the effects of fipronil and its degradates. SPoT is also exploring the possibility of incorporating water column monitoring for imidacloprid and other neonicotinoid pesticides beginning in 2016. In collaboration with DPR and SWAMP, a pilot monitoring project is measuring these pesticides in agricultural streams in 2014 and assessing their effect using *C. dilutus*. Legacy pesticides, PCBs, organophosphate pesticides and metals will be monitored every other year.

In addition to monitoring and assessment activities, SWAMP develops implements and maintains a monitoring infrastructure and associated tools. Key components of this infrastructure include Quality Assurance/Quality Control (QA/QC) protocols, database and data management tools, water quality indicators, methods, and standard operating procedures. These tools are available to SWAMP partners and other interested parties via the SWAMP website. SWAMP leverages limited resources by coordinating with other water quality monitoring efforts on a local, regional and statewide level. SWAMP works with partners to coordinate monitoring efforts among many groups and agencies, and to facilitate the use of data from many sources in statewide assessments.

1.3.2 Department of Pesticide Regulation

The California Department of Pesticide Regulation (DPR) is the lead agency for regulating the registration, sales and use of pesticides in California. This agency oversees pesticide monitoring programs in air, ground and surface waters across the State. The Surface Water Protection Program (SWPP) http://www.cdpr.ca.gov/docs/emon/surfwtr/overvw.html) characterizes pesticide residues, identifies pesticide contamination sources (both agricultural and non-agricultural), determines the mobility of pesticides to surface water, and develops site-specific mitigation strategies. Investigations are done in consultation with other agencies, including the State and Regional Water Boards. In order to promote cooperation, DPR and the SWRCB signed a formal agreement and developed a companion document, "The California Pesticide Management Plan for Water Quality," to coordinate interaction, facilitate communication, promote problem solving, and ultimately assure the protection of water

quality (http://www.cdpr.ca.gov/docs/emon/surfwtr/maaplan.html). Under this plan, DPR investigates pesticides of concern and develops recommended pesticide use practices designed to reduce or eliminate the impact of pesticides on surface water quality. Management practices designed to reduce contamination are usually implemented initially through voluntary and cooperative efforts. If such voluntary practices do not adequately mitigate impacts, DPR can invoke its regulatory authority to impose use restrictions, e.g. by establishing permit conditions to prevent excessive amounts of residues from reaching surface water. If such steps are not adequate, the State and Regional Water Boards may use their authorities to mitigate the adverse effects of pesticides.

To determine if mitigation is effective, the Environmental Monitoring Branch of DPR conducts monitoring studies on pesticides of concern. Two such studies planned for 2014-15 are focused on model watersheds in northern (Emsinger 2014) and southern (Budd 2014) California. Common to these regional studies are the measurement of target pesticides in water and sediment. Pyrethroids (including permethrin and bifenthrin), fipronil and its degradates and chlorpyrifos, identified as high priority CECs by the Panel, are included on DPR's analyte list. Sampling design for these studies focus on characterizing multiple events of dry and wet weather runoff into freshwater systems in suburban and urban neighborhoods.

In addition, DPR has conducted special investigations on the occurrence of pyrethroids in wastewater influent and effluent (Markle et al. 2014, Teerlink 2014). These data may reduce and/or obviate the need to monitor for pyrethroids in WWTP effluent as recommended by the Panel. A third DPR product that may serve useful in future prioritization and monitoring efforts is a model that predicts the mass of pesticides applied in urban landscapes that washoff and enter urban waterways (Luo 2014). Such models can estimate the occurrence of pesticides of concern (i.e. predicted environmental concentrations or PECs) where no measured data are available.

1.3.3 San Francisco Bay Regional Monitoring Program

The San Francisco Bay Regional Monitoring Program (RMP) monitors contamination in the San Francisco Bay Estuary (Estuary). The RMP (http://sfei.org/rmp) is a collaborative effort among the San Francisco Bay Regional Board, the regulated discharger community and the coordinating entity, the San Francisco Estuary Institute (SFEI). The goal of the RMP is to collect data and communicate information about water quality in the Estuary to support management decisions. The RMP, in consultation with stakeholder and technical review committees pose five primary management questions (last refined in 2008), and which closely mirror those posed by SWAMP statewide.

1. Are chemical concentrations at levels of potential concern and are associated impacts likely?
2. What are the concentrations and masses of contaminants in the Estuary and its segments?
3. What are the sources, pathways, loadings, and processes leading to contaminant-related impacts?
4. Have the concentrations, masses, and associated impacts of contaminants increased or decreased?
5. What are the projected concentrations, masses, and associated impacts of contaminants?

To address these questions, the Status and Trends (S&T) monitoring program within the RMP (http://www.sfei.org/content/status-trends-monitoring) is composed of five program elements:

1. long-term water, sediment, and bivalve monitoring
2. episodic toxicity monitoring
3. sport fish monitoring
4. the USGS hydrographic and sediment transport studies
    A. Factors Controlling Suspended Sediment in San Francisco Bay
    B. USGS Monthly Water Quality Data
5. triennial bird egg monitoring (cormorant and tern)

The RMP 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 were the result of recommendations made by the Emerging Contaminants Work Group (ECWG), a panel of internationally renowned scientists coordinated by the RMP. 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. Most recently, the RMP has completed a synthesis of the occurrence of CECs in San Francisco Bay (Klosterhaus et al. 2013) and developed a CEC monitoring strategy (Sutton et al. 2013), which synthesizes existing information on chemical usage, occurrence or toxicity from other locations and best professional judgment); their effects (i.e., from lab and field studies), and occurrence (non-target analyses or fate modeling). The role of the ECWG is to insure the RMP is current with respect to CECs, and to, as needed, recommend, support and implement studies that address the five overarching management questions stated above. The major outcome of this effort will be to provide updates on relevant information to the ECWG, so that they may react and adapt to new information using a tiered risk-management action framework (Klosterhaus et al. 2013).

RMP data, field operations and quality assurance/quality control (QA/QC) documentation can be accessed via on the SFEI website (http://www.sfei.org/programs/rmp-data). Results provided are updated as needed with reanalyzed results and corrections.

1.3.4 Southern California Bight Regional Monitoring Program

Initiated in 1994 as a pilot study, the Southern California Bight Regional Monitoring Program (Bight) is currently conducted in five-year cycles and has involved over 100 different stakeholder organizations. Management of Bight activities is provided by SCCWRP (http://www.sccwrp.org). The goals of this program are to:

1. Establish regional reference conditions
2. Monitor trends over time
3. Develop new environmental assessment tools
4. Standardize regional data collection approaches
5. Provide a platform to support special studies, including those to prioritize CECs for future monitoring.

The monitoring approach utilizes a stratified random sampling design so that data can be statistically extrapolated to estimate conditions across the Bight. Subsections (strata) are selected to distinguish areas of interest such as the coastal ocean, ports, marinas, the Channel Islands, wastewater treatment plant locations, and land-based runoff locations. Each survey revisits some portion of sites sampled in

previous Bight surveys in order to assess trends over the years. The Bight program includes inter-calibration exercises to standardize and improve data quality across participating organizations. An Information Management Committee oversees data structure and reporting requirements, and a centralized database model with a relational database structure was developed to provide easy data access to project scientists.

The current cycle (Bight '13) (http://sccwrp.org/ResearchAreas/RegionalMonitoring/Bight13RegionalMonitoring.aspx) has five components:

1. contaminant impact assessment (offshore sediment condition)
2. nutrient impact (water column condition)
3. microbiology (beach water quality condition)
4. marine protected areas (rocky reef condition)
5. debris assessment

Sampling and laboratory analyses were completed for approximately 400 sites. Hundreds of indicators were measured including sediment chemistry and toxicity; benthic infauna, fish, and invertebrates; contaminant bioaccumulation in bird eggs; trash and debris; physical water column characteristics; nutrients and algae; fecal indicator bacteria; and human pathogens. In 2008, PBDEs and pyrethroids were measured in sediments from at a subset of stations. The Bight Program does not currently target aqueous samples in inland freshwater systems (e.g. Scenario 1) or near marine outfalls (Scenario 3) in the manner specified herein.

The Bight '13 Contaminant Impact Assessment seeks to determine (1) the extent and magnitude of direct impact from sediment contaminants; (2) the trend in extent and magnitude of direct impacts from sediment contaminants; and (3) the indirect risk of sediment contaminants to seabirds. Per the Panel recommendations, new to Bight is the inclusion of PBDEs and PFOS as sediment analytes, and the sampling and analysis of eggs of multiple species of seabirds for contaminants, which includes CECs (PBDEs and PFOS) recommended by the Panel. Also included in the B'13 study are special studies that investigate the application of bioanalytical tools to screen for CECs in extract of B'13 sediments, and trophic transfer of bioaccumulative compounds, including PBDEs, in the coastal Bight marine food web (B'13 CIA Committee 2013).

1.3.5 Bay Area Stormwater Management Agencies Association (BASMAA)

The Bay Area Stormwater Management Agencies Association (BASMAA) is a consortium of eight San Francisco Bay Area municipal storm water programs (http://www.basmaa.org). In addition, other agencies, such as the California Department of Transportation (Caltrans) and the City and County of San Francisco, participate in some BASMAA activities. Together, BASMAA represents more than 90 agencies, including 79 cities and 6 counties, and the bulk of the watershed immediately surrounding San Francisco Bay.

To comply with NPDES permit requirements for stormwater impacts to water quality, six BASMAA agencies collaborated to form the Regional Monitoring Coalition (RMC) and to develop, design and conduct a large scale monitoring and assessment program for Bay Area watersheds (SCVURPPP 2014). The current RMC work plan described 27 individual projects for FY2009-10 and FY2014-15, which are broken down into several primary topical areas, including Bay and Creek status monitoring; pollutant of

concern (POC) loading; long term trends monitoring; and monitoring of emerging pollutants (i.e. CECs). Each of these components utilize a combination of probabilistic and targeted sampling design on selected or model watersheds/waterbodies and a schedule that is optimized for the parameter targeted.

The POC loading study is designed to identify those watersheds draining into the Bay that contribute the majority of mass loading of contaminants. A secondary objective is to determine the effectiveness of management actions in reducing POC loads to the Bay. The current plan targets three of the CECs recommended by the Panel - PBDEs, fipronil and pyrethroids. Pyrethroids were implicated in toxicity observed in water samples tested using *H. azteca* in this study component (SCVURPPP 2014).

