Pilot Study on CEC Monitoring in Aquatic Ecosystems
Contract No. 12-134-250
Summary Minutes from the Stakeholder Advisory Committee Mid-term Meeting

Meeting Date: Friday, May 9, 2014
Location: Southern California Coastal Water Research Project Authority, Costa Mesa, CA
Facilitators: Keith Maruya, Nathan Dodder, Alvina Mehinto
Attendees: Rich Breuer, Phil Friess, Rich Gossett, Ken Landau, Tom Mumley, Deb Smith, Geoff Brosseau (by phone), Jay Davis (by phone), Karen Larsen, Rebecca Sutton
Not in Attendance: Sara Aminzadeh

Section 1.2 Introduction/Questions

(from Brosseau). Questions not stated as management questions.
Action: Change to "pilot study" questions.

(from Smith). Urban (dry weather) runoff applies to occurrence as well as fate
Action: Modify as suggested

(from Friess). Do bioanalytical tools screen for occurrence and potential for effects?
Action: yes, will modify.

(from Davis). Is question "How do concentrations compare to effects thresholds" relevant?
Action: yes, most definitely per the Panel's risk based framework. Will add.

Section 2. Targeted Monitoring Requirements

(from Mumley). Make sure pilot study designs for all scenarios are adaptable.
Action: agree

(from Friess). Scenario 1 Study Questions 2 and 4 are redundant and should not single out POTWs as only source.
Action: Rephrase Q2 to focus on characterizing CECs upstream/away from obvious point source contributions (e.g. POTW discharge).

(from Breuer). Effluent dominated systems (Scenario 1) in the Delta and Central Valley regions should be considered and added as candidates.
Action: agree and will identify appropriate systems with SWB and Delta RMP. WWTPs will be selected based on the level of treatment if differences exist (i.e. secondary vs. tertiary).

(from Mumley). Data for SF Bay (model for Scenario 2) from RMP Status & Trends, not only RMP.
Action: will modify as requested

(unknown). No. of waterways for Scenario 3 (n=3) does not match POTW effluent (n=2).
Action: Change no. waterways to be consistent with effluent (n=2). (Note these are minimum recommended requirements.)

(from Friess, Smith). MS4 Candidate Watersheds. Santa Clara river inputs/hydrology maybe too complex to understand CEC input/fate.
Action: will consider in final selection of watersheds.

(from Group). Concern over inclusion of marine mammals due to lack of credible thresholds.
Action: will take under advisement, although thresholds for bird (eggs) exist, as do thresholds for other contaminants (e.g. PCBs).

(from Landau). Delta and Central Valley are not separate scenarios.
Action: Candidate waterways influenced by CECs in the Delta and Central Valley regions will be identified and added to the existing scenarios (including MS4).

(from Group). QA/QC criteria for RL, set at 1/2 * MTL should be listed as recommended, not prescribed.
Action: RLs should be set to allow for rigorous comparison to existing MTLs, and should be harmonized across different regional and statewide programs/studies.

(from Friess). Because pesticides are regulated and monitored by other agencies (e.g. Dept. of Pesticide Regulation (DPR) and USEPA), CASA asserts that they should be excluded from the statewide CEC pilot study. Deb Smith (LARB) pointed out that protection of beneficial uses from all chemical stressors, including pesticides, is within the jurisdiction of the State/Regional Water Boards, and that she supports their inclusion. SCCWRP added that spatial/temporal coverage and data quality requirements for pilot monitoring of pesticides, regardless of jurisdiction, should be consistent with the CEC Expert Panel's recommendations.
Action: SCCWRP, SWB staff and stakeholders to work collaboratively to identify overlap in monitoring plans and requirements for pesticides. SCCWRP will make a recommendation based on this investigation.

