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 (SWB) 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 SWB 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 stormwater 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 Expert 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.

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

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 MTLs (monitoring trigger levels based on biological effects thresholds) that incorporated safety factors. CECs recommended for initial monitoring exhibited an MTQ (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 SWB for implementation as part of their development of statewide policies, permits and/or guidance.

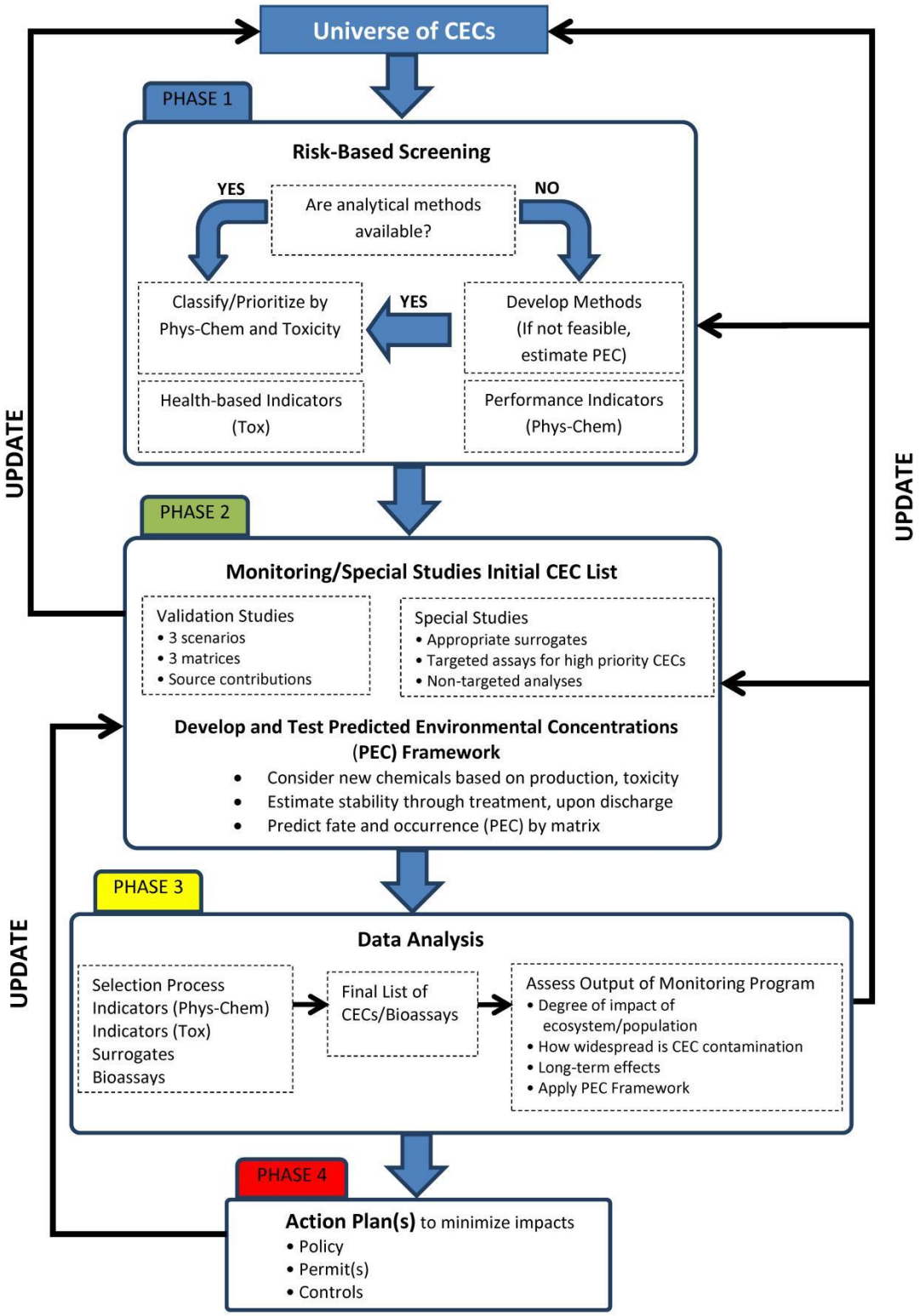

Fig. 1.1-1. The adaptive monitoring strategy for constituents of emerging concern (CECs) developed by the CEC Ecosystems Panel convened to recommend monitoring in California surface waters impacted by NPDES permitted discharges (i.e. treated wastewater effluent and stormwater runoff).

1.1.2 Discharge Scenarios

With guidance from the SWB 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 1.1-1**). 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 1.1-2**), 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 1.1-3**).

Table 1.1-1. 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 1.1-2. 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 1.1-3. 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 1.1-1 and 1.1-2, 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 Quality Assurance Project Plan (QAPP) (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 1.1-3, 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, a quality assurance project plan (QAPP) will be generated as a supplement to this document. The QAPP will provide criteria and guidelines to ensure that robust measurement of targeted monitoring and special study parameters is achieved.

1.3 PAST AND ON-GOING EFFORTS TO PRIORITIZE/MONITOR CECs (UNDER CONSTRUCTION)

1.3.1 Statewide Projects

1.3.1.1 SWAMP (including BOG)

1.3.2 Regional Efforts

1.3.2.1 San Francisco Bay

Regional Monitoring Program

BASMAA

1.3.2.2 Southern California

Bight Regional Monitoring Program

Stormwater Monitoring Coalition

1.3.2.3 Delta Regional Monitoring Program

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 monitoring trigger quotients (MTQs) exceeded unity for the aqueous phase in inland freshwater and coastal embayment 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.

Table 2.1-1. Ecotoxicological data for fipronil.

	Aqueous Freshwater	**Aqueous Saltwater**	**Sediment Freshwater**	**Sediment Saltwater**
Reference	Ali, 1998	USEPA, 1996	Maul, 2008	Chandler, 2004
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**
Inland Freshwater -1	Aqueous	10,004 ng/L (MEC)	240	Gan et al., 2012
Inland Freshwater -1	Aqueous	2110 ng/L (MEC)	50	Ensminger et al., 2013
Inland Freshwater -1	Sediment	1.1 ng/g dw (MEC)	12	Lao et al., 2010
Inland Freshwater -1	Sediment	0.4 ng/g dw (MEC)	4.4	Delgado-Moreno et al., 2011
Embayment -2	Aqueous	1000 ng/L (PEC)	200	Gan et al., 2012
Embayment -2	Aqueous	211 ng/L (PEC)	42	Ensminger et al., 2013

2.2 TARGETED CONTAMINANTS AND REPORTING LIMITS

Reporting limits for the target CECs are based on the monitoring trigger levels (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 no longer necessary to monitor). 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 (**Tables 2.2-1 through 2.2-5)**. 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.