The long term trends monitoring component was integrated into monitoring of creeks performed under SPoT, which measures a number of trace metals and organic chemicals (PAH, organochlorine, pyrethroids and most recently, fipronil) in streams and rivers (see also 1.1.1 SWAMP). The initial projects for CECs will focus on characterization of loading and source identification for endocrine disrupting chemicals, PFCs and nonylphenols and their ethoxylates. In addition, piloting of bioanalytical screening tools consistent with the Panel recommendation is underway. Lastly, the RMC work plan calls for continuing collaboration and coordination with SWRCB efforts to fill data gaps on CECs in Bay receiving waters, e.g. as was recommended by the Panel, and reflected herein.

1.3.6 Southern California Stormwater Monitoring Coalition

The Southern California Stormwater Monitoring Coalition (SMC) was formed in 2001 by cooperative agreement of the Phase I municipal stormwater NPDES lead permittees, the NPDES regulatory agencies in southern California and SCCWRP (http://www.socalsmc.org/AboutUs.aspx). The original 11-member SMC renewed the cooperative agreement for five years commencing June 2008 and added three new member agencies, the California Department of Transportation, the City of Los Angeles and the SWRCB. The current list of SMC members include the stormwater management branches for Los Angeles, Orange, San Diego and Ventura counties, as well as inland empire and city agencies in the region. The SMC also has a cooperative Memorandum of Understanding with USEPA Office of Research and Development to facilitate the development of scientific and technical tools for stormwater program implementation, assessment, and monitoring. The SMC is managed by Steering Committee of its members that meets quarterly to review new projects and assess progress on ongoing projects. Annual reports are available online (http://www.socalsmc.org/Docs).

Despite the success of the SMC, numerous stormwater issues and unresolved problems persist. These remaining challenges, for example, identifying the causative stressor(s) for impacted stream biological communities and the paucity of data on the occurrence of and potential for impact due to CECs, have been especially difficult to address. As part of its 5 year strategic plan, the SMC convened a panel of experts to identify priority issues, which identified CECs as among their top priorities (Schiff et al. 2014). The proposed approach to CECs set forth by the panel was to identify, evaluate and incorporate bioanalytical screening tools to more comprehensively inform the need for more detailed toxicological monitoring. Once the appropriate tools are identified and optimized for stormwater applications, pilot scale evaluation in model MS4 watersheds are planned. The SMC recognizes the implications of SWAMP's CEC efforts (i.e. this pilot study plan), and pledges collaboration with SWAMP and the other monitoring programs described herein (e.g. BASMAA) to best inform SMC's future monitoring strategy for CECs.

1.3.7 Delta Regional Monitoring Program

The Delta Regional Monitoring Program (DRMP) is a pilot stage effort to collaboratively assess the environmental quality and integrity of the Sacramento-San Joaquin River Delta system. The primary agencies coordinating this regional cooperative are the SWRCB (http://www.swrcb.ca.gov/centralvalley/water_issues/delta_water_quality/comprehensive_monitoring_program/), the San Francisco and Central Valley Regional Boards and SFEI (http://www.sfei.org/programs/delta-regional-monitoring-program). The goal of the DRMP is to better define water quality issues of regional concern and to improve the quality and efficiency of water quality monitoring information. Four core management questions were recently identified as guiding principles for the DRMP:

1. status and trends
2. sources, pathways and loadings
3. forecasting the impact of management actions on water quality
4. evaluating the effectiveness of management actions

Initial priorities are an improved understanding of the spatial and temporal distribution of prioritized water quality constituents (i.e. ancillary parameters, methylmercury, nutrients, pathogens, pesticides, and toxicity) in the Delta, improving the efficiency and usefulness of compliance monitoring and data reporting, and fostering large-scale collaborations. Monitoring is expected to begin in 2015.

1.3.8 Other Monitoring Efforts

Pilot and/or special studies on CECs have also been conducted at the regional and local scale in California. Stressor identification in coastal rivers and estuaries along the central California coast have focused on restricted and current use pesticides, including chlorpyrifos, pyrethroids, fungicides and at the current time, neonicotinoid insecticides (Worcester 2011). The Santa Ana Watershed Project Authority (SAWPA) is a collaborative among water agencies and the Santa Ana Regional Board that identifies and addresses water-related issues in the region. The Emerging Constituents Workgroup within SAWPA investigated the occurrence of pharmaceuticals and personal care products in the effluent dominated Santa Ana River watershed (SAWPA 2014). There is currently no known activity or future plans for CEC investigation by SAWPA. In recent years, the Los Angeles Regional Board has commissioned investigations to characterize the occurrence and fate of CECs, including those identified by the Panel, in effluent dominated waterways and their coastal transition zones (i.e. rivermouths). These investigations started with water column occurrence (Sengupta et al. 2014) and are currently targeting priority CECs (e.g. PBDEs, PFOS) in sediment and fish tissue. To address recommendations coming out of this effort, the North Coast Regional Board has plans to conduct a CEC pilot study, focused on the contributions and impacts of WWTP and stormwater associated CECs discharged into the Russian River watershed. This study is tentatively scheduled to commence in 2015.

2 Targeted CEC Monitoring Program Design

2.1 Revisions and Addendums to Panel Recommendations

Subsequent to the Panel's final report (Anderson et al. 2012), the compilation of occurrence and toxicological data for fipronil, a phenypyrazole insecticide whose applications statewide increased during the period 2000-2010, was updated (**Tables 2.1-1 and -2**). The updated MTQs exceeded unity for the aqueous phase in inland freshwaters and coastal embayments (Scenarios 1 and 2). In addition, the MTQ exceeded unity for freshwater sediments, suggesting the need to monitor fipronil in inland freshwater (Scenario 1) sediments, a matrix that was not included for targeted CEC monitoring by the Panel. Since the parent compound is transformed in aquatic systems to several known metabolites, monitoring of these degradates is also recommended.

It is also noted that the monitoring of pesticide analytes, in particular, fipronil and its degradates, bifenthrin, permethrin (and other pyrethroids) and chlorpyrifos is currently planned for freshwater systems across California via existing SWAMP (SPoT) and DPR programs. The current designs for these program carried into the initial 3-year pilot monitoring cycle will obviate the need for monitoring of these analytes as defined in Scenario 1 (Sec 2.2.1) and MS4 (Sec 2.2.4).

Table 2.1-1. Ecotoxicological data for fipronil.

	Aqueous Freshwater	**Aqueous Saltwater**	**Sediment Freshwater**	**Sediment Saltwater**
Reference	Ali et al. (1998)	USEPA (1996)	Maul et al. (2008)	Chandler et al. (2004a,b)
Organism	Chironomid	Mysids	Chironomid	Amphiascus
LC or EC	420 ng/L	<5 ng/L	0.90 ng/g dw	65 ng/g dw
Safety Factor	10	None	10	10
MTL	42 ng/L	5 ng/L	0.090 ng/g dw	6.5 ng/g dw

Table 2.1-2. Monitoring trigger quotients (MTQs) > 1 for fipronil by scenario and matrix. MEC - maximum measured environmental concentration. PEC - maximum predicted environmental concentration. The PECs for embayments (Scenario 2) were calculated assuming a 10-fold dilution factor of MECs representing inland fresh waterways (Scenario 1).

Scenario	**Matrix**	**MEC or PEC**	**MTQ**	**Reference**
1-Inland Freshwater	Aqueous	10,004 ng/L (MEC)	240	Gan et al. (2012)
1-Inland Freshwater	Aqueous	2110 ng/L (MEC)	50	Ensminger et al. (2013)
1-Inland Freshwater	Sediment	1.1 ng/g dw (MEC)	12	Lao et al. (2010)
1- Inland Freshwater	Sediment	0.4 ng/g dw (MEC)	4.4	Delgado-Moreno et al. (2011)
2-Embayment	Aqueous	1000 ng/L (PEC)	200	Gan et al. (2012)
2-Embayment	Aqueous	211 ng/L (PEC)	42	Ensminger et al. (2013)

2.1.1 Targeted Contaminants and Reporting Limits

Reporting limits for the target CECs are based on the MTLs recommended by the Panel. A goal of monitoring is to assess if the MTQ is greater than 1 (indicating it should continue to be monitored) or less than 1 (indicating it is not a high priority for future monitoring). Assuming variance in the measurement accuracy (typically 30%), the required reporting levels should extend below the MTL to ensure confidence the MTQ is greater or less than 1. Thus, the required reporting levels are set at ½ the MTL for each scenario and matrix (**Table 2.1.1-1)**. Reporting limits (RLs) for monitoring of WWTP effluent and in MS4 receiving waters are assumed to be same as for Scenario 1 and 2 receiving waters, respectively.

It is also noted that the RLs for the pesticide analytes, in particular, fipronil and its degradates, bifenthrin, permethrin (and other pyrethroids) and chlorpyrifos recommended herein may not be consistent with those reported for SWAMP (SPoT) and DPR programs that currently measure these analytes. In some cases, the RLs recommended herein (i.e. in Table 2.1.1-1) are lower than those currently reported by SWAMP and DPR.

Table 2.1.1-1. Monitoring trigger levels (MTLs) and reporting limits (RLs) by scenario, compound and matrix. Recommended RLs are derived from MTLs as reported by the CEC Ecosystems Panel. Achievable RLs reflect the current state of art for commercial services laboratories. Recommended RLs for all CECs in wastewater treatment plant (WWTP) effluent and stormwater (MS4) influenced receiving waters are equivalent to Scenario 1 aqueous phase RLs; additional RLs for compounds that are otherwise measured only in sediment or tissues appear at the bottom of the table.

