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Cell Bioassays to Assess Chemical Mixtures in Waters

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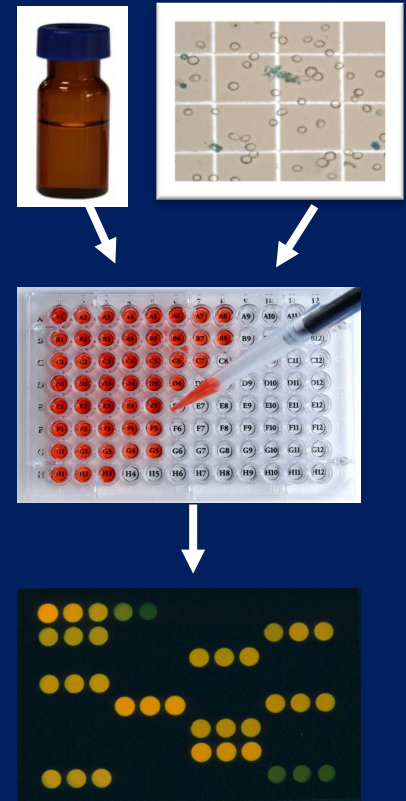


Novel Approach for Chemical Monitoring

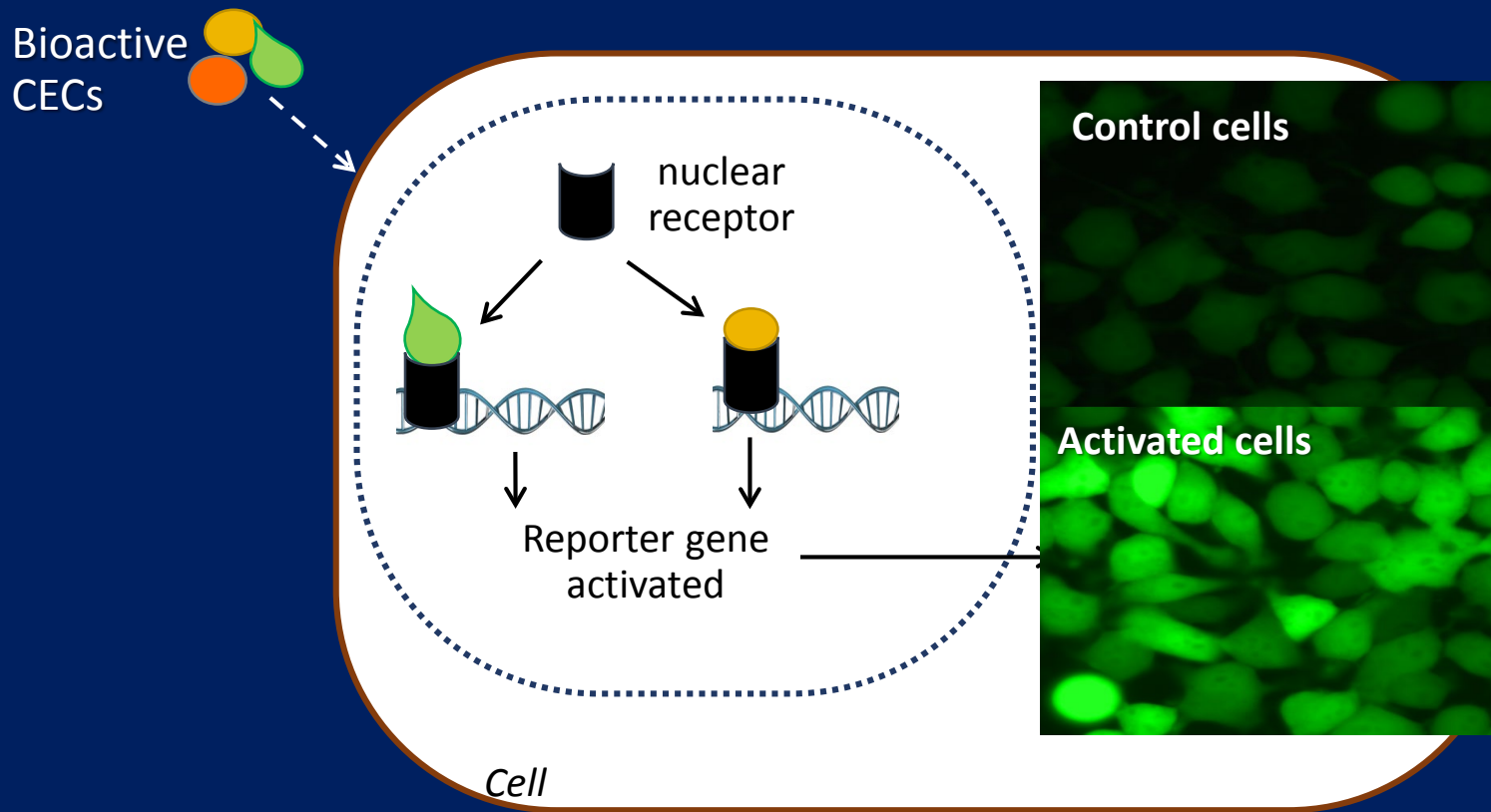
- Targeted analytical chemistry
 - Measure of contaminants prioritized by the State or EPA
- Non-targeted analytical chemistry
 - Broad chemical screening of all detectable chemicals
- Cell bioassays (or bioanalytical tools)
 - Integrated measure of known and unexpected bioactive chemicals

