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Testing A New Framework to Screen for Chemicals and Infer Toxicity

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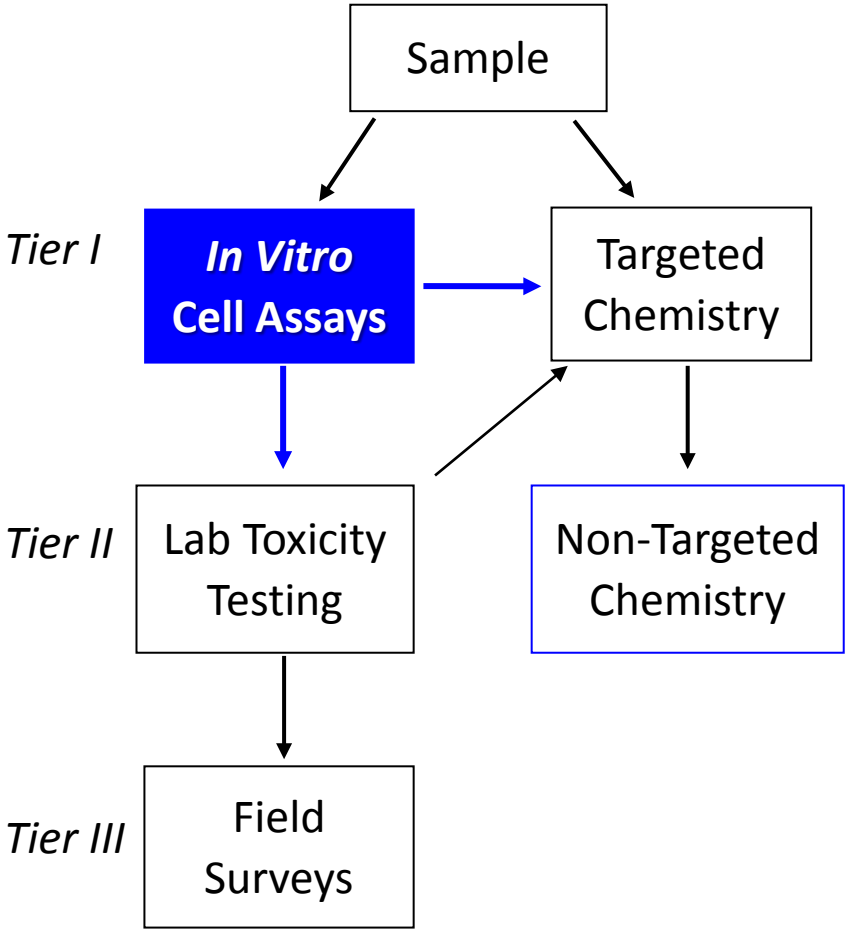
Challenges to Current Monitoring

- Too many chemicals to monitor
  - *Over 100,000 known chemicals*
  - *More discovered every year*

- No standardized analytical methods for unexpected/ unknonws incl. metabolites, byproducts

- Relevant toxicity data often unavailable
  - *Chronic sub-lethal toxicity is of concern*
  - *Toxicity potential of chemical mixtures understudied*

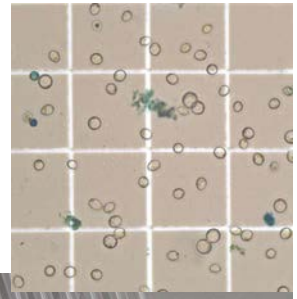
Effect-Based Monitoring as a Solution

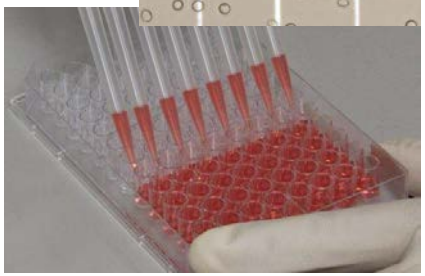


- New tools added to streamline and enhance existing monitoring methods
- Tier I bioscreening to:
  - *Narrow down list of chemicals to measure*
  - *Select relevant species and toxicity endpoints to examine*

