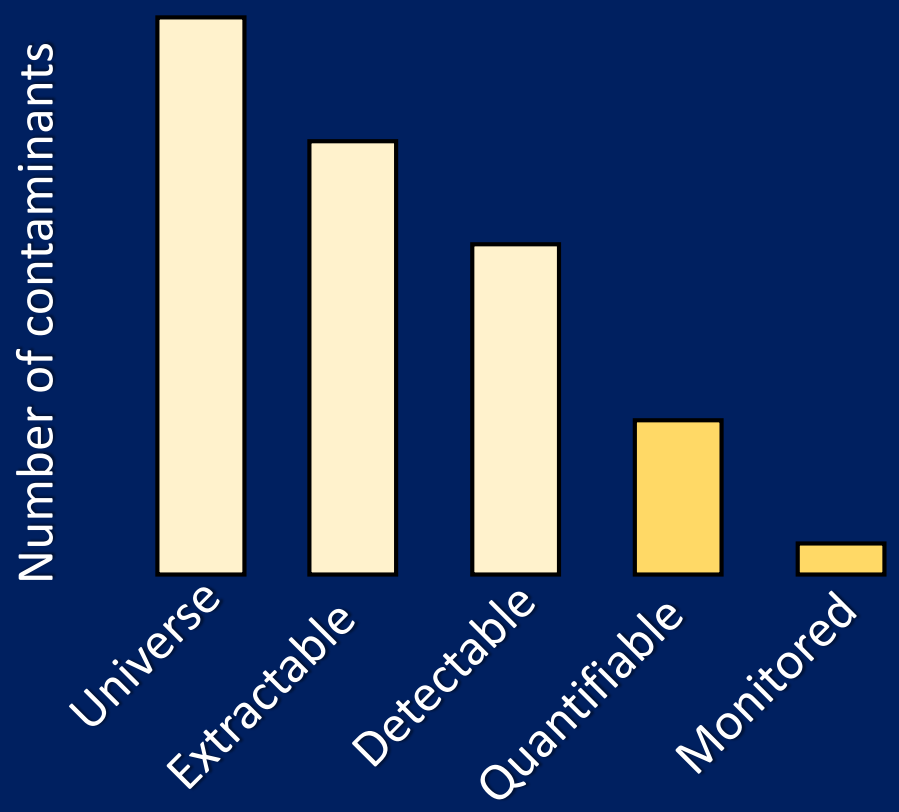
Bioanalytical Technology Transfer to Recycled Water Utilities

Alvina Mehinto

Commission meeting, December 13, 2019

Challenges for CEC monitoring



- Over 150,000 known chemicals, more released every year
- Many occur at levels below analytical detection limits
- No standardized mechanism to address unknowns / unexpected compounds

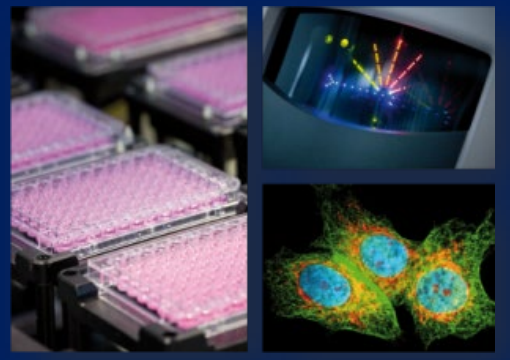
Chemical-by-chemical approach is not sustainable

- Monitoring CECs is a moving target
- Setting relevant monitoring thresholds is difficult
  - Non-lethal long-term toxic effects unknowns
  - Mixture effects are of concern

Bioanalytical tools as an alternative

- Supplement existing chemical monitoring
  - Analytical chemistry for known CECs prioritized by the State or EPA
  - Bioanalytical tools for unknown/unexpected CECs

- Provide integrated measure of all bioactive contaminants
  - Could improve mixture toxicity assessment