Cell Bioassays

- High-throughput method, with rapid turnaround
- Mammalian cells engineered to track cellular effects of chemicals
- Combined measure of all chemicals with same biological activity
- Results expressed relative to a reference chemical -bioanalytical equivalent concentration (BEQ), ng/L



Cell Bioassay Mechanism



Relevant Cell Bioassays

Assay endpoint	Chemicals screened	Associated risk
Estrogen receptor (ER)	Estrogens, alkylphenols	Impaired reproduction
Aryl hydrocarbon receptor (AhR)	Polychlorinated biphenyls, polycyclic aromatic hydrocarbons	Developmental anomalies and tumors
Glucocorticoid receptor (GR)	Anti-inflammatory steroids	Immune-related diseases
Thyroid receptor (TR)	Pesticides, bisphenols	Altered neurodevelopment
Peroxisome proliferator activ. receptor (PPAR)	Pharmaceuticals, phthalates	Metabolic disorders

Commercial Availability

- Several manufacturers with proprietary cell lines
- Full or partial kits:
 - Cells for culture (immortal) or ready to be plated (division-arrested)
 - Fluorescent/luminescence substrate
 - Recommended assay media and reference chemical
 - Instructions for cell handling, substrate addition and bioassay reading parameters

Cell Bioassays to Assess Water Quality

- Technology currently used for pharmacology, food industry and chemical registration
- Must be adapted for unknown chemical mixtures

What is the sensitivity of these assays?

Are the methods reproducible and transferable ?

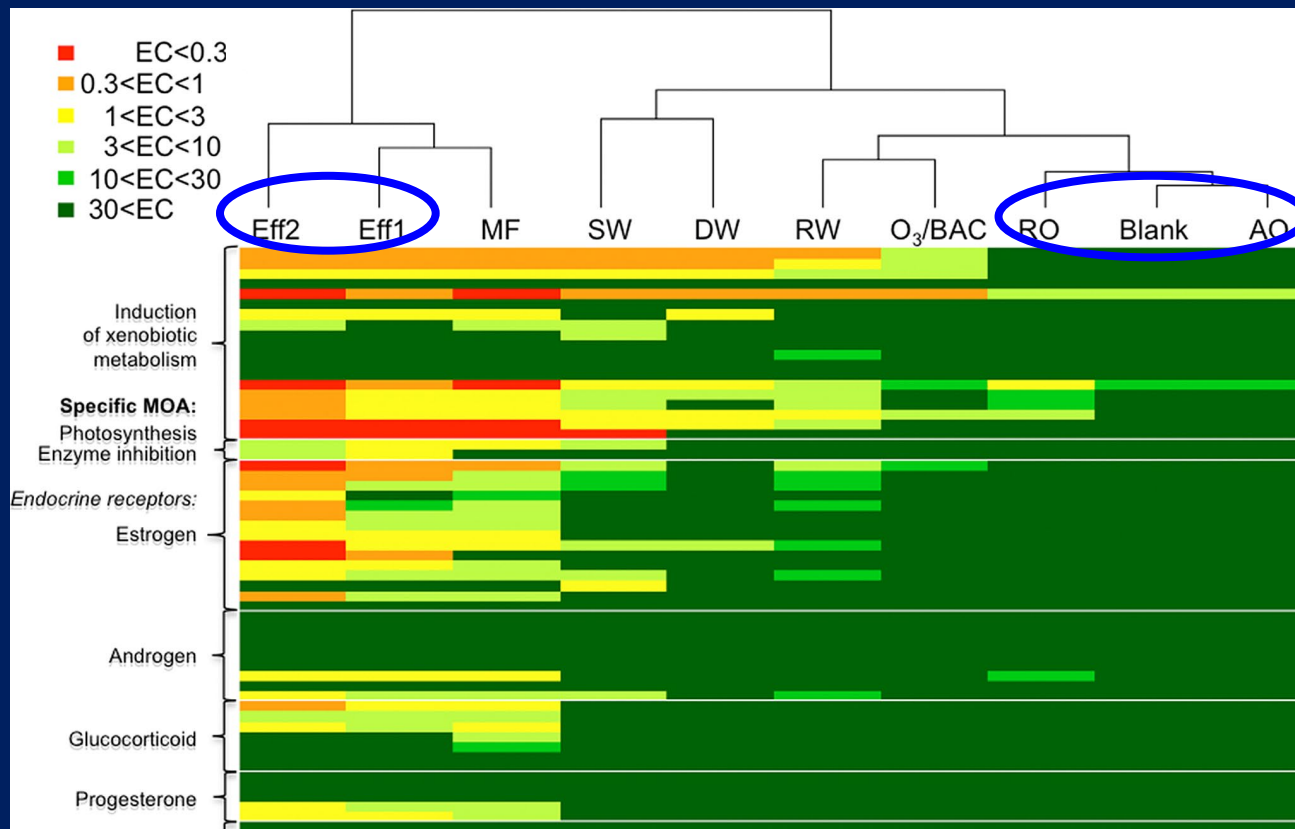
Do the patterns of responses make sense?