Section 3. Special Study Design Requirements

(from Mumley and Larsen). Question was asked how the different tests fit together?
Action: *The different special study components (i.e. toxicity tests and endpoints) are complementary and are envisioned to be implemented in a tiered, sequenced fashion as explained in section 4.1 (Implementation) and illustrated in Fig. 4.1-1.*

(from Friess). Linkage is important and should be emphasized.

Section 4. Implementation

None recorded

Section 5. Research needs

None recorded

Pilot Study on CEC Monitoring in Aquatic Ecosystems
Contract No. 12-134-250
Summary Minutes from the Technical Advisory Committee (Panel) Mid-term Meeting

Meeting Date: Friday, May 2, 2014
Location: Southern California Coastal Water Research Project Authority, Costa Mesa, CA
Facilitators: Keith Maruya, Nathan Dodder, Alvina Mehinto
Attendees: Paul Anderson, Nancy Denslow, Adam Olivieri, Dan Schlenk, Geoff Scott, Shane Snyder, Dawit Tadesse, Jorg Drewes (by phone)

Section 1. Introduction

No comments

Section 2. Targeted Monitoring Requirements

1. (from Anderson). Need to investigate NOECs/LOECs for fipronil; consider endpoint selection, organism sensitivity and fipronil speciation and dosage. In particular, verify above criteria and parameters for 0.9 ng/g freshwater sediment NOEC reported by Maul et al (2008).
Action: compile available toxicity data for fipronil in freshwater and marine systems

2. (from Drewes). Consider "off ramp" in framework if target CEC is banned or use is restricted.
Action: add to decision framework

3. (from Olivieri, Tadesse). In Scenario 1 design, distinguish between Region 2 and others (i.e. Region 5). Investigate possibility of effluent dominated rivers in Region 5 (e.g. Vacaville). For Reg. 2, effluent dominated systems are restricted by policy.
Action: Identify monitoring scenarios and candidate watersheds in the CV/Delta region

4. (from Panel, Schlenk). Is data on aqueous pyrethroids available for San Francisco Bay? If not detected (ND), what is the reporting or method detection limit associated with these data? If ND in the ug/L range, there still could be enough pyrethroids to cause toxicity.
Action: Request and document MDLs from RMP/Reg 2.

5. (from Panel). How are sediment samples to be collected – as grab or composite? The Panel recommends compositing multiple grabs ($n \geq 3$) from one station and homogenizing into a composite.
Action: specify compositing for sediment sampling.

6. (from Panel). Can RMP provide data to clarify disagreement on PPCPs in Bay water? For fipronil and pyrethroids in sediment and water? If not, Panel suggests exploring ways to include the above in pilot monitoring.

7. (from Panel). Please provide a list of questions RMP is not addressing for prioritization
Answer: Gradient study for WWTP outfalls. Sediment gap for shallow water dischargers.

8. (from Panel). Write into procedure to mine current data to prioritize study questions.
Action: add to document as requested

9 (from Panel). For Scenario 3, define multiple (n=3-5) reference sediment locations.
Action: SCCWRP to identify possible reference locations (e.g. Dana Pt.)

10. (from Drewes). For MS4 design, estimating accurate loadings may not be achievable with limited sampling campaign due to annual, intra-storm variability. Suggest answering simple occurrence question first (are target CECs present?). If present, graduate to loading questions, and design pilot studies to populate loading model.
Action: sequence and prioritize MS4 studies as suggested

11. (from Panel, Schlenk). More detail needed on tissue bioaccumulation design. Consider worms to link sediment CECs with water column food web (e.g. via BSAF).
Action: SCCWRP to flush out detail to include additional sentinels based on habitat.

12. (from Tadesse). Consider adding POTW sites for Delta. Consider aquatic life criteria and pesticide runoff as primary issues. Flush out design with Delta RMP representative (K. Landau).
Action: Identify monitoring scenarios and candidate watersheds in the CV/Delta region (see also #3)

13. (from Snyder). Add field blanks to QA/QC
Action: agree, will add

Section 3. Special Study Design Requirements

1. (from Denslow, Schlenk). Remove PXR from in vitro bioassay list.
Action: agreed, will remove.

