Developing statewide guidance for for CEC monitoring – Lessons learned

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SCCWRP

Established 1969

Building consensus

- Provide recommendations based on sound science
  - Find the very best experts
  - CEC Expert Panel, ECWG Advisors

- Encourage (no Demand!) stakeholder engagement
  - Wide representation of interests
  - Invitations to speak/address Panel at public meetings

- Adhere to an open and transparent process
  - Publicize and host public meetings
  - Communicate status/findings for each milestone
  - Seek out peer, stakeholder review of products

Statewide vs. regional needs
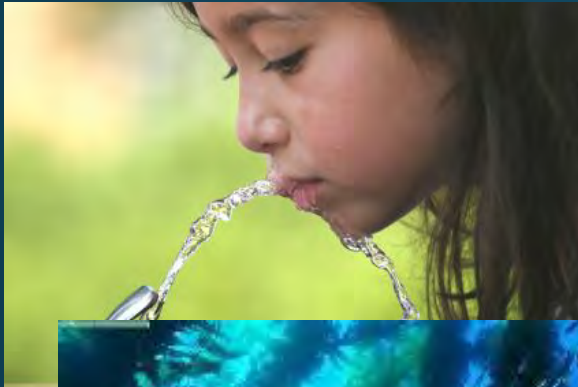
Does the list of CECs change across regions? Land Uses? Dominant sources?

Should biological methods employ indigenous/regionally relevant species?

How low is low enough?

How do we assess mixture toxicity?

*How do we ensure <u>robust</u> monitoring datasets?*

Employ standardized methods

- Data must be comparable across waterbodies, regions

- Data must meet minimum performance criteria
    - Detectability: 10X lower than MTLs
    - Precision/Repeatability: ±20% among reps, or over time
    - Accuracy: ±20% of "true" value

- Data must be usable (translatable) by/for managers
    - Parameters with units amenable to threshold comparison (e.g. concentrations or "BEQs")
    - Translatable into centralized databases
    - Accompanied by relevant metadata

Cell "Bioscreening" Assays

- **Genetically engineered cells**: cultured or used directly from frozen, seeded in multiwell plates

- **Samples**: extracts in solvent, prepared using standard SPE protocols

- **Light intensity**: activation or inhibition of a specific receptor measured based on intensity of light produced
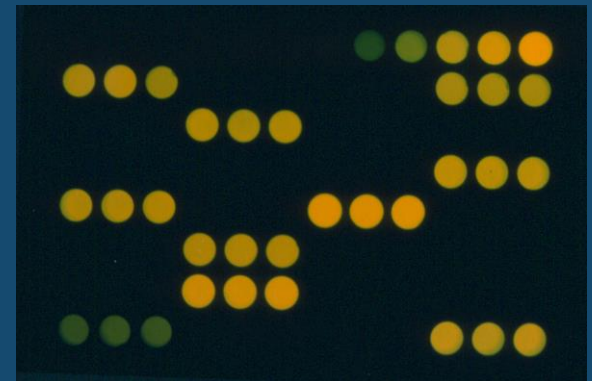
Bioscreening assays integrate exposure & bioactivity


