Incorporating bioanalytical screening and non-targeted analysis for CEC monitoring

Alvine C. Mehinto, Keith A. Maruya and Stephen B. Weisberg
Southern California Coastal Water Research Project Authority (SCCWRP)
Costa Mesa, CA USA

Workshop on CECs and Aquatic Ecosystem Monitoring
Sacramento Regional County Sanitation District
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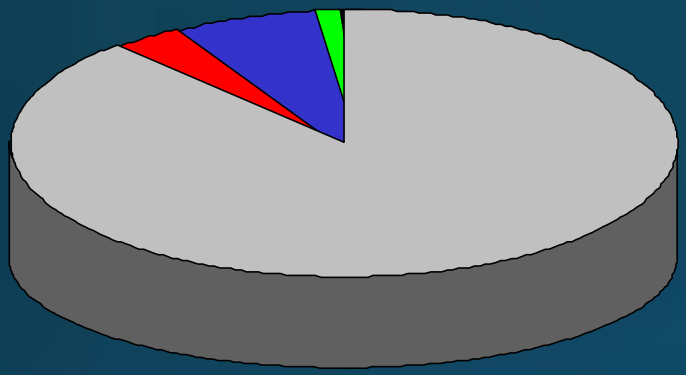
CECs are chemicals that may pose a health risk, but for which limited data is available



Current Targeted Monitoring Lists
- Regional: ~200
- Drinking/Recycled Water: ~500

"Industrial" ~82,000

Food additives ~ 3000

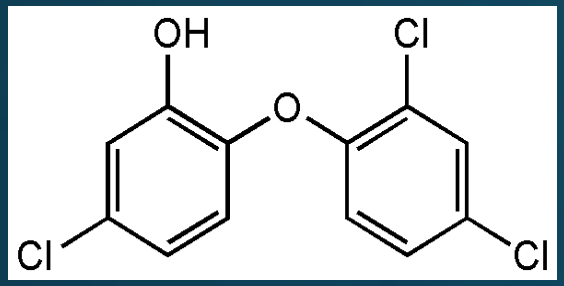
Cosmetics & additives ~6000

Pharmaceuticals ~1000

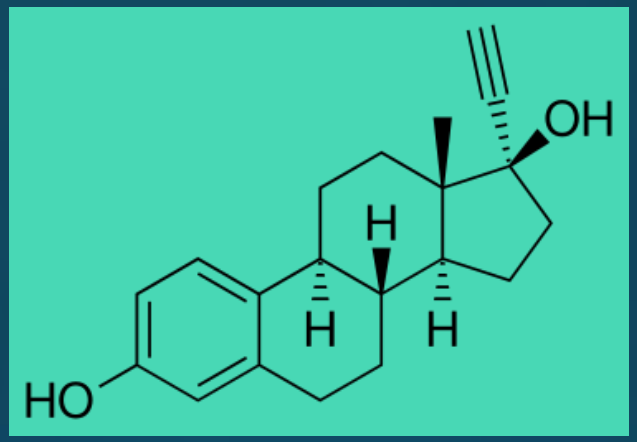
Pesticides ~1000

Muir and Howard (2010)

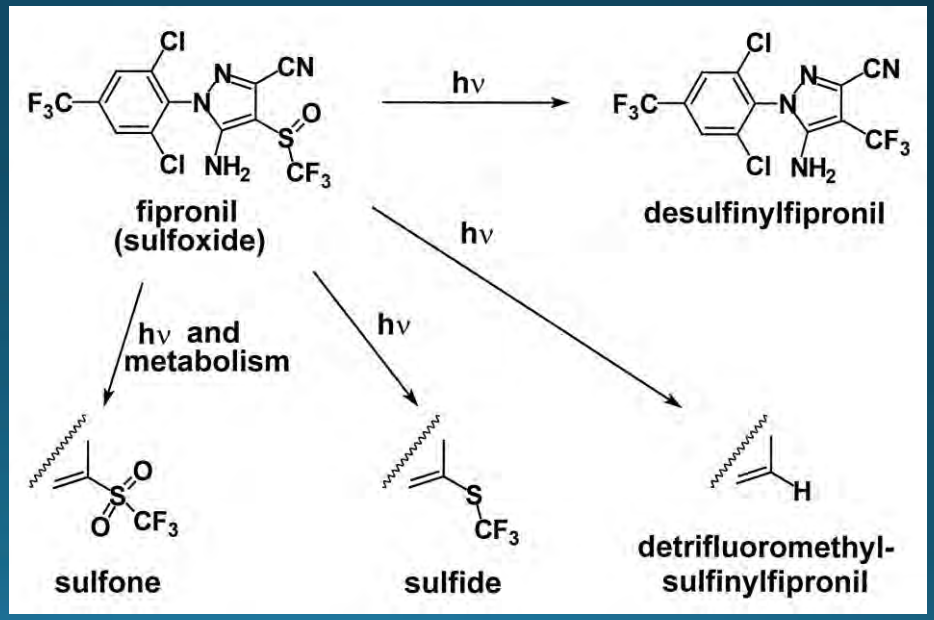
There are CECs we know about…



triclosan -
antibacterial agent

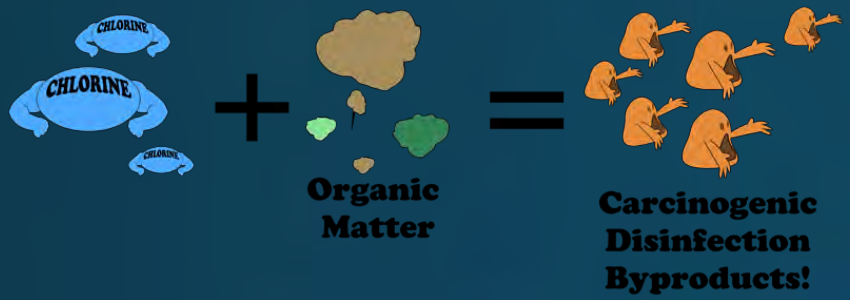


Ethinyl estradiol –
synthetic hormone



fipronil – phenylpyrazole insecticide

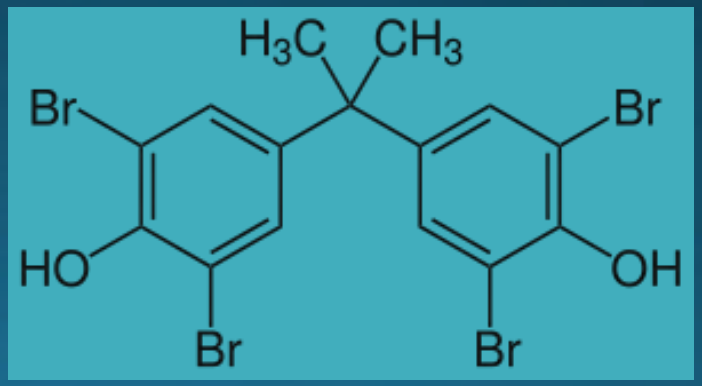
…and those that remain unknown



- Disinfection by-products
  - trihalomethanes (THMs)
  - nitrosamines
  - halogenated CECs
- Drug, pesticide metabolites
- New, replacement chemicals

Treatment practices can lead to unintended CECs

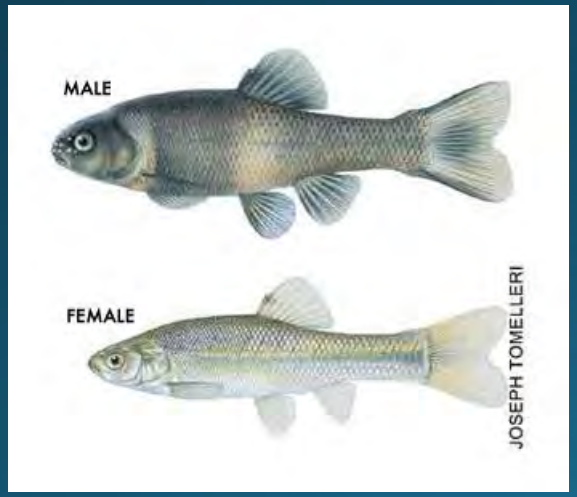
Natural factors (e.g. sunlight) transform CECs



Tetrabromobisphenol A, a flame retardant

CECs are relevant because impacts are possible at low levels, but current methods do not address their effects

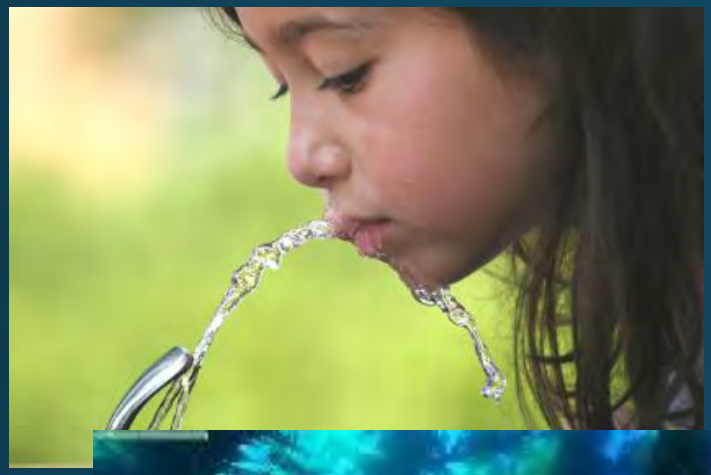
Synthetic estrogens affect fish reproduction at ng/L concentrations (Kidd et al. 2007)



estrogenic CECs

Intersex (ovotestis)

Statewide challenges for monitoring CECs



Which CECs pose the greatest risk to ecological and human health?