2. (from Denslow, Schlenk). Add pros/cons to candidate estuarine fish model table. Contact Brian Anderson & Doug Middaugh concerning applicability of topsmelt reproductive assay.
Action: agreed, will add and consider topsmelt if endpoints/assay are found to be relevant/viable.

3. (from Denslow, Schlenk). Add invertebrate to model species needed for in vivo testing.
Action: Will add if viable candidate can be identified. G. Scott to query patent holders of amphipod life cycle test organism (Amphiascus).

Section 4. Implementation

1. (from Group). Clarify Tier 1 bioanalytical tools are *in vitro*
Action: agree, will clarify

2. (from Panel, Snyder). Add narrative on role and frequency for non-targeted analysis (NTA). Add GC-ICP-MS to list of techniques.
Action: agreed, will add.

3. (from Olivieri, Anderson). Add/develop the on-ramp from Panel's final report.
Action: agreed, will add.

4. (from Panel). How will State pay for implementation of pilot study?
Answer: SCCWRP is charged to design pilot study to address Panel recommendations irrespective of cost; however, the charge includes identifying leveraging opportunities for implementation.

Section 5. Research needs

1. (from Scott). There are NSF funded projects on antibiotic resistance (ABR) administered by UNC Chapel Hill and workshops planned at Univ. Arizona.
No action required

2. (from Snyder). Technology development for real time biosensing will ultimately eliminate sample extraction/concentration steps.
Action: agreed, will add.

Pilot Study on CEC Monitoring in Aquatic Ecosytems

Mid-term Review
Stakeholder Advisory Committee

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)**

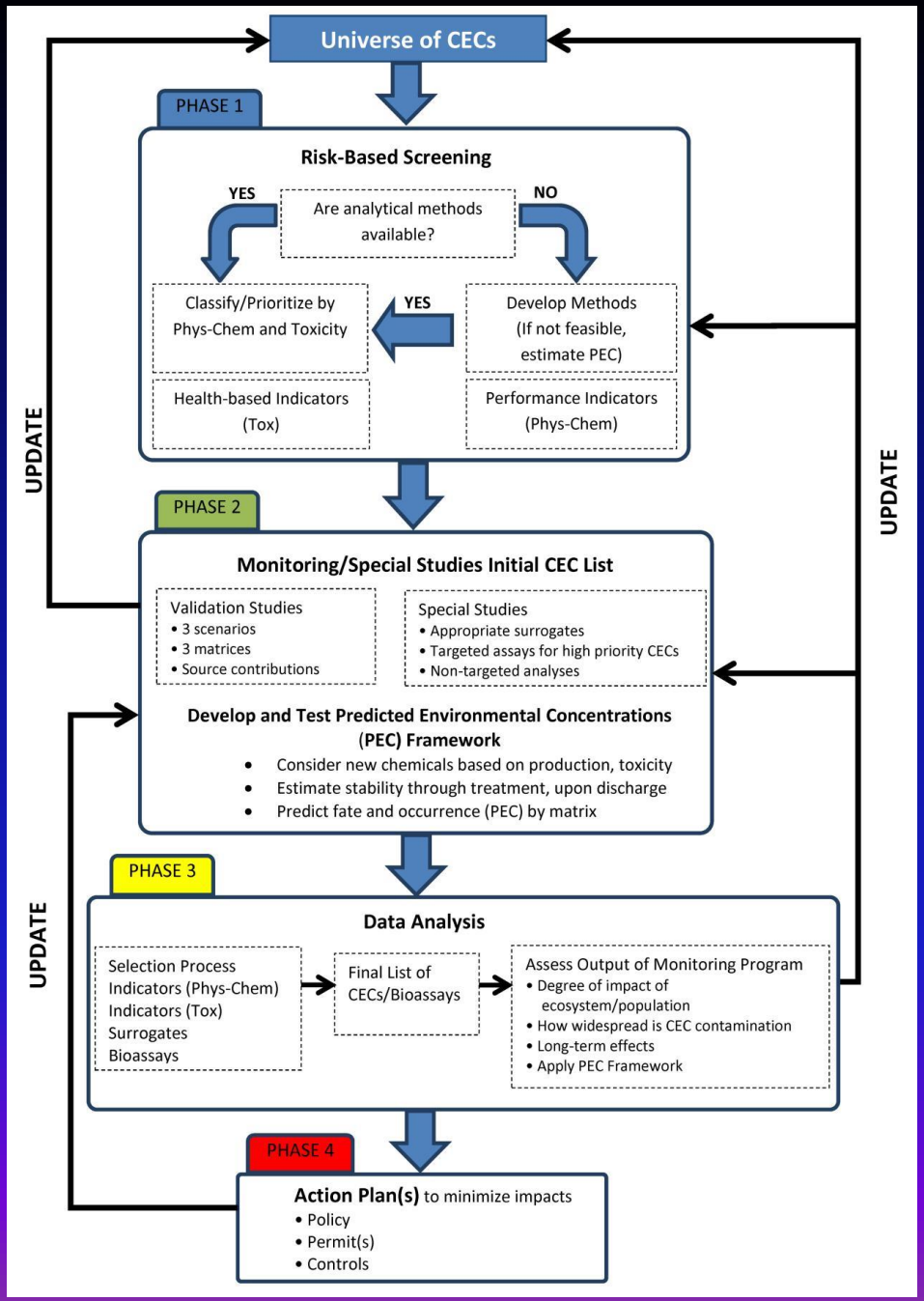
Utilize a transparent, risk-based framework for CECs with adequate data ("knowns") to identify those that should be monitored