Table 2.2-1. Recommended reporting limits (RLs) for aqueous phase CECs in effluent dominated inland waterways (Scenario 1).

Compound	**Freshwater MTL (ng/L)**	**Reporting Limit (ng/L)**
Bifenthrin	0.4	0.2
Permethrin	1	0.5
Fipronil	42	21
Fipronil (sediment)	0.090 ng/g dw	0.045 ng/g dw
Chlorpyrifos	5	2.5
Estrone	6	3
Ibuprofen	100	50
Bisphenol A	60	30
17-beta-estradiol	2	1
Galaxolide (HHCB)	700	350
Diclofenac	100	50
Triclosan	250	125

Table 2.2-2. Recommended reporting limits (RLs) for aqueous phase CECs in coastal embayments (Scenario 2).

Compound	**Estuarine MTL (ng/L)**	**Reporting Limit (ng/L)**
Bisphenol A	6	3
Bifenthrin	0.04	0.02
Permethrin	0.1	0.05
Fipronil	5	2.5
Chlorpyrifos	1	0.5
Estrone	0.6	0.3
17-beta-estradiol	0.2	0.1
Galaxolide (HHCB)	70	35

Table 2.2-3. Recommended reporting limits (RLs) for sediment-associated CECs in WWTP-effluent dominated inland waterways (Scenario 1).

UNDER CONSTRUCTION

Table 2.2-4. Recommended reporting limits (RLs) for sediment-associated CECs in coastal embayments (Scenario 2).

Compound	Estuarine Sediment MTL (ng/g dw)	Reporting Limit (ng/g dw)
Bifenthrin	0.052	0.026
PBDE-47 and -99	0.03	0.015
Permethrin	0.073	0.0365
Fipronil	6.5	3.25

Table 2.2-5. Recommended reporting limits (RLs) for sediment-associated CECs in ocean discharge (Scenario 3).

Compound	Marine Sediment MTL (ng/g dw)	Reporting Limit (ng/g dw)
Bis(2-ethylhexyl) phthalate (BEHP)	130	65
p-nonylphenol	14	7
PBDE-47 and -99	0.30	0.15
Butylbenzyl phthalate (BBP)	6.3	3.15

Table 2.2-6. Recommended reporting limits (RLs) for CECs in tissue (all scenarios).

Compound	Tissue MTL (ng/g dw)	Reporting Limit (ng/g dw)
PBDE-47 and -99	28.9	14.45
PFOS	1000	500

2.3 DESIGN REQUIREMENTS BY SCENARIO

2.3.1 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.

Study 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 POTW, 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?

Examples of waterbodies that represent Scenario 1 are the Los Angeles, Santa Clara, San Gabriel, Santa Ana, and San Diego Rivers. Ideal candidates for this pilot study are waterways with well-characterized

source and flow inputs. The LA River and the Santa Clara River are proposed as candidates in southern California. No similar waterways have been identified in the San Francisco Bay and/or Delta regions.

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) (**Fig. 2.3-1**) . Both the wet and dry seasons will be monitored over a 3 year period (**Table 2.3-1**). For fipronil, sediment analysis is also recommended based on Scenario 1 sediment MTQs > 1.

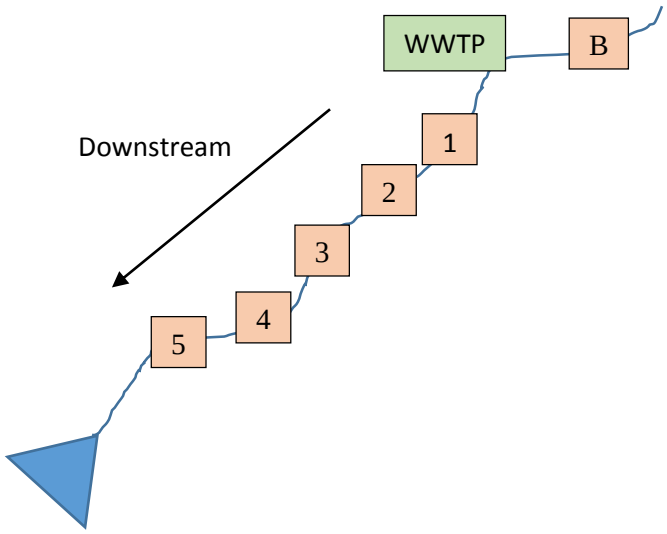

Fig. 2.3-1. Design schematic for monitoring of CECs in a WWTP-effluent dominated inland waterway (Scenario 1).

Table 2.3-1. Aqueous sampling frequency for WWTP-effluent dominated inland waterways (Scenario 1).

Source	Receiving Water	Years	Waterways	Total Samples
POTW effluent 1 station Wet and dry season Samples = 2/yr	River 6 stations Wet and dry season Samples = 12/yr	3	3	Effluent = 18 FW = 108

SEDIMENT SAMPLING FREQUENCY UNDER CONSTRUCTION

2.3.2 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. This scenario is monitored exclusively in San Francisco Bay.

Study Questions:

1. Which CECs are detected in coastal embayment/estuarine water and sediments?
2. What are their concentrations and how quickly (i.e., at what distance) do the CECs attenuate once discharged?
3. Can the CECs be shown to originate from the outfalls, or are they present at background concentrations?
4. Is there a sub-annual change in discharged CECs?
5. Are the concentrations at co-located sediment and aqueous stations correlated?
6. Does the new occurrence data change the estimated MTQs?

UNDER CONSTRUCTION

Fig. 2.3-2. Design schematic for monitoring of CECs in a coastal embayment (Scenario 2).

Table 2.3-2. Aqueous sampling for Scenario 2.

Table 2.3-3. POTW effluent sampling for Scenario 2.

Table 2.3-4. Sediment sampling for Scenario 2.

Table 2.3-5. Tissue (Bioaccumulation) sampling for Scenario 2.

2.3.3 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.

Study Questions:

1. Which CECs are detected in marine waters and sediments adjacent to WWTP and significant stormwater 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 the MS4 study design.)

The effluent and sediments at two POTW ocean outfalls will be monitored, with a grid of to 8 sediment stations at each outfall (**Fig 2.3-2**). The exact locations will consider the oceanic conditions and historic depositional patterns at each 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 2.3-6**).