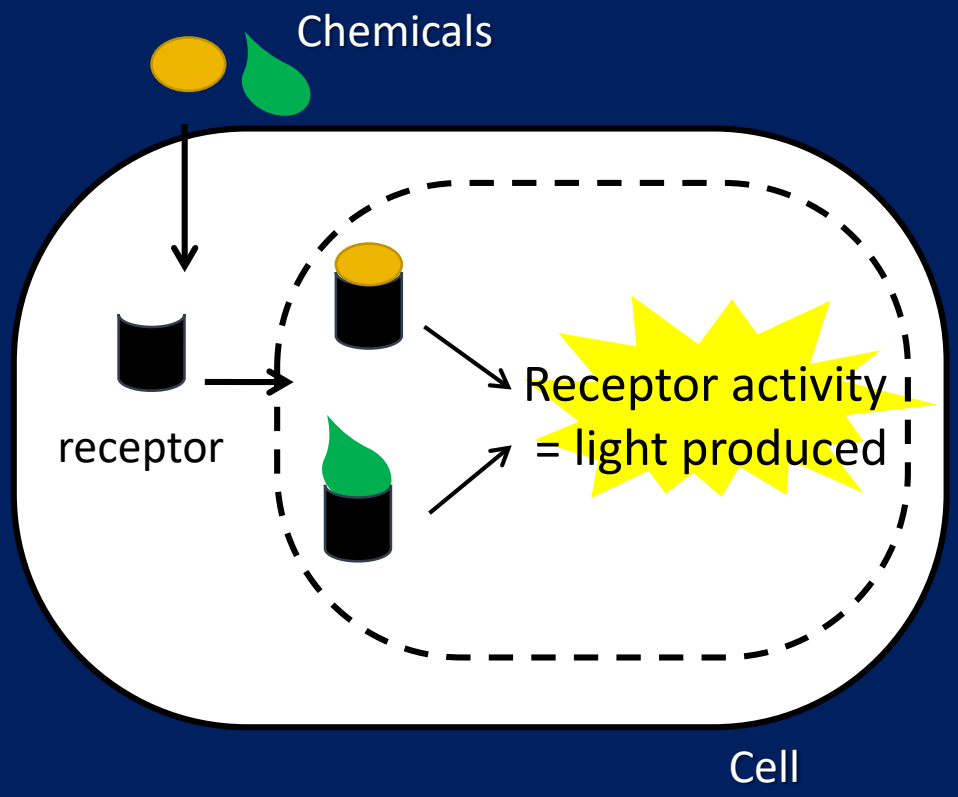


Bioanalytical tools - Background

- Also known as “cell-based assays” or “in vitro assays”
- Currently used in pharmacology, food industry, chemical registration
- High-throughput method with rapid turnaround
  - Data available in 3-5 days
- Screening of hundred of chemicals simultaneously
  - Based on biological activity

Bioanalytical tools - Procedure

- Mammalian cells engineered to track cellular responses
- Cells and sample extracts added to each well, and incubated
- Light intensity is proportional to the concentration of bioactive chemicals
- Data expressed as equivalent concentration (BEQ) relative to a known chemical



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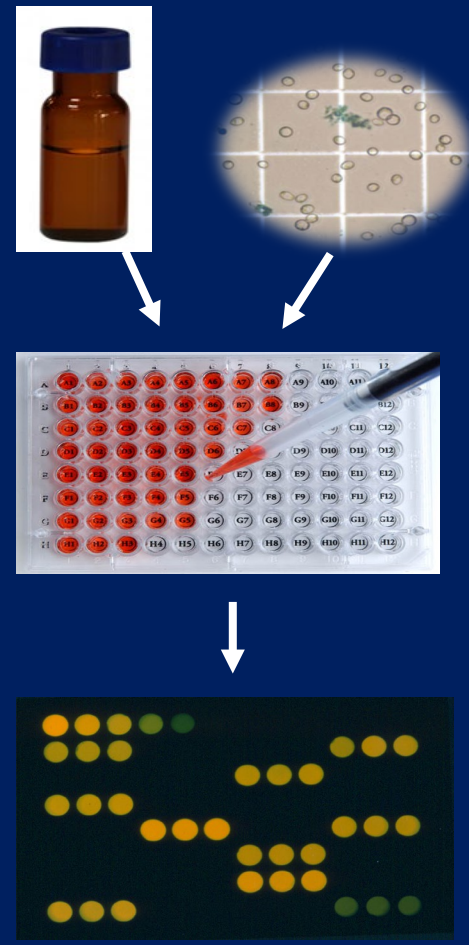


Adapting cell assays for water quality monitoring

1. Identification relevant bioscreening targets
2. Standardization assay protocols
3. Evaluation of assay performance (lab- and field-based studies)
4. Development of relevant bioscreening thresholds
5. Training and certification of laboratories

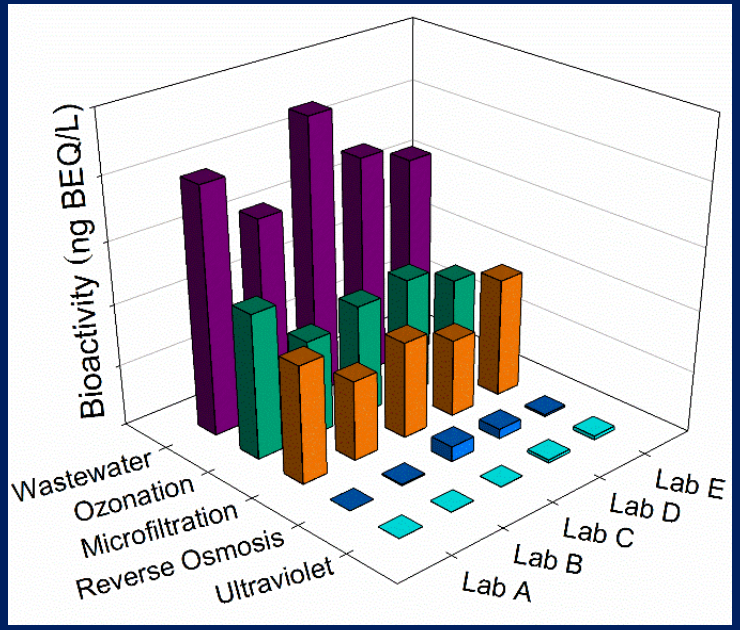
Stages 4, 5
Estrogen receptor alpha (ERα)
Aryl hydrocarbon receptor (AhR)
Stages 2, 3
Glucocorticoid receptor (GR)
Anti-androgen receptor (anti-AR)
Stage 1
Peroxisome proliferator activated receptor (PPAR)
Tumor protein P53 response (P53RE)

Example of cell assay development *(stage 2-3)*

- Performance criteria developed to ensure robustness of data
- Blind analysis of water samples by multiple labs using same protocol
- Pilot study demonstrated successful benchmarking of water qualities