How do we monitor for CECs?

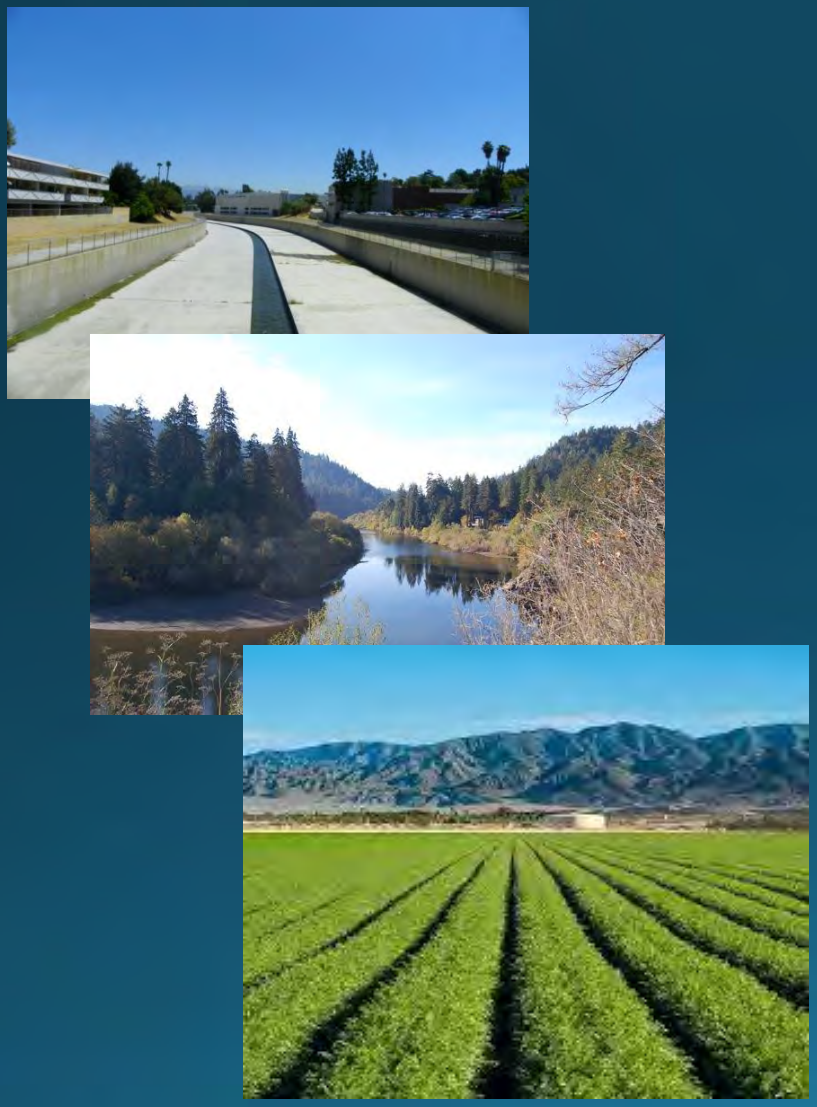
How low is low enough?

How do we assess mixture toxicity?

CA Recycled Water Policy requires monitoring of CECs

Regulators and dischargers need guidance for receiving waters

Regional monitoring questions



Which CECs, if any, are relevant in our watersheds?

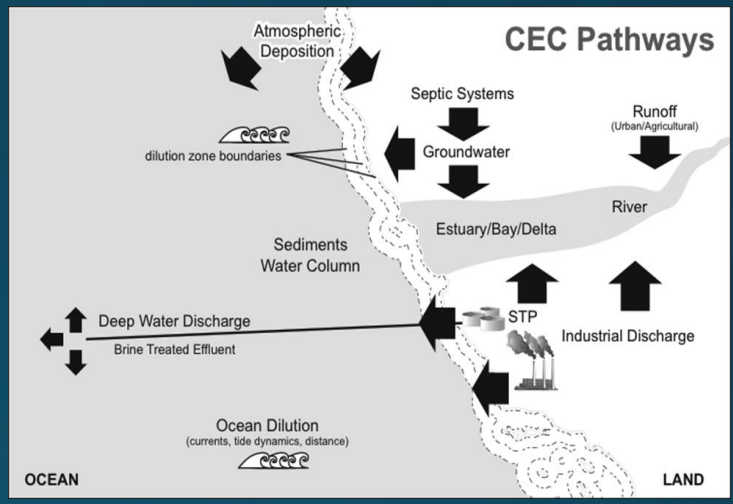
What are appropriate thresholds for priority CECs, and how are they established?

What sources and/or land uses deserve attention?

How do we ensure robust monitoring datasets?

Building scientific consensus

- Expert Panel convened by SWRCB
  - vetted/guided by stakeholders
- Monitor high priority CECs
  - Risk-based list of known chemicals
- Focus on waters receiving WWTP, stormwater discharge
  - agricultural runoff not considered
- Matrix-specific list of CECs
  - drugs, personal care products, hormones, urban pesticides (n=16)



Targeted monitoring for known CECs

In water…

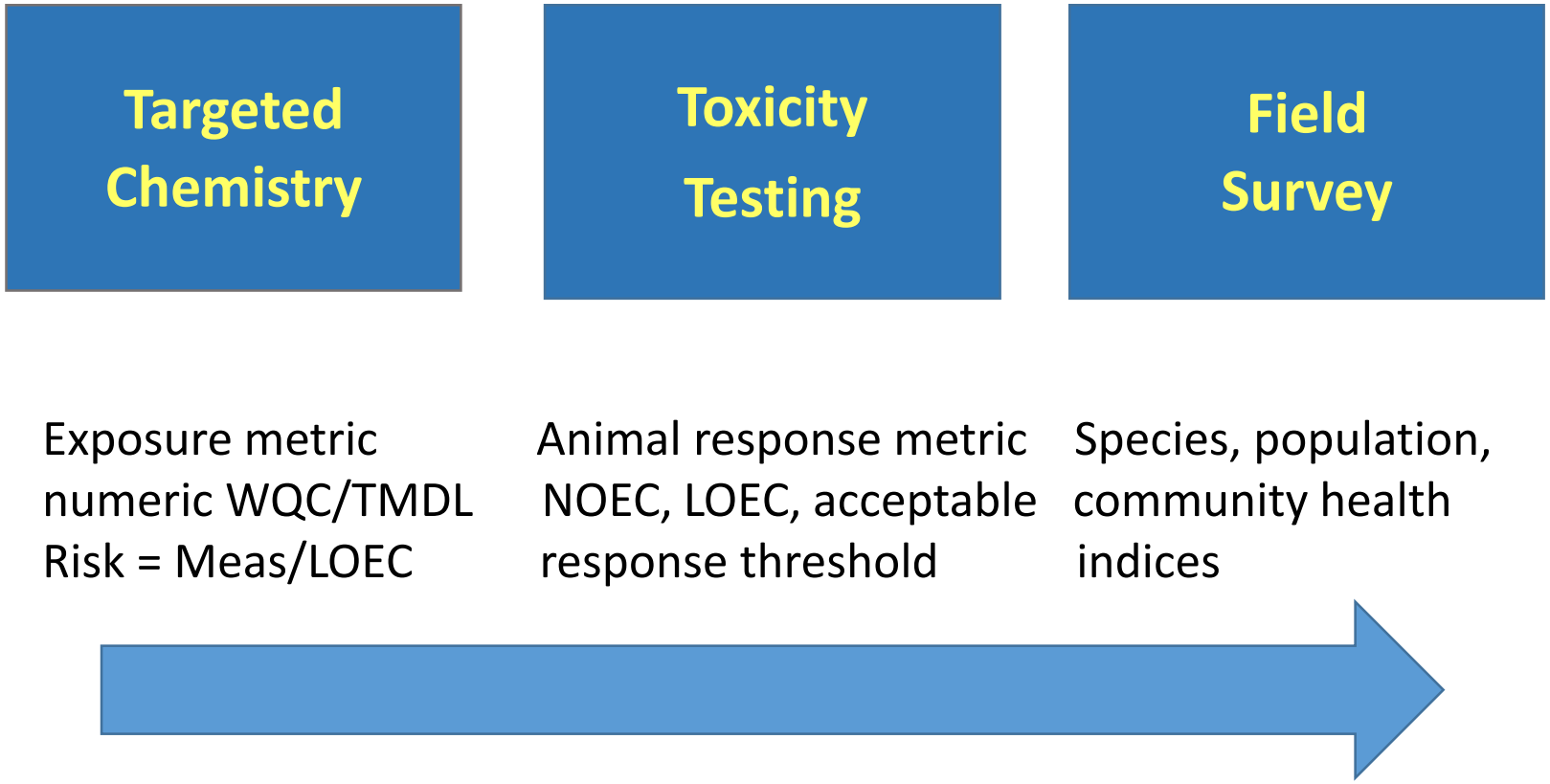
In sediment and fish tissue…

CEC	LA River	SG Rvier	SC River	MTL	MTQ
bpA	**691**	**657**	<25	60	**>10**
estrone	<2.5	<2.5	<5	6	<1
ibuprofen	38.3	40.5	<25	1000	0.04
galaxolide	2619	2753	n/a	7000	0.39
permethrin	<0.17	1.72	n/a	10	0.17
triclosan	10.7	26.0	38.4	92000	<0.01