Compound	Panel Freshwater MTL[1]	Recommended RL[2]	Achievable RL[3]
Aqueous Phase - Effluent dominated inland waterways (Scenario 1) (ng/L)			
Bifenthrin	0.40	0.20	
Permethrin	1.0	0.50	
Fipronil	42	21	
Chlorpyrifos	5.0	2.5	
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	
Sediment Phase - Effluent dominated inland waterways (Scenario 1) (ng/g dw)			
Fipronil	0.090	0.045	1.0
Aqueous Phase - Coastal embayments (Scenario 2) (ng/L)			
Bisphenol A	6.0	3.0	

Bifenthrin	0.040	0.020	0.2
Permethrin	0.10	0.050	0.5
Fipronil	5.0	2.5	
Chlorpyrifos	1.0	0.50	
Estrone	0.60	0.30	2.0
17-beta-estradiol	0.20	0.10	0.4
Galaxolide (HHCB)	70	35	
Sediment - Coastal embayments (Scenario 2) (ng/g dw)			
Bifenthrin	0.052	0.026	0.20
PBDE-47	0.030	0.015	
PBDE-99	0.030	0.015	
Permethrin	0.073	0.036	0.40
Fipronil	6.5	3.25	
Sediment - Ocean discharge (Scenario 3) (ng/g dw)			
Bis(2-ethylhexyl) phthalate (BEHP)	130	65	
p-nonylphenol	14	7.0	
PBDE-47	0.30	0.15	
PBDE-99	0.30	0.15	
Butylbenzyl phthalate (BBP)	6.3	3.15	
Tissues (All Scenarios) (ng/g dw)			
PBDE-47	28.9	14.5	
PBDE-99	28.9	14.5	
PFOS	1000	500	
WWTP Effluent and MS4 Receiving Water (ng/L) [4]			
Bis(2-ethylhexyl) phthalate (BEHP)			3.0
Butylbenzyl phthalate (BBP)			3.0
p-nonylphenol			22 (TBR) [5]
PBDE-47			0.10
PBDE-99			0.10
PFOS			1.0

[1] Monitoring Trigger Level established by CEC Ecosystems Panel (Anderson et al. 2012).
[2] Set at 50% of MTL.
[3] Minimum RL reported by commercial services laboratories. Missing values indicate the achievable value is at or below the recommended RL.
[4] RLs for analytes otherwise measured in sediment or tissues only (no MTL values available). For all other analytes, RLs for WWTP Effluent and MS4 receiving water samples are the same as the aqueous RLs for Scenario 1.
[5] TBR – to be resolved. Estimated from the sediment RL (7.0 ng/g), an estimated sediment-water partitioning coefficient, and assuming 1% organic carbon content of the sediment.

2.2 Design Requirements by Scenario

2.2.1 WWTP Effluent Dominated Inland Freshwater (Scenario 1)

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 considered to be the largest source of CECs during this time period.

Monitoring Questions

1. Which CECs are detected in freshwaters and depositional stream sediments, and in which large 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. Does the new occurrence data change the estimated MTQs?

Design Considerations

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: 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) (**Figure 2.2.1-1**). Both the wet and dry seasons will be monitored over a 3 year period (**Table 2.2.1-1**). For fipronil, annual sediment analysis at three stations (e.g., #1, #5, and background) during the dry season is also recommended based on Scenario 1 sediment MTQs > 1 (**Table 2.2.1-2**).

Ideal candidates for this pilot study are waterways with well-characterized source and flow inputs. Examples of waterbodies that represent Scenario 1 in southern California are the Los Angeles, Santa Clara, San Gabriel, Santa Ana, and San Diego Rivers. The Los Angeles River and the Santa Clara River are proposed as candidates in southern California. In the Delta and Central Valley, proposed candidates are Alamo Creek downstream of the Vacaville Easterly WWTP and Pleasant Grove and Dry Creeks downstream of the City of Roseville Pleasant Grove and Dry Creek WWTPs, see map in **Appendix A**. No similar waterways have been identified in the San Francisco Bay region.

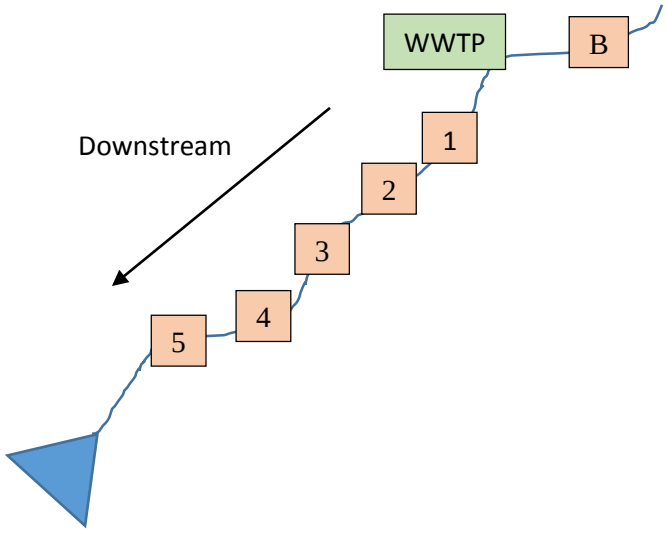

Figure 2.2.1-1. Design schematic for monitoring of CECs in Scenario 1.

Table 2.2.1-1. Aqueous sampling frequency for Scenario 1.

Source	Receiving Water	Years	Waterways	Total Samples
WWTP effluent 1 station Wet and dry season Samples = 2/yr	River 6 stations Wet and dry season Samples = 12/yr	3	4 (two each in SoCal and Delta/CV)	Effluent = 24 FW = 144

Table 2.2.1-2. Sediment sampling frequency for Scenario 1.

Waterway Sediment	Years	Waterways	Total Samples
3 stations Dry season Samples = 3/yr	3	4 (two each in SoCal and Delta/CV)	Sediment = 36

2.2.2 Coastal Embayment (Scenario 2)

Scenario 2 examines coastal embayments that receive CEC inputs at the land-ocean interface, which may originate from upstream WWTP discharge, direct WWTP discharge into the embayment, or stormwater runoff. As by far the largest 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/estuarine water and sediments?
2. Do CECs originate from the outfalls, or are they at background concentrations due to stormwater and other inputs?
3. Is there a sub-annual change in CECs discharged from WWTPs?
4. Does the new occurrence data change the estimated MTQs?

Design Considerations

The Panel's recommendation for Scenario 2 was a 2-D gradient (up to 6 stations) at each of five WWTPs within the San Francisco Bay Estuary. Each station would consist of a sediment sample and an overlying aqueous phase sample, since target compounds for this scenario may occur in both matrices. Monitoring was to be semi-annual over three years. The 2-D gradient design was recommended to measure spatial attenuation of the target contaminants.

Within the Estuary, the Lower South Bay is most strongly impacted by effluent discharge due to its high population and correspondingly high WWTP discharges and lower oceanic dilution. This section of the Estuary is the focus of Scenario 2 monitoring. Due to the multiple WWTP discharges with relatively close outfalls, tidal influences, and multi-directional currents that rapidly distribute contaminants throughout the Lower South Bay, however, the Panel's recommended design will likely not successfully measure stepwise decreases in contaminant concentration (attenuation) moving away from the zone of initial dilution (ZID) of a given outfall.

Instead, paired sediment/aqueous samples will be collected at stations along the interior waters (aka the "spine") from the Lower South Bay to the Central Bay (n = 15 stations) (**Table 2.2.2-1**). This design will integrate influences from multiple WWTPs and will account for mixing. Sampling should take place during the dry season, when dilution from runoff is lowest, and concentrations can be expected to be at their highest. Paired effluent (n = 1) and ZID samples (n = 1 each for sediment and aqueous phase) from at least 5 major WWTPs in the South Bay should also be monitored, to verify contaminants originate from the outfall (**Table 2.2.2-2**). Sediment and receiving water sampling along the spine should occur annually over 3 years. Effluent and aqueous ZID sampling should be performed semi-annually (wet/dry season) over 3 years, and sediment ZID sampling annually over 3 years. Current RMP special studies will inform the selection of WWTPs, and effluent data for the target CEC should be provided.

Table 2.2.2-1. Aqueous and sediment sampling frequency for interior waters (Scenario 2).

Aqueous	Sediment	Years	Total Samples
15 stations Dry season Samples = 15/yr	15 stations Dry season Samples = 15/yr	3	Aqueous = 45 Sediment = 45

Table 2.2.2-2. WWTP effluent and ZID sampling frequency for Scenario 2.

Effluent	ZID Aqueous	ZID Sediment	Years	Total Samples
5 WWTPs Wet/Dry season Samples = 10/yr	5 aqueous Wet/Dry season Samples = 10/yr	5 sediment Dry season Samples = 5/yr	3	Effluent = 30 ZID Aqueous = 30 ZID Sediment = 15

2.2.3 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? (see also Sec 2.2.4)

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 2.2.3-1**). Observation of a stepwise decrease in concentrations away from the ZID verify 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 zone of initial dilution (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 2.2.3-1**). Exact station locations may be assigned based on the results from the Bight '13 Special Study described in **Appendix B**.

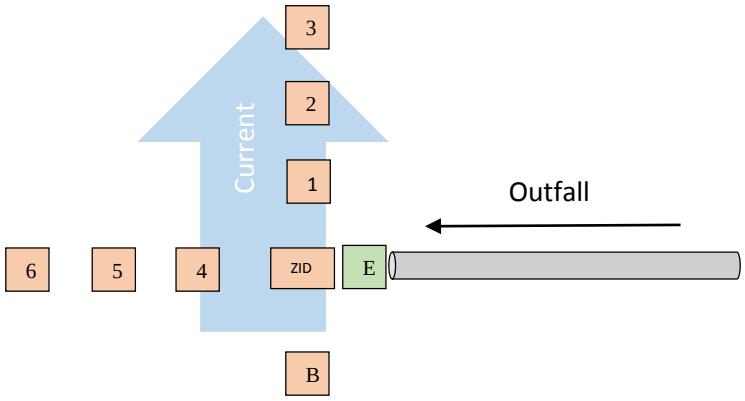

Figure 2.2.3-1. Design schematic for sampling of CECs in Scenario 3.

Table 2.2.3-1. Effluent and sediment sampling frequency for Scenario 3.

