Pilot Study on CEC Monitoring in Aquatic Ecosytems

Mid-term Review

May 2014

SECTION 1 - INTRODUCTION

- **1.0 Background**

  Lack of scientific knowledge and consensus on the impact of unregulated contaminants (constituents of emerging concern or “CECs”)

  In 2009, SWB convened panel of 7 experts to recommend monitoring of CECs in California’s aquatic ecosystems

  Focus on fresh, brackish and marine waters receiving WWTP and stormwater discharge – ag or CAFO not addressed

  Two-year effort to develop CEC monitoring recommendations that can apply statewide

SECTION 1 - INTRODUCTION

- **1.1 Summary of Expert Panel Recommendations (2012)**

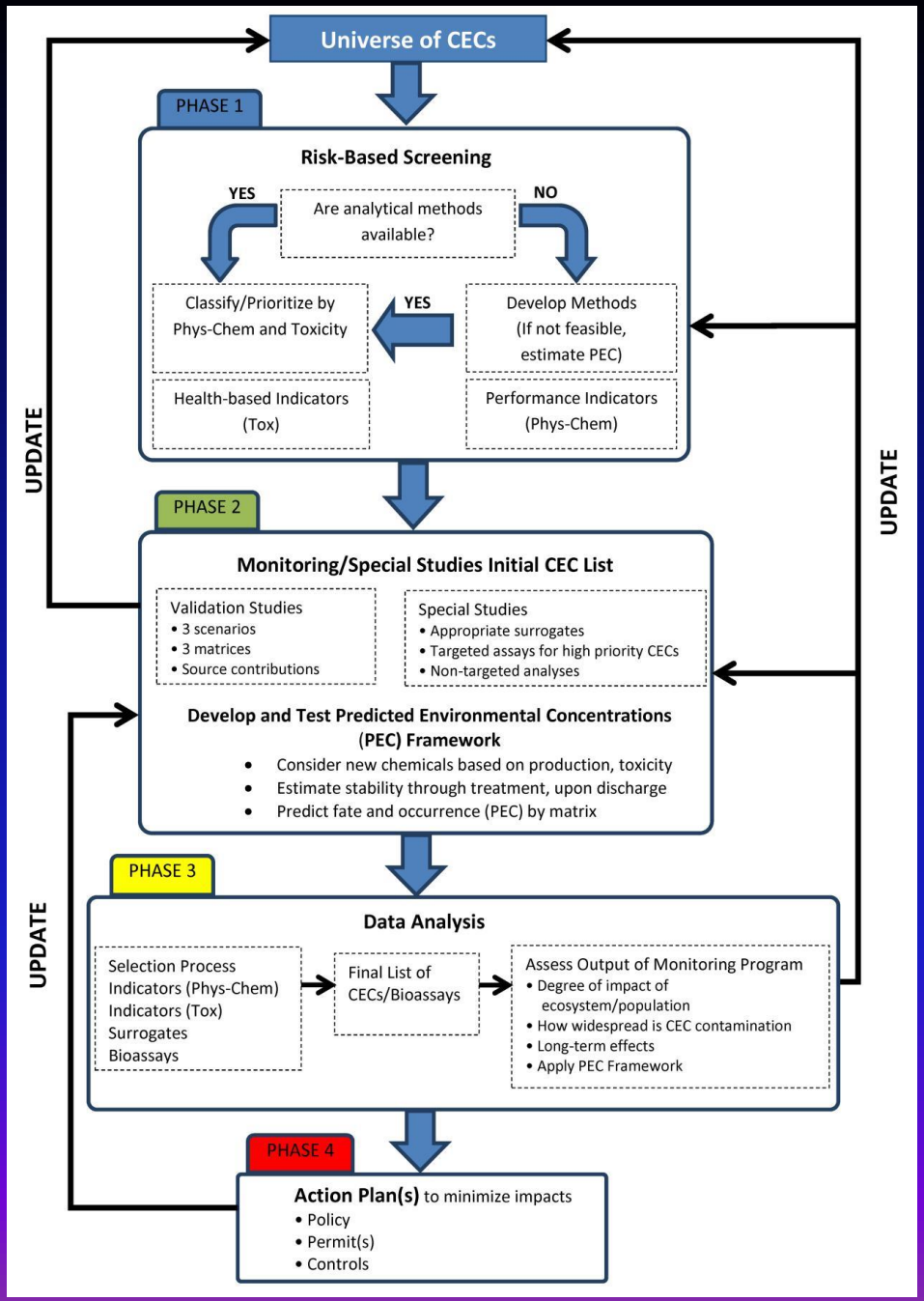
Utilize a transparent, risk-based framework for CECs with adequate data ("knowns") to identify those that should be monitored

Define scenarios where impacts of CECs are most likely to occur

1. effluent dominated rivers
2. coastal embayments
3. marine outfalls

Collect monitoring data for priority CECs (Ph. 2) and re-assess the risk posed (Ph.3) ("adaptive monitoring")

Develop and apply bioanalytical screening tools to address a wider range of CECs, including those lacking robust methods and "unknowns"


Universe of CECs
PHASE 1
Risk-Based Screening
YES
Are analytical methods available?
NO
Classify/Prioritize by Phys-Chem and Toxicity
YES
Develop Methods (If not feasible, estimate PEC)
Health-based Indicators (Tox)
Performance Indicators (Phys-Chem)
UPDATE
UPDATE
PHASE 2
Monitoring/Special Studies Initial CEC List
Validation Studies
• 3 scenarios
• 3 matrices
• Source contributions
Special Studies
• Appropriate surrogates
• Targeted assays for high priority CECs
• Non-targeted analyses
Develop and Test Predicted Environmental Concentrations (PEC) Framework
• Consider new chemicals based on production, toxicity
• Estimate stability through treatment, upon discharge
• Predict fate and occurrence (PEC) by matrix
UPDATE
PHASE 3
Data Analysis
Selection Process
Indicators (Phys-Chem)
Indicators (Tox)
Surrogates
Bioassays
Final List of CECs/Bioassays
Assess Output of Monitoring Program
• Degree of impact of ecosystem/population
• How widespread is CEC contamination
• Long-term effects
• Apply PEC Framework
PHASE 4
Action Plan(s) to minimize impacts
• Policy
• Permit(s)
• Controls

SECTION 1 – INTRODUCTION (cont.)