CEC	Matrix	River	Bay	MTL	MTQ River	MTQ Bay
bifenthrin	sed	**4.9**	**54**	0.052	**94**	**1000**
fipronil	sed	**1.2**	3.7	0.09 (6.5)	**13**	0.57
PBDEs	tissue	20.1	7.0	28.9	0.70	0.24
PFOS	tissue	26	0.59	1000	0.026	<0.01

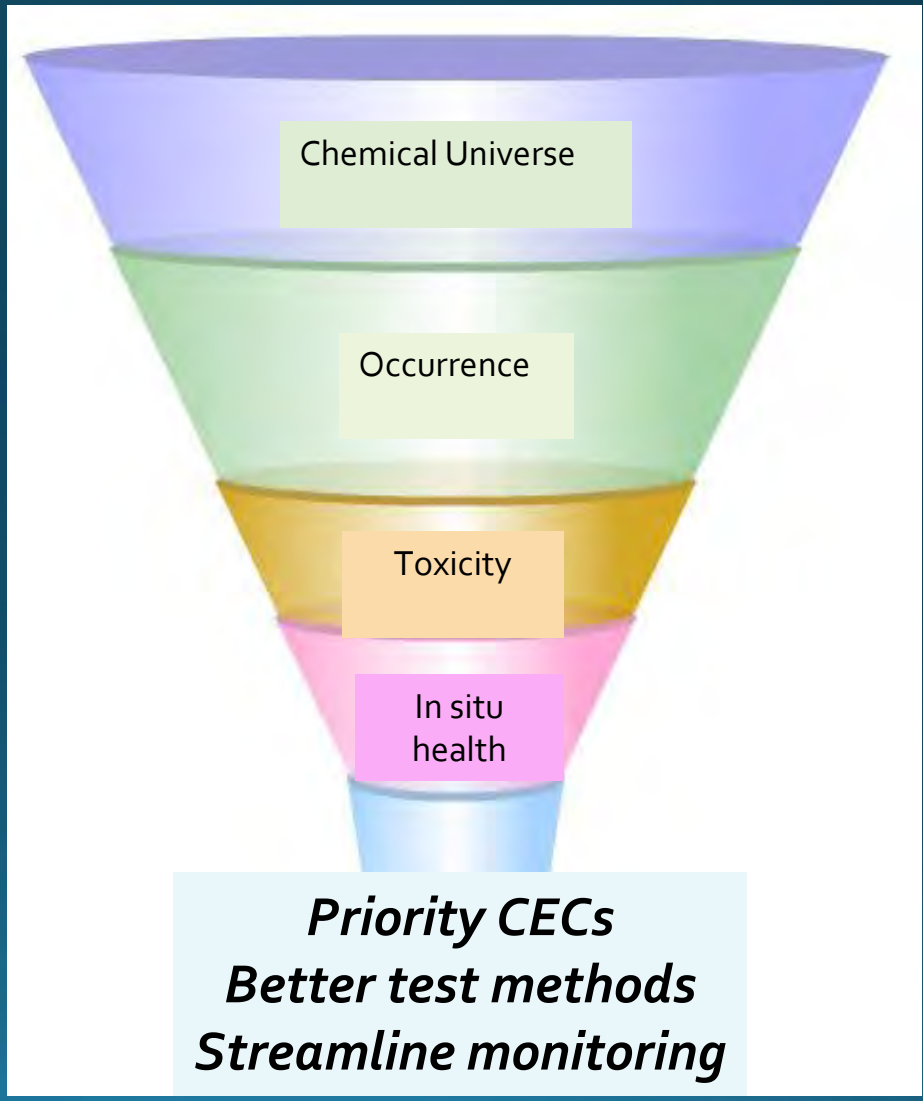
MTL – monitoring threshold
MTQ = trigger quotient = Conc/MTL

Current Water Quality Monitoring



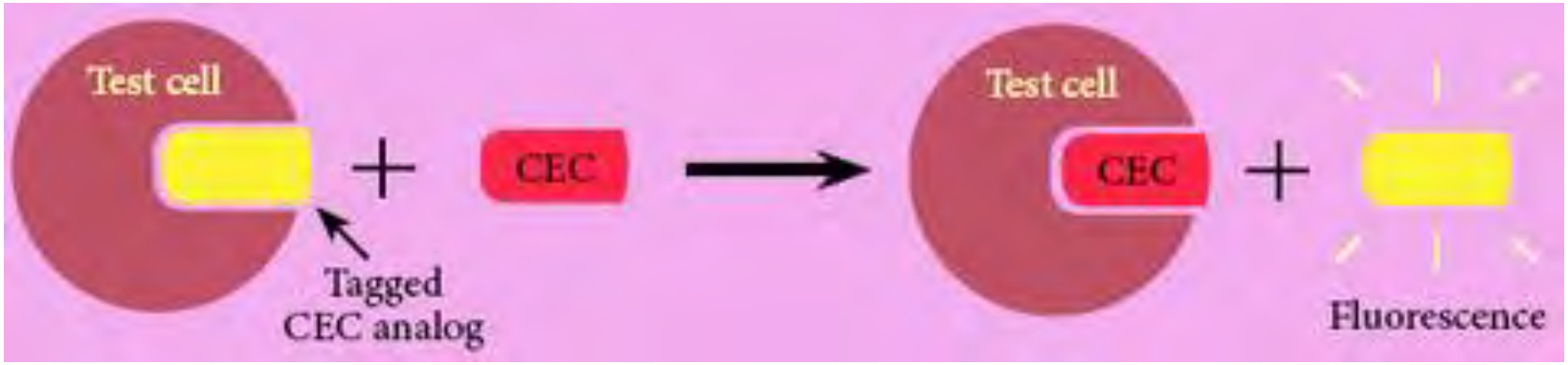
Is there a better way?

- New monitoring tools
  - *bioanalytical tools* to screen for toxicants by mode of action
  - *non-targeted analysis* to identify toxicants that elude targeted methods
- Adaptive management
  - Collect and interpret data
  - *Adjust* target parameters, monitoring effort
  - Test promising *new* technologies