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

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)
- Surface Water and Bioaccumulation Monitoring (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

Nathan Dodder

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

THRESHOLDS FOR FIPRONIL

Panel position: NOECs/LOECs for fipronil do not exhibit consistent or predictable trends

SCCWRP Response or Action:

- Compile published NOECs/LOECs for fipronil
- Compare species, endpoints and toxicant speciation (i.e. are metabolites tested, controlled for?) in published studies
- Synthesize information to confirm the validity of existing NOECs/LOECs on which MTLs are based

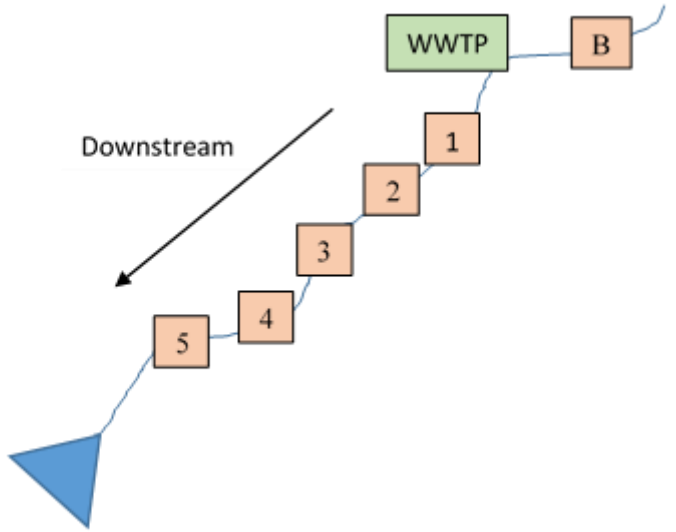
Scenario 1: Freshwater

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

- Includes all Panel recommended analytes + fipronil
- Add sediment matrix (for fipronil)
- Ideal waterways for pilot study have well-characterized source and flow inputs
- The Los Angeles and Santa Clara Rivers are excellent candidates in southern California
- No ED waterways permitted in SF Bay watershed
  - Effluent dominated waterways in the Delta, Central Valley or other regions can be considered, depending on regional regulations.