PERFORMANCE BASED CRITERIA

- Calibration: slope & EC50 range defined
- Cell viability: ≥ 80% compared to control
- Solvent effect: ± 25% of control response
- Assay precision: ≤25% RSD for triplicate
- Data: ER-BEQ as estradiol equivalent, AhR-BEQ as TCDD equivalent

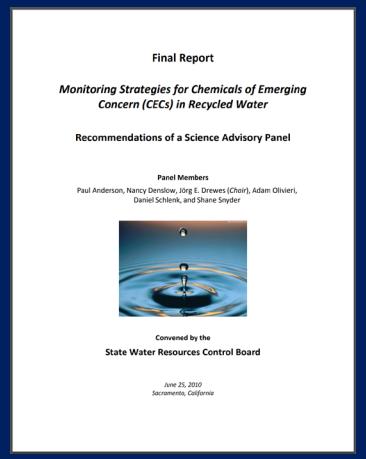


CA is moving forward with bioanalytical tools

2009

Recycled Water Policy adopted

2010


Final Report

Monitoring Strategies for Chemicals of Emerging Concern (CECs) in Recycled Water

Recommendations of a Science Advisory Panel

Panel Members

Paul Anderson, Nancy Denslow, Jörg E. Drewes (*Chair*), Adam Olivieri, Daniel Schlenk, and Shane Snyder

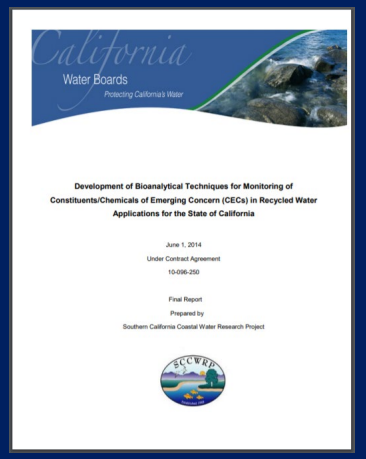
Convened by the

State Water Resources Control Board

June 25, 2010
Sacramento, California

Investigate use of bioanalytical tools

2014


California Water Boards
Protecting California's Water

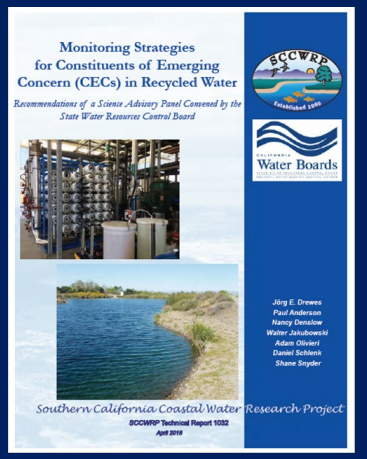
Development of Bioanalytical Techniques for Monitoring of Constituents/Chemicals of Emerging Concern (CECs) in Recycled Water Applications for the State of California

June 1, 2014
Under Contract Agreement
10-096-250

Final Report
Prepared by
Southern California Coastal Water Research Project

Standardize protocols for recycled water

2018


Monitoring Strategies for Constituents of Emerging Concern (CECs) in Recycled Water

Recommendations of a Science Advisory Panel Convened by the State Water Resources Control Board

Water Boards

Jörg E. Drewes
Paul Anderson
Nancy Denslow
Walter Jakubowski
Adam Olivieri
Daniel Schlenk
Shane Snyder

Southern California Coastal Water Research Project

SCCWRP Technical Report 1032
April 2018

RWP amended to include bioanalytical screening

Transitioning technology to water agencies

- Documentation
  - Standardized test methods for sample processing and cell assay screening
  - List of materials and equipment needed
- Training
  - Seminars, workshops
  - Lab demonstration, hands-on practice
- Intercalibration exercises
  - To support laboratory certification

Documentation

- Method papers focus on how to perform the cell assays
- Need for comprehensive document that describes all the steps
  - From collection to data analyses
- Advisory group convened to produce a guidance document
  - Initiative led by water reuse utilities
  - Document will help develop their standard operating procedures

Bioanalytical Implementation Advisory Group

- Facilitated by National Water Research Institute (NWRI)
- BIAG members from different sectors
  - Michael Denison (chair, UC Davis)
  - Dan Schlenk (UC Riverside)
  - Megan Plumlee (OCWD)
  - Shawn Thompson (LACSD)
  - Alvina Mehinto (SCCWRP)
  - Adam Olivieri (EOA, Inc.)
  - Claire Waggoner (SWB)

WATEREUSE CALIFORNIA

Bioanalytical guidance document

- Detailed recommendations for:
  - Water sampling (including QA samples)
  - Preservation method
  - Storage conditions and duration
  - Sample extraction (solid phase extraction)
  - Cell assay plating instructions (# of dilutions, working range of the calibration)
  - Data acceptability criteria
  - Calculation of bioanalytical equivalent concentrations
  - Interpretation guidelines in relation to the RW policy monitoring thresholds

Bioanalytical guidance document

- The document DOES NOT promote the use of one specific cell assay kit
  - Curated list of vendors and service labs will be provided
  - All assays must meet pre-defined set of performance-based criteria
- Stakeholders and peer-review process completed
- Public release expected next week
- Phased bioanalytical monitoring will begin in April 2020