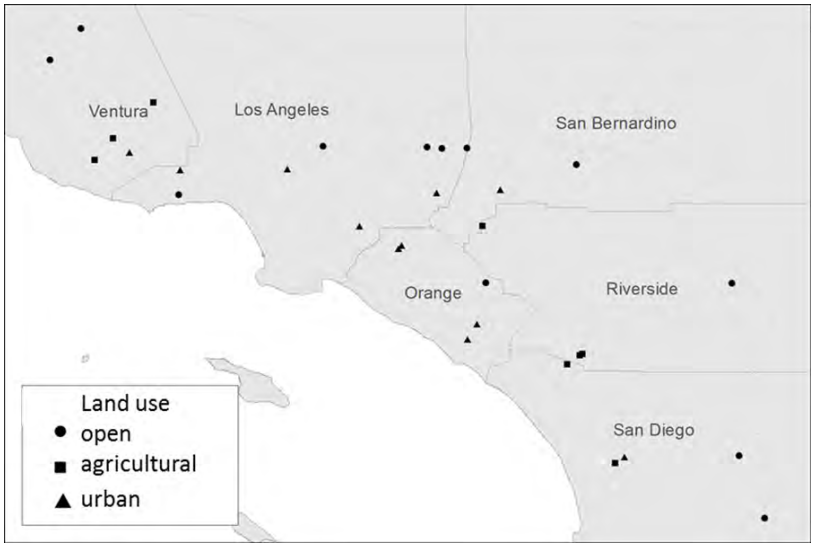
Bioanalytical Screening Tools

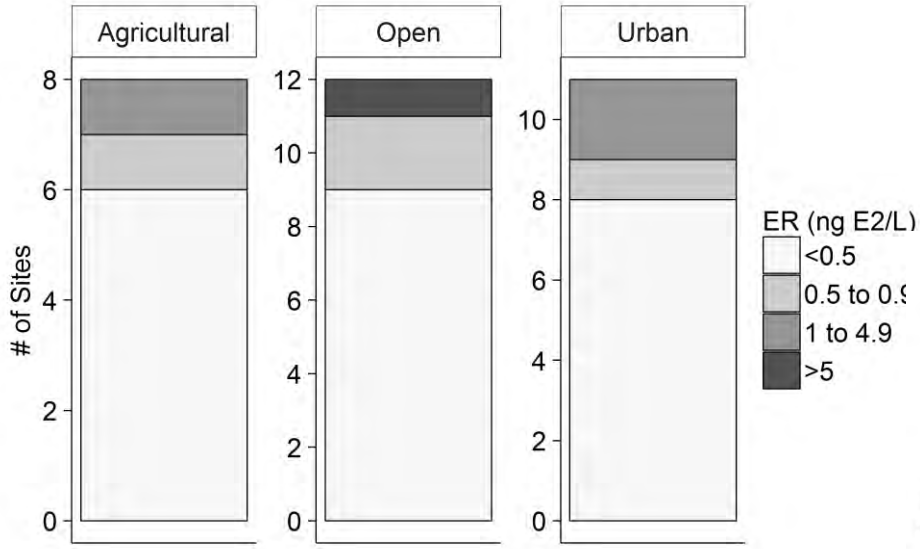
- Integrates the response of all known and unknown chemicals with a common mode of action
- Light intensity is proportional to the concentration of bioactive chemicals
- Cell responses can be linked to whole organism toxicity

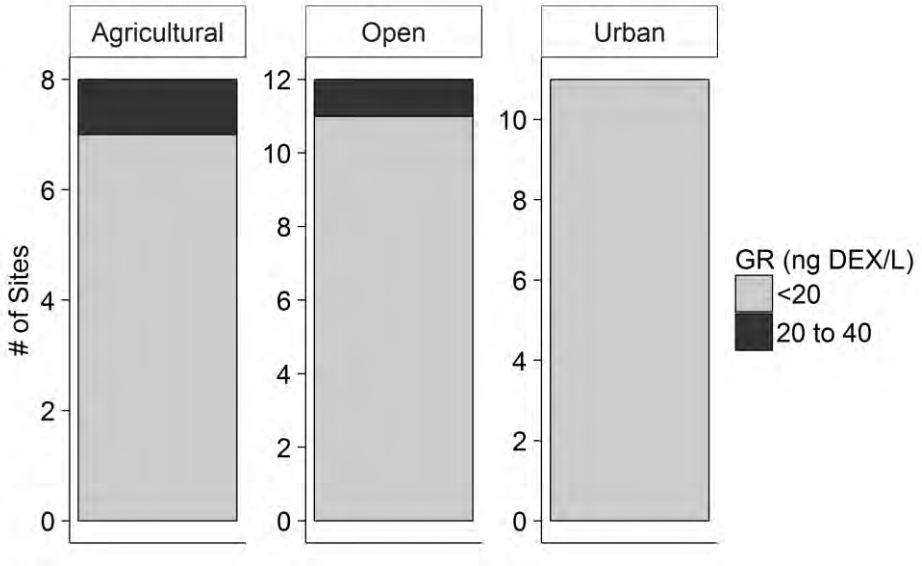


Low endocrine response in SoCal streams

Water collected in 2015 from 33 streams screened for estrogenic, androgenic and glucocorticoid steroidal activity







Bioscreening serves as a proxy for exposure…

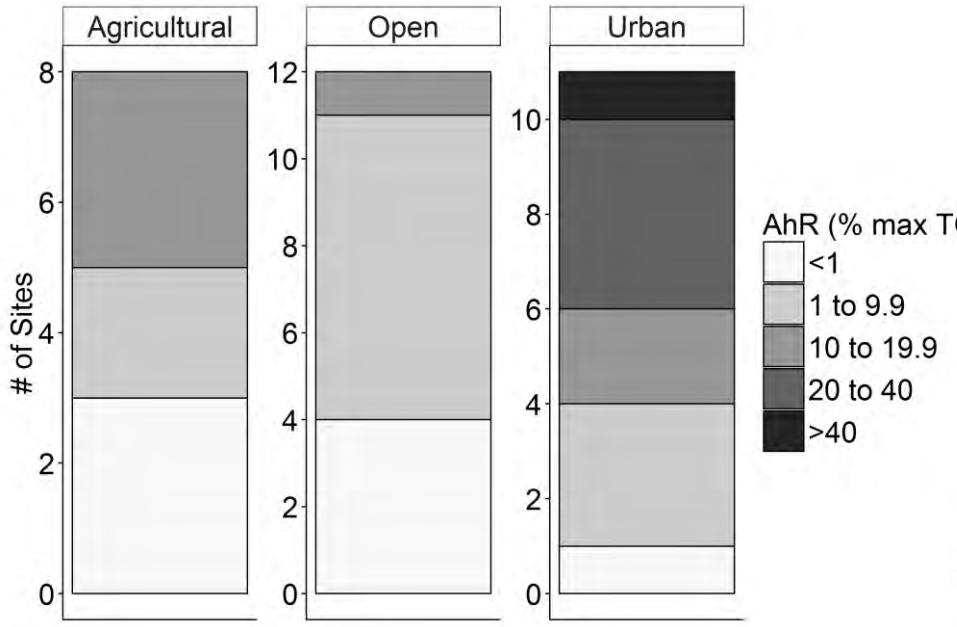
Station ID	Bioscreening (ng E2/L)	LC-MS/MS (ng E2 /L)
114RR0898	BDL: <0.38	BDL: <0.5*
Riverfront	BDL: <0.38	BDL: <o.5*
Mirabel	BDL: <0.38	BDL: <o.5*
Piner Creek	BDL: <0.38	BDL: <o.5*
114LY0010	BDL: <0.38	BDL: <o.5*
Santa Rosa Cr	BDL: <0.44	BDL: <o.5*
Lab Blank	BDL: <0.44	BDL: <o.5*
Field Blank	BDL: <0.44	BDL: <o.5*
114LY0010-Dupl	BDL: <0.44	BDL: <o.5*
WWTP#1 Effluent	BDL: <0.52	BDL: <o.5*
WWTP#2 Effluent	1.90	0.6**

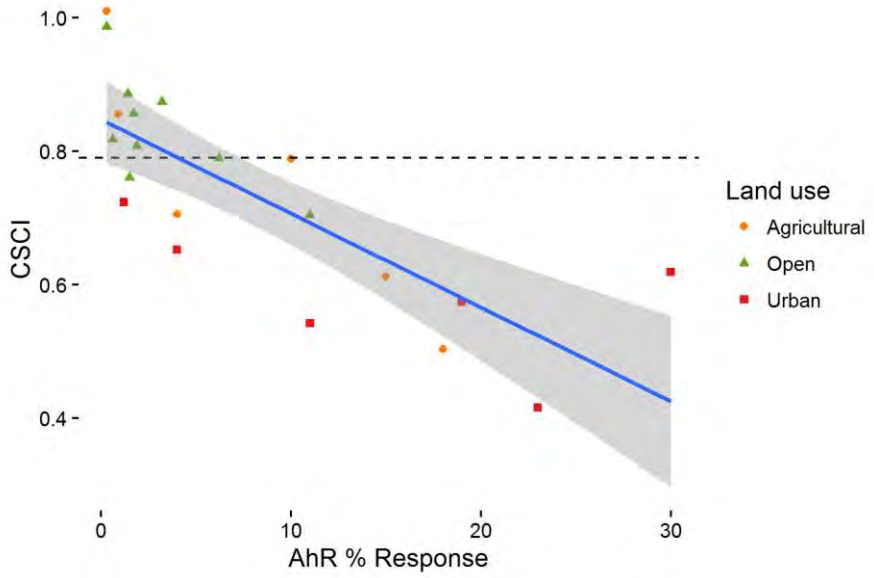
- Estrogen bioscreen applied to Russian River water samples
- Measures total estrogens, expressed as equivalent concentration
- *Bioscreening results in agreement with analysis of known estrogens*