- **1.1.3 Initial List of CECs for targeted pilot monitoring**

16 total CECs representing hormones, pesticides, PPCPs, commercial and consumer chemicals were identified for initial monitoring

Listing of CECs was scenario and matrix dependent

Most CECs identified in aqueous phase, fewer in sediment, two in tissue

Due mostly to dilution, the number of CECs recommended:

Effluent dominated river > coastal embayment > marine outfall

Most CECs should be monitored in WWTP effluent and stormwater receiving waters to address relative source contribution

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

SECTION 1 – INTRODUCTION (cont.)

- **1.1.4 Special studies to improve monitoring/assessment**

  Bioanalytical screening assays – in vitro tests that integrate exposure to and response of chemicals by mode of action (MOA)

  Toxicity testing – develop tests that address endpoints associated with CECs in aquatic systems, e.g. reproductive impairment in fish

  Antibiotic resistance (ABR) – conduct pilot assessment of ABR in effluent, water and sediment

  Passive sampling devices (PSDs) – conduct pilot study on the effectiveness of PSDs to sample and concentrate CECs from water, sediment and/or tissue

SECTION 1 – INTRODUCTION (cont.)

- **1.2 Management questions addressed by pilot studies**

What is the impact (exposure) of CECs on aquatic resources statewide?

What is the occurrence of CECs near WWTP outfalls?
In stormwater impacted receiving waters?

What is the fate of CECs discharged by WWTPs?
In stormwater or urban runoff?

Are levels of CECs increasing or decreasing over time?

SECTION 1 – INTRODUCTION (cont.)

- **1.2 Management questions (cont.)**

What is the impact (effects) of CECs on aquatic resources statewide?

Can bioanalytical tools screen for the occurrence of CECs?

What is the effect of CECs on invertebrate health and fish reproduction ("in vivo" testing)?

What is the linkage between bioanalytical and in vivo test results?

SECTION 1 – INTRODUCTION (cont.)

- **1.2. Scope and Objectives**

Provide uniform guidelines and requirements for generation of CEC occurrence data statewide

1.2.1 Targeted Monitoring Requirements

List of appropriate monitoring questions/objectives
List of target CECs, candidate waterbodies and target media (including sentinel species for tissue monitoring)
Frequency, number and location of sampling stations
Data acceptability (QA/QC) goals and criteria
Data analysis, assessment and management plan
Coordination strategy with existing monitoring programs

SECTION 1 – INTRODUCTION (cont.)

- **1.2. Scope and Objectives (cont.)**

Provide uniform guidelines and requirements for generation of CEC effects data statewide

1.2.2 Special Studies Requirements

List of appropriate monitoring questions/objectives
List of target parameters (i.e. biological endpoints),
methods and measurement goals
List of candidate waterbodies and target media
(including candidate test species)
Frequency, number of location of sampling stations to be evaluated
Acceptability (QA/QC) goals
Rationale for exclusion of studies recommended by Panel (as needed)

SECTION 1 – INTRODUCTION (cont.)

- **1.3 Other CEC Monitoring & Special Studies in CA**

Statewide

- Recycled Water Policy (SWB/Dept Public Health)
- Bioaccumulation Oversight Group or “BOG” (SWB/SWAMP)
- Urban Pesticides “UP3” (Dept Pesticide Regulation)

Regional Studies

- San Francisco Bay Regional Monitoring Program (SFEI, Reg 2)
- Southern California Bight; Stormwater Monitoring Coalition (SCCWRP, Regs 4,8,9)
- Delta Regional Monitoring Program (new, Reg 5)

Local Studies

- Santa Ana Watershed Protection Agency
- Los Angeles Regional Board

SECTION 1 – INTRODUCTION (cont.)

- **1.3 Other CEC Monitoring & Special Studies**

1.3.1 Statewide

Recycled Water Policy (adopted 2012 by SWB/DPH)

Expert Panel convened to recommend CEC monitoring

Adopted risk-based framework; compiled occurrence/tox data

Targeted CEC monitoring and development of bioanalytical tools

Bioaccumulation Oversight Group (BOG)

monitoring of bioaccumulative substances statewide

focused on legacy organics and Hg in fish and shellfish

moving toward assessment of biotoxins

Surface Water Protection Program (DPR)

funds studies on occurrence, fate & effects of pesticides

maintains pesticide occurrence database

focused on freshwater systems

SECTION 1 – INTRODUCTION (cont.)

- **1.3 Other CEC Monitoring & Special Studies (cont.)**

1.3.2 Regional Studies

San Francisco Bay Regional Monitoring Program (RMP)

Investigating CECs since 2000; Working Group established 2006

Preventative monitoring to minimize CEC impacts in Bay

Supports bioeffects and linkage studies

Southern California “Bight” & Stormwater Monitoring Coalition (SMC)

Survey of coastal condition on a 5 y cycle since 1994

Bightwide special studies on CECs starting in 2003

SMC to consider bioanalytical screening in next 10 y cycle

Delta Regional Monitoring Program

design & coordination of local programs established in 2008

address questions of regional management interest

irrigated lands, MS4 and Sac River discharges of primary interest

SECTION 1 – INTRODUCTION (cont.)

- **1.3 Other CEC Monitoring & Special Studies**

1.3.3 Local Studies

Santa Ana Watershed Protection Agency (SAWPA)

effort initiated in 2009 to measure PPCPs in WWTP effluent

selection of target analytes based largely on public perception

results compared to therapeutic doses for humans (non-issue)

Los Angeles Regional Board

required CEC monitoring in regional WWTP effluent (ca. 2010)

supported special studies on CEC occurrence and fate in effluent dominated rivers

special studies designed to yield data for use by Panel in revisiting initial listing of CECs

Section 2

Targeted Monitoring

Outline

- Addition of fipronil to target list
- Scenario 1
- Scenario 2
- Scenario 3
- Stormwater
- Bight ‘13 Pilot Study
- Tissues
- Delta
- Data management and QA

Addition of Fipronil

Revised 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

Monitoring trigger quotients (MTQs) > 1 for fipronil by scenario and matrix.