CEC

Transcription

mRNA

protein
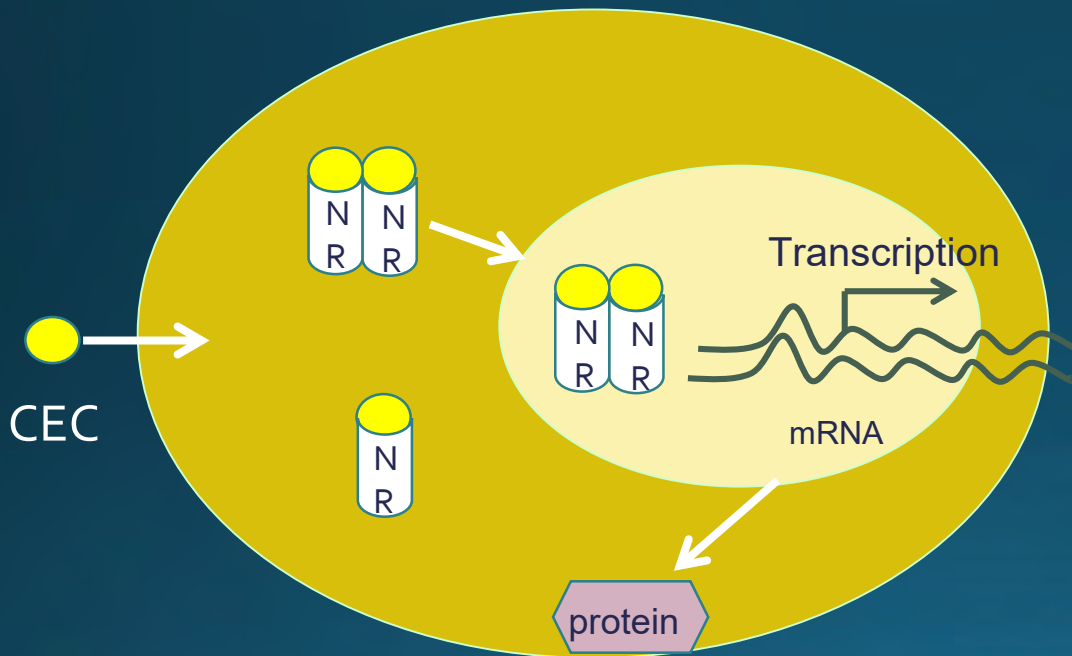
NR= nuclear receptor

- Initiates or propagates genetic interaction leading to a biological response of interest

- Acts through specific receptors
  - Stable vs. transient transfection

- Integrates exposure & bioactivity of chemicals acting via a common *mode of action*,
  - E.g. environmental estrogens

…that can be linked to adverse outcomes
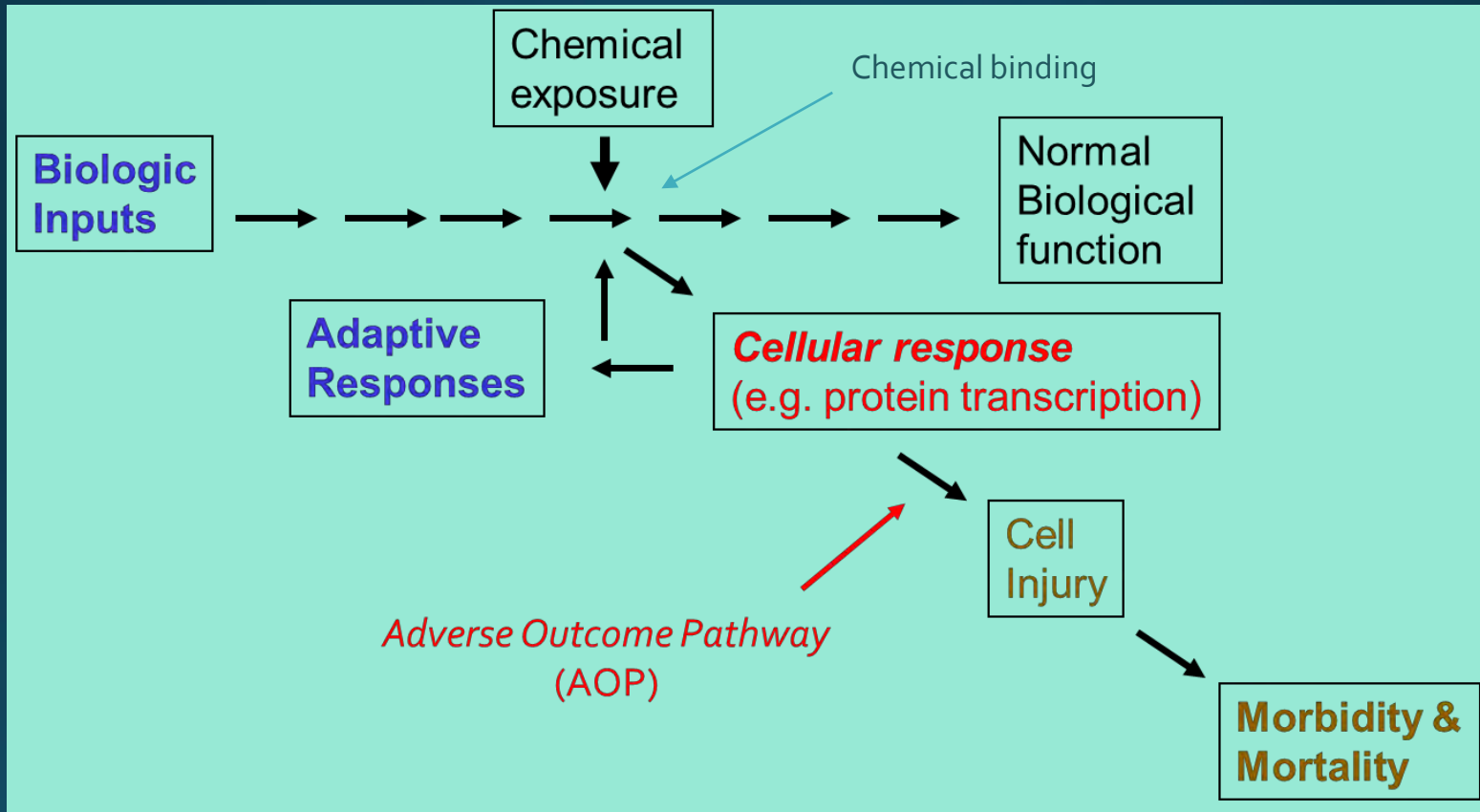
Standard Protocol for Estrogen Bioscreen