* Concentration of estrone ≤ 0.56 ng/L

** Concentration of estrone was 11 ng/L (“CEQ” ~ 1.1 ng/L)

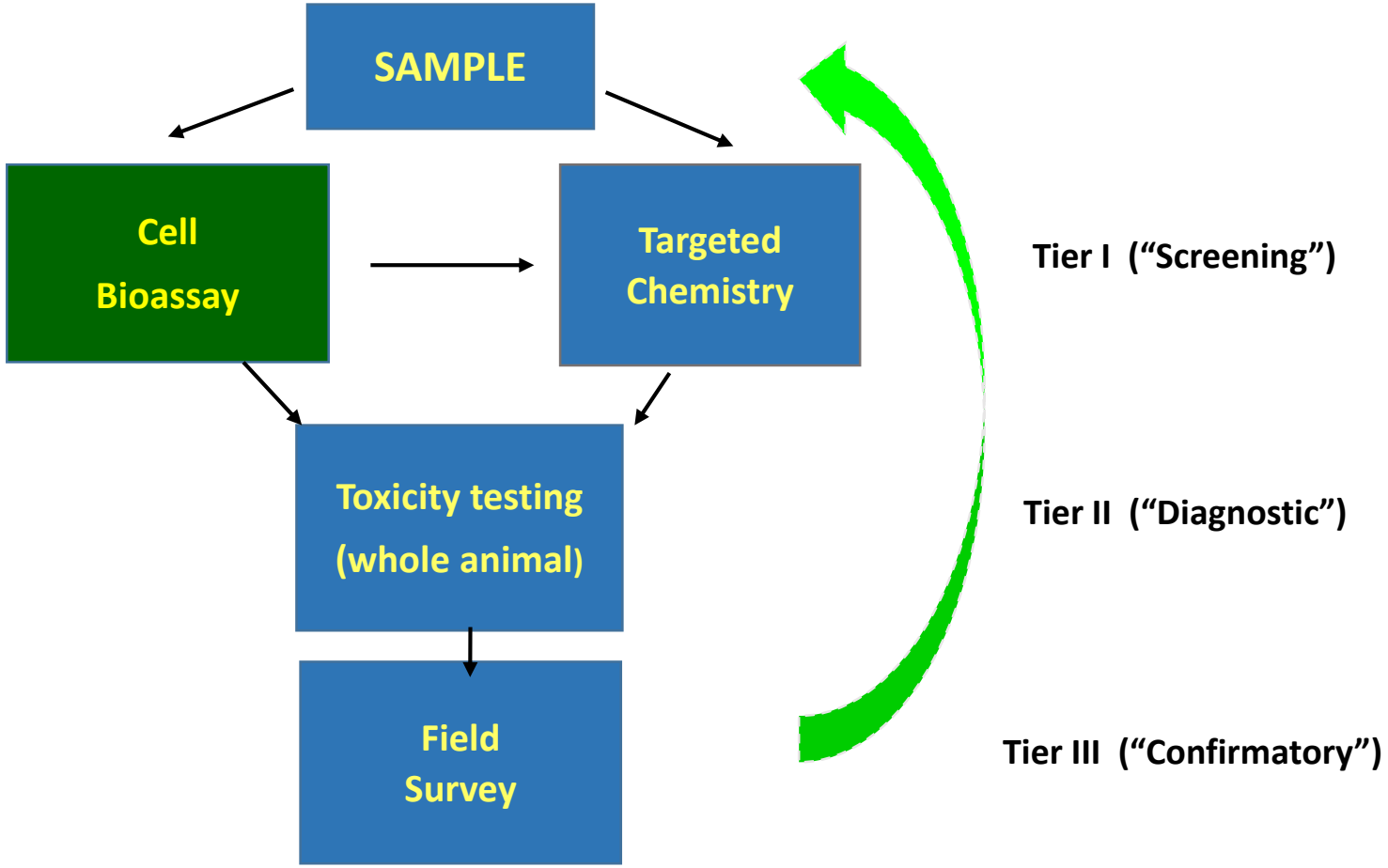
AhR bioactivity reflective of land use





CSCI – index of benthic community condition
AhR % Response – aryl hydrocarbon receptor cell assay response

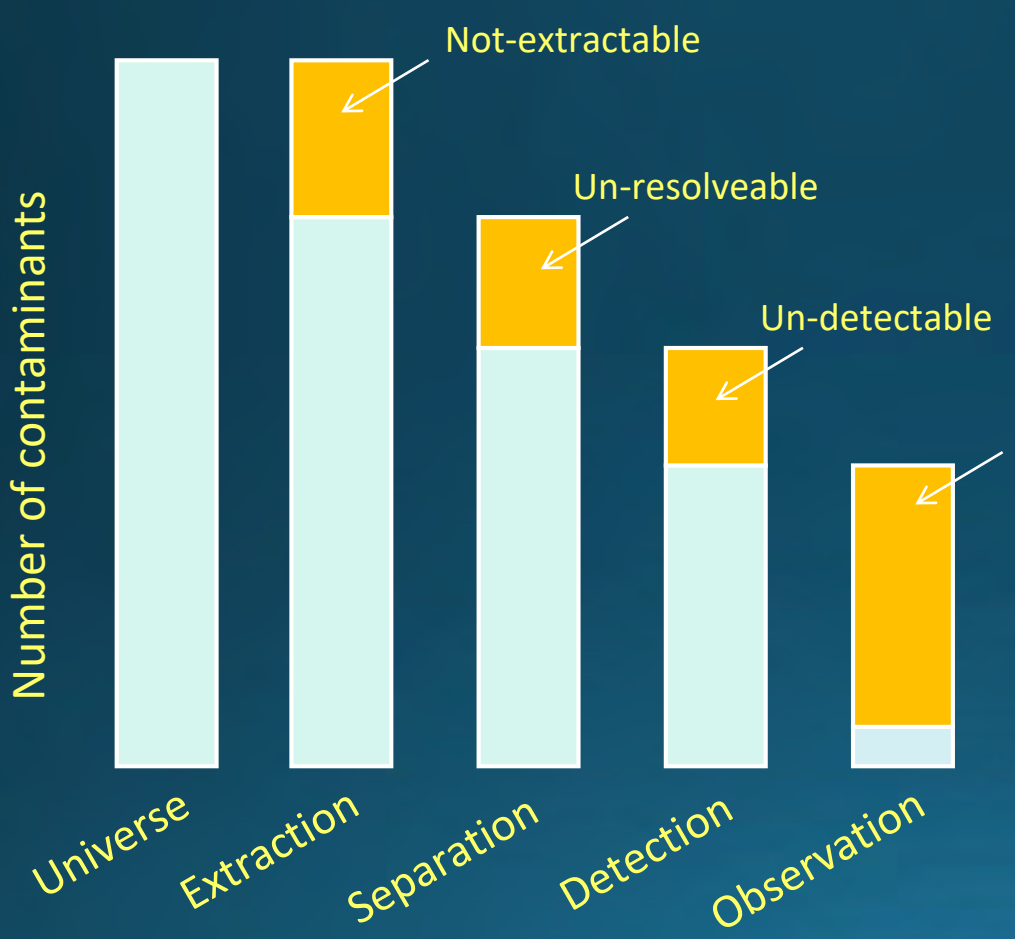
Cell assays screen for a larger suite of CECs that informs which chemicals to analyze and which toxicity tests to run, resulting in greater monitoring coverage and efficiency. This is known as "*effects directed analysis*"



Does targeted chemistry explain biological activity?

	Glucocorticoid Receptor (GR) Transactivation Assay		
	Mean BEQ	Sum CEQ	% BEQ explained
WWTP Effluent	90	0.52	0.6
O3 treated	61	0.49	0.8
MicroFiltration	65	0.46	0.7

