Monitoring contaminants of emerging concern (CECs)

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SCCWRP Commission
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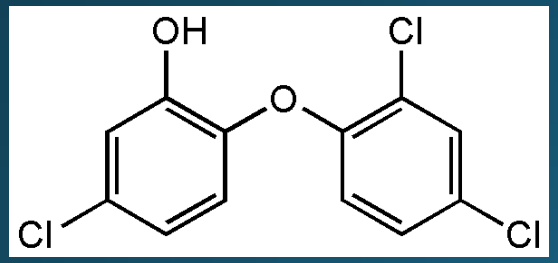
Chemical methods are limited in scope

"Industrial" ~82,000
Food additives ~ 3000
Cosmetics & additives ~6000
Pharmaceuticals ~1000
Pesticides ~1000

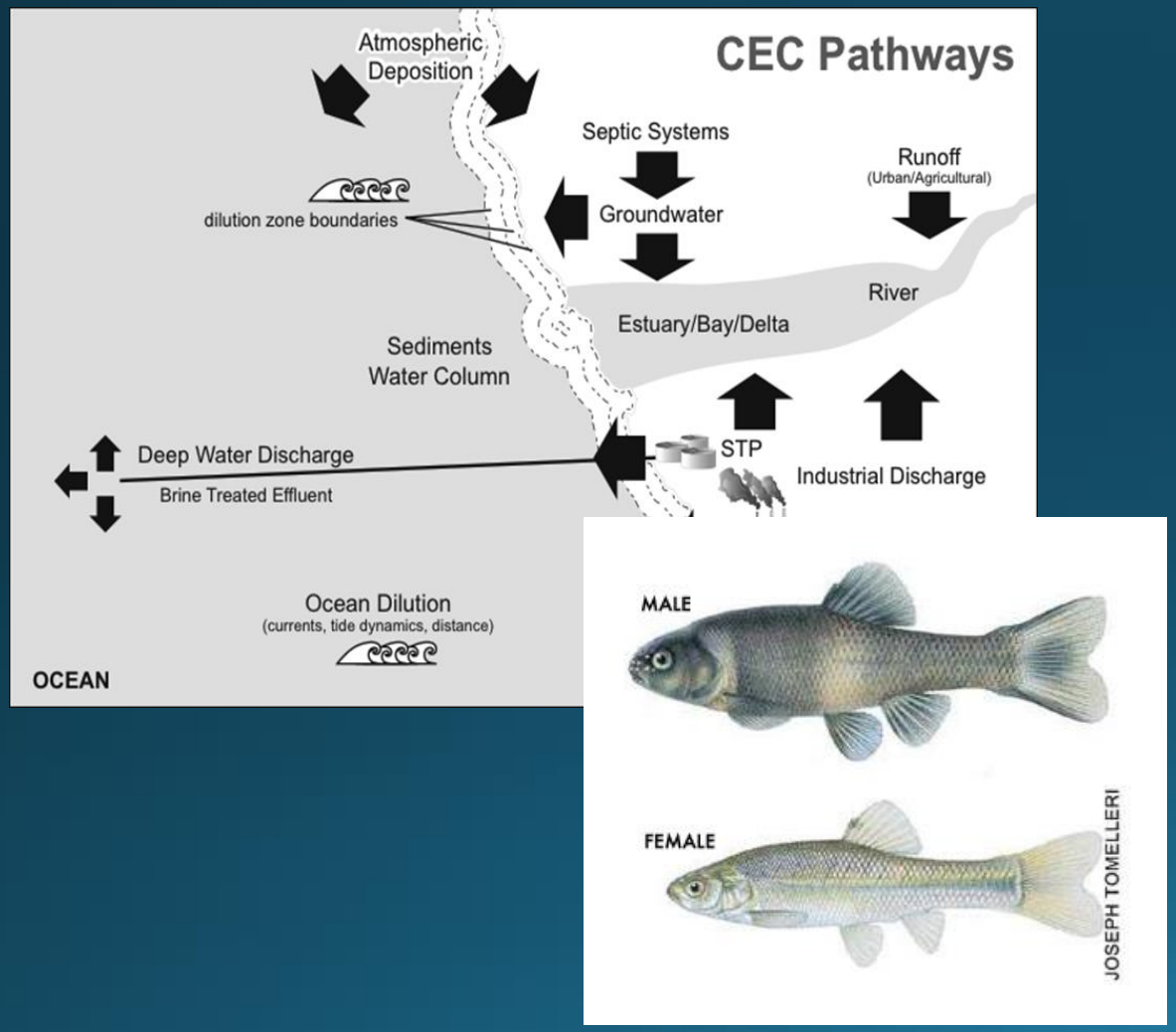


There are thousands of chemicals to consider

Chemical usage will change over time
- new drugs
- replacement chemicals

We have methods for ~500 chemicals
- ~200 priority pollutants
- ~300 "CECs"