Gene-Blazer HTK Division Arrested ("Freeze & thaw") Cells from Life Technologies

Day 1

Cell count

Plate cells @ specific density

Add diluted extracts

Incubate overnight at 37°C, 5% CO_2

Day 2

Add substrate

Incubation (~2 hrs) at room temperature

Measure fluorescence

Export data to spreadsheet calculator

Sample result "BEQ" (ng/L)

Mehinto et al. 2016, 2016. video available on www.sccwrp.org

Bioscreening performance criteria

QA/QC Criteria	Description
Background	Media, solvent blank response $\leq$ 15% of lowest sample
Cell Viability	> 20% cell mortality (corrected for background)
Calibration	Linearity of dose-response curve (R^2 > 0.99).
	Continuing calibration within 10% of initial response.
Sample Response	Serial dilutions (n=4) must show step response
Matrix Spike	Spike sample withing 50-150% of expected response

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

Good precision <u>among</u> labs is achievable

ER, AR, GR cell assays

15 - 35% RSD among labs

Mehinto et al. (2015) *Water Res*
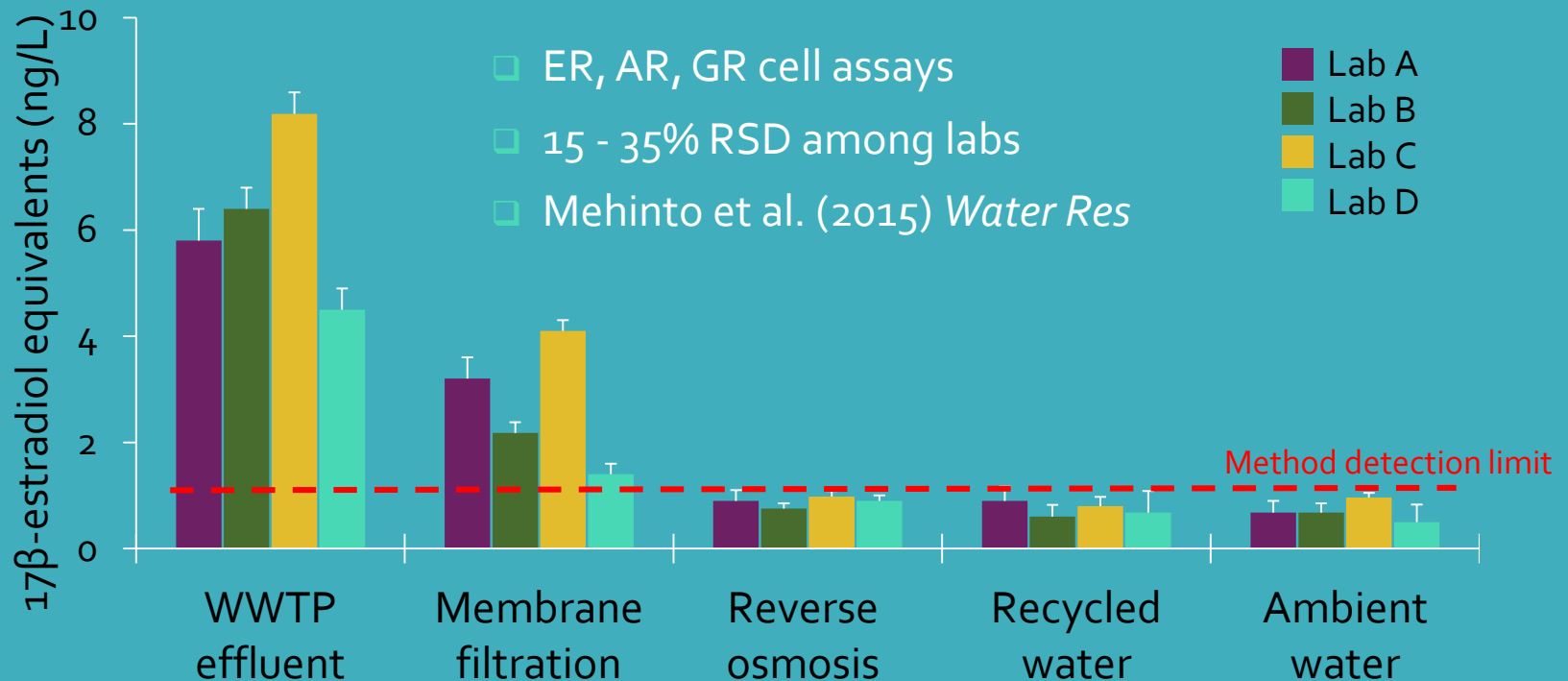
Relevant Cell Receptor Assays