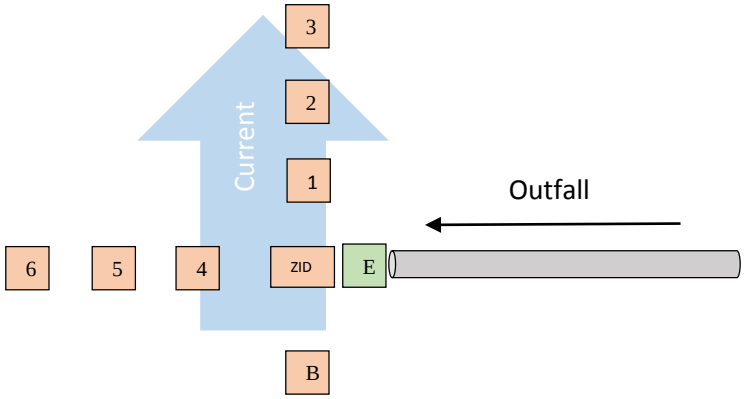

Figure 2.3-3. Design schematic for monitoring of CECs in ocean water receiving WWTP-effluent discharge (Scenario 3).

Table 2.3-6. Aqueous sampling for Scenario 3.

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

2.3.4 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. water soluble 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 MS4 monitoring.

2.3.4.1 Study 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? (See the Scenario 3 study design.)
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)?

2.3.4.2 Design Considerations

Wet Weather: Since annual loading is the main concern during wet weather, a design that focuses on estimating total loads into MS4 receiving waters is the primary goal. Current wet weather monitoring relies on sampling at fixed mass emission (FME) stations located at the bottom of MS4 permitted watersheds. Flow weighted sampling at FME stations for two storms per year per watershed will provide data to address questions 1-3. 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 should be planned during Years 2 and 3, after initial occurrence and loading data have been obtained and analyzed in Year 1. 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 and QAPP).

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 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. 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 Sec 3.x). Such extracts are also amenable, without fortification, for toxicity screening.

2.3.4.3 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 2.2.4.2. Targeted CEC monitoring that require detection limits not readily achievable using conventional or commercially available methodology shall utilize passive sampling devices (PSDs), where such technology is appropriate (e.g. for determination of long term, time-averaged concentrations).

2.3.4.4 Candidate watersheds:

- San Francisco Bay: TBD
- Delta/Central Valley: Steelhead Creek, Morrison Creek, Hood (an integrator site), Arcade Creek, and the Natomas and American Rivers.
- Southern California: watersheds monitored by the Stormwater Monitoring Coalition (SMC), including those in San Diego (San Diego River), Orange (San Diego Creek/Newport Bay), Los Angeles (Ballona Creek) and Ventura (Santa Clara River) counties.

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Fig. 2.3-4. Sampling strategy for MS4 watersheds during (o) dry and (x) wet weather.

Table 2.3-7. Aqueous sampling for stormwater.

Waterway Stormwater	Waterway Sediments	Receiving Water Sediments	Years	Waterways	Total Samples
? stations Wet and dry season Samples = ?/yr	? stations Wet and dry season Samples = ?/yr	? stations Wet and dry season Samples = ?/yr	3	3	FW = ? Sediment = ?

2.3.5 Tissue Monitoring Design

Study Questions:

1. What are the concentrations of CECs in tissues?
2. What is the temporal trend?
3. Are there spatial differences in tissue concentrations (inland vs. coastal vs. marine and northern vs. southern California)?
4. Are there differences among species (i.e., what are the appropriate sentinel species)?
5. What are the concentrations of biomagnifying CECs at the highest trophic levels (i.e.; those species with potentially the greatest risk)?
6. Does the new occurrence data change the estimated MTQs (when NOECs are available)?

2.3.5.1 Design Considerations

UNDER CONSTRUCTION

2.3.5.2 Design Requirements

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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 these methods prior to full implementation (Table 1.1-3). These studies largely address the potential for adverse effects of CECs in aquatic organisms (e.g. animal toxicity; microbial resistance) and will complement the traditional targeted analytical methods (as described in section 2) by providing additional information on the occurrence of known and unknown CECs (e.g. bioanalytical screening assays), and evaluation of emerging technology for sampling of low-level CECs in environmental media (e.g. passive sampling).

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* assays are conducted to screen for the occurrence of chemicals, including CECs, in water and sediment 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 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 treated 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 qPCR or gene microarrays as well as more conventional monitoring and assessment parameters (e.g. tissue histology, species abundance/diversity).

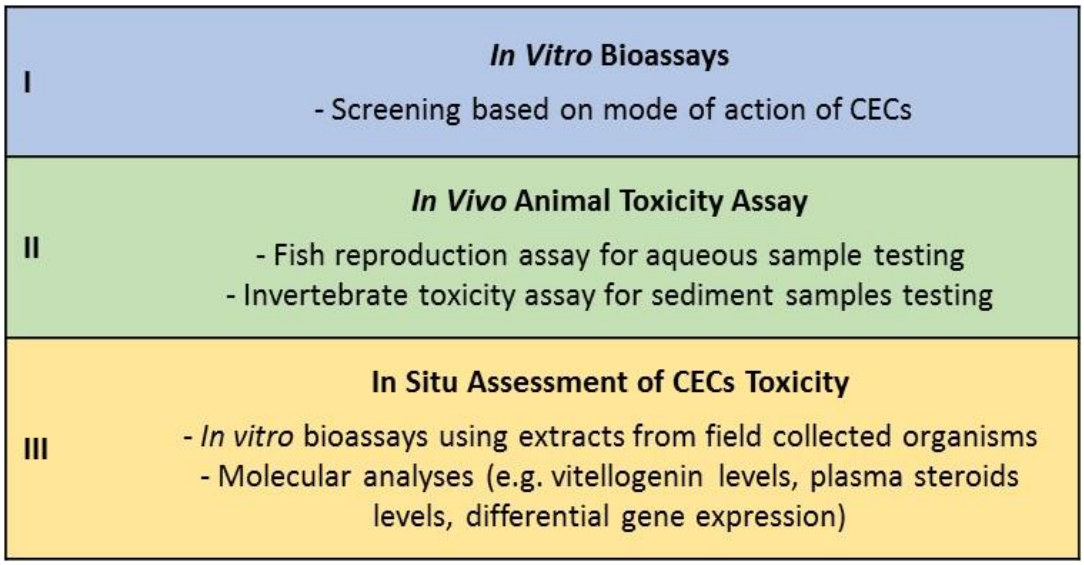

Figure 3.1-1. Proposed framework for biological assessment of CECs in aquatic environments.

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 cell assays 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 cell assays are proposed to assess the capability of environmental CECs to activate endocrine-related receptors, induce xenobiotic metabolism and cause cell death (**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 (Panther 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 (suppression of activity) mode. In some cases, bioassays can screen for exposure to known high priority CECs, but potential adverse outcomes linked to these endpoints are diverse and/or not yet well defined (e.g. AhR and PXR). In other cases, the MOA is known and relevant, but a suitable bioassay is either in the development stage and not yet commercially available (e.g. genotoxicity).