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

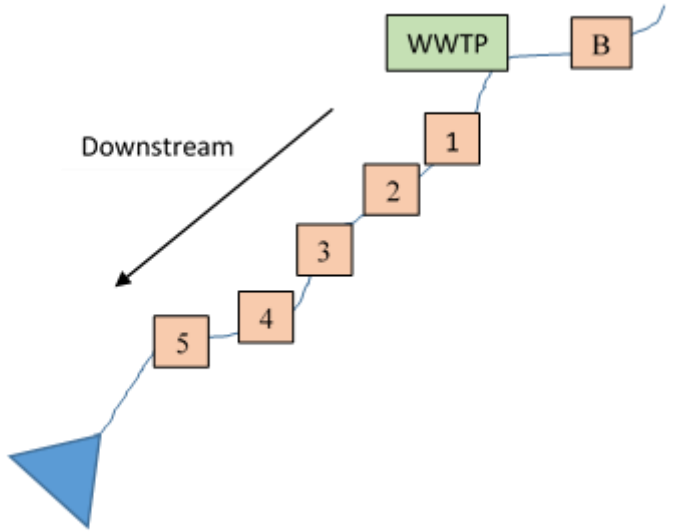
Scenario 1: Freshwater

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.

Scenario 1 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?

Scenario 1 Design



Parameter	Description
Matrix	River (receiving water)
Stations	6
Seasons	Wet and dry
Annual number of samples	12
Total years	3
Number of waterways	3
Total Samples	108

Parameter	Description
Matrix	POTW effluent
Stations	1
Seasons	Wet and dry
Annual number of samples	2
Total years	3
Number of waterways	3
Total Samples	18

Scenario 1 Design

- Analytes are as recommended by the Panel (+ Fipronil)
- 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.

Scenario 2: Embayment

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.

Scenario 2

- This scenario is based on San Francisco Bay.
- SFEI/RMP has been doing CEC monitoring.
- There are similarities and differences between the Panel recommendations and RMP activities.
- Panel decision: Is the RMP's approach compatible with Panel recommendations? If not, how would Panel modify/revise approach?

Panel Scenario 2 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?

Panel Scenario 2 Design

- Five POTWs monitored in in San Francisco Bay.
- 2-D grid of 7 stations at each POTW.
- Co-located sediment and aqueous samples at each station.
- Monitoring frequency for aqueous samples (POTW effluent and receiving waters) is semi-annual (wet and dry season) over 3 years, and sediment annually over 3 years.

Differences Between RMP and the Panel Recommendations

- RMP and the Panel use somewhat different approaches to prioritize targets.
  - RMP monitors contaminants of interest, then assesses risk.
- RMP has already collected CEC data which inform their decisions.
  - This excluded some targets/matrices from further analysis.
- RMP is working within a set budget, which is not yet considered in the Statewide planning process.

RMP CEC Targets

Scenario 2 sediment, water, and tissues

Parameter	RMP	Panel	RMP Justification
PBDE	sediment and tissues	sediment and tissues	
Pyrethroids	sediment	water and sediment	Hydrophobic; expect ND in water
PFOS	tissues	sediment and tissues	RMP may consider sediment based on results from other surveys
Fipronil	sediment	water	ND in pilot water study
17-beta estradiol, estrone, bisphenol A, galaxolide	single water sample as part of bioanalytical tools project	multiple water samples	No SF Bay data

RMP CEC Targets for Other Scenarios

Stormwater

Parameter	RMP	Panel
PBDE, pyrethroids	Measured	Measured
17-beta estradiol, estrone, bisphenol A, galaxolide, diclofenac, ibuprofen, triclosan, PFOS	Not measured (No SF Bay data)	Measured

Effluent

In contrast to the Panel recommendations, RMP does not have planned effluent monitoring activities, except for single measurements taken as part of the RMP bioanalytical tools project. A special study is planned.

RMP Status and Trends Design (excluding stormwater and effluent)

- **Water**: sampling every other year at random sites and historic sites. Don't necessarily run every test every year, or at every site.
- **Sediment**: sample every four years, alternating wet and dry season sampling. Mix of random and historical sites varying by season.
- **PBDE** tests on all ambient sediment samples collected.
- **Pyrethroids**: discontinue ambient sediment testing to focus instead on Bay margins.

Differences in Study Questions

1. Which CECs are detected in coastal embayment/estuarine water and sediments?
   - Addressed by RMP, but the targets have some differences
2. What are their concentrations and how quickly (i.e., at what distance) do the CECs attenuate once discharged?
   - Not addressed by RMP (random sampling design)
3. Can the CECs be shown to originate from the outfalls and/or stormwater, or are they present at background concentrations?
   - Not currently addressed by RMP. Special study on effluent being considered. (stormwater addressed elsewhere)
4. Is there a sub-annual change in discharged CECs?
   - Not addressed by RMP
5. Are the concentrations at co-located sediment and aqueous stations correlated?
   - Not addressed by RMP
6. Does the new occurrence data change the estimated MTQs?
   - Addressed by RMP for monitored targets

Panel Decision

- Are the RMP's differences acceptable?
- Are design changes recommended to address Panel concerns?
- Does SF Bay represent Scenario 2 statewide?

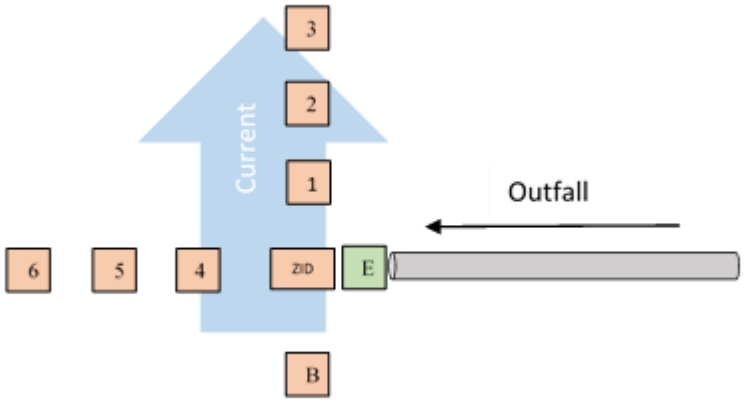
Scenario 3: Marine

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.

Panel Scenario 3 Study Questions

1. Which CECs are detected in 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 the MS4 study design.)

Scenario 3 Design



Parameter	Description
Matrix	Sediment
Stations	8
Annual number of samples	8 (sampling once per year)
Total years	3
Number of waterways	3
Total Samples	48

Parameter	Description
Matrix	POTW effluent
Stations	1
Annual number of samples	2 (sampling twice per year)
Total years	3
Number POTWs	2
Total Samples	12

Stormwater

Stormwater 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
    1. Between storm variability during the wet season
    2. In stream attenuation rate during low flow dry season

Stormwater: Wet Weather Design

- Annual loading is main goal.
- Flow weighted sampling at fixed mass emission stations for two storms per year per watershed.
- Minimum of three watersheds statewide assessed over a 3-year pilot study period.
- Sampling during and/or between storm events to address variability.
- Non-filtered, whole water analyzed when addressing loading.
- Filtered water samples may be adequate for effects/toxicity evaluation.