Training

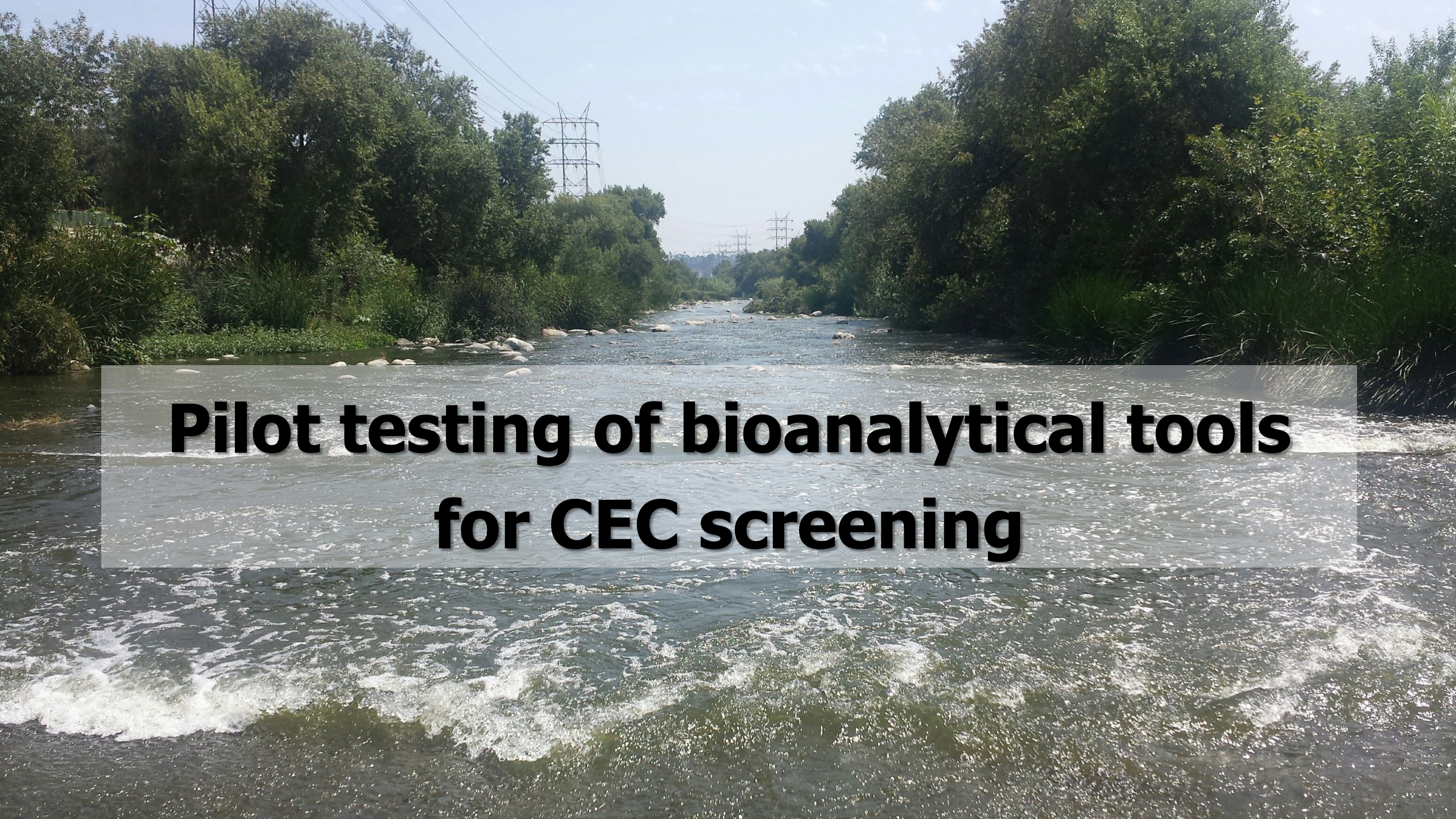
- SCCWRP has conducted trainings to support member agencies
  - Workshops on lab equipment, lab demonstration
  - Individual trainings to help with lab set-up and train staff (LACSD, San Diego, OCSD)

- Additional trainings will be conducted next year
  - Hands-on training in Spring 2020
  - Priority to member agencies
  - Could include reuse agencies and testing labs