Current toxicity testing has low relevance



Complex chemical mixtures are present in scenarios of interest

Current toxicity tests target lethality, growth

For CECs (e.g. synthetic hormones), *latent effects at sub-lethal exposure levels* are of concern

A new CEC monitoring approach is needed

Tools that can screen for various chemicals with high sensitivity

Analytical methods that identify problematic CECs

Biological tests that target plausible "CEC outcomes"

intersex, reproduction

immunosuppression

CEC	Class	Reporting Limit (ng/L)
17-β estradiol	Natural hormone	**•0.09 (1.0*)**
Caffeine	Food product	•35 (50*)
Triclosan	Pers care prod	•50
NDMA	Rxn by-product	**•0.1 (2.0*)**

from Anderson et al (2010).
Monitoring strategies for recycled water applications in California.

Our strategy

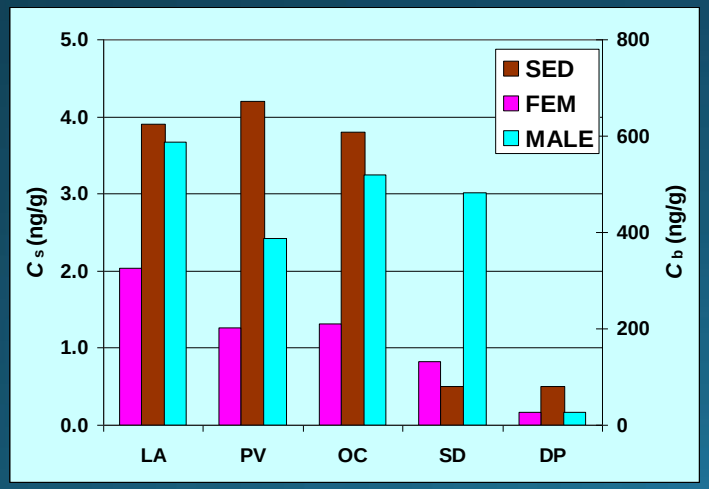
- *Define chemicals we should and can monitor now*
  - Pervasiveness
  - Potency
  - Availability of robust methods
- *Develop an approach to screen for the larger universe of CECs*
  - Bioanalytical tools
- *Identify methods to interpret screening (bioassay) results*
  - Non-targeted chemical analysis

Which CECs should we be monitoring?

SCCWRP convened a panel of experts to give us a process to identify chemicals for pilot monitoring:

- Step 1: measure or predict occurrence (i.e. concentration)
  - investigative monitoring (e.g. regional, special studies)
- Step 2: establish threshold that is protective of resource
  - toxicity tests (no/low observable effects concentrations)
- Step 3: does occurrence exceed toxicity threshold?
  - If no, do not monitor at this time
  - If yes, add to monitoring list

We are collaborating to fill data gaps



- CECs are prevalent in urban waterways
  - monitor effluent, in stream
- Few CECs accumulate in coastal bivalves
  - monitor to ensure levels aren't increasing
- Marine fish are exposed to CECs, but show little evidence of impact (so far)
  - We will see changes in effluent quality?

The long term solution features bioanalytical screening…

Cellular ("*in vitro*") bioassays

Efficient screening for ALL chemicals of interest, including CECs

Whole animal *("in vivo")* toxicity testing

Linking screening bioassays response to higher order effects

Field *("in situ")* monitoring

Assessing health of resident/sentinel species

Adapting cell assays for water quality monitoring

- Collaborated with international consortium of investigators
- Perform literature review to identify endpoints of concern
- Evaluate and optimize performance of *off-the-shelf* products
- Establish a protocol for water samples
- Analyze samples representing a range of water quality
- Compare accuracy, precision across laboratories