Develop non-targeted analysis to identify bioactive chemicals



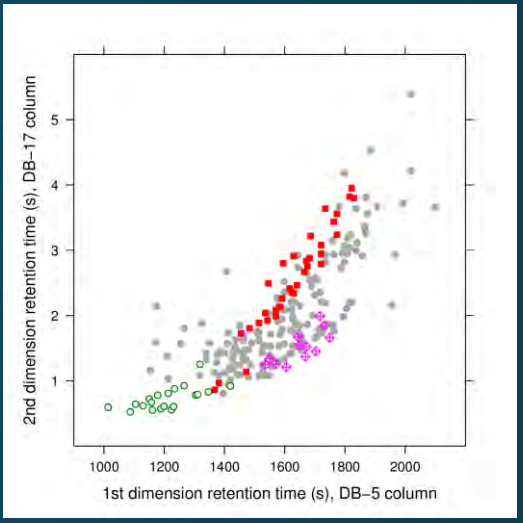
Uses latest instrumental technology

Generates large amounts of data

Requires high degree of expertise

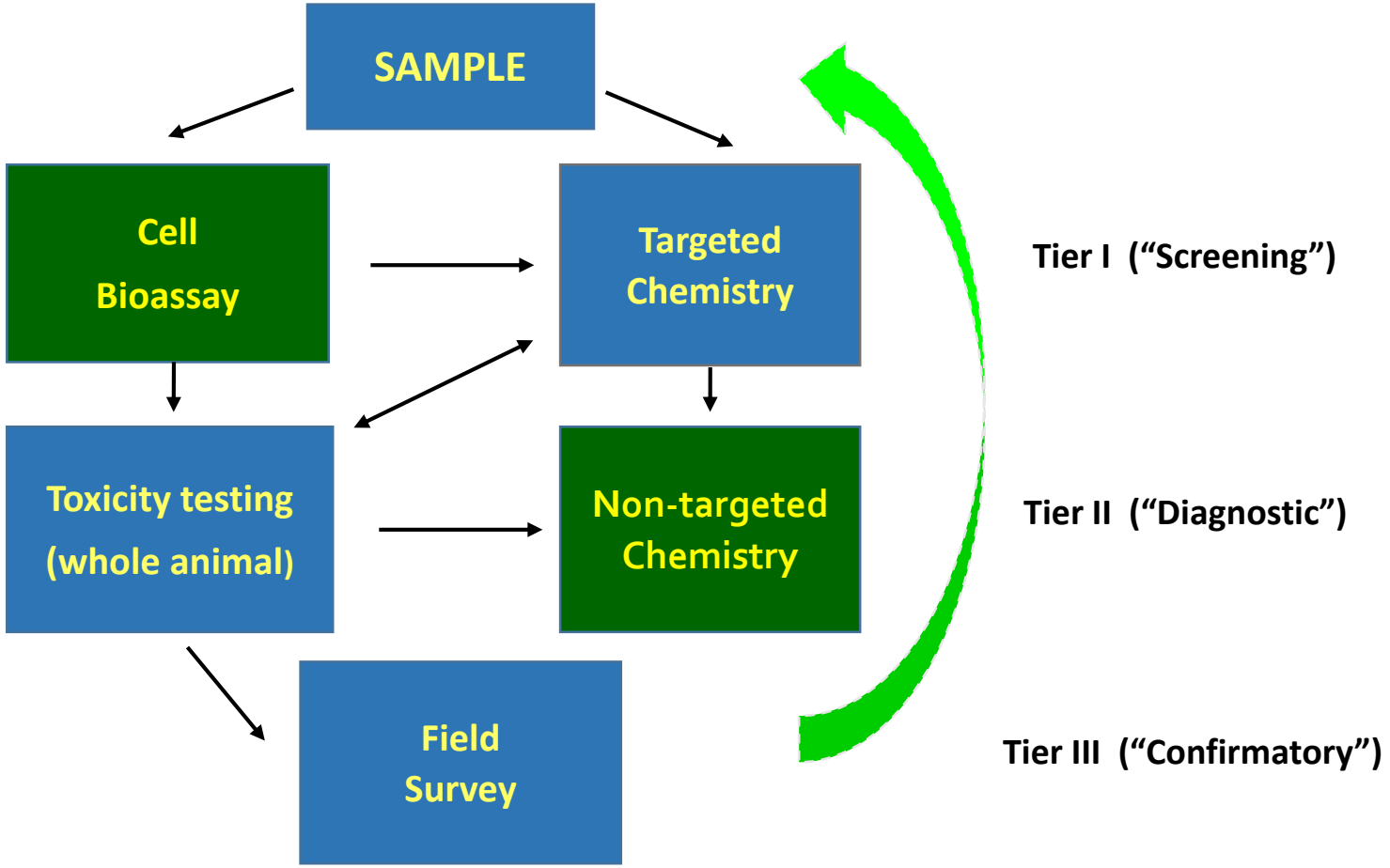
NTA fingerprinting

- Leco Pegasus 4D GCxGC-TOF/MS
  - Dimension 1: Rtx-5MS
  - Dimension 2: Rxi-17
- Rank and group SoCal stream samples by AhR response
  - control group (2 open, 1 urban) with non-detectable AhR response
- Greatest no. compounds in urban samples w/highest AhR response
- *Results are preliminary*

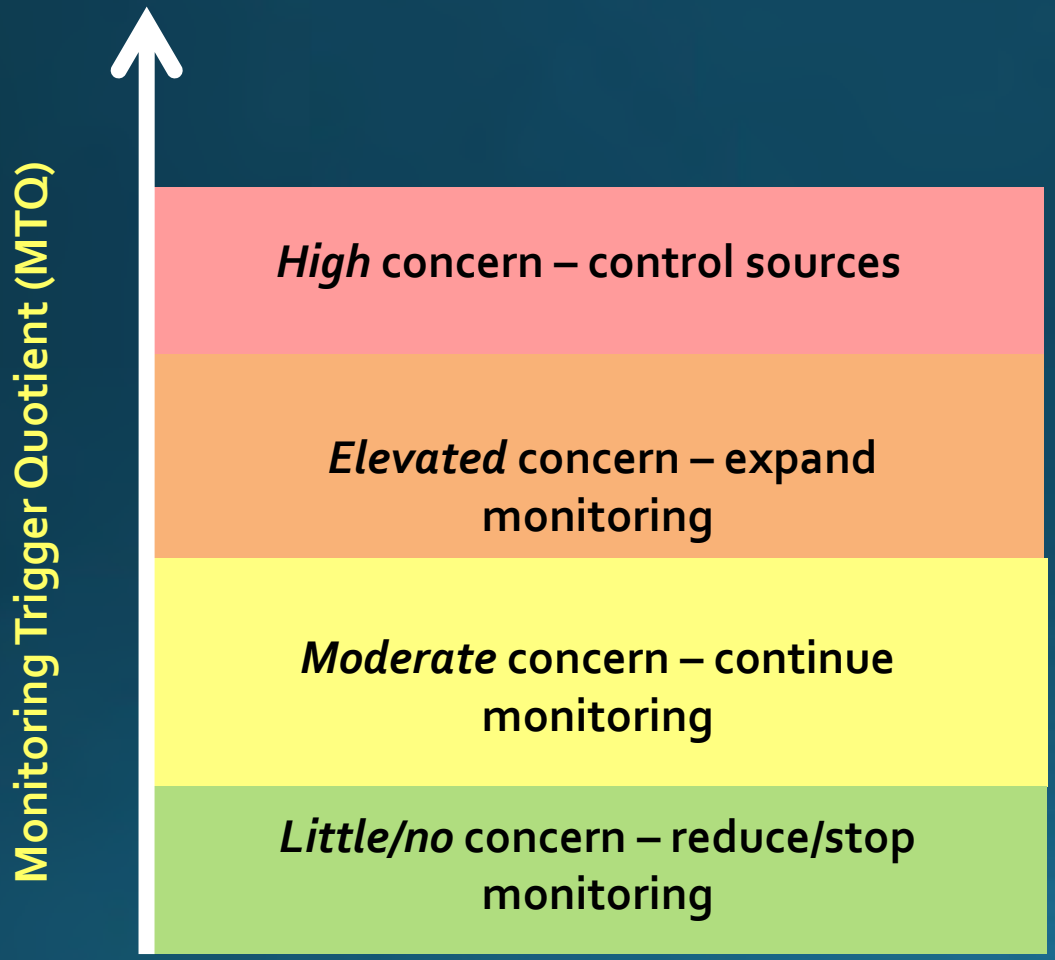


Group	AhR Range (% of max)	No. Cpds
1 (all urban)	28-49	280
2 (urban, ag)	15-23	24
3 (mixed)	8.4-11	65
4 (mixed)	4.0-6.7	2

Non-targeted analysis can identify toxicants that are missed by targeted analysis



Adaptive Decision-Making



- SFB RMP has been monitoring CECs since mid-90s
- Identify CECs of interest using "BPJ"
- Perform targeted monitoring (e.g. PBDEs)
- Adaptively manage using tiered response scheme

Limitations

- **The number of bioscreens standardized for water quality is *limited***
  - Our current toolbox has 5 endpoints that are ready for piloting
- **The number of labs performing bioscreening is *small***
  - Mostly academic/research
  - Utilities have had some success
- **The number of bioscreening thresholds is *even smaller***
  - Only one (estrogen receptor) has a credible threshold

Limitations (cont.)

- **Threshold development requires *investment* in *science***
  - coupling molecular, cellular and organism responses via AOPs
  - national and international efforts in progress
- **NTA is expensive, time-consuming and requires expertise**
  - spectral data analysis is tedious
  - rapidly emerging field
- **Sample collection, isolation and concentration is cumbersome, slow**
  - Direct analysis will be a major breakthrough
  - Passive sampling looks promising

Moving forward

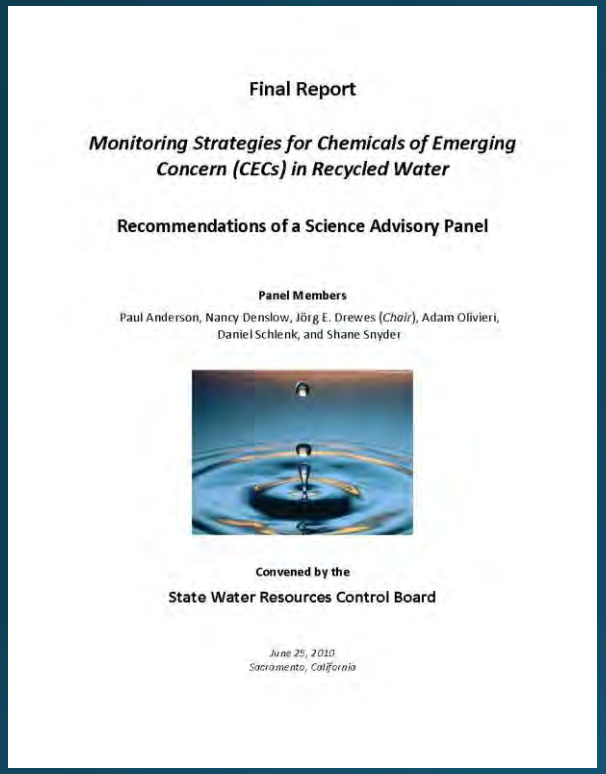
BIOSCREENING (AND NTA) *ENHANCES*, AND DOES NOT REPLACE EXISTING MONITORING TOOLS!