Intercalibration exercises

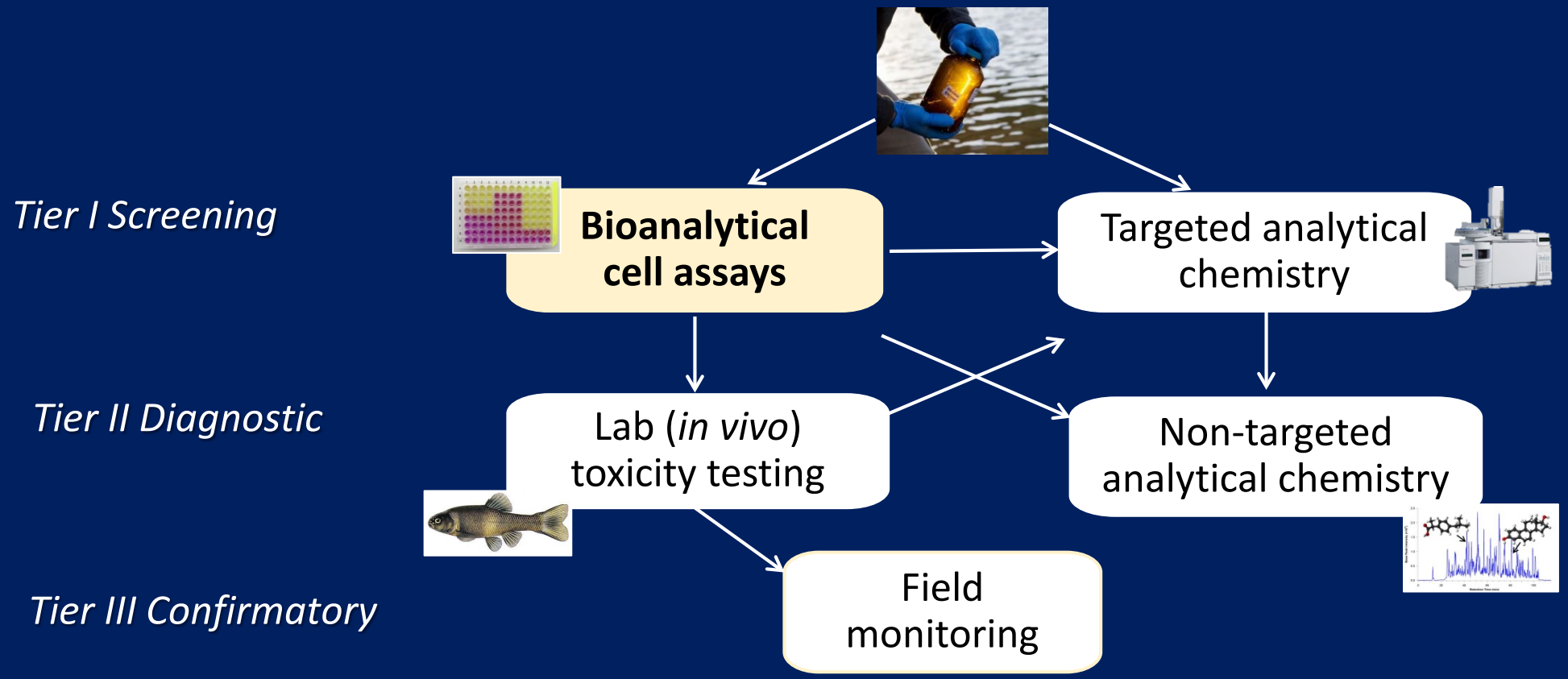
- Previous exercises limited to expert labs and academic groups
- No immediate plan to do this with water agencies …
  But we are open to do so!
- In absence of external funds, we will plan this exercise for Bight 23

Questions?



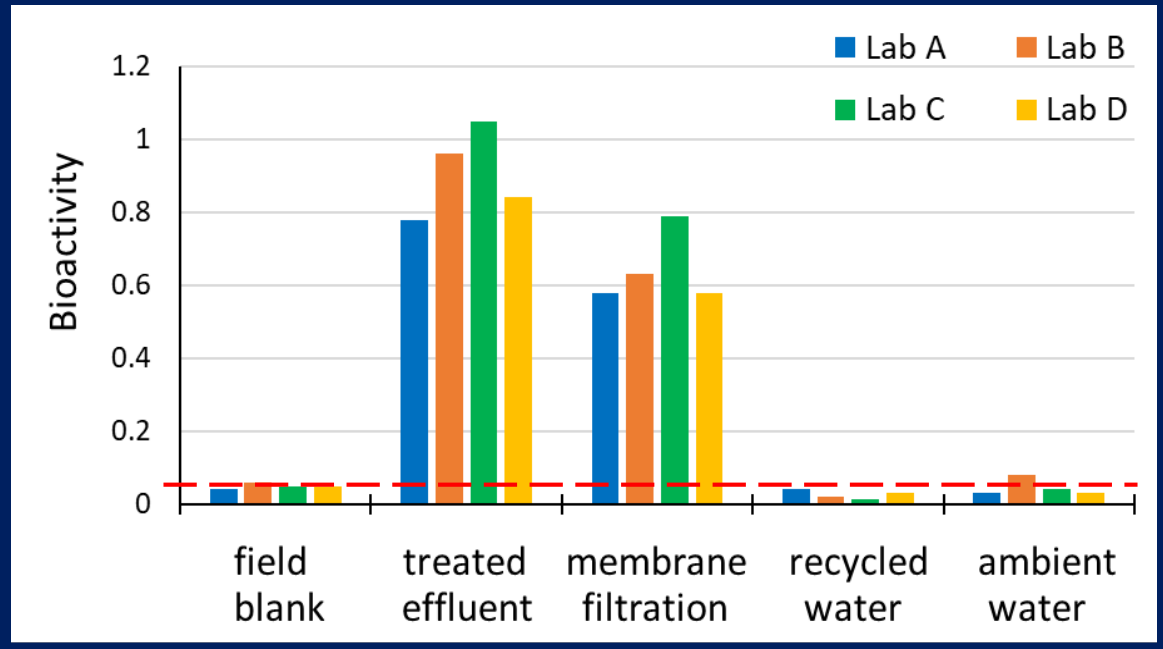
Pilot testing of bioanalytical tools for CEC screening

California is using a multi-dimensional approach



Initial lab-based studies were successful

- Standardized protocols available for two assays
- Reproducibility demonstrated through interlaboratory comparison exercises
- Capable of discriminating between "clean" and "contaminated" samples



Transitioning from lab to field application

Question 1: What is the sensitivity of these assays?

Are test samples always in exceedance?

