Bioanalytical Tool Development

Nancy D. Denslow, Ph.D.
Center for Environmental and Human Toxicology
University of Florida, Gainesville, FL 32611

Sources of Stressors

Natural Stressors

Atmospheric Disposition

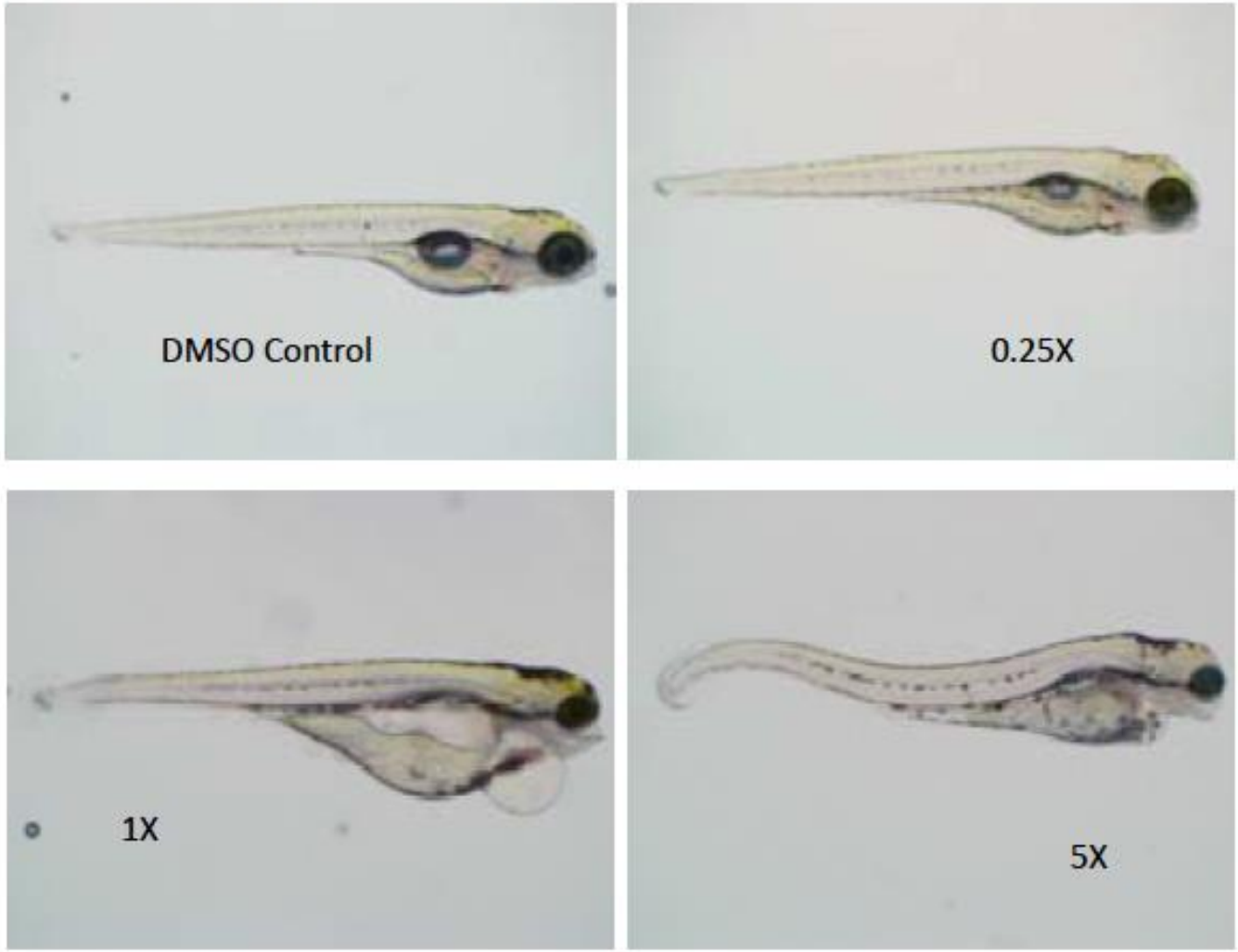
Septic Tanks → Groundwater

Runoff
(Urban, Agriculture)

Industrial Discharge

Sewage treatment Plants

Five Day Embryonic Exposure to Organic Constituents in Tucson WWTP Effluent

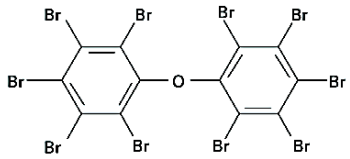


(slide from Shane Snyder)

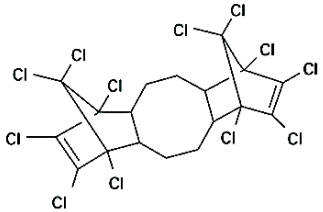
Fish consumption advisories

(EPA, Fish consumption advisories)

