Bioanalytical Tool Development

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Sources of Stressors

- Septic Tanks
- Groundwater
- Runoff (Urban, Agriculture)
- Industrial Discharge
- Sewage treatment Plants

Atmospheric Disposition

Natural Stressors
Five Day Embryonic Exposure to Organic Constituents in Tucson WWTP Effluent

DMSO Control

0.25X

1X

5X
Fish consumption advisories

(Ela et al. EHP, 2010)

What about our water?

(Flame retardants, Perfluorinated chemicals, Siloxanes)

(EPA, Fish consumption advisories)
Nearly All Substances are Detectable in Water

Chemical origins

Pharmaceuticals
Pesticides
Industrial chemicals
Natural chemicals
Transformation products
Personal care products
Household chemicals

Microbial origins

Anions
Cations
Metals

Bacteria
Viruses
Protozoa
Helminths

(slide from Shane Snyder)
TACKLING THE CHEMICAL UNIVERSE

CHEMCATS 65,768,974 Commercially available chemicals


http://www.cas.org/
Contaminants of emerging concern

Universe of Chemicals

Known Knowns

Known Unknowns

Unknown Unknowns

Mixtures
Concentration Addition Synergy
Transformation Products

Searching for unknowns in water

Chromatograms

Very Similar

Extraction of Molecular Features Reveals Almost 1,000 compounds in each chromatogram

Further Data Processing Requires Specific Software

(slide from Shane Snyder)
Analytical Chemistry VS Bioassay

Targeted Analytical
- Known compounds
- Quantitative
- Individual compounds

Mechanistic Bioassay
- Knowns/unknowns
- Semi-quantitative
- Synergism/Antagonism

(Slide from Shane Snyder)
Estrogen equivalency

Correlation between chemically estimated EEQs and bioassay EEQs (Bulloch et al., 2010).

$r^2 = 0.7888$
Biomarker sensitivity

Percent Measured Effect

Concentration of Toxicant X Time of Exposure

Molecular
Tissue
Population

nM uM mM

100 50 0

Reversible Irreversible
High throughput assays – InVitrogen

Estrogen or CEC → Receptor → Reporter

Signal

Human Cell Line

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96-well assay

17β - Estradiol dose response

Blue/green ratio (480/534 Ratio)

17β - Estradiol concentration (M)

1.0E-14 1.0E-13 1.0E-12 1.0E-11 1.0E-10 1.0E-09 1.0E-08 1.0E-07
Antagonist activity

GeneBLaZer ERα Assay - Agonist and Antagonist Assay

GAL

Bifulthrin dose response with or without 0.2nM 17β-Estradiol

0.2nM E2

Bifulthrin

Bifulthrin & E2

Estrate dose response with or without 0.2nM 17β-Estradiol

0.2nM E2

Estrate

Estrate & E2

4-Nonylphenol dose response with or without 0.2nM 17β-Estradiol

0.2nM E2

4-Nonylphenol

4NP & E2

Bisphenol-A dose response with or without 0.2nM 17β-Estradiol

0.2nM E2

BPA

BPA & E2

Activity
Signaling systems affected by BPA

- Androgen signaling
- Glucocorticoid signaling
- Estrogen signaling
- Adipogenesis
- Thyroid signaling

BPA (bisphenol A) interactions with various signaling pathways.
Linkage from bioassays to higher endpoints

- Development of Adverse Outcome Pathways

Key Events
Gene and protein changes

Molecular Biomarkers

Exposure 1 $\rightarrow$ R 1

Exposure 2 $\rightarrow$ R 2

Exposure 3 $\rightarrow$ R 3

Phenotype

Adversity at the Population level
Choice of species: *Menidia beryllina* (*silverside*)

- Sensitive estuarine fish – similar fish exist in CA
- Related to the topsmelt
- EPA assays used for regulatory purposes: early life stage (ELS) and juvenile stage (JS)
Menidia gonadal differentiation