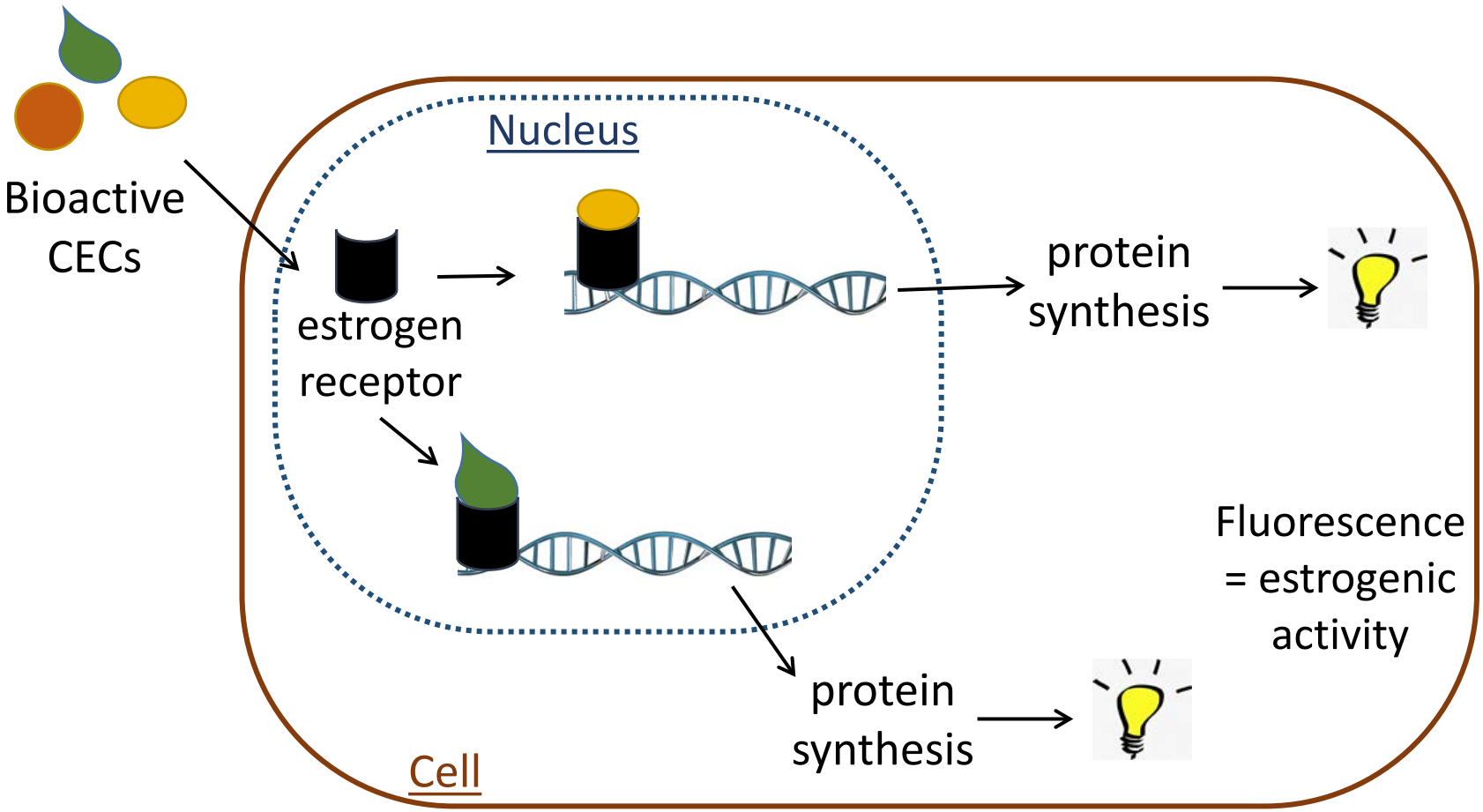
Cell Assays as Bioscreening Tools

- High-throughput methods with rapid turnaround
  - *Data available within days*
- Integrated measure of known and unknown bioactive chemicals with a common mode of action
- Results calibrated against a reference chemical
  - *Bioanalytical equivalent concentration (BEQ in ng/L)*
- Technology adopted by pharmaceutical, cosmetic and industrial companies to develop their products