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 suppression	Estrogen signaling	Feminization of males. Impaired reproduction, cancer
Androgen Receptor (AR)	Activation and suppression	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	
Pregnane X Receptor (PXR)	Activation	CYP3A metabolism induction	
TBD	Activation	Genotoxicity	
Cytotoxicity	-	General cell toxicity	Tissue damage, death

TBD – to be determined

Two types of investigations are recommended. First, a battery of candidate *in vitro* bioassays 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 bioassays 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 traditional 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 the extract in the bioassay)	
Data analyses	Effect concentration (EC)	Induction ratio (IR)
Output parameter	Bioanalytical equivalent concentration (BEQ)	

3.2.1 *In vitro* screening of targeted CECs

Study Questions:

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, PXR, TR)?
3. What are the effects (additive or antagonist) of priority CECs mixtures using the selected cell assays?

Seventeen CECs (including fipronil) have been selected for target monitoring in water, sediment and/or tissue. The objective of this study is to identify the most sensitive and reliable 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 2.2-1 through 2.2-6). A mixture of the selected CECs will also be tested at MTLs to determine the additive or antagonist effects that may occur.

Table 3.2-3. *In vitro* assays for screening of priority CECs.

Endpoint	Priority CECs	Other CECs
ERa	BEHP, BBP[1] Galaxolide (Anti-ER)[2] Chlorpyrifos[3], PFOS[4] 17-beta estradiol – known strong ER agonist Estrone – known moderate ER agonist BPA, nonylphenol – known weak ER agonist	
AR	Galaxolide (Anti-AR)[2] No AR activation data for CECs of interest	
AhR	PBDE-47 and -99 Chlorpyrifos[5]	PCBs
GR	No GR activation data found for CECs of interest	
PR	No PR activation data found for CECs of interest	Progestins (e.g. levonorgestrel)
PXR	All[6]	

[1]Harris et al. 1997; [2]Schreurs et al. 2005; [3]Juberg et al. 2013; [4]Kjeldsen and Bonefeld-Jorgensen 2013; [5]Long et al. 2003; [6]Moore and Kliewer 2000.

3.2.2 *In vitro* screening of environmental extracts

Study Questions:

1. How sensitive and precise are the candidate in vitro bioassays in detecting CECs in aqueous samples of interest (e.g. WWTP effluent and receiving waters from all Scenarios)?
2. 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 different classes of CECs can be quantitatively screened for using the selected cell assays. 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 of this document) and all *in vitro* assays (**Table 3.2-4**). Prior to *in vitro bioassay* screening, the extracts will be solvent exchanged to DMSO.

Table 3.2-4. Sampling locations and frequency for *in vitro* testing

	Sample Type	Location	Sampling Frequency
Scenario 1 Freshwater	WWTP effluent	Outfall	2/year (wet & dry season)
	River water	Station #2 and 5 (section 2.2.1)	2/year (wet & dry season)
Scenario 2 Estuaries	WWTP effluent	Outfall	1/year
	Receiving water	TBD	1/year
Scenario 3 Oceans	WWTP effluent	Outfall	1/year
	Receiving water	Station #ZID, 3 & 6 (section 2.2.3)	1/year
Scenario 4 MS4	Stormwater run-off	TBD	2/year (wet & dry season)
	Watershed	TBD	2/year (wet & dry season)

3.2.3 *In Vitro* Assays Parameters and Optimized Methods

A number of commercially available cell assays have been identified for screening CECs in the environment. Among those, the GeneBLAzer assays (Life Technologies) and the CALUX assays (BioDetection Systems) have shown promising results (Escher et al., 2014), however, differences in operating procedures exist among the endpoints and manufacturers.

UNDER CONSTRUCTION

Table 3.2-5. Summary of validation parameters for each endpoint

	Reference chemical	# cells/well	DMSO content	Relative enrichment factor (REF)
Estrogen receptor alpha (ERa)	17-beta estradiol (+) 4-hydroxy-tamoxifen (-)		0.5 % per well	5 to 20 X
Androgen receptor (AR)	Methyltrienolone(R1881) (+) flutamide (-)		0.5 % per well	20 to 50 X
Progesterone receptor (PR)	Levonorgestrel (+)		0.5 % per well	20 to 50 X
Glucocorticoid receptor (GR)	Dexamethasone (+)		0.5 % per well	20 to 50 X
Aryl hydrocarbon receptor (AhR)	PCB 126		0.5 - 1% per well	TBD
Pregnane X receptor (PXR)	TBD (+)		0.5 - 1% per well	TBD
Genotox endpoint	TBD (+)		TBD	
Cytotoxicity	15% DMSO (+)			

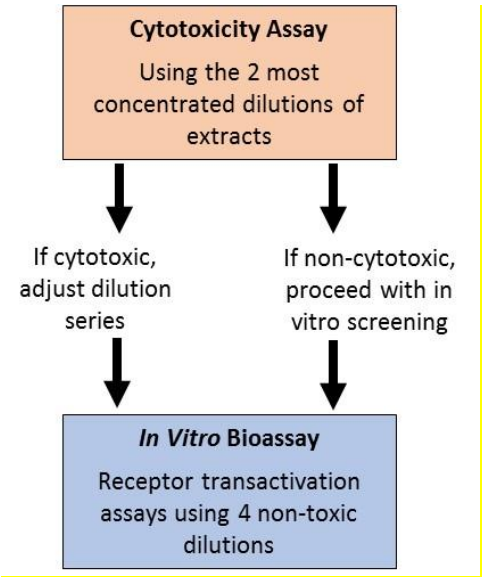

Figure 3.2-2. In vitro bioassay endpoints are sequenced to screen for cytotoxicity prior to testing for specific mode of actions.

Table 3.2-6. Test conditions for all *in vitro* bioassays

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 (BEQ) - 9 dilutions in duplicate in first assay plate - 4 dilutions in duplicate in subsequent plates (sample precision)
QA/QC	on each plate - blank response – assay media only - negative control – cells only, no DMSO - positive control – cells and DMSO, no water extract
Acceptability criteria	Cytotoxicity assay- 80% or more survival compare to control; % response for blank sample, negative and positive control response should be less than 10% of sample 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, growth, reproduction and behavior at the tissue and organism level. 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 and effects on fish reproduction using model compounds*

Study Questions:

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?

This study 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 fathead minnow (*Pimephales promelas*) reproductive assay will be performed following the EPA guidelines (see section 3.3.3). 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. Test parameters for linkage study of in vitro and in vivo responses to model compounds

	Test parameters - ER agonist
Chemicals	17-beta estradiol, concs 5, 50, 500 ng/L 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, conc TBD 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 - ER antagonist
Chemicals	TBD
In vitro endpoint	ER receptor suppression
	Test parameters - AR antagonist
Chemicals	Flutamide, conc TBD Solvent control (TEG or ethanol, less than 0.05%) Water control (no solvent)
In vitro endpoint	AR receptor suppression
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 Toxicity of complex mixtures of CECs in environmental aqueous samples on fish reproduction.