Stormwater: Dry Weather Design

- Short term maximum concentrations resulting in acute toxicity is the main concern.
- Target known or suspected incidental runoff sources (e.g. system that drains golf course)
- Depositional area sediments sampled at the start and end of the dry season
  - What has been washed in during the previous wet season?
  - Degree of attenuation occurring during the dry season?

Stormwater: Dry Weather Design

- Non-filtered aqueous samples should be sufficient for monitoring and assessment during dry weather
- 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.

Candidate Stormwater Systems

- San Francisco Bay: TBD

- Southern California: watersheds monitored by the Stormwater Monitoring Coalition (SMC)
  - San Diego County (San Diego River)
  - Orange County (San Diego Creek/Newport Bay)
  - Los Angeles County (Ballona Creek)
  - Ventura County (Santa Clara River)

Bight ’13 Special Study

Integrates Marine and Stormwater scenarios. Results inform the design of future monitoring.

Bight ‘13 Pilot Study

- A Bight ’13 Special Study was implemented to address Scenario 3 monitoring.
- **Aim 1.** Compare CEC sediment concentrations impacted by three sources:
  - Marine outfalls, storm water, and inland waste water.
- **Aim 2.** Verify CECs originate from the outfalls and are not simply at background concentrations.
  - Use grid design at outfall.
- APs, PFCs, pyrethroids/fipronil, and PBDEs 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.

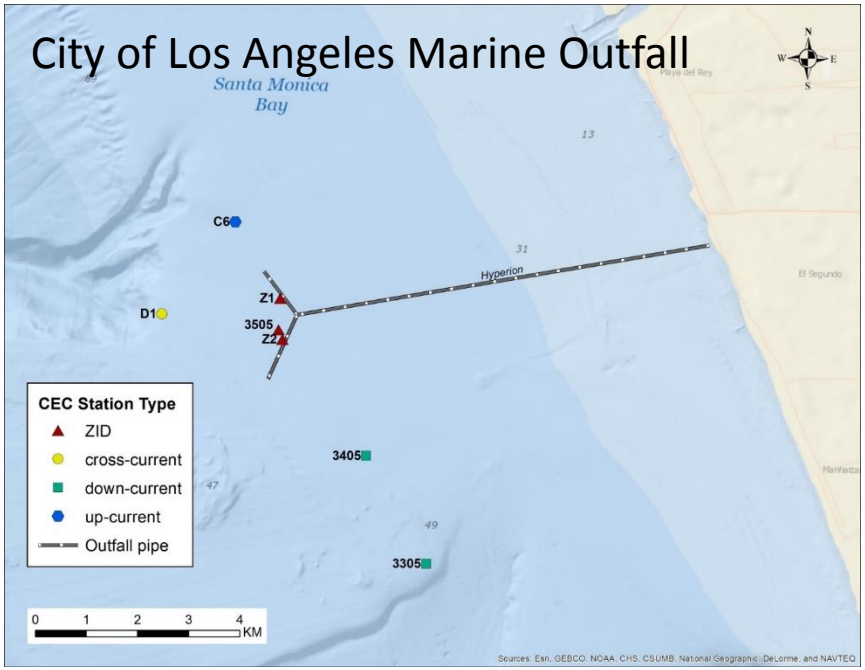
Bight ‘13 Pilot Study Design: Stormwater and Wastewater Receiving Stations