Scenario 2: Embayment

Coastal embayments that receive CEC inputs at the land-ocean interface, which may originate from WWTP discharge (directly or within watershed) and stormwater/urban runoff

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

- Utilizes San Francisco Bay as model embayment
- Five WWTPs discharging to Bay monitored
- 2-D grid of 7 stations for each WWTP
- Sediment and aqueous samples collected at each station
- Monitoring frequency (3 yr pilot study)
  - Semi-annually for aqueous (WWTP effluent & Bay water)
  - annually for sediment

RMP vs. Panel Recommendations

- RMP and Panel agree on risk-based prioritization framework
- RMP focuses on forward-looking CEC assessment, Panel selects based on existing data (occurrence and toxicity).
- RMP has collected CEC data since 2000 to inform regional priorities
- RMP prioritizes funding for CEC data collection in a landscape where several water quality issues are in need of attention

RMP vs Panel Target CEC List

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, fipronil	Measured	Measured
17-beta estradiol, estrone, bisphenol A, galaxolide, diclofenac, ibuprofen, triclosan, PFOS	Not measured (No SF Bay data)	Measured

Effluent

Special study proposed for 2014-15. Target analytes are not synchronized at this time.

RMP Status and Trends Design

- **Receiving (Bay) Water**: bi-annual sampling at random and historic sites. Don't necessarily run every test every year, or at every site.

- **Sediment**: every 4 yrs, alternating wet/dry season. Mix of random & historical sites varying by season.
  - PBDEs analyzed on all samples

- **WWTP Effluent: special study being considered**

TARGET CEC LIST

- Panel position: *data is needed to address occurrence and source comparison questions for all recommended CECs*

SCCWRP Response and Action:

- Agree with Panel
- Investigate stormwater database for target CECs
- If data exists, work with stakeholders to provide to Panel for assessment
- If data does not exist (or is too scarce), ask stakeholders to explore options for filling data gaps through existing program(s) (e.g. RMP, MS4)

TARGET MATRICES

- Panel position: *data on aqueous, sediment and WWTP effluent needed to address exposure, effects testing, loading and source comparison. (effects can occur near "ND")*

SCCWRP Response and Action:

- For aqueous, unfiltered water should be targeted
- If warranted, particulate vs. dissolved fractions can be specified (e.g. for some toxicity endpoints)
- For sediment, are shallow water discharges impacting sediments?
- Request harmonization of target CEC list for WWTP effluent special study

IS SF BAY REPRESENTATIVE OF EMBAYMENTS STATEWIDE?

- Panel position: if recommendations on SF Bay are not translatable to other CA embayments, additional case studies should be identified for pilot evaluation

SCCWRP Response and Action:

- San Diego Bay represents a contrast with SF Bay (no/little direct WWTP influence)
- Embayments representing a gradient of urbanization (e.g. Newport, Morro, Tomales, Humboldt/Arcata Bays)
- Query stakeholders on inclusion of more case studies

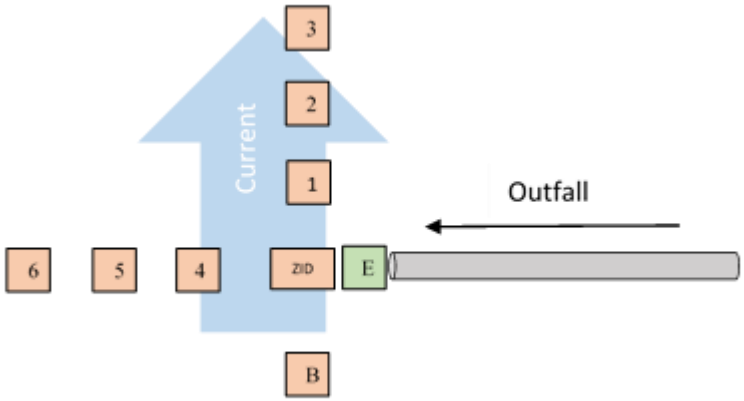
Scenario 3: Marine

Effluent from large (> 100 mgd design capacity) WWTPs discharged via outfalls on the continental shelf (50-100 m depths). Discharged CECs are diluted by ambient seawater, transformed into breakdown products and/or are transported by prevailing currents. This scenario is monitored at five marine outfalls in 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 do these levels change with distance from the outfall?
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