Study Questions:

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 using targeted analyses and Tier I *in vitro* analyses, according the schedule in **Table 3.3-2**.

Table 3.3-2. Aqueous test samples for fish reproduction assay

Scenario	Sample and location	Dilutions	Sampling Frequency
Freshwater	3 POTW effluents	1x – undiluted effluent	
	Receiving river water Station #2 & 5 (section 2.2.1)	1x – undiluted samples	
Estuaries*	2 POTW effluents	1x – undiluted effluent 10x – worst case 100x – best case	
Oceans*	2 POTW effluents	1x – undiluted effluent 50x – worst case > 1000x – best case	

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

3.3.3 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 chemicals (EPA, http://www.epa.gov/endo/pubs/att-f_fish_assay_protocol.pdf). Test parameters of the assay are presented below (**Table 3.3-3**).

Table 3.3-3. 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 – 6 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 ± 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

3.4 TIER III (IN SITU)

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3.5 PASSIVE SAMPLING

UNDER CONSTRUCTION

3.6 ANTIBIOTIC RESISTANCE

UNDER CONSTRUCTION

3.7 NON-TARGETED CHEMICAL ANALYSIS

UNDER CONSTRUCTION

4 STATEWIDE CEC MONITORING PROGRAM

4.1 Relationship between targeted monitoring and special studies

A comprehensive, tiered monitoring strategy for aquatic ecosystems combines elements of targeted and special study monitoring as described in this pilot study (**Fig. 4.1-1**). In Tier 1, newly developed in *vitro* transactivation bioassays screen for known and unknown CECs in concert with conventional targeted chemical analysis. Because all possible MOA and/or effects at the organism level are not addressed by currently available in vitro bioassay endpoints, in vivo testing is also recommended in Tier 1. If, however, screening level in vitro bioassay results are below pre-established thresholds deemed protective, the frequency of in vivo testing can be reduced. If in vitro bioassay results exceed thresholds, confirmatory evaluations (Tier II) using appropriate sentinel species and more advanced diagnostic (non-targeted) chemical analysis are undertaken to determine the likelihood and severity of impact, as well as the likely causative stressors. The information gleaned in Tiers I and II are used to reconcile observations from routine or periodic surveys of environmental condition performed in situ.

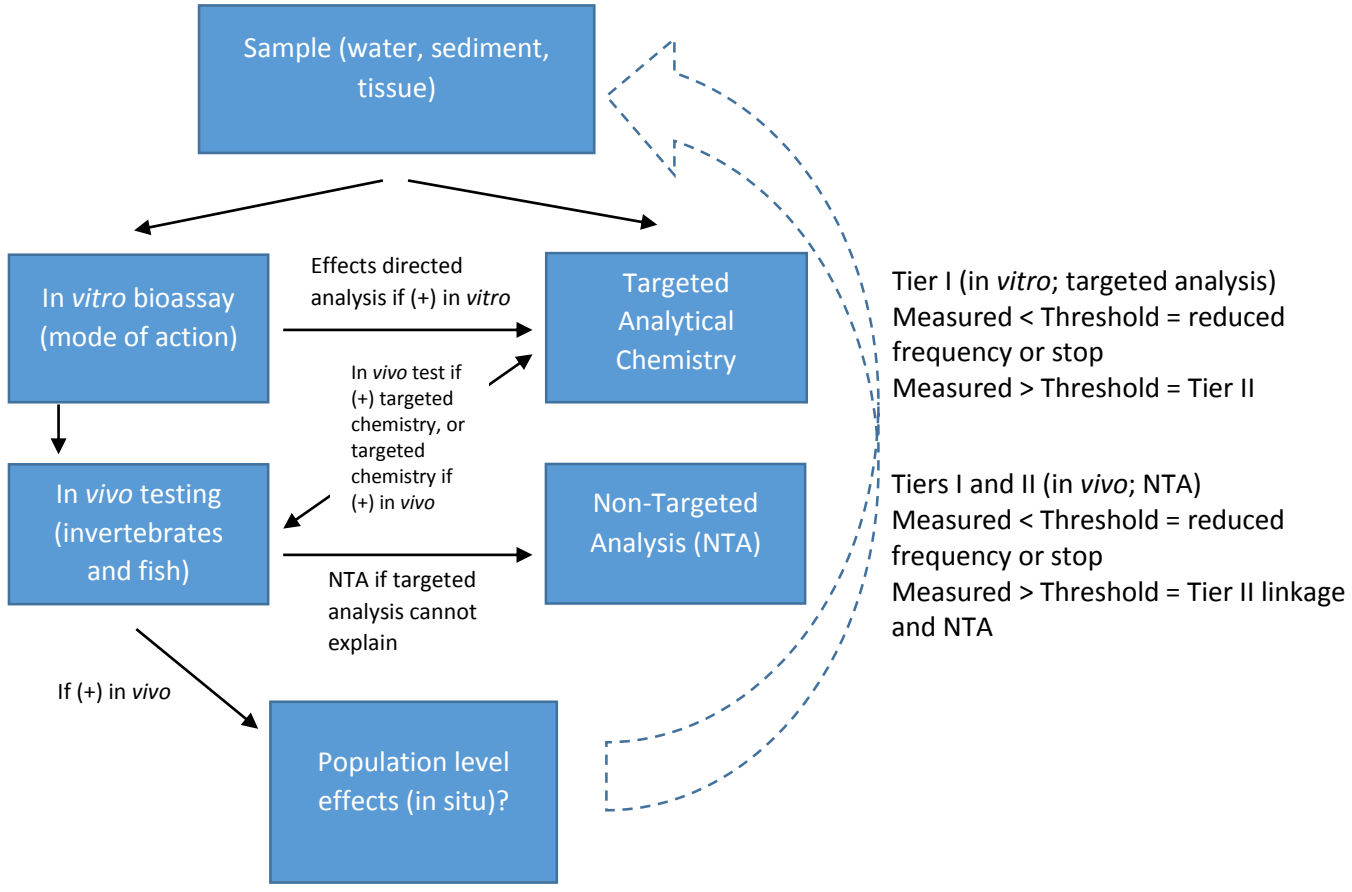

Figure 4.1-1. A comprehensive, tiered monitoring approach utilizes the results of targeted and special study components to efficiently screen for CECs and identify potential causative agents when biology is impacted.

4.2 Coordination with statewide, regional and local monitoring efforts

UNDER CONSTRUCTION

5 RESEARCH NEEDS

UNDER CONSTRUCTION

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APPENDICES

UNDER CONSTRUCTION

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.