Source	Sediment	Years	WWTPs	Total Samples
WWTP effluent 1 station Samples = 2/yr	Grid 8 stations Samples = 8/yr	3	2	Effluent = 12 Sediment = 48

2.2.4 Stormwater Discharge to Receiving Waters (MS4)

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. Materials from various sources/surfaces (e.g. road dust, topsoil, sediments) are mobilized during wet weather events, transporting suspended particulates and associated contaminants, including some CECs, into receiving waters. Thus, annual loading (on a mass per year basis) of particle reactive CECs into receiving waters is highly seasonal. Receiving water impacts resulting from such loading can be direct, e.g. release of pesticide residues from sediments transported into receiving waters resulting in invertebrate or fish toxicity, or indirect, e.g. bioaccumulation of sediment-associated CECs (e.g. PBDEs) by benthic organisms and subsequent trophic transfer into higher biota (e.g. fish and humans). During the dry season, in contrast, incidental runoff (e.g. due to excess irrigation of gardens and/or parks) may contain CECs (e.g. pesticides) at higher concentrations, since runoff volume and base flow to the receiving water are relatively small. Moreover, particulate loading is typically negligible under these conditions, directing attention to dissolved, aqueous phase (i.e. more water soluble) CECs. Thus, it is critical to address both short term toxicity vs. 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 Considerations

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. Current wet weather monitoring relies on sampling at fixed mass emission (FME) or integrator stations located at the bottom of MS4 permitted watersheds. Integrator stations identified and monitored in other monitoring programs (e.g. RMC, SMC, SPoT, DPR) should be utilized for the candidate watersheds. Flow weighted sampling at FME stations for two storms per year per watershed will provide data to address monitoring questions 1-3 (**Table 2.2.4-1**). Ideally, the storms sampled will include an early ("first flush") and late season event. A minimum of three watersheds statewide should be assessed over a 3-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 3 year screening, should be planned during subsequent pilot study cycles. Non-filtered, whole water samples should be analyzed when addressing loading. Filtered water samples maybe adequate for effects/toxicity evaluation. Sufficient sample size and analytical methods should be specified to meet target detectability of CECs (see also Sec 2.1.1 and Supplemental Guidance for QA/QC).

Dry Weather. Since short term maximum concentrations resulting in acute toxicity is 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 (river mouths, oxbows, retention basins) should 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 2.2.4-1**). Unless unexpectedly high total suspended solids (TSS) samples are encountered, non-filtered aqueous samples should be sufficient for monitoring and assessment during dry weather. To address chronic exposure of CECs, base flow conditions over longer time periods (weeks to months) can be assessed using emerging technology, e.g. passive sampling devices that provide a time-average concentration of CECs that have been pre-calibrated in the laboratory (see also Sec 5). Such extracts are also amenable, without fortification, for toxicity screening.

Coordination with Special Studies

Samples collected for targeted chemistry will also be evaluated for toxicity parameters as specified in section 3. Bioanalytical screening assays will be adapted and evaluated on organic extracts of water and sediment samples collected as part of this scenario. Targeted CEC monitoring that require RLs not readily achievable using conventional or commercially available methodology shall utilize passive sampling devices (PSDs), where such technology has been validated and is amenable for deployment (e.g. conditions and timing for continuous submerged conditions are available).

Candidate Watersheds

- San Francisco Bay: watersheds monitored by the RMC, SWAMP/SPoT and DPR, including Coyote Creek and the Guadalupe River (Santa Clara County) [1,3,4]; Grayson Creek (Contra Costa County)[4]; Arroyo de la Laguna (Alameda County) [4]
- Delta/Central Valley: watersheds monitored by the DRMP, SWAMP/SPoT and DPR, including Arcade Creek[4], Steelhead Creek, Morrison Creek, American River[3] and the Sacramento River at the Hood integration site[3] (Sacramento County); Pleasant Grove Creek (Placer County) [4] ; see map in **Appendix A**.
- Southern California: watersheds monitored by the SMC, SWAMP/SPoT and DPR, including Ballona Creek[2,3,4] and Bouquet Canyon Creek[3,4] (Los Angeles County); San Diego Creek[2,3] and Salt Creek[4] (Orange County); Chollas Creek[4] and San Diego River[2,3,4] (San Diego County).

1 scheduled for monitoring by RMC (SCVURPPP 2014)
2 scheduled for monitoring by SMC (SMC/BWG 2007)
3 scheduled for monitoring of toxicity stressors by SPoT (Phillips et al. 2014)
4 scheduled for monitoring of pesticides by DPR in 2014-15 (Emsinger 2014)

Table 2.2.4-1. Sampling matrix for MS4 watersheds. Monitoring of a minimum of 3 watersheds over a 3 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	54

Aqueous concentration, dry weather	Whole water (unfiltered)	3 (source-related)	1/yr	1	27
Sediment concentration, dry weather	Whole (sieved) sediment	3 (depositional)	twice/yr	1	54

2.2.5 Tissue Monitoring

Wildlife living in receiving waters can be exposed to CECs by direct uptake via the aqueous phase and through ingestion of contaminated prey. Chemicals that are hydrophobic (log K_{ow} >3), remain un-ionized in either freshwater or saltwater environments, and that are persistent 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 by the Panel have the potential to bioaccumulate, only two (PBDE and PFOS) have NOECs from which body burden-based MTLs could be derived. The Panel used studies on birds (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). The Panel was not able to identify allowable concentrations of PBDEs in fish for protection of marine mammals. The Panel believes such marine mammal-based MTLs could be derived in the future.

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 Considerations

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. Both the RMP and Bight programs are currently collecting this data. Since 2006, RMP has monitored bird eggs for PBDEs and PFCs every 3 years, addressing the temporal trend question. Bight is performing bird egg measurements on PBDEs and PFOS for the first time in 2014. Therefore, data from the RMP and Bight programs may be used to re-assess tissue MTQs. Recommended species are the cormorant, caspian tern, western gull, and the California least tern. Within the regional programs, we recommend bird egg temporal monitoring to continue in the future, particularly in key urban areas such as covered by the RMP and Bight. To our knowledge, bird egg monitoring does not currently occur in the Delta/Central Valley region, and is therefore recommended. A sample size of n = 10 for a single bird sentinel species is recommended over the 3-year pilot study cycle (**Table 2.2.5-1**). If the recommended target species listed above are not feasible for the Delta/Central Valley, alternate species as recommended by the DRMP shall be substituted.

Marine Mammals. Marine mammals such as pinnipeds and cetaceans occupy high trophic positions and thus can have relatively high concentrations of bioaccumulative CECs (e.g. PBDEs). The Panel was unable to establish MTLs for marine mammals, but recognized the potential for risk associated with biomagnification and discussed possible future methods for determining marine mammal MTLs. Therefore, collection of occurrence data in marine mammals is warranted. Live-capture harbor seal blubber will be measured for PBDEs in 2014 as part of a RMP special study, and PFCs will be measured in the blood. Although some specific studies have been carried out, contaminants in marine mammals are not routinely monitored in southern California, e.g., within the Bight program. It is recommended that southern California sea lions and/or bottlenose dolphins be measured for PBDEs (blubber) and PFOS (blood). A minimum sample size of n = 10 for each matrix (blood and blubber) that can be a composite total for both species, or of a single species, is recommended over the 3-year pilot study cycle (**Table 2.2.5-1**). As data exist for PBDEs in these two species, comparisons to current and future conditions can be made to obtain temporal trends (Meng et al., 2009; NOAA, unpublished). Live biopsies are recommended to obtain fresh tissue representative of a healthy population, however fresh dead strandings could be considered in the absence of access to tissues from live biopsies.

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 and to identify localized contamination sources. Bivalves in particular are sessile and there is substantial historical bivalve tissue data for comparison (Dodder et al. 2014; Klosterhaus et al. 2013; Sutton et al. 2014). However, these filter feeding organisms indicate exposure to waterborne CECs, as opposed to bioaccumulation and/or biomagnification potential. For example, PFCs (including PFOS) were sporadically detected at low levels in California coastal mussels (*Mytilus* spp.) (Dodder et al. 2014), in direct contrast to elevated PFC concentrations in bird eggs (Sedlak and Greig 2012). Fish, on the other hand, occupy a higher trophic position and may have higher body burdens of target CECs. Therefore, monitoring of both bivalves (for PBDEs) and fish (for PBDEs and PFOS) is recommended. Sampling of fish and bivalves should occur annually over the 3 year pilot study cycle (**Table 2.2.5-2**).

Candidate fish species will vary in availability by location. Species that exhibit high spatial fidelity and are suspected to accumulate relatively high levels of PBDEs and PFOS should be selected for monitoring. Candidate bivalve species are *Corbicula fluminea* (freshwater) and *Mytilus spp. (californianus or galloprovicialis)* for marine habitats. Fish may be individuals (provided enough sample mass is available) or composites, and bivalves should be composites. Whole bodies for small fish, and filets of larger fish should be analyzed. The final selection of sentinel species shall be made in coordination with SWAMP/BOG.

- For freshwater systems (e.g. Scenario 1 and MS4 monitoring), it is recommended that fish (PBDEs and PFOS) and bivalves (PBDEs) be sampled in one system each in the San Francisco Bay watershed, southern California and the Delta/Central Valley region. The selection of these systems can coincide with those identified for sediment and aqueous phase monitoring in Sections 2.2.1 and 2.2.4. Based on historical sampling and results from SWAMP/BOG, recommended fish species for freshwater systems are large and smallmouth bass, Sacramento or Santa Ana sucker, and channel catfish. An additional recommended species for the Delta is striped bass.

  - For Scenario 1, bivalves and fish should be collected from a location in close proximity to the WWTP outfall, during the period of highest effluent loading.
  - For MS4 watersheds, bivalves and fish should be 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.

- For San Francisco Bay (Scenario 2), the RMP measures PBDEs in bivalves every 2 years, and PBDEs and PFCs in sportfish every 5 years. Forage fish are not part of RMP Status and Trends. Therefore, embayment tissue monitoring can be carried out through RMP. Recommended fish species are shiner perch, white croaker, topsmelt, and California halibut.
- For marine outfall tissue monitoring (Scenario 3), it is recommended that fish 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 should be selected. The Bight program does not currently monitor fish for PBDEs and PFOS, therefore sampling is recommended annually over the 3 year pilot study cycle (Table 2.2.5-2). Recommended species include those collected in abundance historically at these outfalls, e.g. hornyhead turbot, Dover sole and scorpionfish.

Table 2.2.5-1. Recommended sampling of bird eggs and marine mammals for the 3-year pilot study cycle. Additional tissue samples are to be analyzed through regional programs, as noted in the text.