Mechanism of Action



Development of Bioscreening Tools

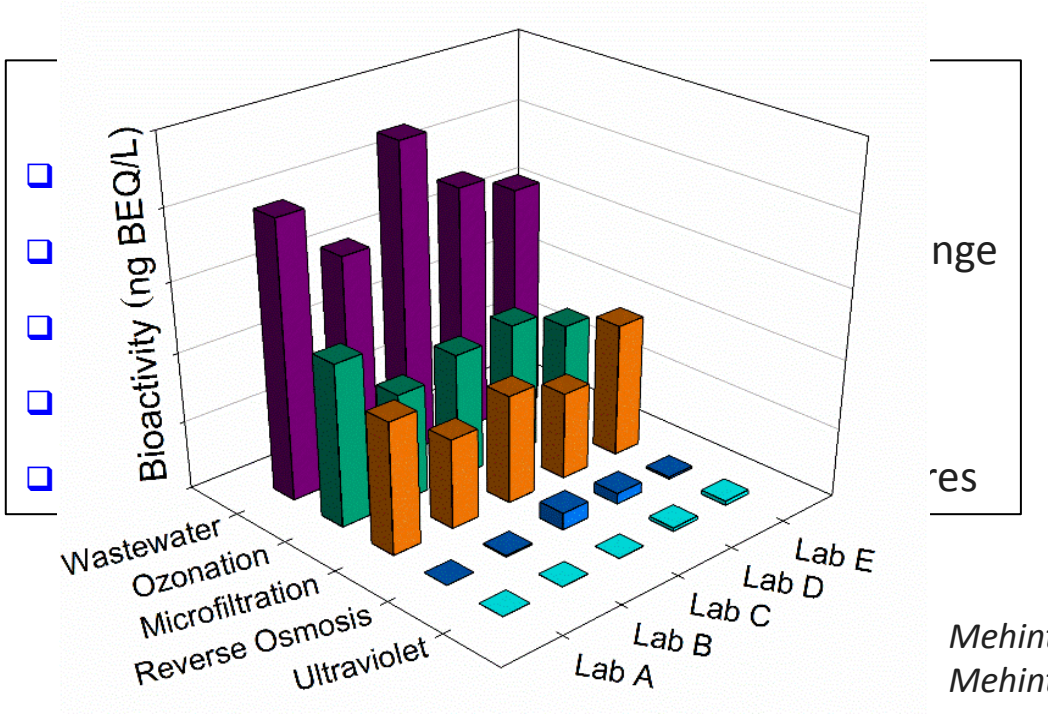
1. Identify promising cell lines and endpoints
2. Standardize protocols and evaluate performance
3. Develop effect thresholds to link cell assay responses to relevant toxicity outcomes
4. Conduct pilot evaluation to determine how results can inform management decisions

Endpoints of Interest

Assay endpoint	Chemicals screened	Potential toxicity
Estrogen receptor (ER)	Estrogens, alkylphenols	Feminization, reduced reproduction
Aryl hydrocarbon receptor (AhR)	Dioxin-like chemicals, PAHs, pesticides	Developmental anomalies, tumor
Glucocorticoid receptor (GR)	Anti-inflammatory steroids	Diabetes, immune diseases
Androgen receptor (anti-AR)	Musks, phthalates	Demasculinization
Thyroid receptor (TR)	Pesticides, bisphenols	Poor immune functions, metabolic disorders
Acetylcholine receptor (AChR)	Neonicotinoid and other pesticides	Neurotoxicity, altered behavior

Standardization of Protocols

- Standardized protocols exist for a handful of assays
- Robustness of protocols demonstrated using QA/QC criteria
- Data comparability through interlab exercises



Mehinto et al. 2015. Water Res. 83
Mehinto et al. 2016: JoVE 118

Evaluating Cell Assays Performance

Can we use bioscreening tools to reliably identify contaminated samples?