SCCWRP Established 1969

entox national research centre for environmental toxicology

Griffith UNIVERSITY Queensland, Australia

UF UNIVERSITY of FLORIDA

THE UNIVERSITY OF ARIZONA

USF UNIVERSITY OF SOUTH FLORIDA

CALIFORNIA Water Boards STATE WATER RESOURCES CONTROL BOARD REGIONAL WATER QUALITY CONTROL BOARDS

WATEREUSE FOUNDATION

UC RIVERSIDE UNIVERSITY OF CALIFORNIA

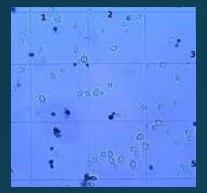
Products that screen for CECs are *commercially* available

ENDPOINT	SIGNIFICANCE
Estrogen Receptor (ER)	Feminization of males
Androgen Receptor (AR)	Masculinization of females
Glucocorticoid Receptor (GR)	Impaired development, immunosuppression
Progesterone Receptor (PR)	Cancer, hormone resistance syndrome
Aryl hydrocarbon Receptor (AhR)	Dioxin-like toxicity, tissue damage, cancer
Umu or p53	Genotoxicity, DNA damage, cancer
Cytotoxicity	Cell --→ tissue damage --→ death

We established a water testing protocol

Day 1

Thaw frozen cells



Cell count

Plate cells @ specific density

Extract water sample

Add diluted extracts

Overnight incubation (~16 hrs) at 37°C, 5% CO_2

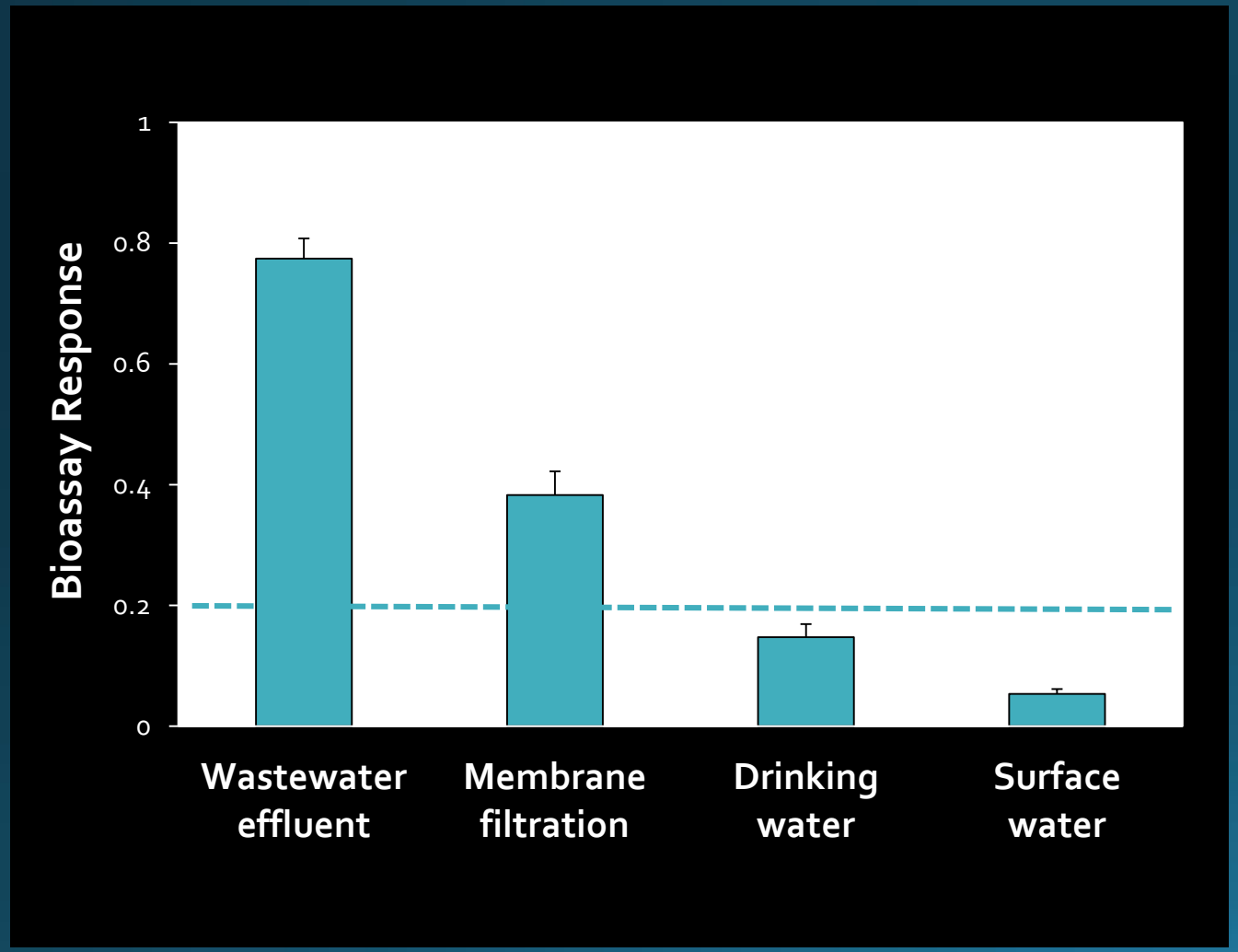
Day 2

Add substrate

Incubation (~2 hrs) at room temperature

Measure fluorescence

Our tests screened by water quality…



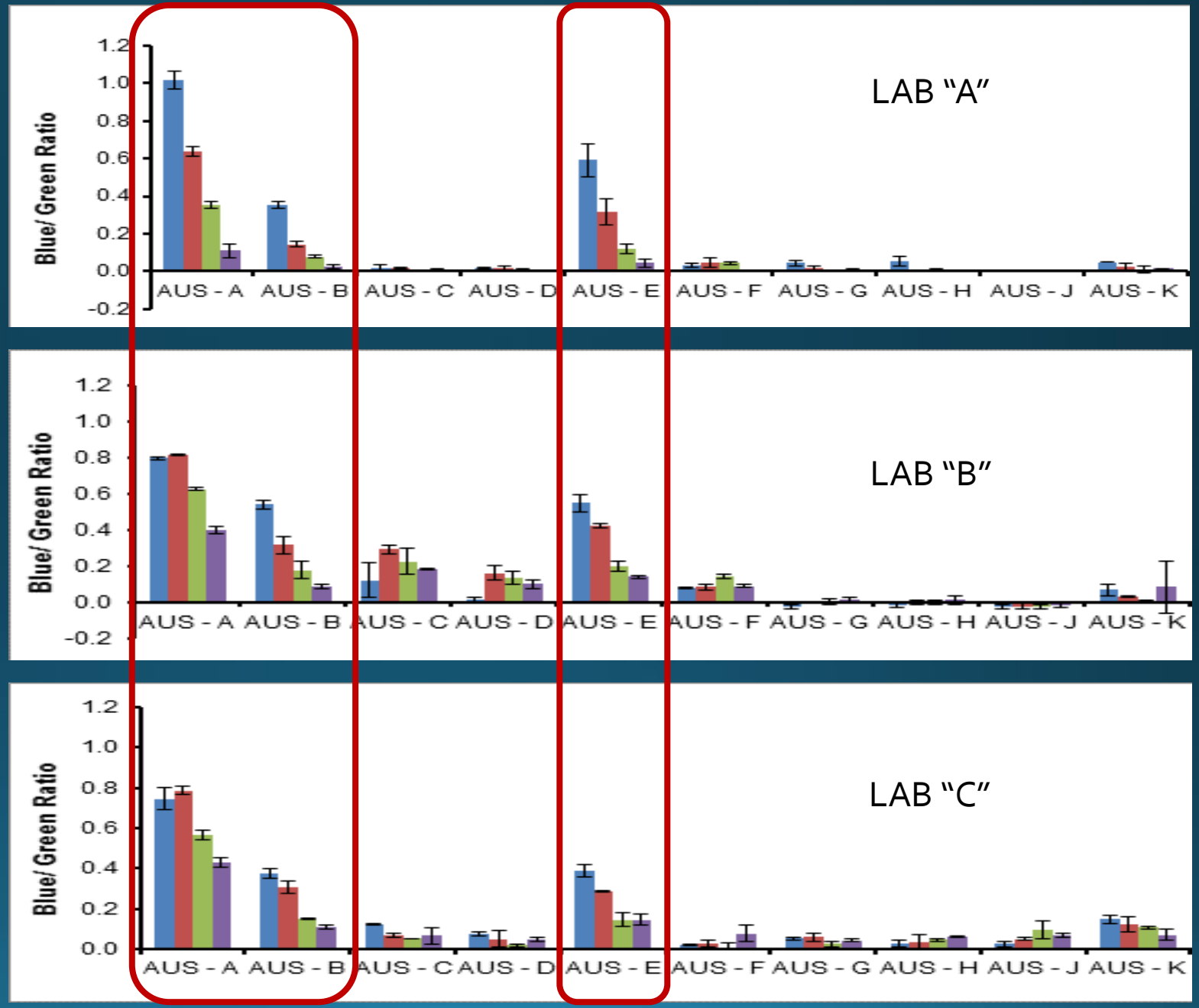
- blind analysis of samples from water recycling treatment train
- 20 labs measuring > 100 bioassay endpoints
- *Test for estrogenic chemicals ranked samples by water quality*
  - *WWTP effluent > filtered effluent > drinking, surface water*