Or are they always below reporting limits?

Question 2: Do the patterns of responses make sense?

Are bioactivity data in agreement with biological and chemical data?

Approach

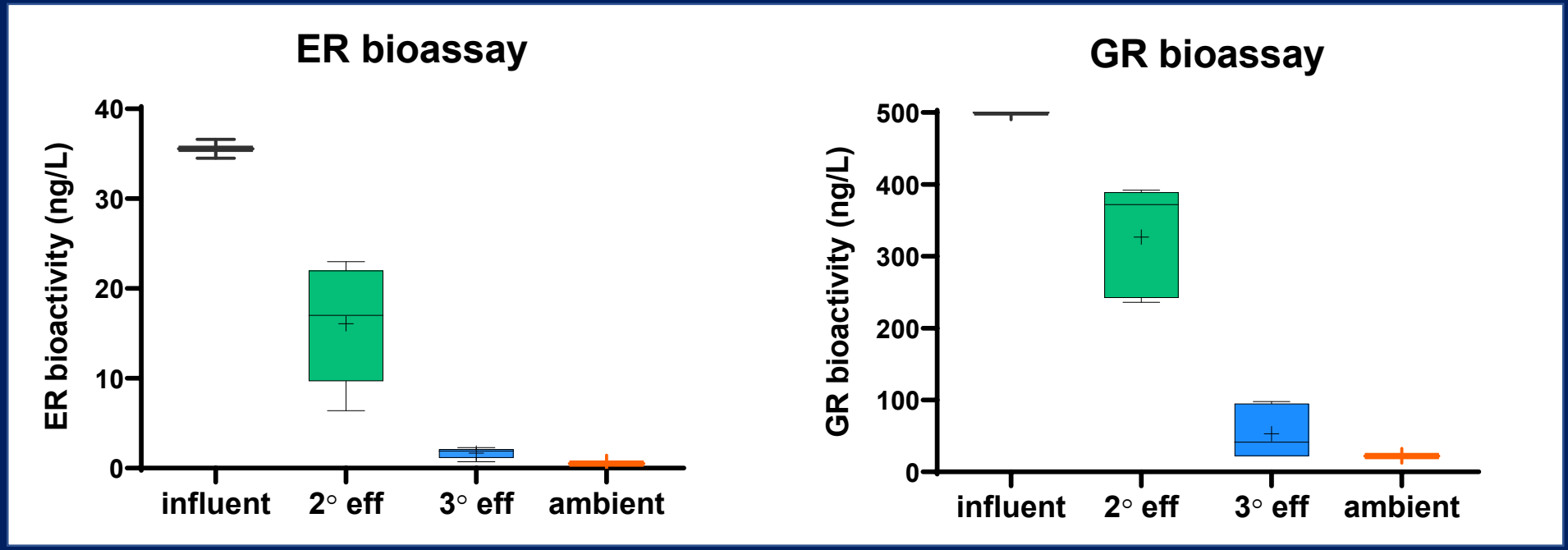
- Compare different samples types:
  - Influent (1 plant)
  - Secondary effluent (3 plants)
  - Tertiary effluent (5 plants)
  - Ambient water (stream, river, seawater)
  - Sediment and fish

- Cell assay endpoints tested:
  - ER assay for estrogens
  - AhR assay for dioxin-like chemicals
  - GR assay for glucocorticoids




Bioscreening results in ambient waters

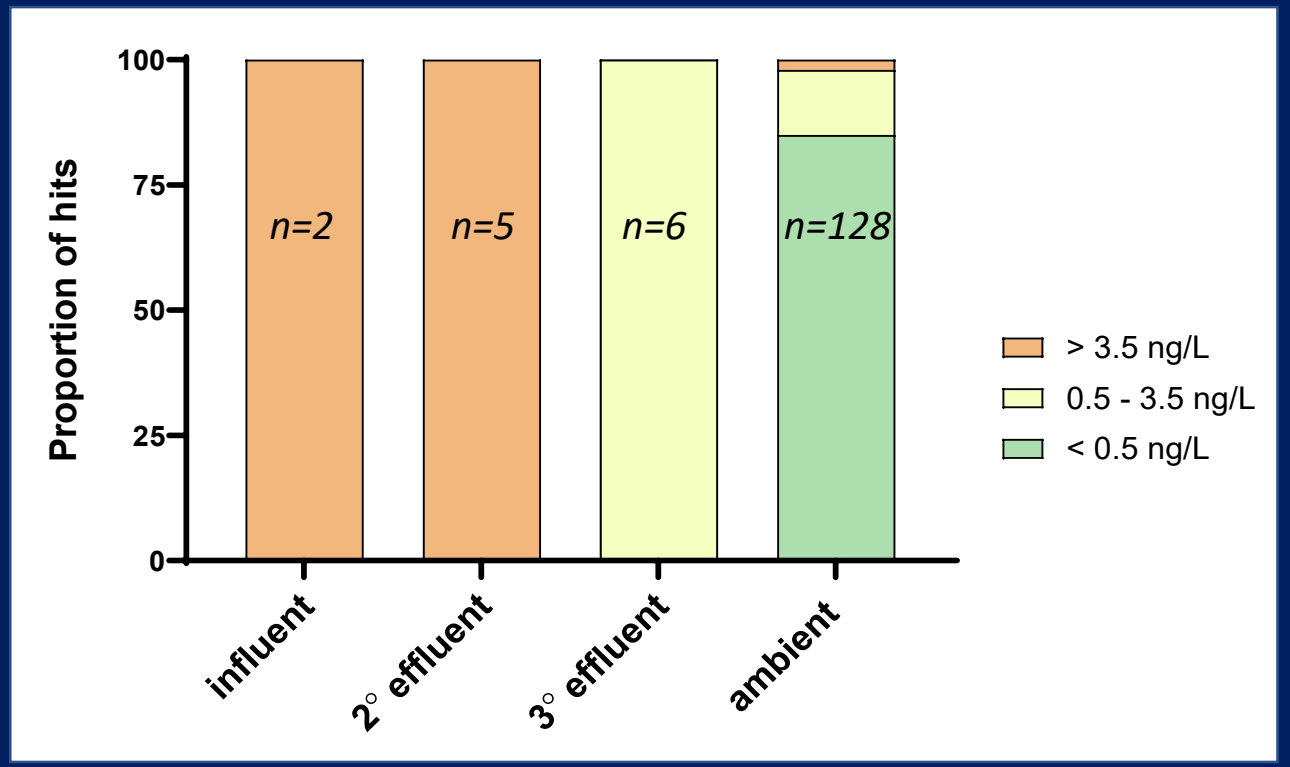
- Patterns of bioactivity make sense
- Promising as a rapid method to assess water quality and guide further testing