In Vitro Assay	Chemicals Screened For	Potential Adverse Effects
Estrogen receptor (ER)	Estrogens, bisphenols, alkylphenols	Impaired development and reproduction
Androgen receptor (AR)	Musks, phthalates, pesticides	Impaired reproduction, cancer
Glucocorticoid receptor (GR)	Glucocorticoids	Development, immune diseases, diabetes
Progesterone receptor (PR)	Progestins	Cancer, hormone resistance syndrome
Aryl hydrocarbon receptor (AhR)	Dioxin-like chemicals, PAHs, pesticides	Cancer, liver toxicity, impaired reproduction

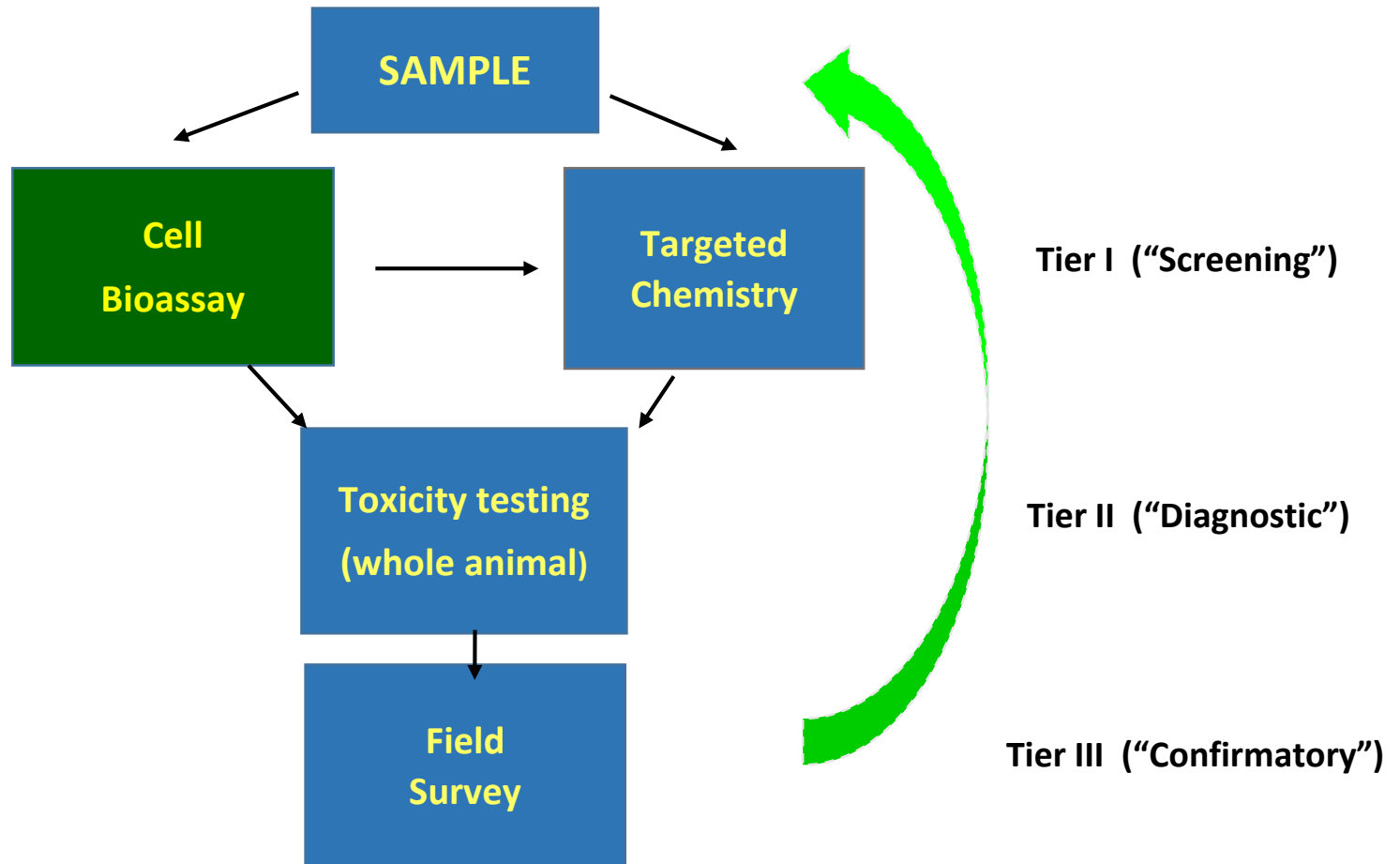# Bioscreening serves as a proxy for exposure...