…and were reproducible across labs

Five CA team labs used standardized protocol for estrogenicity test kit

Some variability in absolute response observed

Test response across samples was similar



These tests are ready for trial

- State-of-the-art cell assays can screen for a wide variety of relevant environmental contaminants, including CECs
- Off the shelf product that tests for *estrogenic chemicals* was
  - adapted for analysis of recycled water samples
  - successful in ranking samples according to quality
  - precise in the hands of capable (research) labs
  - vetted by international scientific community
- Other cell-based test products (e.g. genotoxicity) were less successful

Better chemical diagnostics will explain bioassay results

- If screening bioassay response is below threshold, keep monitoring
- If screening bioassay response is above threshold
  - confirm response, isolate affected area or unit process

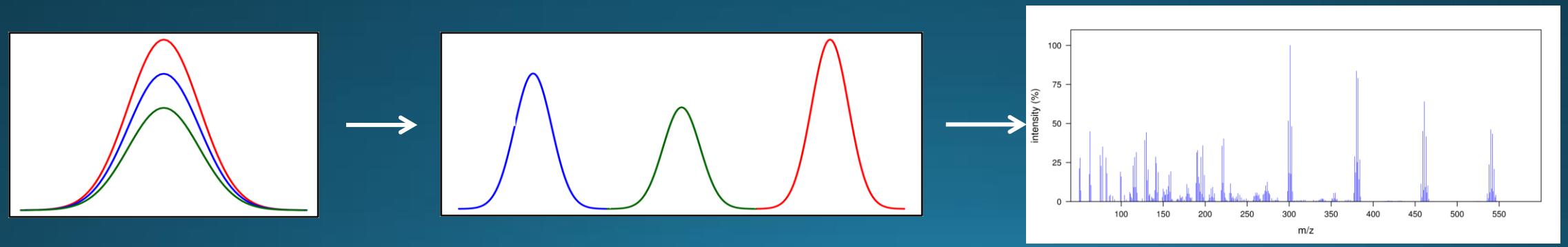
- Initiate whole organism testing (does exposure result in a real effect?)
- Perform targeted monitoring for likely candidate stressors

- ***What do we do when targeted monitoring is inconclusive?***

  Non-targeted analysis (in toxicity identification mode)

What is non-targeted chemical analysis?

- Integrated method that investigates all compounds in a sample
- Current mass spectrometric technology allows for
  - excellent resolution (separation) of co-eluting peaks (“2-D” chromatography)
  - precise chemical identification using “full scan“ mass spectrum
  - Low detection limits for chemicals of interest
- Requires development of data handling and analysis tools (custom libraries)