SEDIMENT REFERENCE CONDITION

Panel position: (1) Because currents are variable, near outfall stations may not define no/low impact. (2) Variable or patchy sediment characteristics prohibits direct comparison of CEC concentrations on a dry or wt weight basis.

SCCWRP Response or Action:

- Agree with both (1) and (2)
- (1) Recommend sampling and analysis of target CECs at historic reference stations (e.g. WWTP far field sites and/or regional reference stations)
- (2) Recommend co-measurement of organic carbon (TOC) and grain size on all collected sediment samples.

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 Scenario 3 design)
4. What are the spatial and temporal variabilities 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

- Estimate of annual loading is the 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.
- Unfiltered samples to be analyzed for loading
- Filtered samples may be needed for toxicity testing

Stormwater: Dry Weather Design

- Short term maximum concentrations (acute toxicity) is of concern
- Target known or suspected incidental runoff sources (e.g. system that drains golf course)
- Non-filtered water samples to be collected and analyzed
- Base flow conditions over longer time periods (weeks to months) can be assessed using passive sampling devices that provide a time-average concentration of CECs
- 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?

Candidate MS4 Watersheds

- San Francisco Bay: To be provided by stakeholder advisory committee

- 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)

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?

- Invertebrates (mussels, worms)
  - Bottom of food web
  - Historical data exists (Mussel Watch)
  - 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
  - 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 archives)
  - San Francisco Bay RMP & Bight 13 (bird egg study)
- Design is still evolving

Delta

Design Considerations

- Included in Panel's MS4 recommendations
- Waters receiving WWTP discharged requested by SWB/Delta RMP
- Aquatic life impacts from CECs in runoff (e.g. urban pesticides, PPCPs)
- Five WWTPs identified as possible pilot study watersheds
  - Sac Regional, Turlock, Tracy, Dry Creek, Easterly Creek
- Several MS4 monitoring stations identified for pilot study
  - Steelhead, Morrison, Hood and Arcade Creeks
  - Natomas, American Rivers
- Design is evolving
  - frequency and actual locations still to be determined

General Sampling Considerations

- Grab vs. composite
  - Effluent should be 24 h composite
  - Receiving water can be grabs (mid-channel, just below surface)
  - Sediment – composite of 3 grabs per station preferred
  - Tissue – composite for lower food web; individuals for higher biota
- Filtered vs. unfiltered
  - Depends on question (loading vs. toxicity?)
  - Depends on matrix (stormwater vs. clarified WWTP effluent)

QA/QC

Strive for compatible data quality objectives (DQOs) across collaborating/contributing programs

Need to harmonize and agree on minimum DQOs that address regional and statewide goals.

Most programs (e.g. SWAMP, RMP, Bight) have established QA/QC criteria that are adaptable to common uses of data

QA/QC 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 and Field Blanks	Minimum number per field samples analyzed; 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
- Participating laboratories should demonstrate acceptable performance prior to work initiation
- Inter-laboratory comparison exercises have been used by, e.g. Bight, for chemistry and toxicology

Data Management

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

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 BIOASSAYS

Endpoint	Response	Mode of Action	Potential Adverse Outcome
Estrogen Receptor Alpha (ERa)	Activation/ suppression	Estrogen signaling	Impaired reproduction, feminization of males, cancer
Androgen Receptor (AR)	Activation/ 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
- Pregnane X receptor (PXR): indicative of CYP3A metabolism

INCLUSION OF PXR ENDPOINT

Panel Response: Because PXR can be activated by numerous chemicals and is associated with several metabolic pathways, there is no credible potential adverse outcome (i.e. toxic effect) that can be positively linked to CECs for this endpoint.