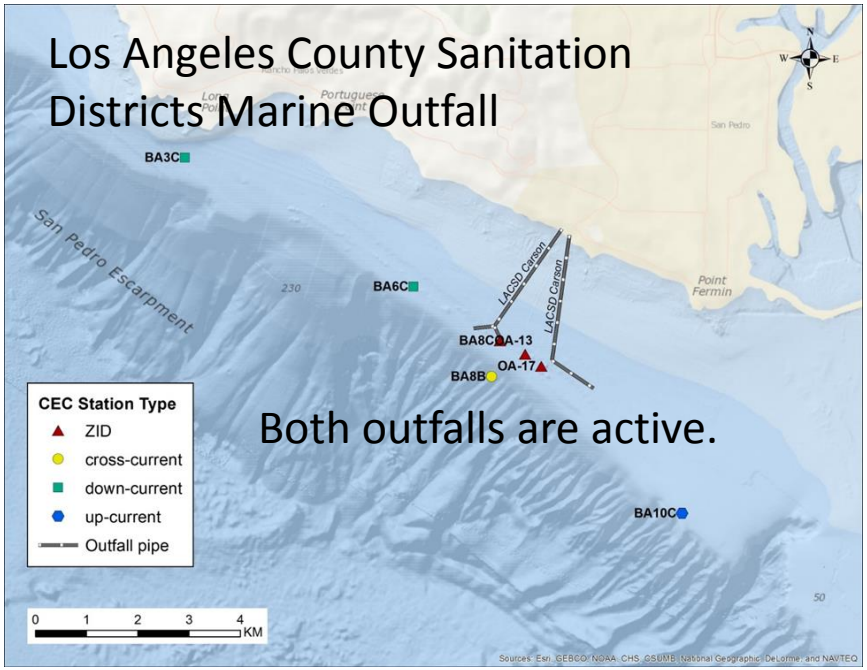
15 stations at River Mouths

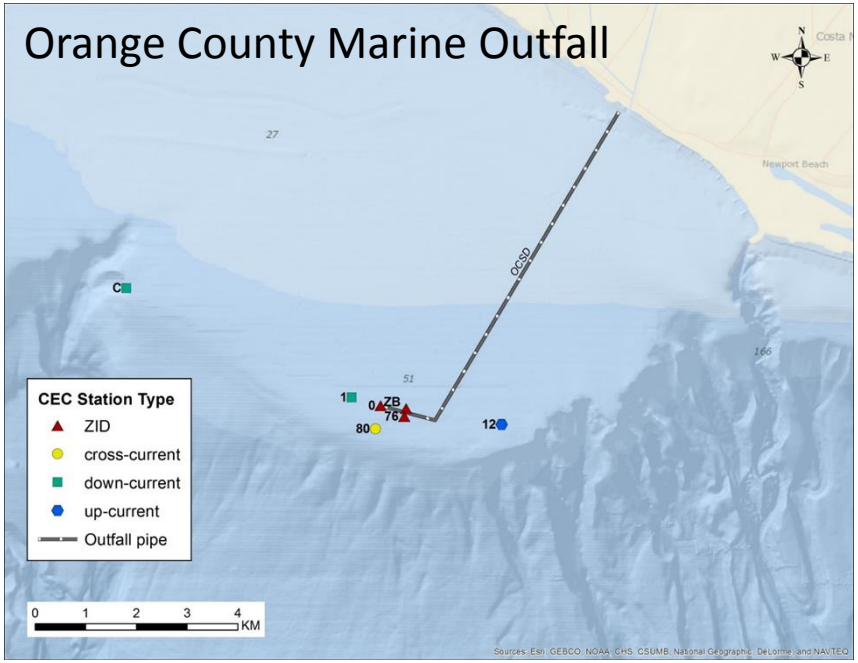
B '13 Station ID	Region	Source
8040	San Diego Bay	storm water
8077	San Diego Bay	storm water
8136	San Diego River	storm water
8163	Mission Bay	storm water
8169	Los Penasquitos Lagoon	storm water
8187	San Dieguito Lagoon	storm water
8189	San Elijo Lagoon	storm water
8202	Batiquitos Lagoon	storm water
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

Bight ‘13 Pilot Study Design: Outfalls

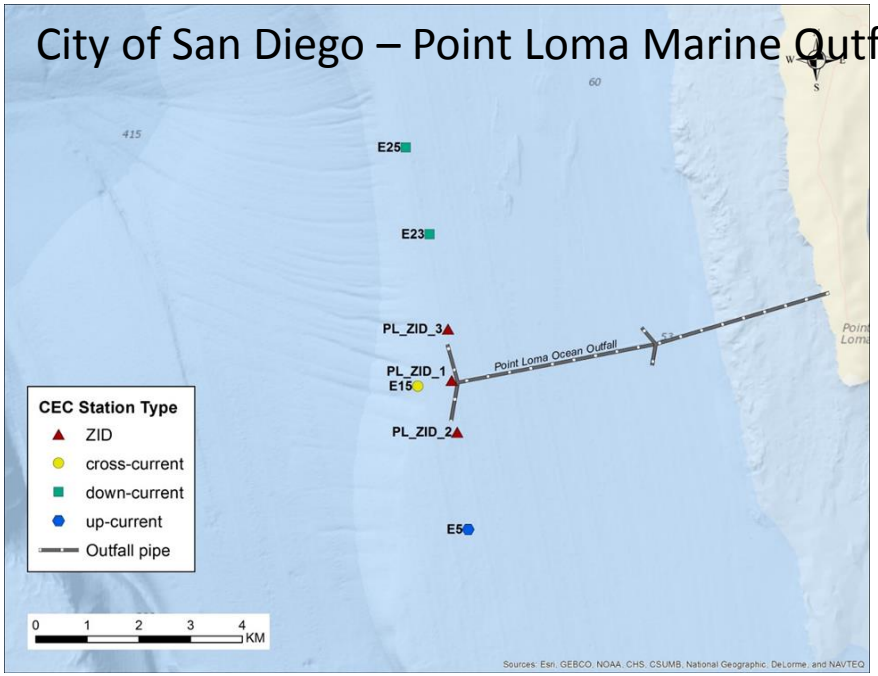
- We expanded the number of outfalls from 2 to 5.
- This required a reduction in the number of stations per outfall from 7 to 5.
- Increasing the number of outfalls provides more ZID stations for comparison to the river-mouth concentrations, and provides information on CEC occurrence at all major ocean outfalls in the region.
- Outfalls: 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.


City of Los Angeles Marine Outfall
Santa Monica Bay
C6
D1
Z1
3505
Z2
Hyperion
3405
3305
CEC Station Type
ZID
cross-current
down-current
up-current
Outfall pipe
0 1 2 3 4 KM


Los Angeles County Sanitation Districts Marine Outfall
BA3C
San Pedro Escarpment
BA6C
LACSD Carson
LACSD Carson
BA8C
OA-13
OA-17
BA8B
Point Fermin
BA10C
CEC Station Type
ZID
cross-current
down-current
up-current
Outfall pipe
0 1 2 3 4 KM
Both outfalls are active.

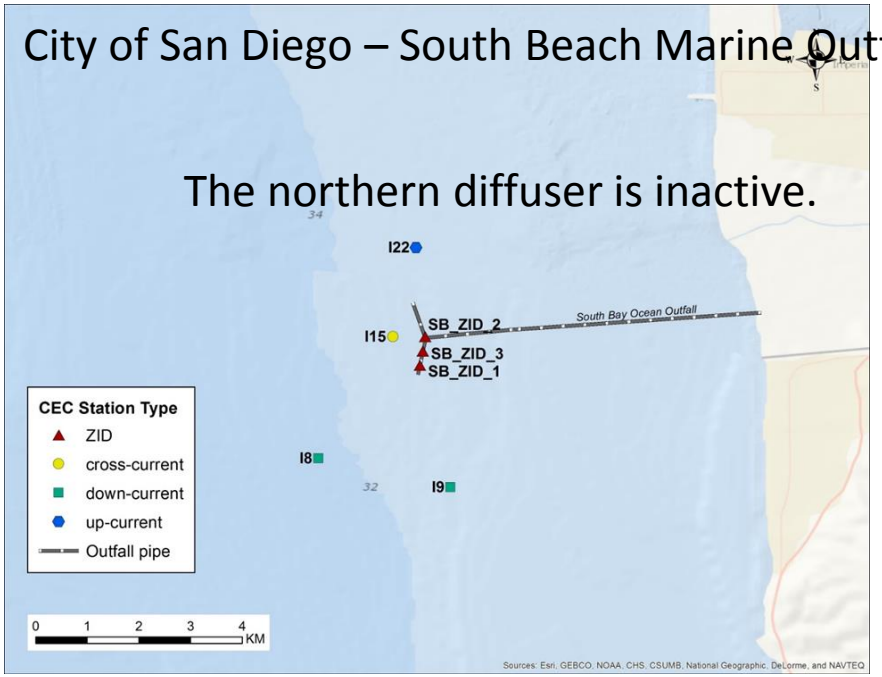

Orange County Marine Outfall
C
OCSD
1
0
ZB
76
80
12
CEC Station Type
ZID
cross-current
down-current
up-current
Outfall pipe
0 1 2 3 4 KM

City of San Diego – Point Loma Marine Outfall



City of San Diego – South Beach Marine Outfall

The northern diffuser is inactive.