Evaluating Bioassay Sensitivity

- Bioassay responses should reflect level of treatment and/or amounts of chemicals in a sample
- Bioscreening analyses conducted on various sample types
 - Influent
 - Secondary and tertiary treated effluents
 - Advanced treated water (microfiltration, reverse osmosis, UV...)
 - Ambient water (stream, river, stormwater...)

Benchmarking Water Quality Is Possible

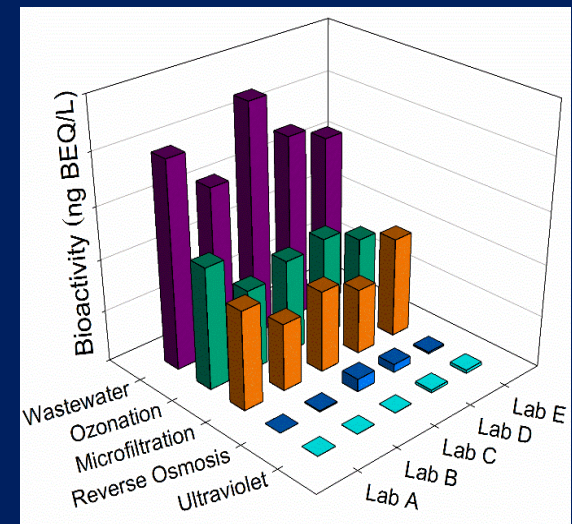
- 20 laboratories tested over 100 different bioassays



Standardizing Assay Protocols

- Performance-based criteria developed to ensure robustness and comparability of data
- Reproducibility of protocols demonstrated through inter-laboratory exercises

Parameter	Acceptance criteria
Cell viability	≥80% viability compared to control wells
Calibration	Hill slope, EC50, Z' within expected range, $R^2 > 0.95$
Matrix spike	Recovery of spiked chemicals between 70 - 130%
Precision	RSD/CV of triplicate measurements ≤30%