SCCWRP Action/Response:

- *Remove PXR from list of endpoints*

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% (TBR) 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% (TBR) of sample response
Dose response fitting curve	Response of the reference toxicant run on replicate plates should be within 10% (TBR) 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

TBR – to be resolved/finalized

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 chemical monitoring data?

STUDY 2- DESIGN

- Sampling location selected based on study design for targeted monitoring (Sec 2)
- Linkage between in vitro responses and profiles and concentrations of CECs obtained via targeted monitoring (Sec 2)

	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 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 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 freshwater (Scenarios 1 and MS4) and WWTP effluents discharging to estuaries and ocean
- 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

FATHEAD MINNOW TOXICITY ASSAY FOR MODEL CECS

Study is being 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	

ASSESSING TOXICITY OF EFFLUENT AND RECEIVING WATERS USING FHM

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

FHM STUDY DESIGN - 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 NEED- ESTUARINE/ MARINE FISH MODEL

	Sheepshead minnow	Atlantic killifish	Threespine stickleback	Inland silverside
Location	Atlantic coast of USA, Gulf of Mexico	Atlantic coast of USA (CA species exists)	Found in California	Found in California
Validation	EPA	- -	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)

COMPARISON OF FISH MODELS

Panel Response: (1) Discuss pro/cons to each candidate fish model

(2) Consider topsmelt (*Atherinops affinis*) reproductive assay as a viable candidate

SCCWRP Action/Response:

- (1) Discuss pros/cons in final document
- (2) Contact developers of topsmelt assay to determine relevance/feasibility. If promising, include as a candidate test.

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?

INVERTEBRATE TESTING

Panel Response: There is no mention of specification of an invertebrate toxicity testing strategy for CECs.

SCCWRP Action/Response:

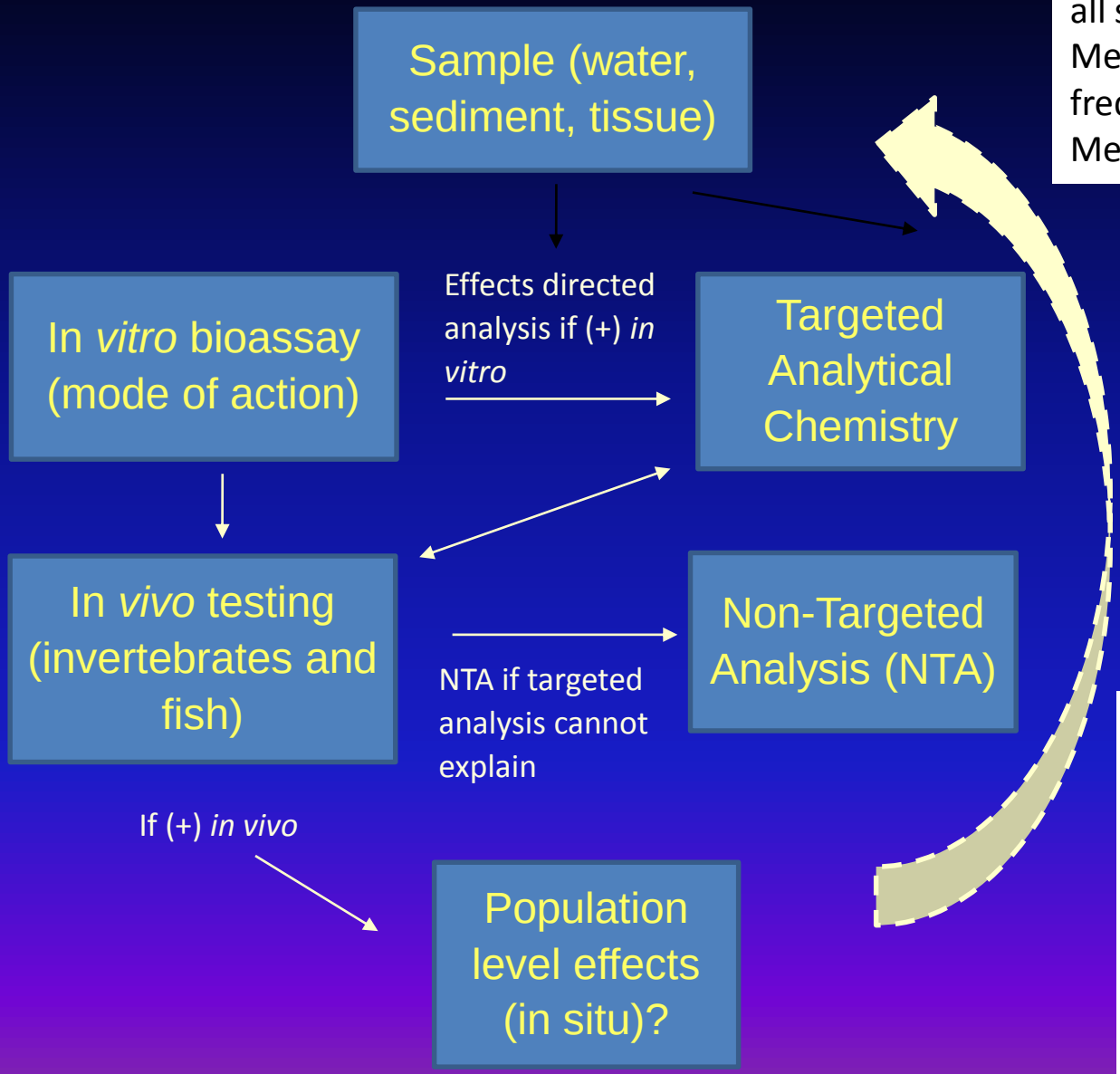
- (1) Discuss pros/cons of available test methods (similar to fish model table)
- (2) Recommended development of invertebrate test methods that addresss management questions for CECs.