Tissues

Tissue 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)?

Design – which trophic levels?

- Bivalves
  - Bottom of food web
  - Historical data exists
  - Known spatial accuracy
- Fish
  - Forage fish provide biomagnification data
  - Some spatial accuracy (smaller habitats)
  - Sportfish provide data for human risk
- Birds (eggs)
  - Top of food web
  - Possibly sensitive species (PFCs)
  - Investigate both freshwater and saltwater habitats; (partially) Scenario-specific habitats
- Marine Mammals
  - Pinnipeds and dolphins
  - Have the highest concentrations of biomagnifying contaminants
  - Integrate contaminants from multiple Scenarios.

Design – Species, Location, Frequency

- Selection of sentinel species
  - Known life history
  - Abundance and distribution
  - Availability of historical data
- Frequency
  - Depends on response time and life history/expectancy of sentinel
  - Bivalves, fish – annually or semi-annually
  - Birds, marine mammals – every 3-5 years
- Locations
  - Targeted vs. probabilistic vs. opportunistic
  - Historic/revisited (i.e. is time trend data available?)
- Coordination with existing programs
  - State and NOAA Mussel Watch
  - BOG (e.g. sportfish study)
  - San Francisco Bay RMP
  - Bight 13 bird egg study
  - Delta RMP

Delta

Addition of the SF Bay Delta as a Scenario

- Included at the request of the SWB
- Proposed Stormwater monitoring at Steelhead Creek, Morrison Creek, Hood (an integrator site), Arcade Creek, and the Natomas and American Rivers.
- What are the Panel's CEC concerns in the Delta?
- What would be a recommended study design for the Delta?

Data Management and QA

Two Situations

1. Data collected as part of an existing regional program
2. Data collected specifically for the CEC statewide monitoring pilot

- The data format can be the same for both (CEDEN), and is already used by many contract labs within California
- QA is either set by the regional program or by the statewide pilot
  - Labs likely use similar criteria, but this may require more coordination prior to field monitoring (e.g., RLs)

QA Criteria

Laboratory Quality Control	Measurement Quality Objective Basis
Reporting Level	½ the Panel recommended MTL
Instrument Calibration (initial and ongoing)	Variation in response factor, or r^2 value, or relative percent difference
Method Blank	Value less than a factor of the reporting level or detection limit
Sample duplicate	Relative percent difference
Reference Material	Percent difference from certified value
Matrix Spike and Duplicate	Recovery of spiked mass and relative percent difference between duplicates
Standard Recovery (surrogate and internal standards)	Percent recovery

Inter-Laboratory Comparisons

- Statewide CEC monitoring will likely involve several laboratories
- Analytical methods for CECs may not be as robust as for legacy contaminants
- Recommend that contract laboratories are required to pass an inter-calibration exercise prior to bidding
- For example, Bight uses this procedure for chemistry and toxicology

(E = coastal embayment; F = inland freshwater, O = ocean) and matrices of interest (i.e., aqueous, sediment, tissue). M = monitor; NA = not applicable.

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

Addition of Fipronil

- The updated monitoring trigger quotients (MTQs) exceeded unity for the aqueous phase in inland freshwater and coastal embayment scenarios
- MTQ exceeded unity for freshwater sediments, a matrix that was not previously 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.

STATEWIDE CEC PILOT MONITORING STUDY

SECTION 3- SPECIAL STUDIES

Alvina Mehinto

Technical Advisors Mid-Term Meeting
Friday, May 2nd 2014

SPECIAL STUDIES RECOMMENDED BY THE PANEL

- Bioanalytical screening assays
- In vivo toxicity assays
- Antibiotic resistance assays
- Passive sampling

DESIGN AND REQUIREMENTS

- List of target parameters, preferred methods and desired measurement goals
- List of candidate waterbody(ies) for each special study
- List of target media (e.g. water, sediment, tissue), and candidate target species
- Frequency, number and location of sampling stations to be evaluated within each candidate waterbody
- QA/QC goals for measurement of specific parameters
- Rationale for exclusion/inclusion of studies that differ from the Panel's final recommendations

BIOANALYTICAL SCREENING

- High throughput methods
- Screen a large number of chemicals based on their mode of action
- Assess the ability of CECs to activate cellular receptors
- Use by EPA for chemical registration (ToxCast™)
- Pilot studies needed to evaluate potential for use to screen environmental samples (water, sediment, tissues)

SELECTION OF IN VITRO CELL ASSAYS

Endpoint	Response	Mode of Action	Potential Adverse Outcome
Estrogen Receptor Alpha (ERa)	Activation and suppression	Estrogen signaling	Impaired reproduction, feminization of males, cancer
Androgen Receptor (AR)	Activation and suppression	Male sexual phenotype	Androgen insensitivity, impaired reproduction, masculinization of females
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 (Umu or p53)	Activation	Genotoxicity	Cancer
Cytotoxicity	-	General cell toxicity	Tissue damage, death

RATIONALE FOR INCLUSION/EXCLUSION

Biological response monitored is specified

- Transactivation and inhibition assays for ER and AR
- Some environmental chemicals are known to suppress cell receptor activity
- Linkage exist between suppression of receptor activity and physiological/phenotypic endpoints

Exclusion of peroxisome proliferator activated receptor gamma (PPARg)

- Commercially available assays are not sensitive (i.e. effect conc. higher than environmental conc.)
- Tests with GeneBLAzer assay were not able to screen for gemfibrozil

Inclusion of xenobiotic metabolism endpoints

- Aryl hydrocarbon receptor (AhR): indicative of CYP1A metabolism, activation by PCBs and PBDEs
- Pregnane X receptor (PXR): indicative of CYP3A metabolism

DESIGN REQUIREMENTS AND OUTPUT PARAMETERS

	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) (Product of 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 in ng/L)	

ASSAY-SPECIFIC STUDY PARAMETERS

Endpoint	Estrogen receptor alpha (ERa)	Androgen receptor (AR)	Progesterone receptor (PR)	Glucocorticoid receptor (GR)
Reference toxicant	17beta estradiol	R1881	levonorgestrel	dexamethasone
REF	5 to 20 X	20 to 50 X	20 to 50 X	20 to 50 X