Assessing frequency of exceedance

- Ambient thresholds are still in development
- But the State has established thresholds for recycled water
- ER Bioassay
  - Reporting limit = 0.5 ng E2/L
  - Monitoring threshold = 3.5 ng E2/L

ER bioassay ambient data



Explaining measured bioactivity

- Relationship exists between bioactivity and chemical concentrations measured
- E.g. of ER bioscreening data and detection of known estrogens

  E2 = 17β-estradiol (potency =1); E1 = estrone (potency = 0.1)

Site ID	ER-BEQ (ng E2/L)	LC-MS/MS (ng /L)	Chem EQ (ng E2/L)
Riverfront	< 0.4	E2 <0.5; E1 < 0.6	< 0.5
Piner Creek	< 0.4	E2 <0.5; E1 < 0.6	< 0.5
WWTP effluent	**1.9**	**E2= 0.6; E1= 11**	**1.7**
Field blank	< 0.4	E2 <0.5; E1 < 0.6	< 0.5

SCCWRP Established 1969

SFEI AQUATIC SCIENCE CENTER
SAN FRANCISCO ESTUARY INSTITUTE & THE AQUATIC SCIENCE CENTER

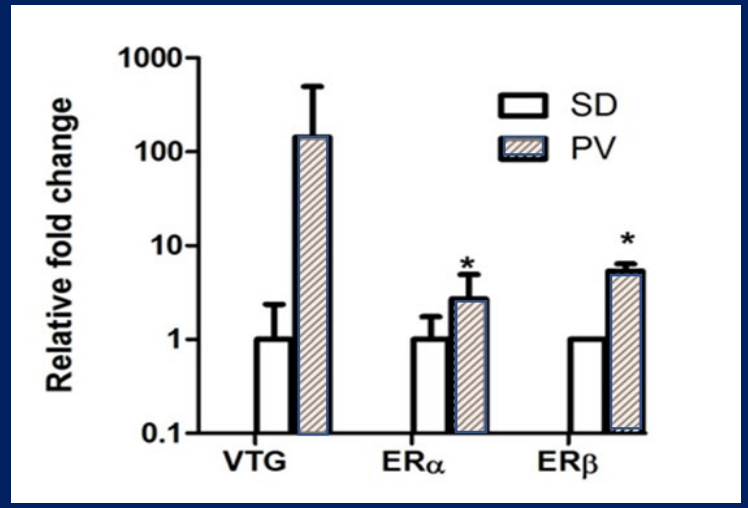
Linking bioactivity to aquatic health

- Palos Verdes – known contamination of estrogenic chemicals (DDTs, PCBs)
- Good agreement between tissue chemistry, cell assay and gene biomarkers
- Study highlights potential as surrogate measure of endocrine related effects