Sample	Region	Number per 3 yr cycle	Total Samples
Bird eggs	Delta/Central Valley	10 egg composites	10
Marine Mammals Blubber (PBDEs) Blood (PFOS)	Southern California Bight	5 sea lion 5 bottlenose dolphin	Blubber = 10 Blood = 10

Table 2.2.5-2. 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	3 Waterways ea. scenario	3	90
Marine fish	Scenario 3	5	2 WWTP outfalls	3	30
Bivalves	Scenario 1 and MS4	3	3 waterways ea. scenario	3	54

Non-Targeted Analysis**.** Targeted analytical methods will used to quantify the Panel-recommended CECs. However, these methods are not designed to screen for new or unexpected contaminants; i.e., unknown CECs. 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 and 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. Directly linking non-targeted mass spectrometry and in-vitro bioassays to identify contaminants contributing to the biological response is discussed as a research need in Section 5.2. (**Table 2.2.5-3**)

Table 2.2.5-3. Recommended non-targeted analysis of tissue samples collected for monitoring of PBDEs and PFOS.

Sample	Scenario/Region	Number per 3 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)	3	n/a	3
Marine fish	Scenario 3	2	2 WWTP outfalls	4
Marine mammal blubber (2 species)	Southern California Bight	3	n/a	6

3 Special Studies Design Requirements

3.1 Introduction

The Panel recommended that a number of special studies be conducted as part of a statewide CEC pilot monitoring program in order to evaluate and where possible, validate the methods evaluated in these studies prior to full implementation (**Table 1.1.4-1**). These studies largely address the potential for adverse effects of CECs in aquatic organisms (e.g. animal toxicity; microbial resistance) and will complement traditional targeted chemical monitoring (described in section 2) by providing additional information on the occurrence of known and unknown CECs (e.g. bioanalytical screening assays).

Moreover, the special study bioassay components target and/or link the responses across increasingly complex levels of biological organization, and thus can be integrated in a multi-tiered interpretive framework (**Figure 3.1-1**). 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. The Panel also recommended whole organism toxicity testing to determine if CECs present in aquatic ecosystems can have adverse effects at the organism level (Tier II), 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 focus on in situ evaluation, e.g. field collection of biological samples of sentinel organisms (e.g. invertebrates, fish, birds and/or mammals), specifically to investigate whether such MOAs identified using Tier 1 *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).

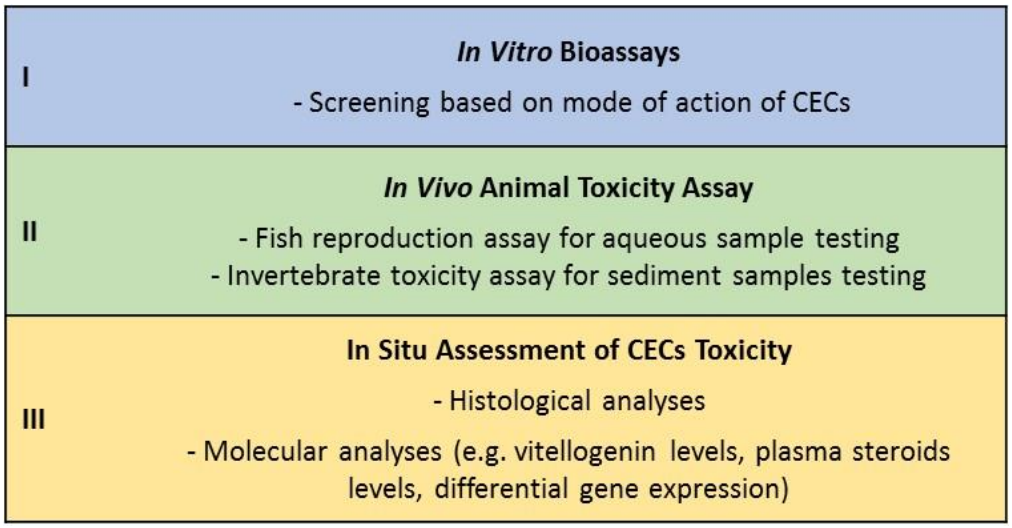

Figure 3.1-1. Proposed framework for biological assessment of CECs in aquatic ecosystems.

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

In vitro bioassays can be used to screen a large number of chemicals based on a MOA paradigm. Selected IVBs are currently being evaluated for screening of recycled and drinking water quality (Leusch et al. 2010; Escher et al. 2014), with encouraging results for the detection of endocrine disrupting CECs. To address the Panel's recommendations, a number of commercially available IVBs are proposed to assess the capability of environmental CECs to activate endocrine-related receptors, induce xenobiotic metabolism and cause cell damage (**Table 3.2-1**). Some chemicals are also known to suppress the activity of endocrine-related receptors causing adverse effects. For example, male fish exposed to anti-androgenic compounds or females exposed to anti-estrogenic compounds can cause reproductive impairment via alteration of plasma sex steroids levels and subsequent reduction in fertility and fecundity (Panter et al. 2004; Filby et al. 2007). To screen for these outcomes, estrogen receptor (ER) and androgen receptor (AR) assays will be conducted in agonist (receptor activation) as well as antagonist (inhibition of activity) mode.

Table 3.2-1. In vitro bioassays that screen for endocrine disruption, xenobiotic metabolism 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	Androgen insensitivity, masculinization of females, impaired reproduction
Glucocorticoid Receptor (GR)	Activation	Cortisol binding, regulation of gene transcription	Development, immune diseases, diabetes
Progesterone Receptor (PR)	Activation	Embryonic development, cell differentiation	Cancer, diabetes, hormone resistance syndrome
Aryl Hydrocarbon Receptor (AhR)	Activation	CYP1A metabolism induction	No known adverse outcome. Indicates exposure to dioxin-like chemicals
Cytotoxicity	-	General cell toxicity	Tissue damage, death

Two types of investigations are recommended. First, a battery of candidate IVBs will be evaluated to determine their response to the list of Panel recommended CECs at exposure concentrations of monitoring relevance (see section 2). Second, the IVBs will be evaluated 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. Because the output parameters resulting from bioassays are not directly comparable with individual chemical concentrations, translation of bioassay into equivalent concentrations, or bioassay equivalents (BEQs), is necessary (**Table 3.2-2**).

Table 3.2-2. Output parameters of *in vitro* assays.

	In vitro assays with reference toxicant	**In vitro assays without reference toxicant**
Calibration	Dose response curve with reference toxicant	N/A
Concentration effect assessment	Relative Enrichment Factor (REF) (enrichment factor of extraction process and dilution of extract in the IVB)	
Data analyses	Effect concentration (EC)	Induction ratio (IR)
Output parameter	Bioassay equivalent concentration (BEQ)	Toxic unit

3.2.1 *In Vitro* Screening of Targeted CECs

Questions to be addressed:

1. Which priority CECs are detectable at environmentally relevant RLs using the endocrine-related cell assays?
2. Which priority CECs are detectable at environmentally relevant RLs using other relevant endpoints (e.g. AhR)?
3. What are the responses (additive or antagonist) of priority CECs mixtures using the selected cell assays?

Seventeen CECs (see Table 2.2-3) have been selected for target monitoring in water, sediment and/or tissue. The objective of this study is to identify the most robust cell assays to screen for priority CECs at environmentally relevant levels (**Table 3.2-3**). For each chemical, four concentrations will be selected based on their monitoring trigger levels (MTLs – lowest test concentration) (Tables 2.1.1-1). 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 3.2-3. *In vitro* assays for screening of priority CECs.

Endpoint	**Priority CECs**	**Other environmental chemicals**
ERa	BEHP, BBP[1] Galaxolide (Anti-ER)[2] PFOS[3] 17-beta estradiol – known strong ER agonist Estrone – known moderate ER agonist BPA, nonylphenol – known weak ER agonist	Musks
AR	Galaxolide (Anti-AR)[2] No AR activation data for priority CECs of interest	

AhR	PBDE-47 and -99 Chlorpyrifos[4]	PAHs, PCBs
GR	No GR activation data found for CECs of interest	Glucocorticoid steroids
PR	No PR activation data found for CECs of interest	Progestins (e.g. levonorgestrel)

[1]Harris et al. (1997), [2]Schreurs et al. (2005), [3]Kjeldsen and Bonefeld-Jorgensen (2013), [4]Long et al. (2003).

3.2.2 *In Vitro* Screening of Environmental Extracts

Questions to be addressed:

- How sensitive and precise are the candidate *in vitro* bioassays in detecting CECs in aqueous samples of interest (e.g. WWTP effluent and receiving water)?
- How do cell assay responses correlate with analytical chemistry data?

Aqueous environmental samples contain complex mixtures of CECs. Thus, it is important to determine if the occurrence of different classes of CECs can be quantitatively assessed using the selected IVBs. This pilot study will be conducted over a three-year period. Water samples will be collected, extracted and split on an annual schedule for targeted monitoring (see section 2) and testing using the IVBs (**Table 3.2-4**). Prior to *in vitro* screening, the 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. 3.2-2**).

Table 3.2-4. 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)	3
	River water	Stations # B, 1, 3 and 5 (section 2.2.1)	2/year (wet & dry season)	
Scenario 2 Embayment	WWTP effluent	Outfall	1/year	1
	Receiving water	Every third station for SF Bay interior waters (section 2.2.2)	1/year	
Scenario 3 Ocean	WWTP effluent	Outfall	1/year	3
	Receiving water	Stations # B, ZID, 3 and 6 (section 2.2.3)	1/year	
Scenario 4 MS4	Watershed	1 FME 3 source-related (section 2.2.4)	2 storms/year dry weather 1/year	3

3.2.3 *In Vitro* Assay Parameters and Optimized Methods

A number of commercially available cell assays have been identified for screening CECs in environmental samples. Among those, the GeneBLAzer assays (Life Technologies) and the CALUX assays (BioDetection Systems) have shown promising results. It should be noted, however, that differences in operating procedures exist among the endpoints and manufacturers. Based on the performance of these assays in screening of potable and surface water samples (Escher et al. 2014), the minimum requirements for reference chemicals and enrichment (i.e. pre-concentration) of aqueous samples relative to their collecting sample volume (denoted as REF) are provided in **Table 3.2-5**. Key cell bioassay conditions and QA/QC requirements are summarized in **Table 3.2-6**.

Table 3.2-5. Aqueous sample enrichment requirements for candidate *in vitro* screening assays.