Gonadal differentiation between day 50 - 60 post hatch

Oogonia

Undifferentiated

Spermatogonia

A

B

C

D

E

F

G

H

I

20X

40X

60X
Gene expression in 70 dph fish

ERα

**E2**
ERα mRNA expression in the liver samples of E2 or EE2 treated Menidia juveniles

**E1**
ERα mRNA expression in liver samples of E1 treated Menidia juveniles

**NP**
ERα mRNA expression in liver samples of NP treated Menidia juveniles

**BPA**
ERα mRNA expression in liver samples of BPA treated Menidia juveniles
Gene expression in 70 dph fish

**Chg**

E2  Chg-L mRNA expression in the liver samples of E2 or EE2 treated Menidia juveniles

![Graph showing fold change in Chg-L mRNA expression for E2 treatments.]

E1  Chg-L mRNA expression in liver samples of E1 treated Menidia juveniles

![Graph showing fold change in Chg-L mRNA expression for E1 treatments.]

NP  Chg-L mRNA expression in liver samples of NP treated Menidia juveniles

![Graph showing fold change in Chg-L mRNA expression for NP treatments.]

BPA  Chg-L mRNA expression in liver samples of BPA treated Menidia juveniles

![Graph showing fold change in Chg-L mRNA expression for BPA treatments.]

**Gene expression in 70 dph fish Chg**
Gene expression in 70 dph fish

Vtg
Next Steps in Project

- Get a better experimental handle on changes in apical endpoints for Menidia
  - Growth
  - Tissue differentiation

- Test 1-2 wastewater treatment plants in CA

- Write a final report – available by next fall
Experiment we plan to do
ER assay and evaluation of water

Standard curve

Water samples

Legend for samples
A= Effluent 2
B= Effluent 1
C= Ozonation
D= Storm water
E= Membrane
F= RO
G= River Water
H = AO
J= Blank
K= Drinking water
CA= SCCWRP proj
Activity of nuclear receptors/transcription factors by WWTP effluent
73 receptors/TFs tested

<table>
<thead>
<tr>
<th>Nuclear Receptors/transcription factors</th>
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<tr>
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<td>+++</td>
<td>PPARa Peroxisome proliferator-activated receptor</td>
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<td>AR Androgen receptor</td>
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<td>PPARg Peroxisome proliferator-activated receptor</td>
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<td>+++</td>
<td>PXR Pregnan-X-receptor</td>
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<tr>
<td>ERa Estrogen receptor alpha</td>
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<td>RARa Retinoic Acid receptor, alpha</td>
<td>++++++</td>
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<tr>
<td>ERb Estrogen receptor beta</td>
<td>+++</td>
<td>RARb Retinoic Acid receptor, beta</td>
<td>+++++</td>
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<tr>
<td>ERRg Estrogen receptor related gamma</td>
<td>++</td>
<td>RARg Retinoic Acid receptor, gamma</td>
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<tr>
<td>FXR Farnesoid X Recepctr</td>
<td>+</td>
<td>RORb Retinoid related orphan receptor beta</td>
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<td>RXRa Retinoic-X receptor, alpha</td>
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<td>RXRb Retinoic-X receptor, beta</td>
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<td>VDR Vitamin D receptor</td>
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<td>NRF2 Nuclear factor erythroid 2-related factor 2</td>
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23 total hits with 11 strong hits
Attagene factorial assay

Escher et al, EST 48(3):1940-56, 2014
Conclusions

- Bioanalytical approaches (cell based assays) are complementary to analytical chemical approaches and can be used for monitoring.
- Bioanalytical approaches give clues to the molecular initiating events and thus to mechanism.
- Biomarkers are useful to depict biochemical pathways but must be linked to higher order population level effects to be useful.
- Considerable effort is being expended on developing adverse outcome pathways to link biomarkers with adversity.
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