Non-targeted chemical analysis can identify bioactive CECs

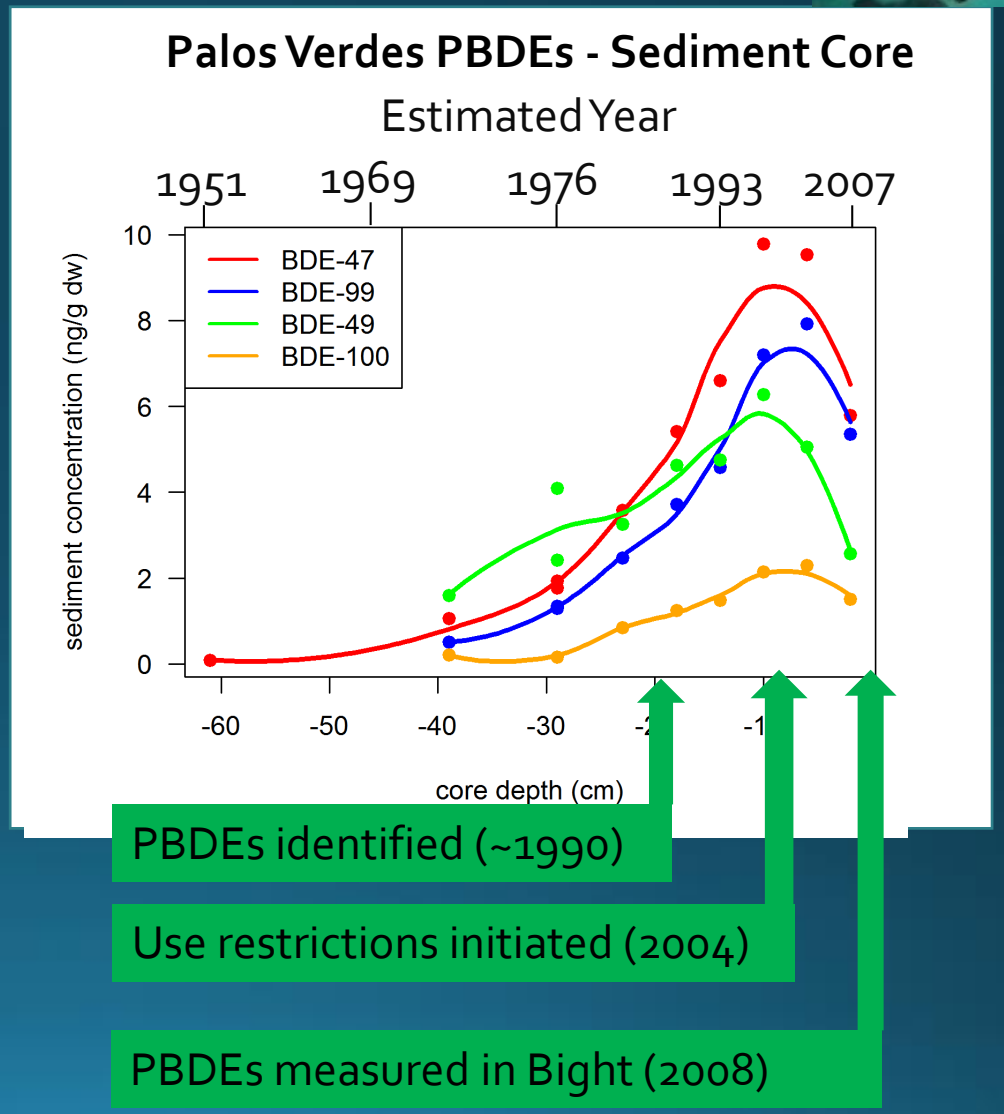
- There are hundreds of chemicals that can cause toxicity
- Targeted chemical methods are limited as diagnostic tools (i.e. for identifying causative chemicals)
- Non-targeted analysis broadens the scope of CECs that can be identified in toxic samples
- We are testing the "linkage" between non-targeted data and toxicity results
  - bioanalytical screening
  - whole organism (CEC) toxicity tests

Are we missing relevant contaminants?

- CECs are often discovered after they have become pervasive
  - flame retardants (PBDEs)
  - perfluorinated compounds

- Non-targeted analysis can identify CECs ***BEFORE*** they become an issue

- We are testing this concept by cataloguing ALL chemicals in marine top predators