Endpoint	Aryl hydrocarbon receptor (AhR)	Pregnane X receptor (PXR)	Genotox endpoint
Reference toxicant	PCB 126	N/A	TBD
REF	20 to 50 X	5 to 20 X	TBD

QA/QC CRITERIA

QA/QC criteria	Description
Blank response	Response in media only wells should be less than 10% (?) of sample response
Solvent effect	Cytotoxic effects of DMSO (positive control) must be within 15% of the standard deviation of the negative control (cells only)
Background adjustment	Negative and positive control samples should be less than 15% (?) of sample response
Dose response fitting curve	Response of the reference toxicant run on replicate plates should be within 10% (?) of the standard deviation of the calibration curve
Extract toxicity	Sample extracts should not cause more than 20% cell mortality (i.e. $\geq$ 80% survival) compared to the positive control

SEQUENCE OF ENDPOINTS

1. Cytotoxicity Assay

    Test 2 most concentrated dilutions of extracts

    If toxic, adjust dilution

    Otherwise proceed to in vitro testing

2. In vitro bioassay with 4 non toxic extracts (in DMSO)

STUDY 1 – BIOSCREENING OF TARGETED CECS

Questions addressed:

1. Which priority CECs identified by the Panel 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)?
3. What are the effects (additive or antagonist) of priority CECs mixtures using the selected cell assays?

STUDY 1- DESIGN

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.

STUDY 2- BIOSCREENING OF ENVIRONMENTAL SAMPLES

Questions addressed:

1. What is the response of environmental aqueous samples using selected cell assays?

2. How do cell assay responses correlate with targeted chemistry data?

STUDY 2- DESIGN

- Sampling location selected based on study design for targeted chemistry
- this pilot study will explore the linkage between in vitro responses and identification of contaminants by targeted monitoring

	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)

IN VIVO TOXICITY TESTING

- Evaluate effects of CECs on key biological processes and predict adverse outcomes at organismal or population level
- Endpoints of interest: development, growth, reproduction, behavior
- The Panel recommended to conduct toxicity assays for all 4 scenarios (freshwater, estuaries, marine and stormwater)
- Existing EPA or OECD validated assays will be used whenever possible
- Need to optimize and validate the assays for some of the scenarios

FRESHWATER TOXICITY TESTING

- 21-day recrudescence fathead minnow assay
- Validated by EPA and OECD for environmental samples testing
- Used in Tier I of EPA Endocrine Disruptor Screening Program
- Applicable for Scenario 1 (FW), Scenario 4 (MS4) and WWTPs effluents discharging in estuaries and oceans
- Multiple lines of evidence - phenotypic, physiological and molecular endpoints
- Potential for linkage study

QA/QC CRITERIA

Water control and solvent control:

- 90% survival
- 1 spawning event every 2-4 days per replicate aquarium
- 15 eggs/female/day/replicate
- 95% fertility

We recommend using a positive control for the pilot study

- Potent estrogen – conc. should cause significant induction of vitellogenin in males
- Potent androgen – conc. should cause significant changes in female sex characteristics

FHM TOXICITY ASSAY FOR MODEL COMPOUNDS

Study designed in collaboration with LACSD

Question 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 and behavior
3. How reliable and reproducible is the fathead minnow test?

FHM STUDY DESIGN FOR MODEL COMPOUNDS

	ER DISRUPTION	AR DISRUPTION
Test solutions	Water control, vehicle control 17-beta estradiol and antagonist TBD	Water control, vehicle control trenbolone and flutamide
Bioscreening	GeneBLAzer ER transactivation/inhibition	GeneBLAzer AR transactivation/inhibition
Chemistry	Solid phase extraction and quantification by LC-MS	
Endpoints	% survival and changes in behavior relative to controls No. eggs laid and fertilized Gonadosomatic index, histopathology Levels of plasma steroids relative to controls Molecular analyses - qPCR (e.g. vtg, CYP19, ER and AR) and microarrays	

FHM STUDY FOR TOXICITY OF EFFLUENTS AND FW ENVIRONMENTS

Questions 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?

Samples selected based on study design for targeted chemistry and bioanalytical screening

STUDY DESIGN FOR FHM STUDY WITH ENVIRONMENTAL SAMPLES

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	

RESEARCH NEEDS

Optimization and validation studies are needed for :

- aqueous toxicity testing of brackish and marine water environments
- Sediment toxicity testing

RESEARCH NEED- ESTUARINE/BRACKISH FISH MODEL

	Sheepshead minnow Cyprinodon variegatus	Atlantic killifish Fundulus heteroclitus	Threespine Stickleback Gasterosteus aculaetus	Inland silverside Menidia beryllina
Location	Atlantic coast of USA, Gulf of Mexico	Atlantic coast of USA (CA species exists)	Found in California	Found in California
Validation	EPA	No	considered by OECD	EPA
Fish stage	Reproductive adults	Reproductive adults	Reproductive adults	10-day old larvae
Test duration	180 days	15 days	21 days	7 days
MOA targeted	Estrogenicity	Estrogenicity Anti-estrogenicity	Estrogenicity Anti-androgenicity	General toxicity
Endpoints	Fecundity, fertility, GSI Plasma sex steroids and vitellogenin Hatching success Larval morphology and development	Plasma sex steroid Vitellogenin GSI Egg production	Vitellogenin Spiggin levels Histopatology	Growth (biomass) Survival
References	Raimondo et al. 2009	MacLatchy et al. 2009	Bjorkblom et al. 2009, Katsiadaki 2009	EPA report (section 13, method 1006.0)

VALIDATION OF ESTUARINE/MARINE FISH MODEL

Proposed study questions:

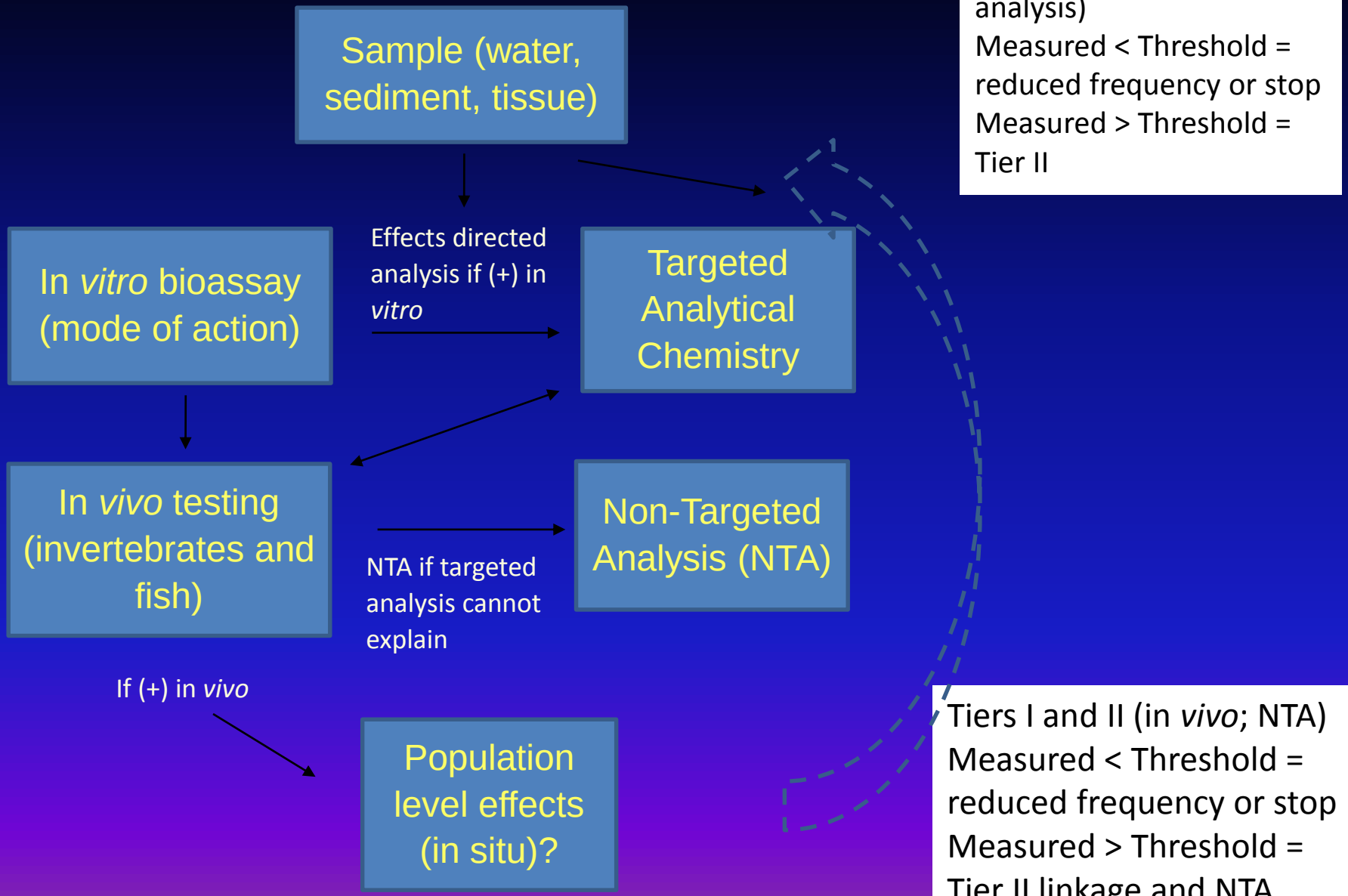
1. How does the fish species selected respond to exposure to model compounds?
2. How do changes in salinity affect toxicity responses?
3. Which apical and molecular endpoints are the most responsive to exposure to model toxicants (strong ER and AR agonists)?
4. How robust is the assay compared to the fathead minnow assay?

SECTION 4 – IMPLEMENTATION

4.1 Integrating targeted monitoring & special study results

- **Tier 1 (most frequent, more stations)**
  - Bioanalytical tools can screen (i.e. with high sensitivity) for some but not all potential harmful chemicals
  - Targeted chemical analysis is needed to screen for known toxicants (i.e. those with MTQ > 1) not addressed by bioscreening endpoints (e.g. pesticide toxicity)

- **Tier II (less frequent; fewer stations)**
  - In vivo toxicity testing (e.g. fish reproduction assay) that captures whole organism response
  - Non-targeted chemical analysis to assist TIE and identify “new” contaminants


Sample (water, sediment, tissue)
In *vitro* bioassay (mode of action)
Effects directed analysis if (+) in *vitro*
Targeted Analytical Chemistry
In *vivo* testing (invertebrates and fish)
NTA if targeted analysis cannot explain
Non-Targeted Analysis (NTA)
If (+) in *vivo*
Population level effects (in situ)?
Tier I (in *vitro*; targeted analysis)
Measured < Threshold = reduced frequency or stop
Measured > Threshold = Tier II
Tiers I and II (in *vivo*; NTA)
Measured < Threshold = reduced frequency or stop
Measured > Threshold = Tier II linkage and NTA

SECTION 4 – IMPLEMENTATION (cont.)

4.2 Coordination with existing programs

- Work toward compatible designs for water, sediment, tissue, effluent
- Establish sampling stations that provide adequate spatial coverage
- Coordinate sampling schedules to address principal questions of management concern
- Harmonize data collection requirements
  - Data quality objectives, e.g. reporting limits (RLs), precision
  - Data formatting (CEDEN) and reporting

SECTION 5 – RESEARCH NEEDS

- **Antibiotic Resistance**
  - Knowledge on environmental occurrence and consequences remains scarce
  - Recommend convening panel of experts on ABR to collate and synthesize state of the science
- **Non-targeted Analysis**
  - GC for persistent, bioaccumulative “unknowns”
  - LC for water soluble, transient “unknowns” (metabolites, intermediates)
- **Passive sampling**
  - Develop devices that can sample target CECs at environmentally relevant concentrations (i.e. 50% of MTL)
  - Assess dosing capability for bioanalytical tools and non-targeted analysis, replacing extraction of large volume water samples

SECTION 5 – RESEARCH NEEDS (cont.)

- **Development and validation of a broader suite of bioanalytical tools**
  - Non endocrine endpoints and toxicity pathways (e.g. genotoxicity)
  - Those that can incorporate metabolic activation
  - Interlaboratory comparison of mature bioassay endpoints
- **Development and validation of in vivo test protocols**
  - Saltwater fish (Menidia spp.)
  - Evaluation of robustness and repeatability
- **Linkage between in vitro and in vivo response**
  - Integrated studies measuring both elements to address predictive capability of bioanalytical tools in screening mode (i.e. (+) in vitro--→ (-/+) in vivo is OK; (-)in vitro -→ (+) in vivo is not OK