	Reference chemical	Relative enrichment factor (REF)
Estrogen receptor alpha (ERa)	17-beta estradiol (+) 4-hydroxy-tamoxifen (-)	5 to 20 X
Androgen receptor (AR)	Methyltrienolone(R1881) (+) flutamide (-)	20 to 50 X
Progesterone receptor (PR)	Levonorgestrel (+)	20 to 50 X
Glucocorticoid receptor (GR)	Dexamethasone (+)	20 to 50 X
Aryl hydrocarbon receptor (AhR)	PCB 126 (+)	TBD

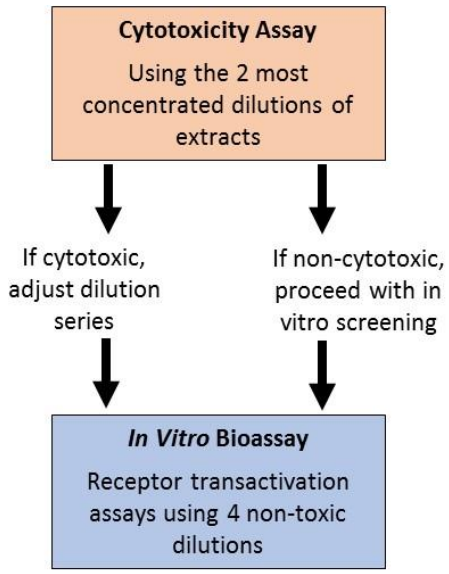

Figure 3.2-2. In vitro bioassay endpoints are sequenced to screen for cytotoxicity prior to testing for specific modes of action.

Table 3.2-6. Test conditions and QA/QC requirements for candidate *in vitro* screening assays

Parameters	***In Vitro* Bioassays Test Conditions**
Assay plates	96- or 384-well plates, black wall clear-bottom
Test samples	4 non-cytotoxic dilutions run in triplicate
Test solvent	Extracts in DMSO
Reference chemicals (if appropriate)	Potent chemical used to calculate bioassay equivalent concentration (BEQ) - Initial calibration : 9 concentrations minimum in triplicate in first plate - Calibration verification: 4 concentrations minimum in duplicate in subsequent plates (sample precision)
QA/QC	Per plate - Cell free media blank response – assay media only - Vehicle free response – cells only, no DMSO - Vehicle blank response – cells and DMSO only - Matrix spike response
Acceptability criteria	Cytotoxicity assay- 80% or more survival compare to control; Cell free and vehicle blank responses shall be < 15% of lowest calibration response

3.3 Tier II – Toxicity Testing Using Whole Organisms

The Panel recommended that *in vivo* tests 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.

3.3.1 Linkage of In Vitro Responses with Effects on Fish Reproduction

Questions to be addressed:

1. What are the NOECs and LOECs of model CECs *in vivo*?
2. What is the relationship between *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 will be performed following the USEPA guidelines (Appendix C, http://www.epa.gov/endo/pubs/att-f_fish_assay_protocol.pd). Specific parameters for this study are described in **Table 3.3-1**. The toxicity of model compounds known to affect ER and AR receptors will be investigated. Water samples from the exposures will be extracted and analyzed using the appropriate cell receptor assay.

Table 3.3-1. 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 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
	Test parameters - AR agonist
Chemicals	Trenbolone Solvent control (TEG or ethanol, less than 0.05%) Water control (no solvent)
In vitro endpoint	AR receptor transactivation
Fish assay endpoints	- % survival and changes in behavior relative to controls - No. eggs laid and fertilized - Levels of vitellogenin (in females) and plasma steroids and relative to controls - Appearance of nuptial tubercles in females - Gonadosomatic index - Gonad histopathology (possible ovo-testis in females) - qPCR (e.g. vtg) and/or microarrays
	Test parameters - AR antagonist
Chemicals	Flutamide Solvent control (TEG or ethanol, less than 0.05%) Water control (no solvent)
In vitro endpoint	AR receptor activity inhibition
Fish assay endpoints	- % survival and changes in behavior relative to controls - No. eggs laid and fertilized - Levels of plasma steroids and relative to controls - Reduction of the number of nuptial tubercles in males - Gonadosomatic index - Gonad histopathology (possible testis-ova) - qPCR and/or microarrays

3.3.2 Effects of CECs in Complex Environmental Matrices on Fish Reproduction

Questions to be addressed:

1. How sensitive and reliable is the 21-day fathead minnow assay in identifying presence of CECs in complex mixtures?
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, following to the design in **Table 3.3-2**.

Table 3.3-2. 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

* Dilutions of the WWTP effluents will be tested using the Fathead Minnow Assay until an estuarine/marine fish model is developed.

3.4 Tier III – In Situ Toxicity Assessment

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

The SRWCB has developed guidelines to sample and measure environmental chemicals (e.g. metals, PCBs, alkylphenols) in fish and invertebrates (Davis et al. 2014, SWAMP 2014). For this pilot study, Tier III analyses will be conducted using fish species selected for tissue monitoring (section 2.2.5). 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, striped bass can serve as a sentinel fish species. For each waterway, a minimum of 3 species and 5 fish per species will be collected and histopathological analyses of the liver and gonads (and kidney if possible) will be conducted to assess the health of the organisms.

4 Statewide CEC Monitoring Program Framework

4.1 Relationship Between Biological and Chemical Monitoring

A comprehensive monitoring strategy for aquatic ecosystems combines biological and chemical monitoring elements in a multi-tiered framework to determine if beneficial uses are compromised and intervening management action is needed (**Figure 4.1-1**). In Tier I, *in vitro* transactivation bioassays (see Section 3) screen for known and unknown CECs in concert with conventional targeted chemical analysis (see Section 2). Because all relevant MOAs and/or effects at the organism level are not addressed by currently available IVBs, periodic *in vivo* testing is also recommended in Tier I. If, however, screening level IVB results are below pre-established thresholds deemed protective, the frequency of *in vivo* testing in Tier I can be reduced. Should IVB results exceed thresholds, Tier II diagnostic evaluation using appropriate sentinel species and non-targeted chemical analysis (NTA) are undertaken to determine the likelihood and severity of impact, as well as to broaden the scope of pollutants targeted by chemical analysis in identifying likely causative stressors. If Tier II *in vivo* testing indicates a level of toxicity that is of concern, confirmatory monitoring (Tier III) is accelerated to determine if resources *in situ* are being impacted. Tier III monitoring is also necessary as an additional safeguard because Tier I and II monitoring tools are not entirely fail safe. The monitoring tools in Tiers I and II can also be utilized to identify MOAs and apical endpoints as well as chemical stressors in the case that *in situ* monitoring reveals an unacceptable level of impact.

4.2 Adaptive Management

The state of knowledge on CEC sources, fate and effects in aquatic ecosystems is continually evolving. To keep pace with new information and availability of new tools, the four-step adaptive process recommended by the Panel (**Figure 1.1-1**) is key to maintaining an up-to-date, relevant monitoring approach. Phase II constitutes the data gathering step, as described in this 3-year pilot study plan, in this cyclical process. Plans should be made in Year 4 of this 5-year cycle for the subsequent evaluation of monitoring data and the efficacy of new monitoring tools and models that predict occurrence, effects and the linkage between *in vitro* and *in vivo* endpoints (Phase III). This evaluation should include a review and modification, as necessary, of the:

1. Updated monitoring trigger quotients (MTQs)
2. Scenarios and model watersheds sampled
3. Sampling design (sample size, frequency, spatial coverage)
4. CEC analyte list and matrix specific RLs
5. Performance of tools evaluated as part of the special studies, e.g. bioanalytical screening assays, non-targeted chemical analysis

The final year of the 5-year cycle (Phase IV) should be devoted to initiating management actions, as needed and as informed by the monitoring data. This step also provides an opportunity to revisit and revise, as necessary, the management and monitoring questions of importance regarding CECs, in preparation for initiation of the next monitoring cycle (Phase I).