- ER, AhR, and GR bioactivity
- Nearly 100 sites sampled across California
  - *Water, sediment and/or fish collected at each site*
- SPE extraction within 72 hours of collection using Oasis HLB columns; final extract in DMSO




Russian River Pilot Study (NorCal)

- Water and sediment collected
- ER bioactivity in WWTP effluent only
- Good agreement with targeted chemistry (E2-estradiol; E1- estrone)
- Potential as a reliable measure of exposure

Site ID	ER-BEQ (ng E2/L)	LC-MS/MS (ng /L)
WWTP effluent	**1.90**	**E2: 0.6; E1: 11**
Riverfront	< 0.4	E2 <0.5; E1 < 0.6
Mirabel	< 0.4	E2 <0.5; E1 < 0.6
Piner Creek	< 0.4	E2 <0.5; E1 < 0.6
Santa Rosa Cr	< 0.4	E2 <0.5; E1 < 0.6
Field blank	< 0.4	E2 <0.5; E1 < 0.6

Study of SoCal Coastal Environments

Σ DDT related chemicals (ng/g)

	Sediment	Fish liver
San Diego	0.05	1,650
Palos Verdes	1,610	11,700

ER-BEQ (ng/g E2)

	Sediment	Fish liver
San Diego	0.3	3.3
Palos Verdes	1.3	90

- Sediment and fish collected
- PV – known contamination of DDT related chemicals, PCBs
- Cell assays in agreement with chemistry for both sediment and fish tissue

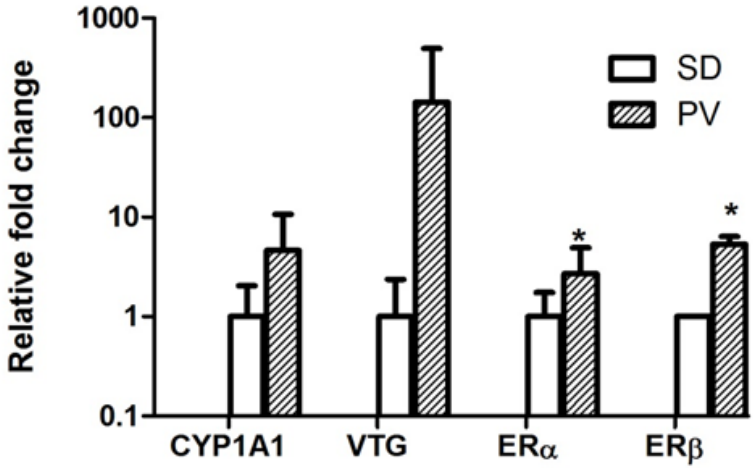
Crago et al. 2016: Environ Pollu 213

Study of SoCal Coastal Environments

ER-BEQ (ng/g E2)