- Targeted chemical analysis
- (Whole animal) toxicity testing
- Field monitoring/bioassessment

Future Work

- Compile and assess first round of pilot data
  - what results were expected? unexpected?
- Expand the current bioscreening toolbox
- Establish bioscreening thresholds via linkage studies
  - identify best animal, in silico models
- Provide education and training for bioscreening tools
- Develop, optimize and standardize NTA methods
  - identify bioactive unknowns and establish databases
- Develop more efficient sample introduction methods

Questions?


Final Report

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

Recommendations of a Science Advisory Panel

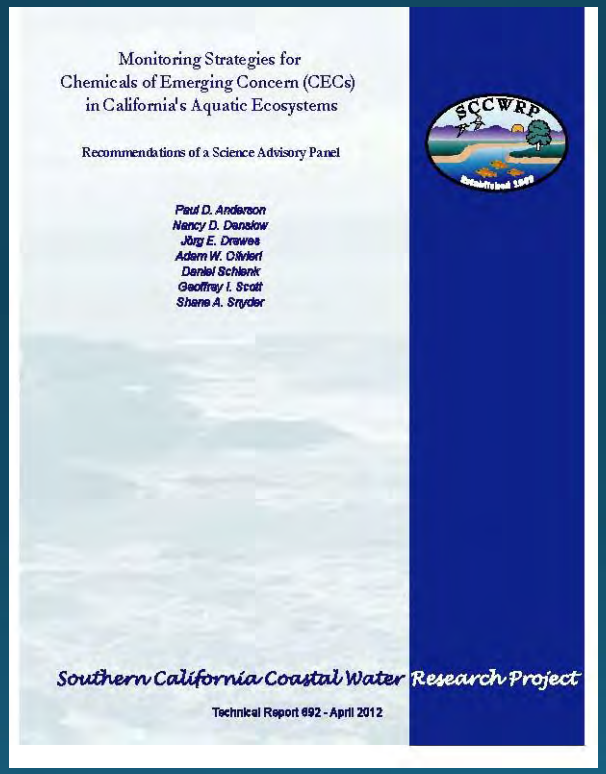
Panel Members

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


Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems

Recommendations of a Science Advisory Panel

Paul D. Anderson
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Jörg E. Drewes
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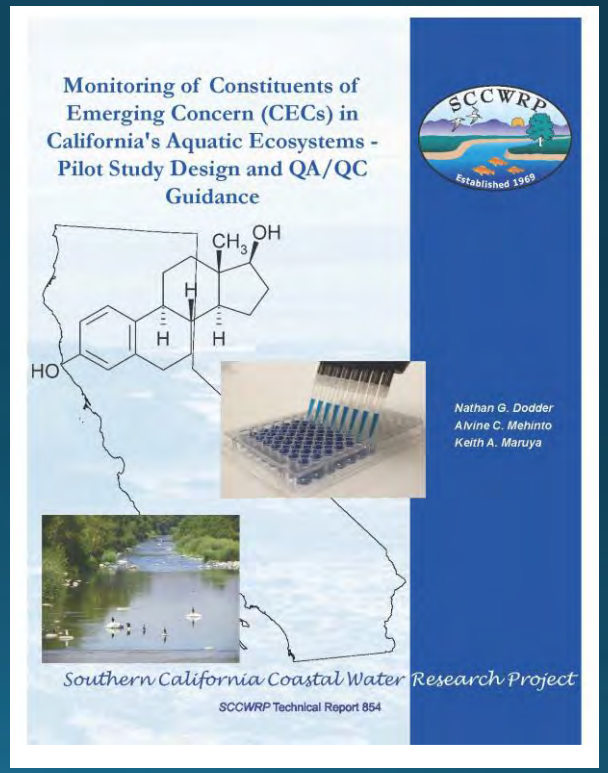
Daniel Schlenk
Geoffrey I. Scott
Shane A. Snyder

SCCWRP Established 1969

Southern California Coastal Water Research Project

Technical Report 692 - April 2012


Monitoring of Constituents of Emerging Concern (CECs) in California's Aquatic Ecosystems - Pilot Study Design and QA/QC Guidance

SCCWRP Established 1969

Nathan G. Dodder
Alvine C. Mehinto
Keith A. Maruya

Southern California Coastal Water Research Project

SCCWRP Technical Report 854

- keithm@sccwrp.org
- sccwrp.org/ResearchAreas/Contaminants

SCCWRP's "bioscreening" toolbox

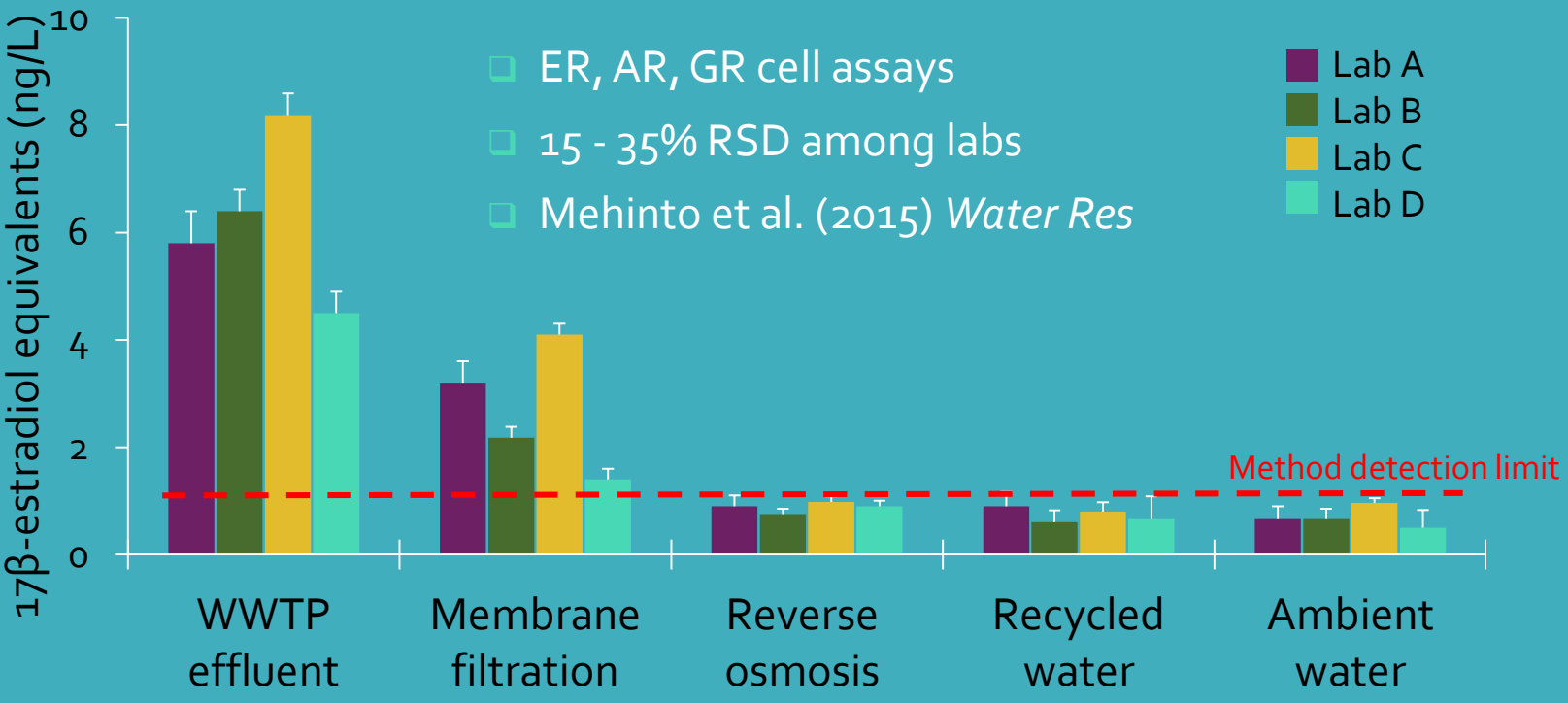
ENDPOINT	SIGNIFICANCE
Estrogenicity (Estrogen Receptor or ER)	Impaired reproduction, feminization of males
Androgenicity (Androgen Receptor or AR)	Impaired reproduction, masculinization of females
Glucocorticoid Activity (Glucocorticoid receptor or GR)	Impaired development, immune diseases
Aryl Hydrocarbon Receptor (AhR)	Dioxin-like toxicity, cancer, tissue damage
Tumor suppressor protein response element (p53RE)	DNA damage, mutagencity, cancer
Peroxisome proliferator activated receptor (PPAR)	Metabolic disoders, impaired immune function, cancer