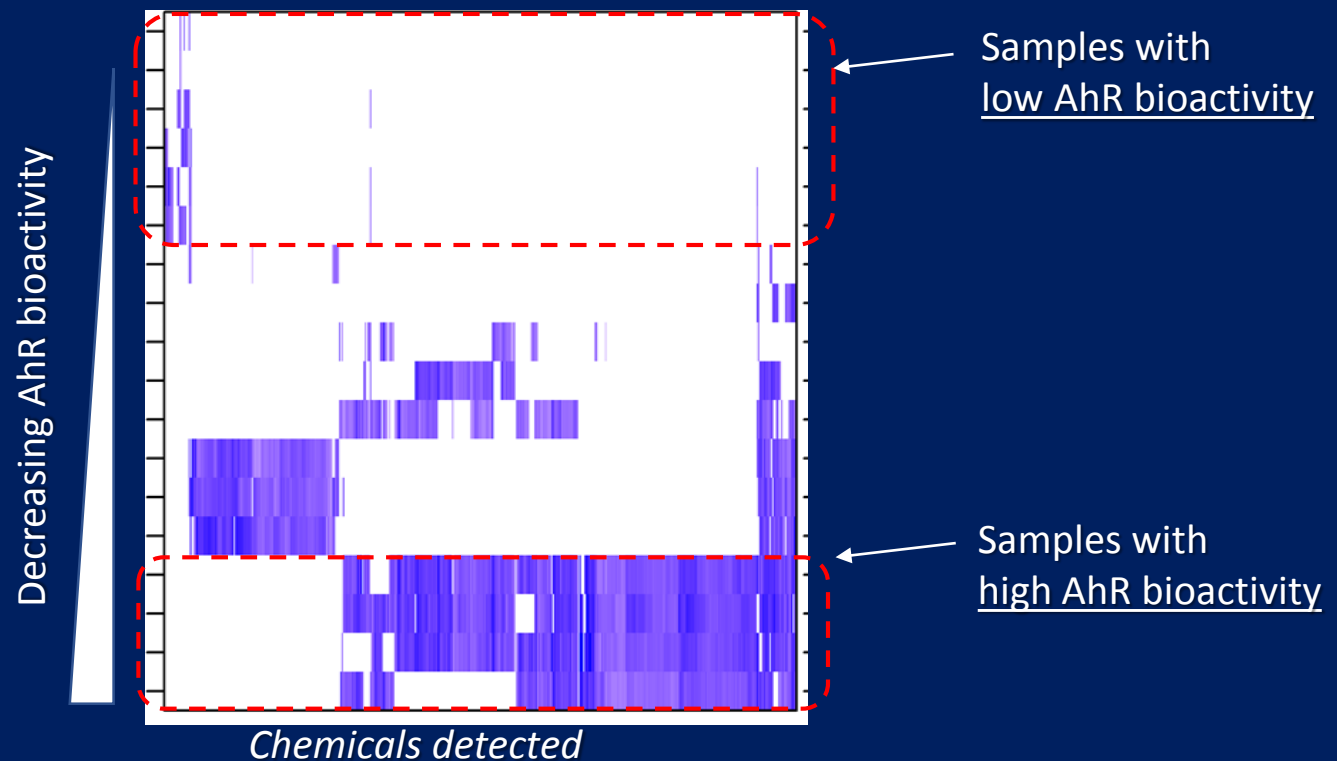
Explaining Measured Bioactivity

- Targeted analyses to measure known chemicals
 - Mass balance based on relative potency of individual chemicals
estradiol > estrone > bisphenol A > nonylphenol

	Santa Rosa	Mirabel	Piner Crk	Effluent
ER- BEQ (ng E2/L)	<0.5	<0.5	<0.5	1.9
Targeted chemical analyses (ng/L)				
17 β -Estradiol (E2)	<0.5	<0.5	<0.5	0.6
Estrone	<0.5	0.5	0.6	11
Bisphenol A	16	<10	55	12
4-Nonylphenol	63	25	53	247

Explaining Measured Bioactivity

- Non-targeted MS analyses to measure unexpected chemicals
 - Promote discovery of emerging chemicals



Transitioning Cell Bioassay to Water Quality Agencies

- Results are encouraging
 - Bioscreening patterns are indicative of water quality
 - Standardized bioassay protocols exist for a handful of endpoints
- Next steps
 - Develop better testing guidelines (from sample collection to data analyses)
 - Conduct interlaboratory exercises to assess lab proficiency and bioassay comparability
 - Establish relevant bioscreening thresholds for data interpretation

Bioanalytical Implementation Advisory Group

- Convened by CA WaterReuse, led by NWRI
- Members include cell assay experts and stakeholders
- Goal is to produce a guidance document with detailed recommendations for:
 - Collection (incl. QA), preservation, storage
 - Extraction procedure
 - Samples plating instructions
 - Data acceptability criteria
 - BEQ calculation and data interpretation



Questions?



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Principal Scientist in Ecotoxicology

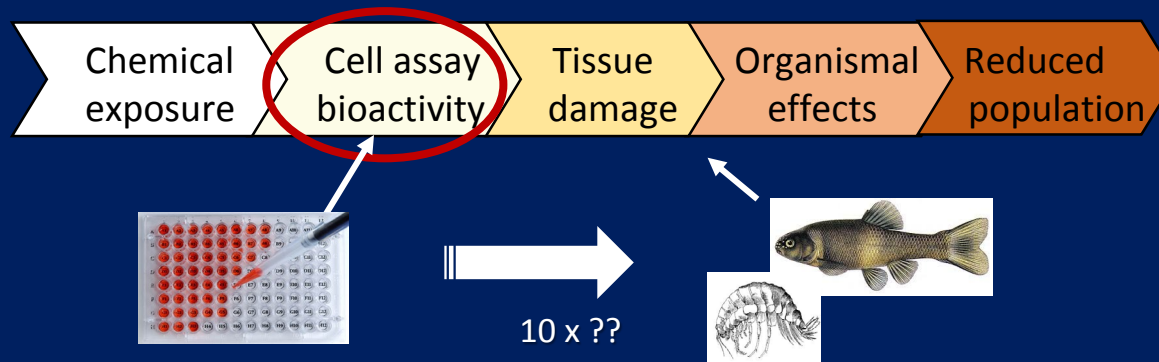
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Back up slides

Developing Bioscreening 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



Developing Bioscreening Thresholds

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