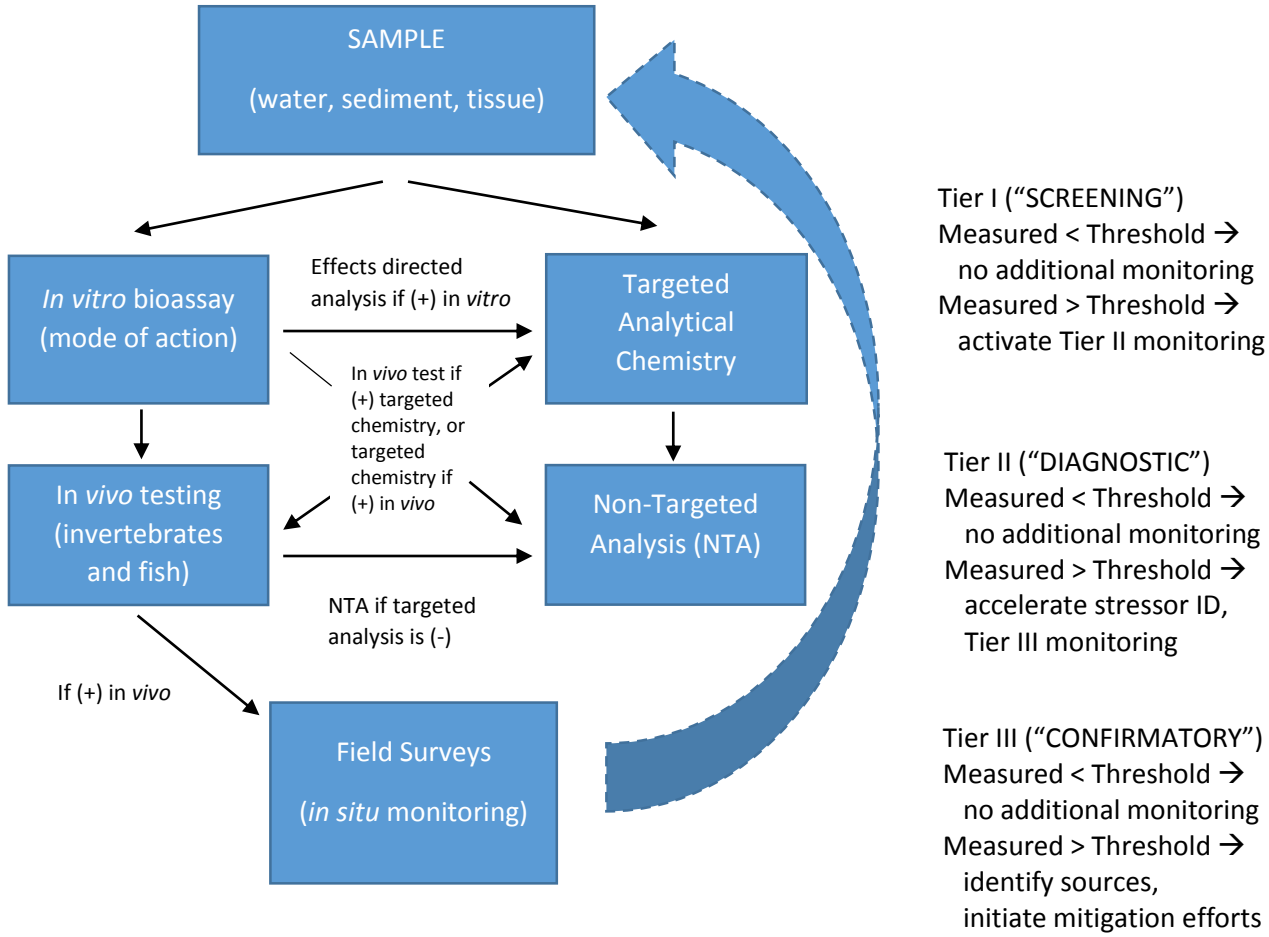

Figure 4.1-1. A comprehensive monitoring framework utilizes the results of tiered biological and chemical monitoring tools of increasing breadth, complexity and relevance to efficiently screen for CECs and identify potential causative agents when cell-based, whole organism and field-scale impacts are observed.

5 Research Needs

5.1 Toxicity Testing

Development of *in vivo* test species across habitats (fresh, marine, water column, sediment)

The Panel recommended that whole organism toxicity tests focused on reproductive and/or developmental endpoints be conducted for all scenarios (except MS4) and matrices. The fathead minnow reproductive assay, proposed and described in section 3, can only be applied to evaluate aqueous freshwater samples. Toxicity assays must be optimized and validated for other scenarios and matrices (**Tables 5.1-1, 5.1-2** and **5.1-3**)

Table 5.1-1. Candidate fish species for estuarine/marine aqueous toxicity testing.

	Sheepshead minnow ***Cyprinodon variegatus***	**Atlantic killifish** ***Fundulus heteroclitus***	**Inland silverside** ***Menidia beryllina***
Test duration	180 days	15 days	15 – 20 days
Endpoints	- Fecundity, fertility, GSI - Plasma sex steroids and vitellogenin - Hatching success - Larval morphology	- Plasma sex steroid - Vitellogenin - GSI	- Fecundity, fertility - Molecular markers - Hatching success - Gonad histology
Strengths	- EPA validated protocol	- Killifish species are widespread	- EPA validated species - found in state waters
Limitations	- Long test duration - Species known to be less responsive to CECs than other fish	- Species known to adapt in polluted environments - No egg output endpoint	- Reproductive endpoints have not been validated
References	Raimondo et al. (2009)	MacLatchy et al. (2003)	Personal communication (S. Brander, UNCW)

Table 5.1-2. Candidate invertebrate models for freshwater sediment toxicity testing.

	California blackworm ***Lumbriculus variegatus***	**Amphipod** ***Hyalella azteca***	**Midge** ***Chironomus species***
Test duration	28 days	42 days	44 days (*C. riparius*) 65 days (*C. tentans*)
Endpoints	- No. surviving worms - Growth (biomass) - Behavior (e.g. sediment avoidance)	- No. offspring/female - No. surviving adults - Sex ratio of surviving adults	- Development rate - Adult survival - Sex ratio of emerging adults - Fecundity and fertility
Comments	Asexual reproduction by regeneration	USEPA protocol currently optimized to include guidance on feeding and water quality	Shorter 28-day test is available with developmental endpoints
References	USEPA (2000), OECD (2007)	USEPA (2000)	OECD (2010)

Table 5.1-3. Candidate invertebrate models for estuarine/marine sediment toxicity testing.

	Polychaete ***Neanthes arenaceodentata***	**Amphipod** ***Leptocheirus plumulosus***	**Copepod** ***Amphiascus tenuiremis***
Test duration	28 days	28 days	16-17 days
Endpoints	- Survival - Growth - Bioaccumulation	- Survival - Growth rate - No. offsprings/adult - Behavior (sediment avoidance)	- Growth - Survival - Sex ratio - Fertility
Comments	No egg output endpoint	High variability often reported for reproduction	Patent rights on lab-cultured test organism
References	Farrar and Bridges (2011)	USEPA (2001), ASTM (2010)	Chandler et al. (2004b)

Development of *in vitro* assays for all relevant modes of action

For effective bioanalytical monitoring, a comprehensive suite of *in vitro* endpoints is warranted. *In vitro* assays recommended for pilot CEC monitoring are commercially available and screen mostly for

endocrine disrupting chemicals. Other environmentally relevant endpoints exist and need to be optimized for CEC monitoring (**Table 5.1-4**).

Table 5.1-4. *In vitro* assays to develop for CEC monitoring

Endpoint	Mode of Action/ Adverse outcome
P53 or Umu	Genotoxicity
Peroxisome proliferator activated receptor (PPARa and PPARg)	Fatty acid storage, glucose metabolism
Acetylcholine receptor	Neurotoxicity
*Thyroid receptor (TR)**	*Metabolism, growth*

* Commercial assays exist but performance is highly variable.

Development of in situ endpoints

In situ analyses conducted during routine environmental monitoring programs often focus on bioaccumulation of chemicals in tissues and the damages caused in tissues (histopathology). Special studies have also investigated the effects of environmental pollution on the population, but these studies can be expensive and time-consuming. Additional *in situ* endpoints indicative of early signs of exposure and toxicity should be developed. New molecular technologies measuring changes in gene expression (qPCR, microarrays, direct sequencing), protein levels (proteomics) and metabolite levels (metabolomics) have shown promising results (Biales et al. 2013; Martinovic-Weigelt et al. 2014; Skelton et al. 2014). Further research should be conducted using resident organisms to identify sensitive and reliable molecular endpoints.

5.2 Effect Directed Chemical Analysis

Environmental chemical mixtures inducing an *in vitro* assay response can be elucidated with a combination of targeted and non-targeted analysis. Targeted priority chemicals may explain a portion of the assay response, with the remaining unknown but responsible compounds identified through non-targeted analysis. This application is essentially a TIE methodology designed around the IVBs that utilizes recent advances in analytical instrumentation for non-targeted screening. Either gas-chromatography based (for hydrophobic compounds, e.g., GCxGC-TOF) or liquid chromatography based (for aqueous phase compounds, (e.g., LC-Q/TOF) non-targeted methods may be applied to the identification of bioactive compounds. The two primary research lines that must be addressed prior to implementing are the development of (1) libraries containing mass spectra and retention time information of chemicals with known *in vitro* and *in vivo* responses and (2) effects directed analytical methods that directly link bioassay response with chemical fractionation, which reduces mixture complexity and informs analytical method choice.

5.3 Passive Sampling Methods

As new science pushes monitoring thresholds lower, conventional environmental sampling and analytical methods become antiquated, incapable and cost-ineffective in concentrating high priority CECs from environmental media. Passive sampling methods (PSMs) show promise in sampling chemical constituents at very low occurrence in water, sediment and even biological tissue (sub-parts per billion concentrations). For hydrophobic CECs (e.g. PBDEs), PSMs that employ low density polyethylene films or polysiloxane (silicone) thin film coatings supported on hollow glass fibers or jars can pre-concentrate target analytes from freshwater, seawater, sediment and lipid-poor fish tissue. PSMs that employ sorbents that can concentrate both hydrophobic and hydrophilic CECs have been utilized is freshwater and coastal marine environments, however calibration of such samplers for estimation of concentration is incomplete. As the science on PSMs matures, and new approaches are developed and validated, these methods should be considered for future CEC monitoring programs in California water bodies.

5.4 Antibiotic Resistance

As identified by the Panel, antibiotics may adversely affect bacteria resulting in death at high clinical, therapeutic doses whereas at lower doses bacteria may survive and adapt to exposure by mutations which may result in development of antibiotic resistance (ABR). It remains unknown whether ABR in receiving waters of California is widespread, and if so, what implications for environmental quality and protection of beneficial uses would result from such occurrence. This is in large part due to the lack of definitive methods to quantify ABR in environmental media. Thus, development of standardized biological screening assays for quantitation of ABR in receiving water samples (water, sediment and tissue) for antibiotics that have been measured in monitoring studies conducted in California and throughout the US is recommended. To determine what risks due to ABR are plausible in California receiving waters, it is recommended that the SWRCB convene an expert panel of microbiologists, microbial ecologists, aquatic ecotoxicologists and water quality scientists, to define such risks, and to provide advice and oversight on the development and implementation of the ABR methods that can be employed in future monitoring studies.

5.5 Other

Additional research needs that would improve the monitoring of CECs statewide include:

1. Additional development and pilot evaluation of a statewide CEC Monitoring Framework (Fig. 4.1-1)
2. Bioaccumulation and trophic transfer factors for high priority bioaccumulative CECs, including PFOS and PBDEs, for freshwater, estuarine and marine food webs.
3. Measured or predicted half-lives of high priority CECs in aqueous (fresh and seawater) and sediment.
4. Development and validation of predictive models for source input (loading), concentrations (fate) and effects (by MOA, QSAR) of high priority CECs.