Chemical screening in fish

	Conc. DDTs (ng/g)	ER-BEQ (ng/g)
Palos Verdes	11,700	90
San Diego	1,650	3.3

Biological responses



SCCWRP Established 1969

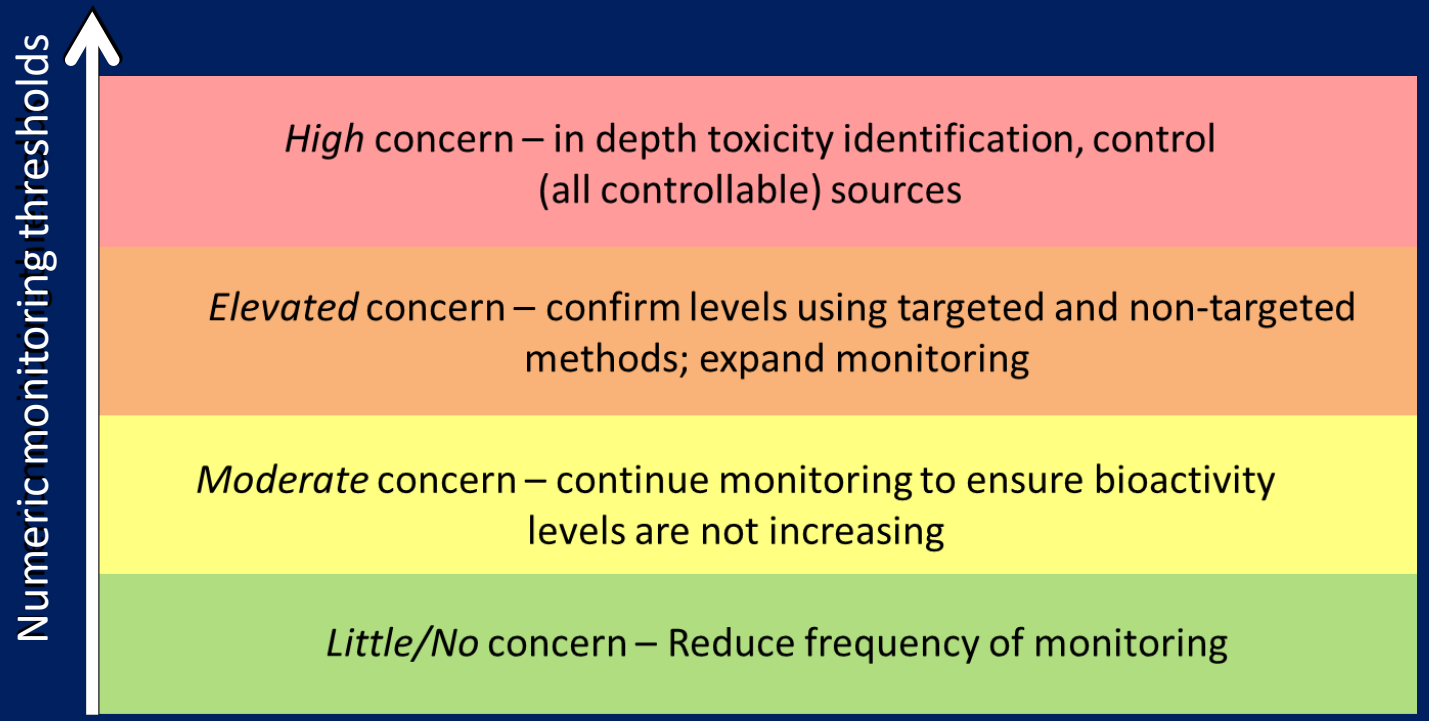
UNIVERSITY OF CALIFORNIA UCRIVERSIDE

What is next?

- Investigate causes of exceedance
  - e.g. targeted and non-targeted chemical analyses
- Develop better testing guidelines
  - e.g. dilutions credits for 2° effluents
- Establish screening thresholds for ambient environment
- Optimize protocols for new cell assay endpoints

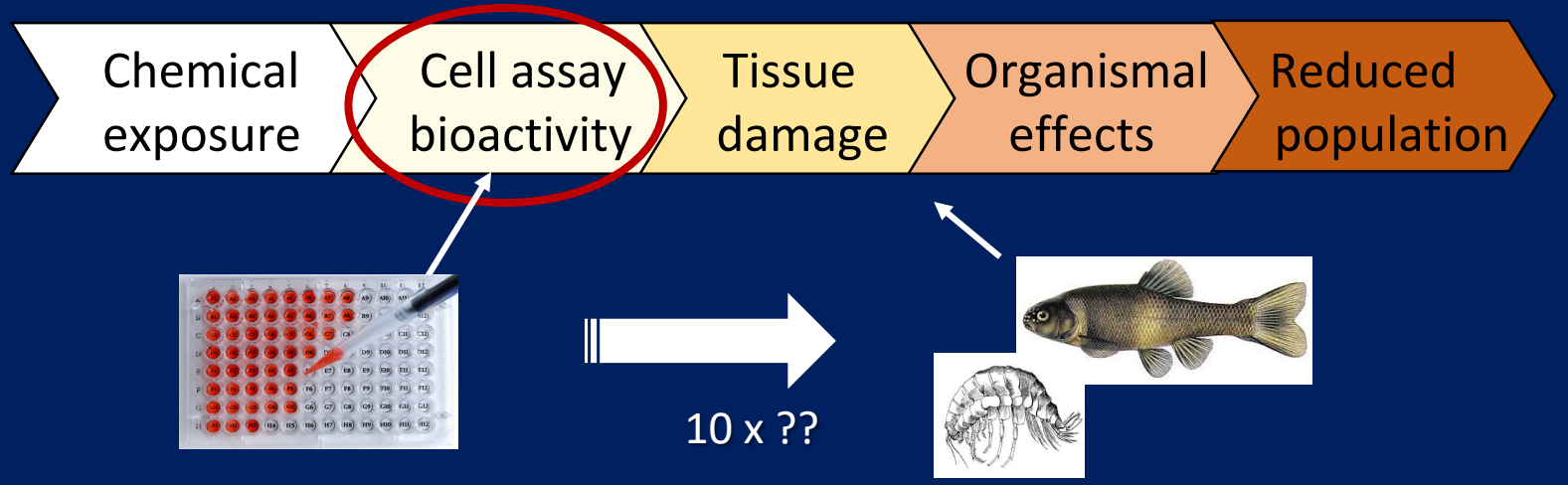
Our plan for developing thresholds

- We envision four thresholds that could inform management actions



Our plan for developing thresholds

- We envision four thresholds that could inform management actions
- This is achieved through lab and field-based studies to quantify the relationship between cell assay response and animal response



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