Table 1. River Mouth Samples in the Bight '13 Special Study

B '13 Station ID	Region	Source
8040	San Diego Bay	storm water
8077	San Diego Bay	storm water
8136	San Diego River	storm water

8163	Mission Bay	storm water
8169	Los Penasquitos Lagoon	storm water
8187	San Dieguito Lagoon	storm water
8189	San Elijo Lagoon	storm water
8202	Batiquitos Lagoon	storm water
8219	Agua Hedionda Lagoon	storm water
8411	Ballona Creek	storm water
8250	Santa Margarita Estuary	wastewater and storm water
8292	Upper Newport Bay	wastewater and storm water
8378	San Gabriel River Estuary	wastewater and storm water
8390	Los Angeles River	wastewater and storm water
8421	Mugu Lagoon-South	wastewater and storm water

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. The outfall stations are shown in Figures 1-5.

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.

Figure 1. City of Los Angeles Marine Outfall

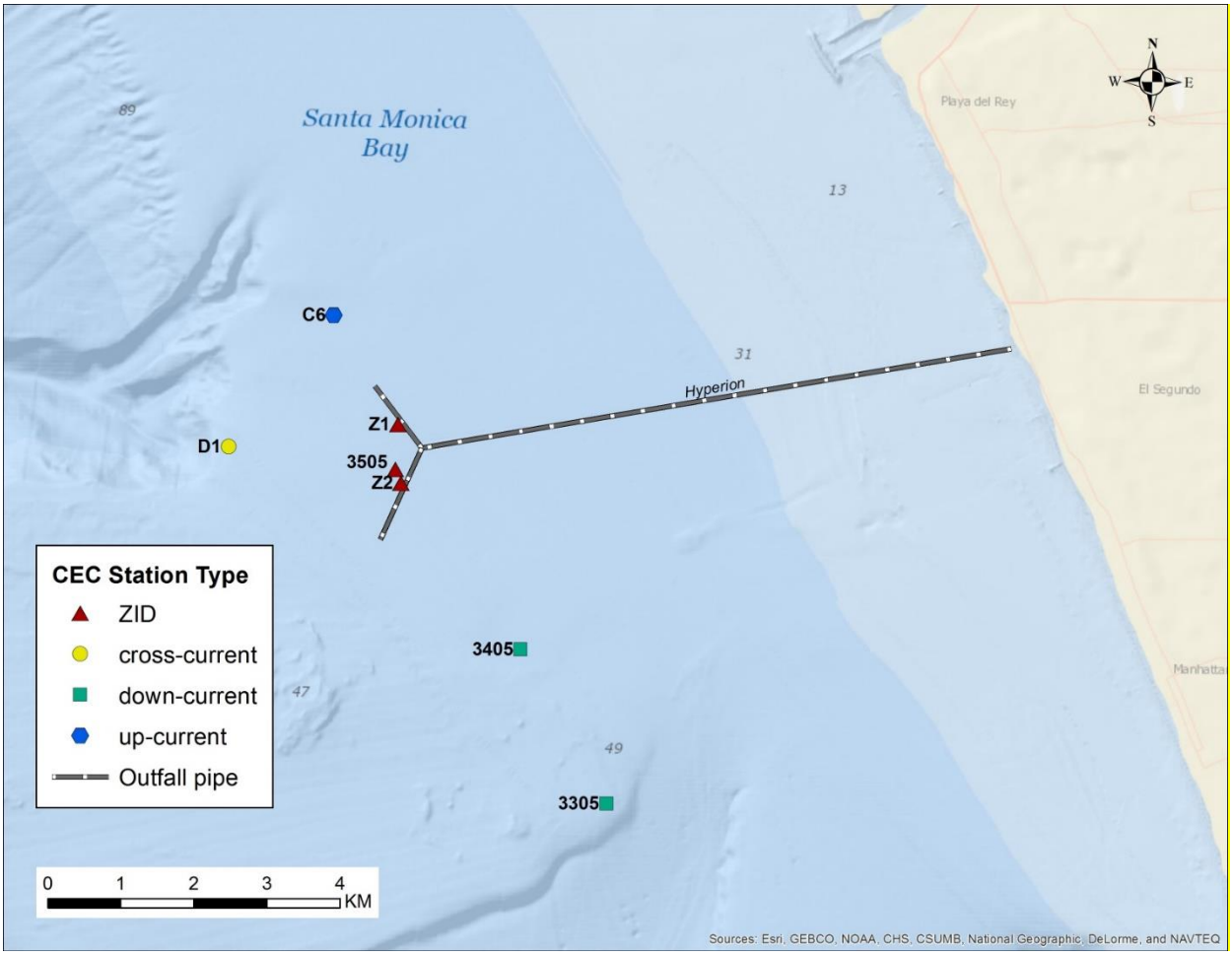
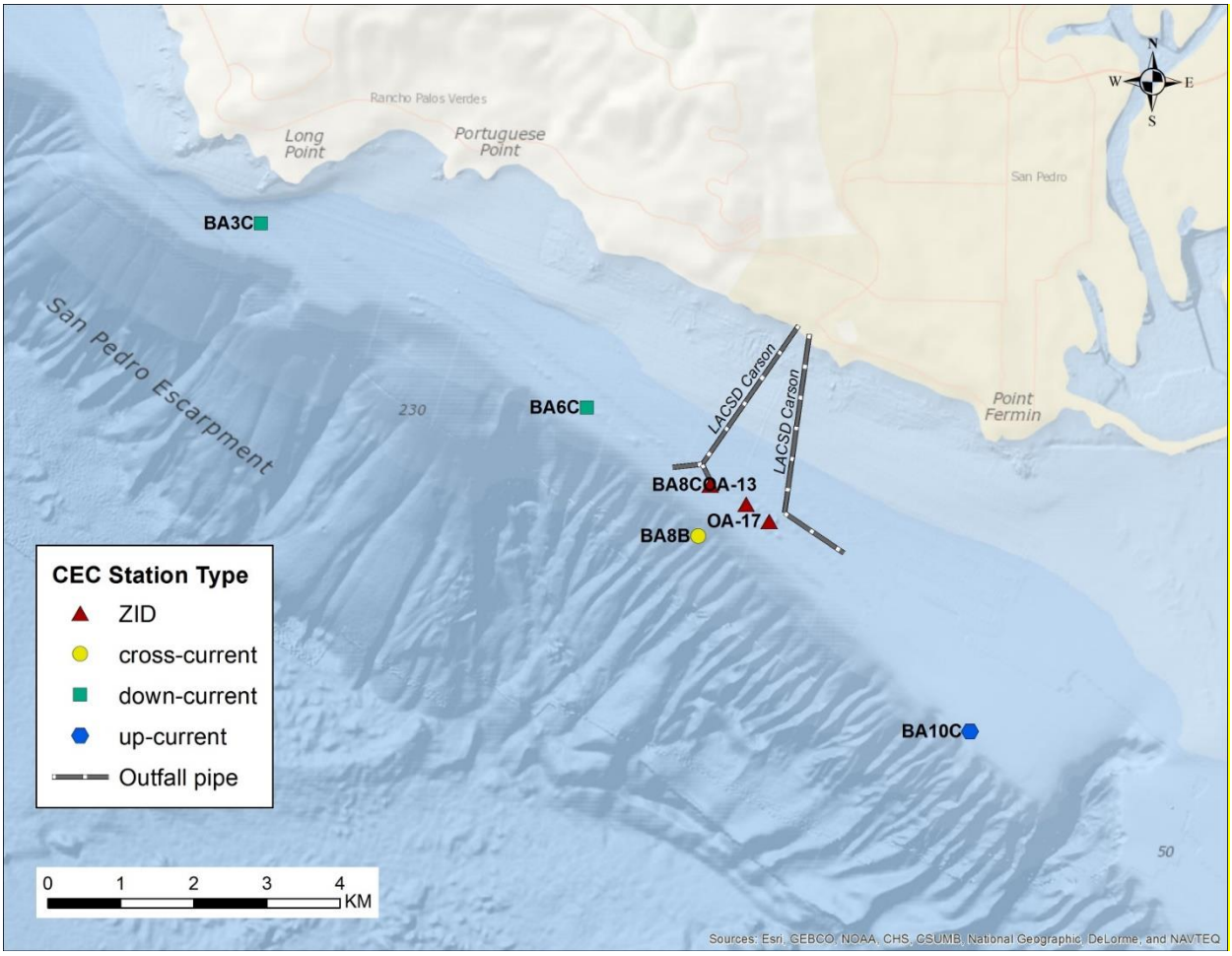