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 <u>total</u> estrogens, expressed as equivalent concentration

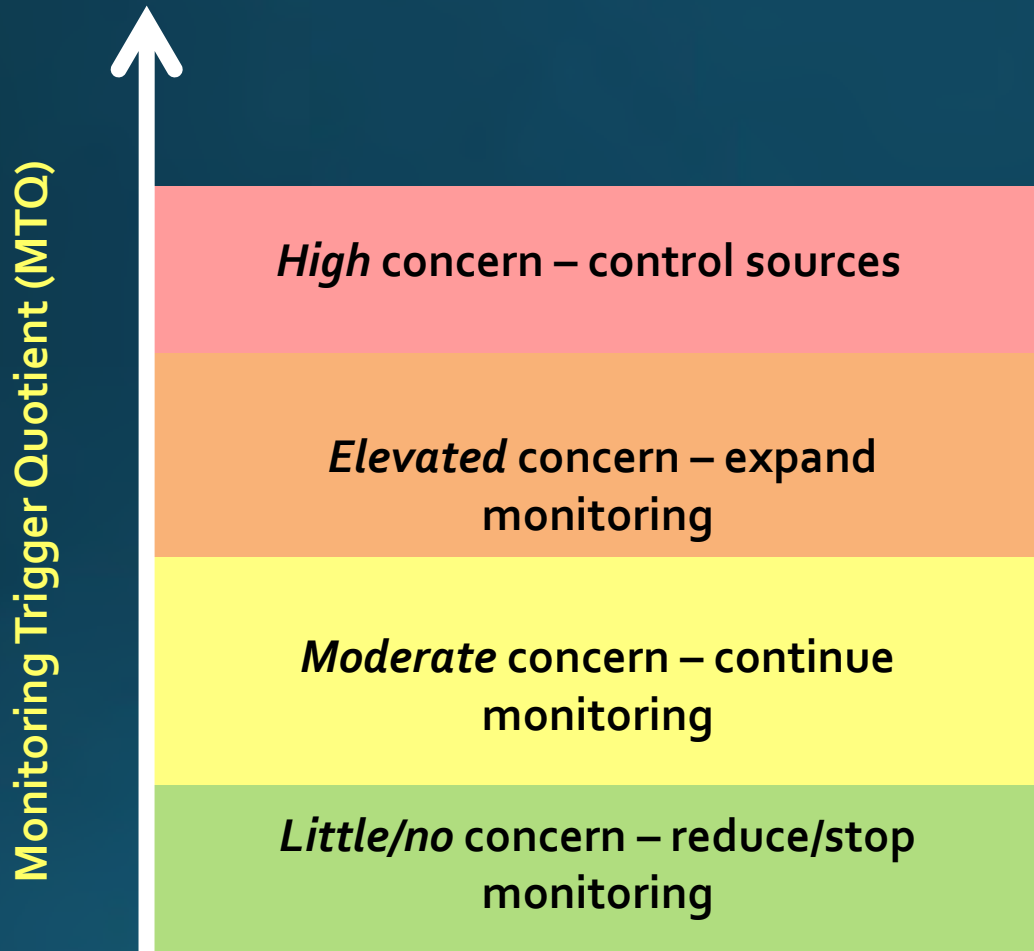- *Bioscreening results in agreement with analysis of known estrogens*

* Concentration of estrone $\leq$ 0.56 ng/L
** Concentration of estrone was 11 ng/L ("CEQ" ~ 1.1 ng/L)

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

Adaptive Decision-Making

Monitoring Trigger Quotient (MTQ) →

High concern – control sources

Elevated concern – expand monitoring

Moderate concern – continue monitoring

Little/no concern – reduce/stop monitoring

- 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

Questions?

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

Sample processing & analysis

Filter (GF/A) → **Solid Phase Extraction (Oasis HLB)** → **Elute Organics of Interest (MeOH)**

Solvent Exchange (DMSO) → **Analyze** →

- ER, GR (Mehinto et al. 2015)
- AhR (He et al. 2013)
- Zebrafish HCS (Yozzo et al. 2013)
- GC-MS for AhR agonists

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