…and guidelines for data quality*

QA/QC Criteria	Description
Background	Media, solvent blank response shall be ≤ 15% of lowest sample response
Cell Viability	Samples shall not cause > 20% cell mortality (corrected for background)
Calibration	Dose-response curve for reference toxicant shall be linear ($R^2 > 0.99$).
	Continuing calibration shall be within 10% of mean initial calibration response.
Test Response (Spiked sample)	Test response of sample spiked with reference toxicant shall be within 50-150% of expected response

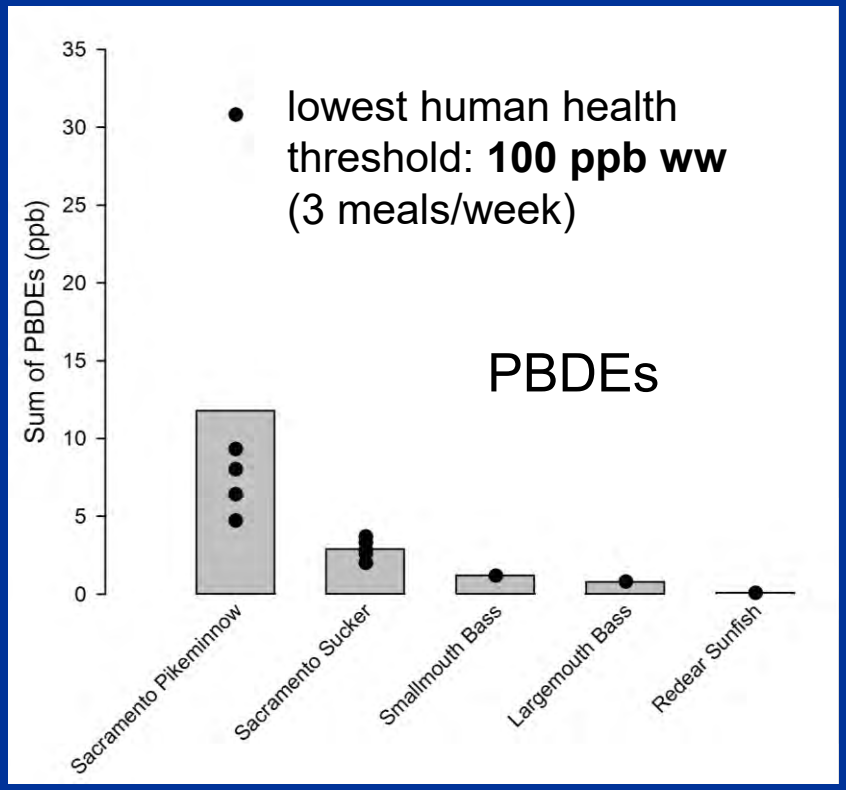
Mehinto et al. (2015), *Wat Res*

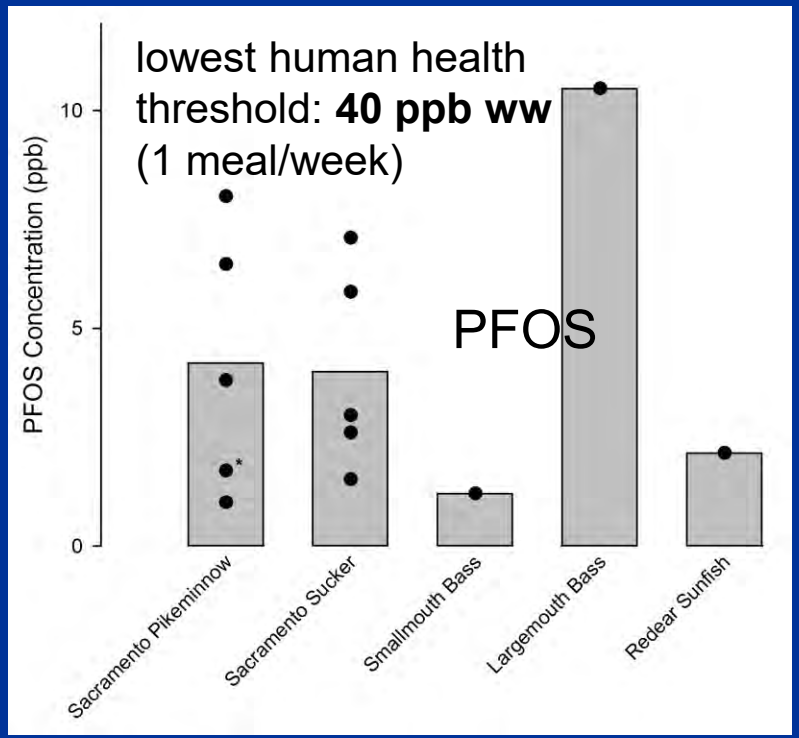
Good precision among labs is achievable



CECS IN FISH TISUE – RUSSIAN RIVER

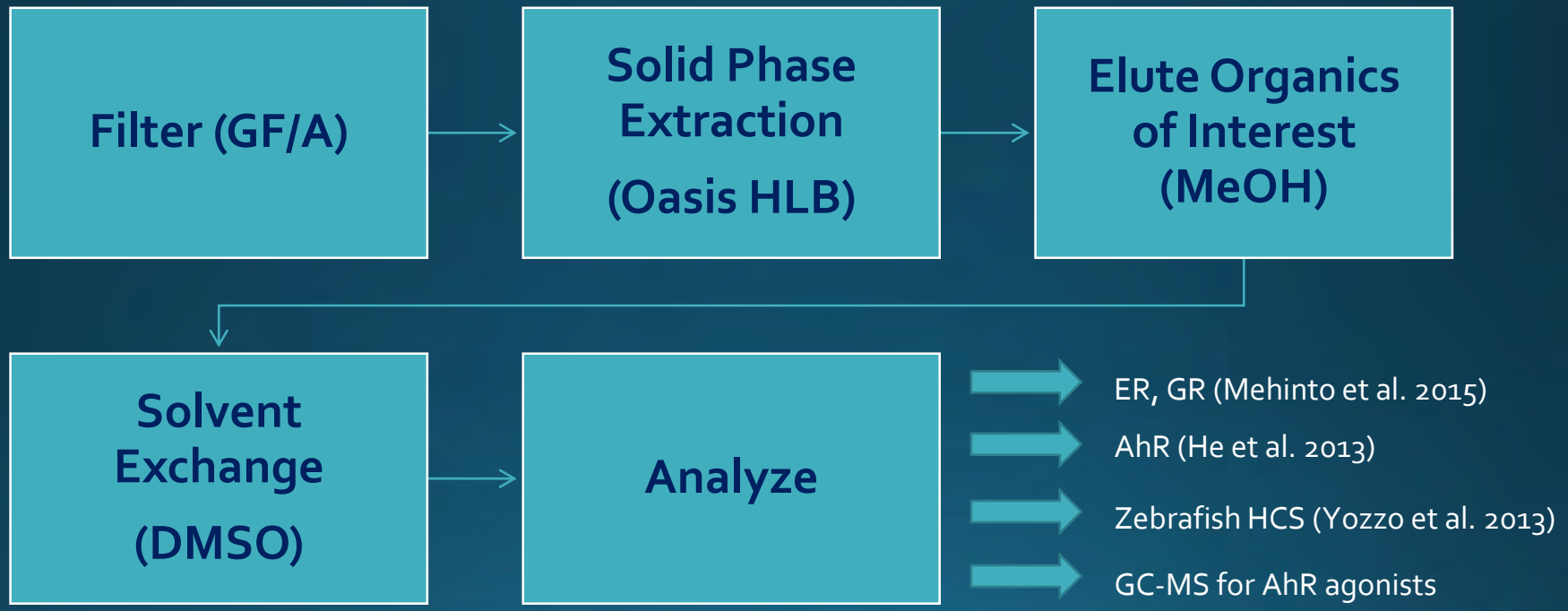
- Fish (multiple species) collected in August 2015 in 5 sections of the River
- Tissue composited by species and location analyzed for PBDEs, PFOs
- ***PBDEs, PFOs levels were < available human health thresholds***





CEC Initiative

Sample processing & analysis



Targeted monitoring is a short term fix

Scenario	WWTP Effluent	Storm Water (MS4)	Effluent Dominated River	Coastal Embayment		Ocean Outfall	All Scenarios
Matrix	Aqueous	Aqueous, Sediment	Aqueous	Aqueous	Sediment	Sediment	Tissue
Bis(2-ethylhexyl) phthalate	O	NA	NA	NA	NA	M	NA
Butylbenzyl phthalate	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 -99	E F O	M	NA	NA	M	M	M
PFOS	E F O	M	NA	NA	M	M	M