Figure 2. Los Angeles County Sanitation Districts Marine Outfall



Both outfalls are active.

Figure 3. Orange County Marine Outfall

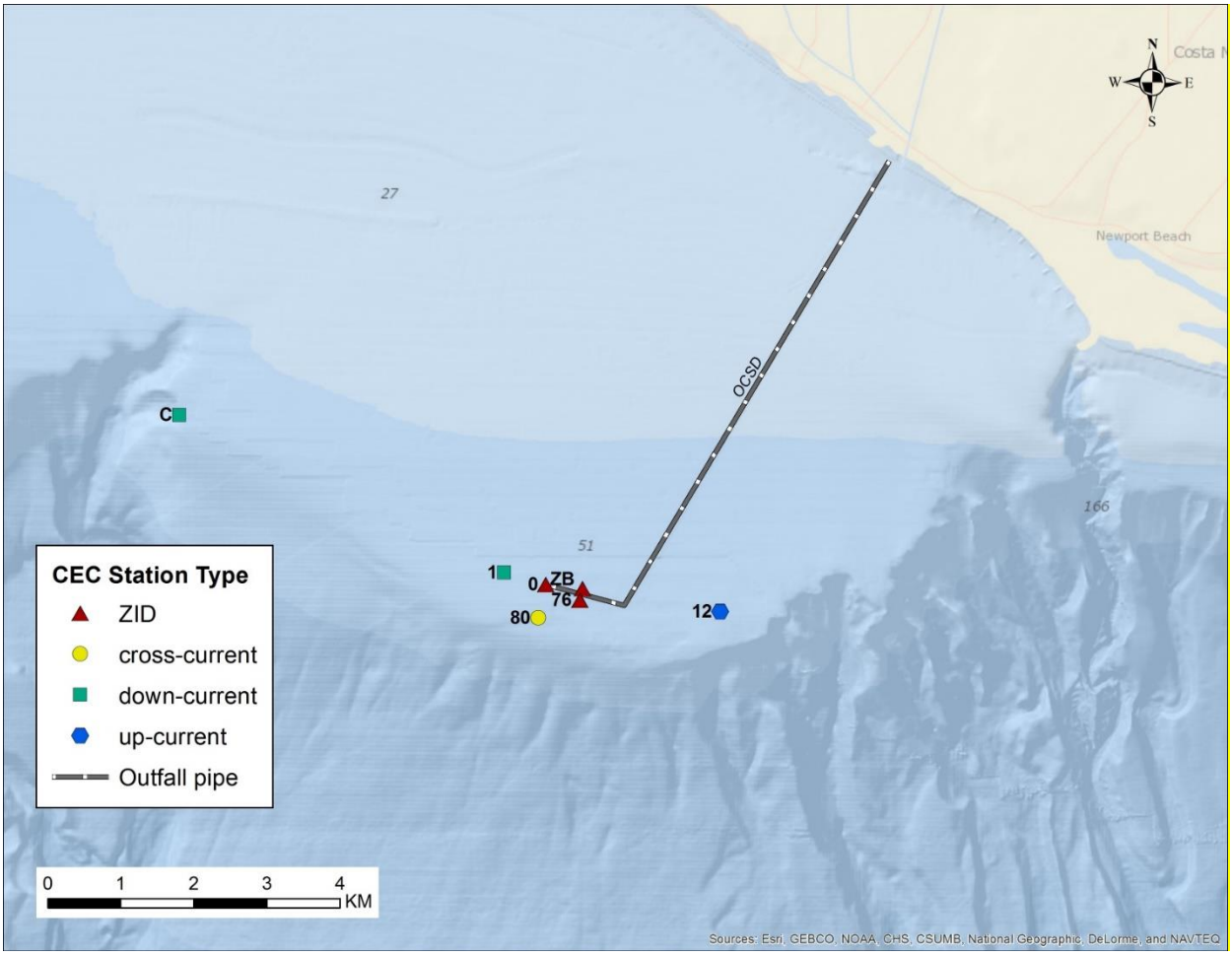