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7 Glossary of Terms

ABR	Antibiotic Resistance
AhR	Aryl hydrocarbon receptor
AR	Androgen Receptor
BASMAA	Bay Area Stormwater Management Agencies Association
BBP	Butylbenzylphthalate
BEHP	Bis(2-ethylhexyl)phthalate
BEQ	Bioassay equivalent concentration
BOG	Bioaccumulation Oversight Group
CECs	Chemicals of Emerging Concern
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethylsulfoxide
DPR	Department of Pesticide Regulation
DRMP	Delta Regional Monitoring Program
Dw	Dry weight
E2	17β-estradiol
EDC	Endocrine Disrupting Chemical
ECWG	Emerging Contaminants Work Group
FME	Fixed mass emission
GC-MS	Gas Chromatography-Mass Spectrometry
GCxGC/TOF-MS	Two Dimensional Gas Chromatography-Time of Flight Mass Spectrometry
GR	Glucocorticoid Receptor
IVB	In vitro bioassay
LC-MS	Liquid Chromatography-Mass Spectrometry
LOEC	Lowest Observed Effect Concentration
MEC	Measured Environmental Concentration
mgd	Million gallons per day
MOA	Mode of Action
MS4	Municipal Separate Storm Sewer System
MTL	Monitoring Trigger Level
MTQ	Monitoring Trigger Quotient
NIST	National Institute of Standards and Technology

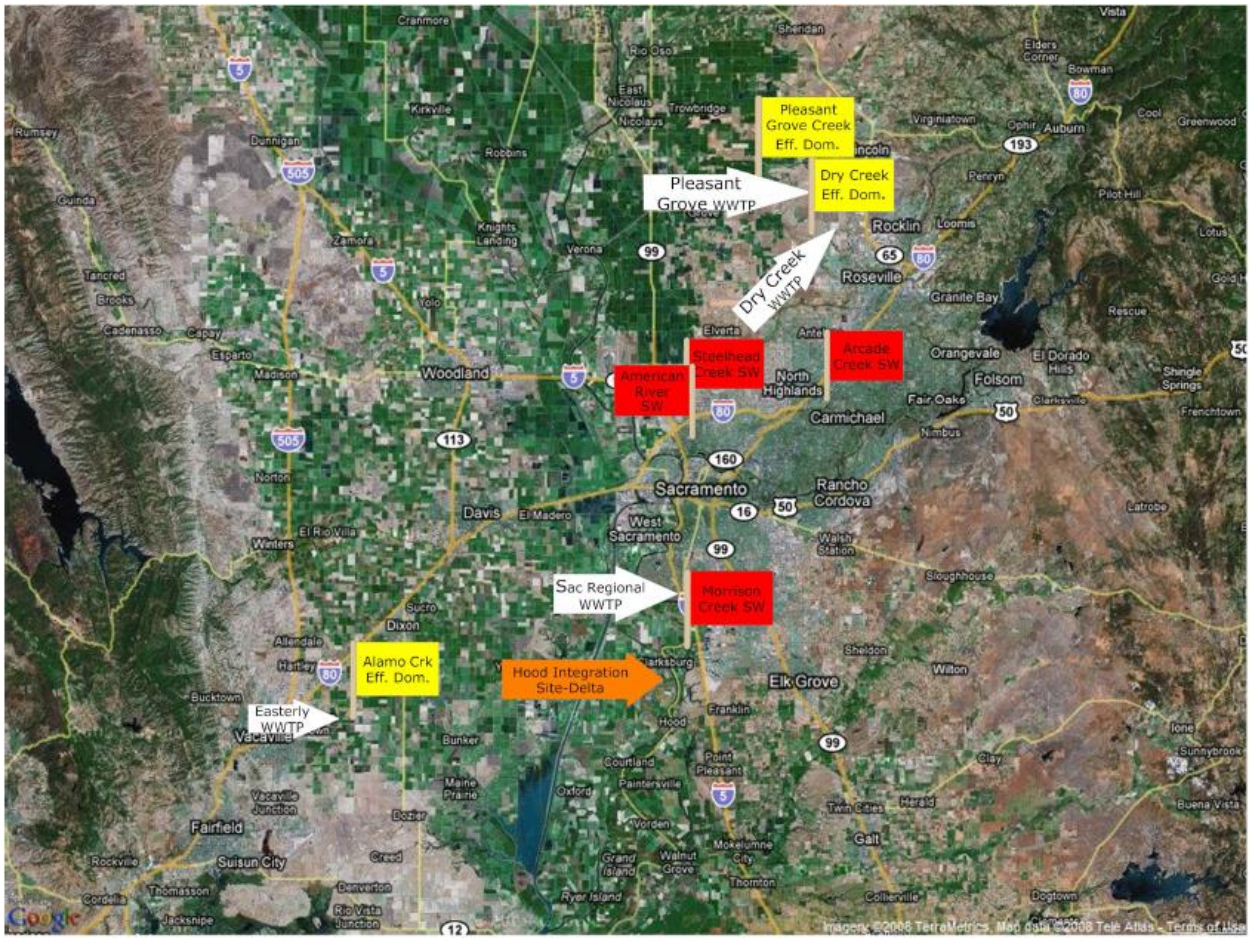
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NTA	Non-targeted chemical analysis
PAH	Polycylic Aromatic Hydrocarbon
PBDE	Polybrominated Diphenyl Ether
PCB	Polychlorinated Biphenyl
PEC	Predicted Environmental Concentration
PFC	Perfluorinated Compound
PFOS	Perfluorooctane Sulfonate
PNEC	Predicted No Effect Concentration
POC	Pollutant of concern
POTW	Publicly Owned Treatment Works
PR	Progesterone Receptor
PSD	Passive sampling device
PSM	Passive sampling method
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
QSAR	Quantitative Structure Activity Relationship
REF	Relative enrichment factor
RL	Reporting limit
RMC	Regional Monitoring Coalition
RMP	Regional Monitoring Program
RW	Receiving Water
RWQCB	Regional Water Quality Control Board
SCCWRP	Southern California Coastal Water Research Project
SFEI	San Francisco Estuary Institute
SMC	Stormwater Monitoring Coalition
SPoT	Stream Pollution Trends Monitoring Program
SRM	Standard Reference Material
S&T	Status and Trends
SWAMP	California Surface Water Ambient Monitoring Program
SMC	Stormwater Monitoring Coalition
SWPP	Surface Water Protection Program

SWRCB	State Water Resources Control Board
TIE	Toxicity Identification Evaluation
TSS	Total Suspended Solids
USGS	United States Geological Survey
USEPA	United States Environmental Protection Agency
VTG	Vitellogenin
WET	Whole Effluent Testing
WWTP	Wastewater Treatment Plant

8 Appendices

8.1 Appendix A: Delta Station Map

Candidate northern California Delta Scenario 1 WWTP (white) and Stormwater (red) station locations.



8.2 Appendix B: Bight ’13 Outfall Special Study

Southern California Bight 2013 Targeted CEC Survey

A Bight ’13 Special Study was implemented to address Scenario 3 monitoring. This study is intended as a pilot project, and future surveys may be modified based on the results of this initial monitoring. The design addresses Scenario 3 questions regarding marine outfall discharge, as also compares marine outfall receiving stations with storm water receiving stations. All samples are sediments.

Aim 1. Compare CEC sediment concentrations impacted by the three sources (marine outfalls, storm water, and inland waste water). Only marine outfall zone-of-initial-dilution (ZID) stations will be used for this purpose. Outfall contaminant concentrations are expected to be highest in the ZID and are

potentially more variable than stations further out. To account for this potential variability, three sub-stations within the ZID were be sampled, and the composite will be analyzed as a single sample.

Aim 2. Verify CECs originate from the outfalls and are not simply at background concentrations. Decreasing CEC concentrations down-current away from the outfall will indicate the compounds originate at the outfall. Also, stations up current (presumably at background), and cross-current station will indicated if the outfall is the source. Outfall stations were assigned in consultation with the dischargers and based on 1) the predominant current direction throughout the year, and 2) spatial trends of legacy contamination. The main gradient direction relative to the outfall varied among locations. For example, the LACSD outfall is perpendicular to the current in that region, but the OCSD outfall is parallel the current. The selected station distance is expected to show a decrease in CEC concentrations away from the outfall, based on legacy data.

Target Compounds

The four analyte classes are alkylphenols (APs), perfluorinated compounds (PFCs), pyrethroids/fipronil, and polybrominated diphenyl ethers (PBDEs). They will be measured at all stations in the survey. Phthalates, recommended by the Panel for Scenario 3 monitoring, will not be measured due to resource limitations.

Survey Design

Fifteen river-mouth samples throughout southern CA were obtained as part of the regular Bight ’13 sediment survey (sampled July – September 2013). There was 1 station per river-mouth. Ten stations receive storm water and 5 receive both storm water and waste water discharge.

The 5 outfalls were City of LA Hyperion (CLA), LA County Sanitation District’s outfall off Palos Verdes (LACSD), Orange County Sanitation District (OCSD), and the two City of San Diego (CSD) outfalls Point Loma and South Bay. There are 5 stations at each outfall, and three sub-stations within the ZID station. Samples were collected in January 2014.

Relationship to the Panel’s original marine outfall design. For this pilot survey we expanded the number of outfalls from 2 in the original design to 5. This required a reduction in the number of stations per outfall from 7 to 5. Increasing the number of outfalls provides more ZID stations for comparison to the river-mouth concentrations (see Aim 1), and provides information on CEC occurrence at all major ocean outfalls in the region.

8.3 Appendix C: Freshwater Fish Reproduction Assay

Protocol for Aqueous Toxicity Testing Using Freshwater Fish Reproduction Assay

The 21-day fathead minnow reproduction assay has been developed and vetted to assess the toxicity of endocrine disrupting). Test parameters of the assay are presented below (Table 3.3-3).

Table 8.3-1. EPA validated methods for short term toxicity testing using fathead minnow.

Parameters	Test Conditions
Test type	Flow-through system
Test chamber size	10 or 18L glass tank
Test volume	8 or 10L
No exchanges of test solutions	6 per day
No. replicate chambers	4 per test condition
Age of organisms	5 – 7 months old reproductive fathead minnow
No. fish per chamber	2 males and 4 females
Feeding regime	Brine shrimp twice a day
Water quality	Temperature 25 $\pm$ 2°C, pH 6.5 - 9 D.O. > 4.9 mg/L (60% of saturation)
Test controls	Dilution water (e.g. clean dechlorinated tap water) Solvent control (if solvent used)
Pre-exposure period	14 days
Test sample exposure period	21 days
Endpoints	- % survival and changes in behavior relative to controls - No. eggs laid and No. eggs fertilized - Levels of plasma sex steroids and vitellogenin relative to controls - Changes in secondary sex characteristics (nuptial tubercles) - Gonadosomatic index (GSI) and gonad histopathology