	Fish liver
San Diego	3.3
Palos Verdes	90

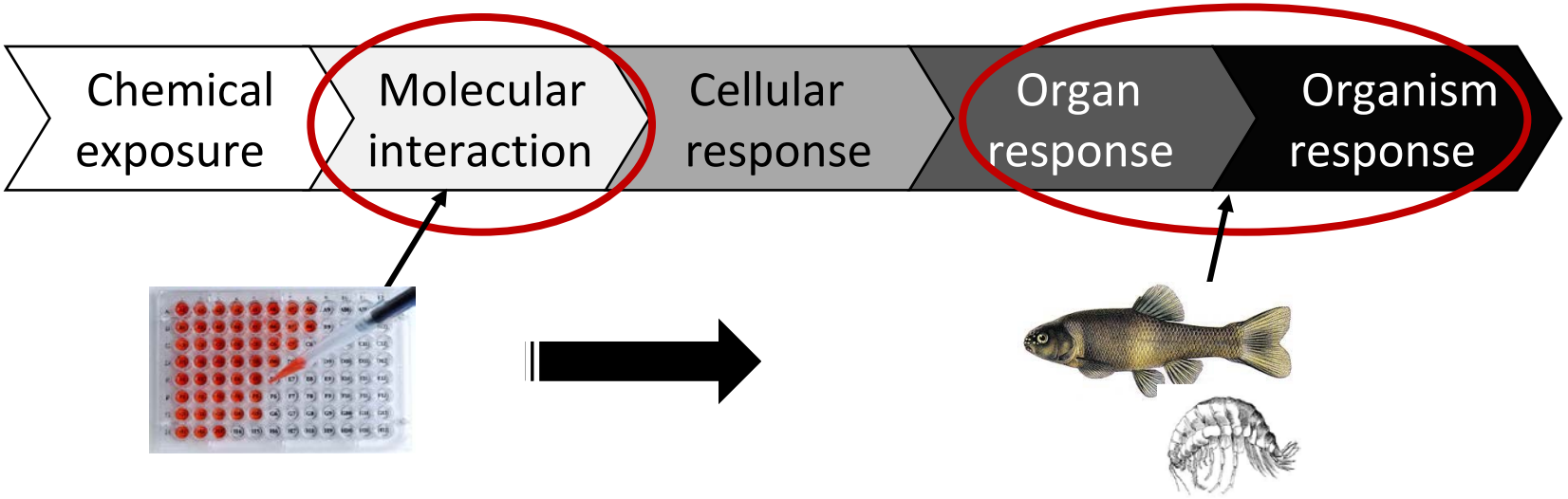
- Agreement between *in vitro* and *in vivo* ER responses
- Biomarkers of exposures were elevated in PV fish tissues



Crago et al. 2016: Environ Pollu 213

What Bioactivity Levels Are of Concern?

- Effect thresholds required to interpret cell assay results
- Linkage between cell assays and animal toxicity is key
- Approach based on Adverse Outcome Pathway (AOP)



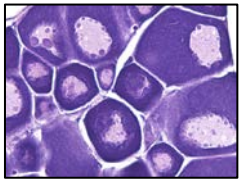
Linking Estrogen Bioscreen to Fish Toxicity

- Test samples
  - *Model estrogens incl. estradiol (E2), estrone, nonylphenol*
  - *Final wastewater effluent*

- Test species
  - *Inland silverside Menidia (larvae and juveniles)*
  - *Fathead minnow (adults)*

Estrogen receptor cell assay

Gene expression (qPCR assay)



Histology of gonads

Fish health (e.g. weight)

Linking Estrogen Bioscreen to Fish Toxicity

Chemical/ species	In vitro ER activity EC_{50}	Vitellogenin increase	Tissue feminization	Weight/ survival
Estradiol/ 	**1 X** (~ 20 ng E2/L)	**1 X** (18 ng/L)	**≤ 10 X** (180 ng/L)	**> 10 X** (180 ng/L)
Estradiol/ 	**1 X** (~ 20 ng E2/L)	**≤ 8.5 X** (170 ng/L)	**≤ 8.5 X** (170 ng/L)	**> 28 X** (550 ng/L)
WWTP effluent 	*NA* BEQ < EC_{50}	**No effect observed**	**No effect observed**	**No effect observed**

Lessons Learned

- Cell assays can serve as a proxy for exposure and improve our ability to identify contaminants of concern
- Establishing effect thresholds protective of aquatic life is possible
- Gene biomarkers can be useful indicators of exposure before more severe damages occur
- Pilot studies in different environments (e.g. stormwater, estuary) will help us determine the value and limitations of cell assays for monitoring

Questions?

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