Figure 4. City of San Diego – Point Loma Marine Outfall

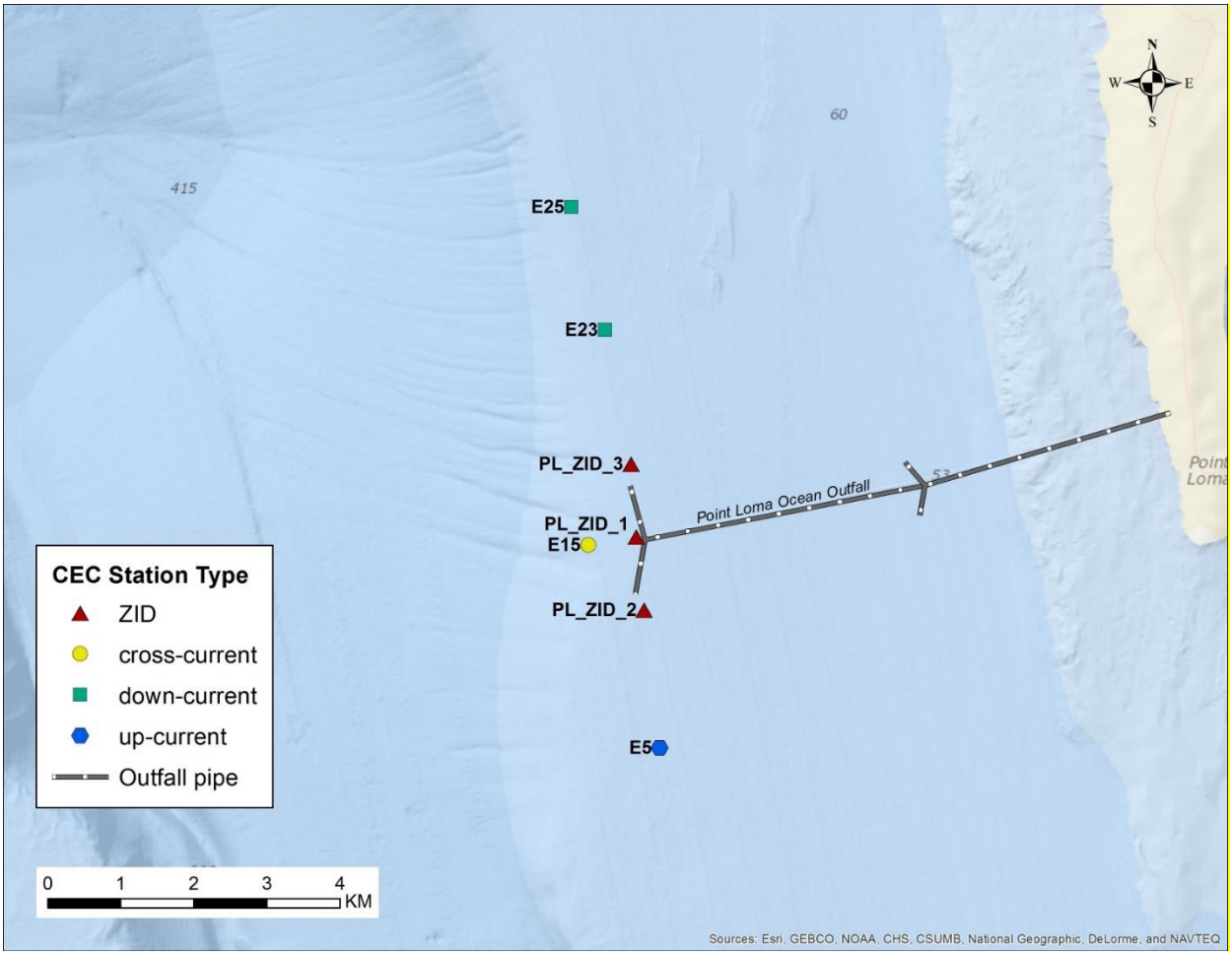
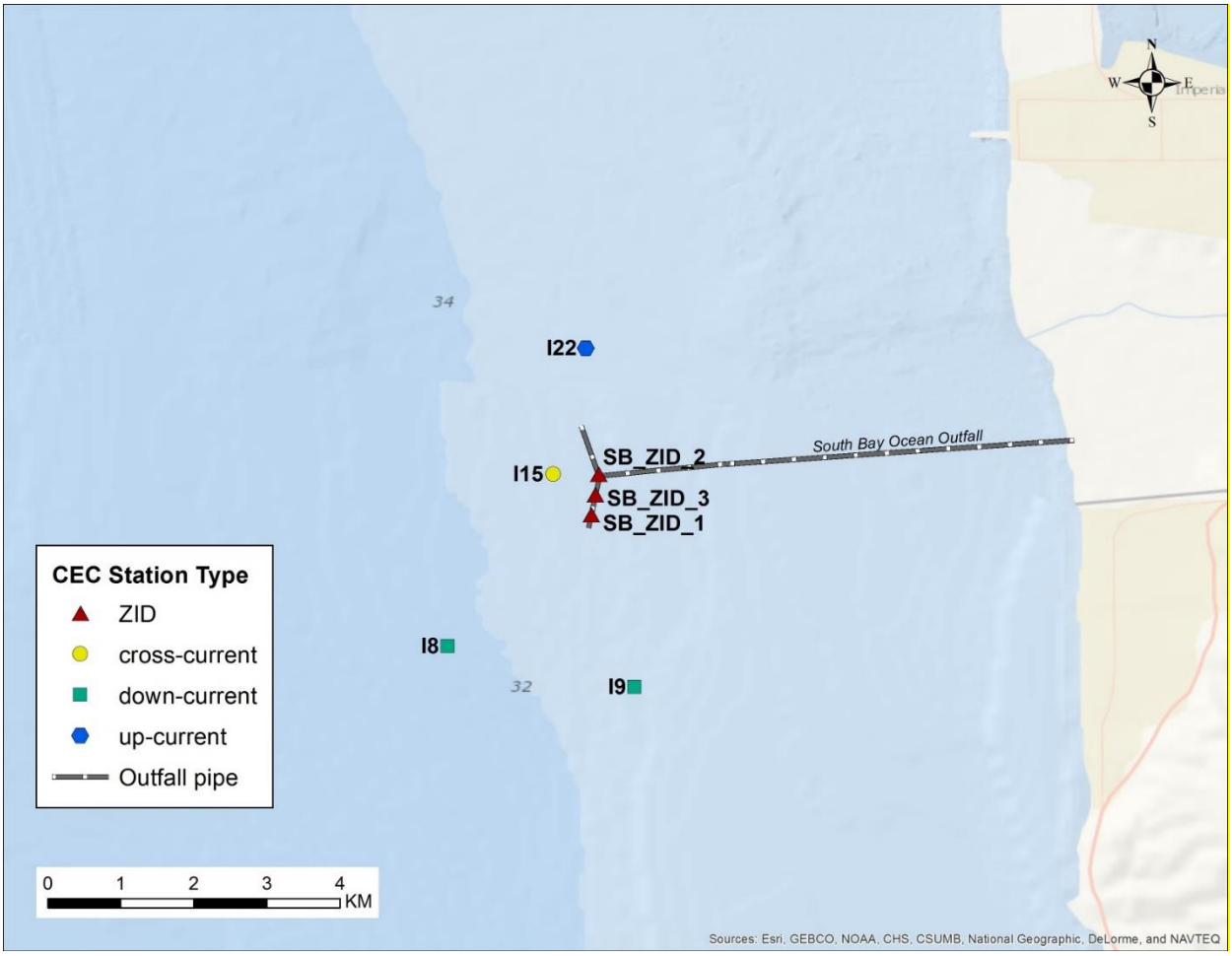


Figure 5. City of San Diego – South Beach Marine Outfall



The northern diffuser is inactive.