Non-targeted analysis identifies problematic CECs…

Targeted chemical analysis

Develop & implement methods for individual, high priority CECs

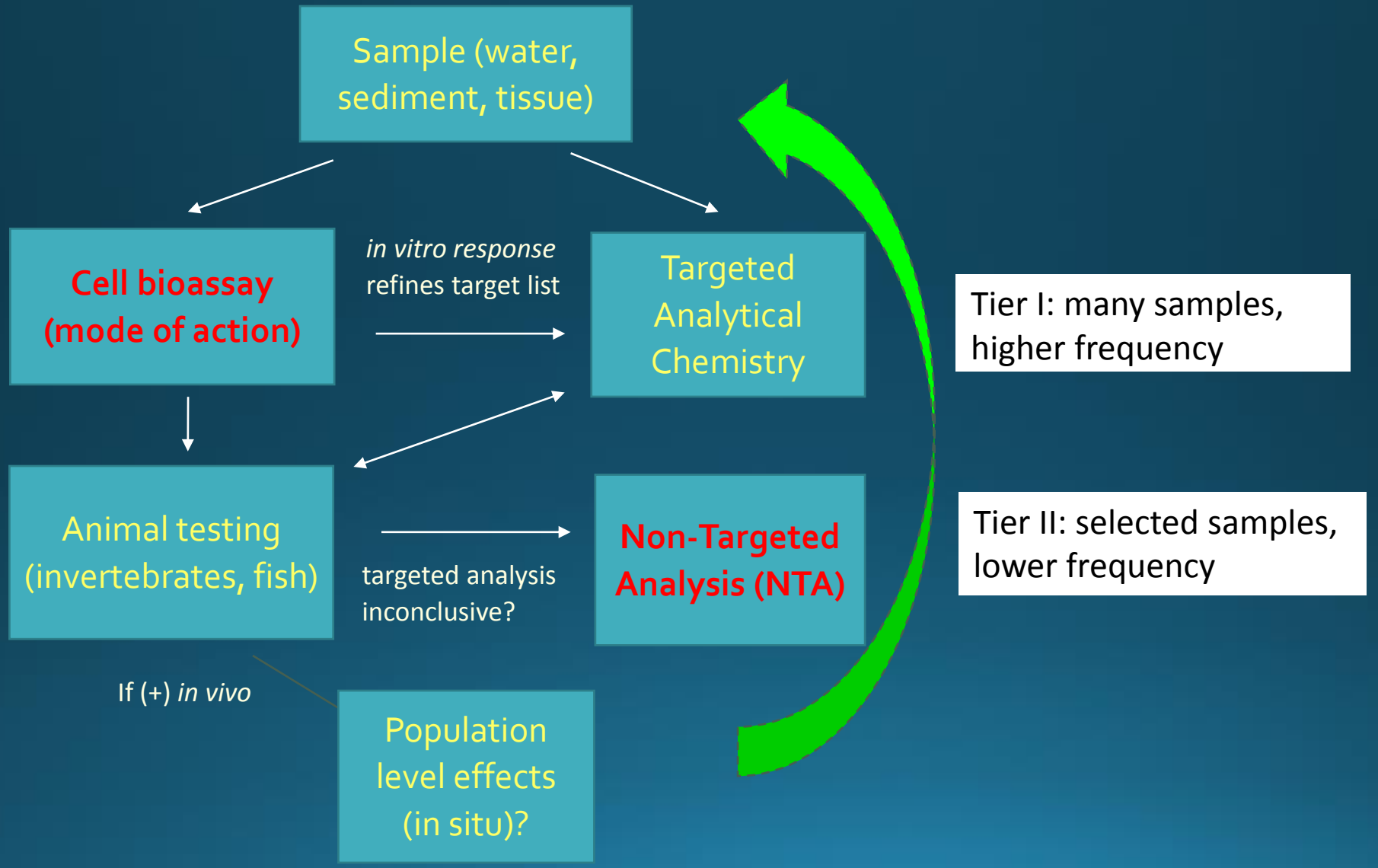
Non-targeted chemical analysis

Broadens window to include unexpected, bioactive contaminants

Field *("in situ")* monitoring

Assessing CEC exposure in resident/sentinel species

New tools are critical pieces of our CEC monitoring strategy



We need your help…

- continued participation in CEC occurrence studies
- pilot testing of bioanalytical screening tools
- proof of concept for non-targeted analysis in diagnosing toxicity

Current mechanisms for testing of our CEC strategy

- Statewide CEC Pilot Monitoring Study
- Stormwater Monitoring Coalition
- Regional special studies
- Bight activities

- Please contact us
  - Framework/Strategy: keithm@sccwrp.org
  - Bioanalytical screening: alvinam@sccwrp.org
  - Non-targeted analysis: nathand@sccwrp.org;

Three questions

- Do you see merit in moving from a chemically-based monitoring approach to one based on bioassays?
- What do you see as the biggest impediments to adoption of a bioassay approach?
- Are we ready to start a pilot implementation?
  - If so, where would be the best place to do so?