SECTION 4 – IMPLEMENTATION

4.1 Integrating targeted monitoring & special study results

- **Tier 1 (most frequent, more stations)**
  - *In vitro* 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



Tier I (in *vitro*; targeted analysis)
all samples, high frequency
Measured < Threshold = reduced frequency or stop
Measured > Threshold = Tier II

Tier I or II (in *vivo*; NTA)
Selected samples, low frequency
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
- Pool available resources to address highest priority gaps

SECTION 4 – IMPLEMENTATION (cont.)

4.3 (proposed) Track Progress of CEC Monitoring Statewide

- Are recommendations of Statewide Panel and Regional Guidance Workgroups being implemented?
  - Develop and implement more efficient monitoring tools
  - Fill data gaps on occurrence and toxicity via monitoring and modeling
  - Prioritize CEC monitoring relative to other water quality issues
- Establish and maintain Statewide technical and stakeholder advisory groups
- Foster/coordinate opportunities for scientific and management exchanges
  - Expert Panels
  - Regional Monitoring Conferences
  - QA/QC workgroups

SECTION 5 – RESEARCH NEEDS

- **Antibiotic Resistance**
  - Knowledge on environmental occurrence and consequences is scarce
  - Recommend convening panel of experts to collate and synthesize state of the science
- **Non-targeted Analysis**
  - Establish regional “fingerprints” by matrix, source
  - Distinguish persistent (bioaccumulative) from transient unknowns
- **Passive sampling**
  - Develop devices that can sample target CECs at relevant concentrations (i.e. 50% of MTL)
  - Assess dosing capability for bioanalytical tools and non-targeted analysis, replacing extraction of large volume water samples
- **Real time monitoring**
  - Sensor networks that deliver continuous monitoring output
  - Obviates need for costly sample extraction/processing

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**
  - Invertebrate test species
  - Saltwater fish (Menidia spp.)

- **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