Flame retardants

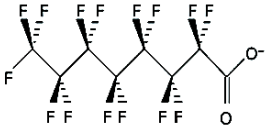


DBDE

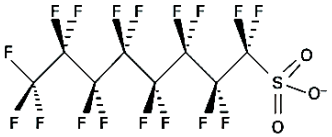


Dechlorane Plus

Perfluorinated chemicals

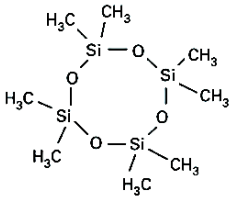


PFOA

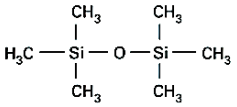


PFOS

Siloxanes



D4

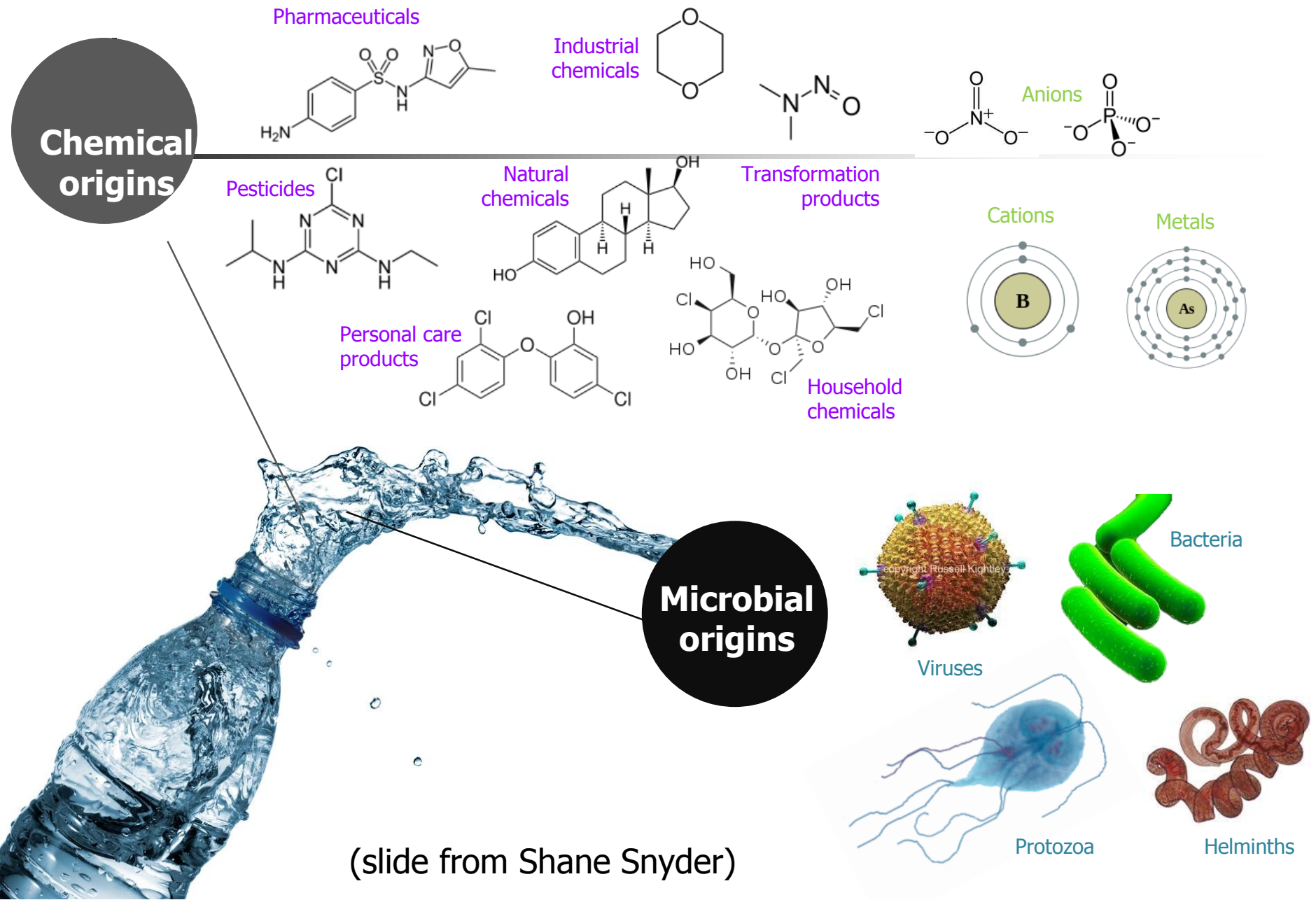


HMDS

(Ela et al. EHP, 2010)

What about our water?

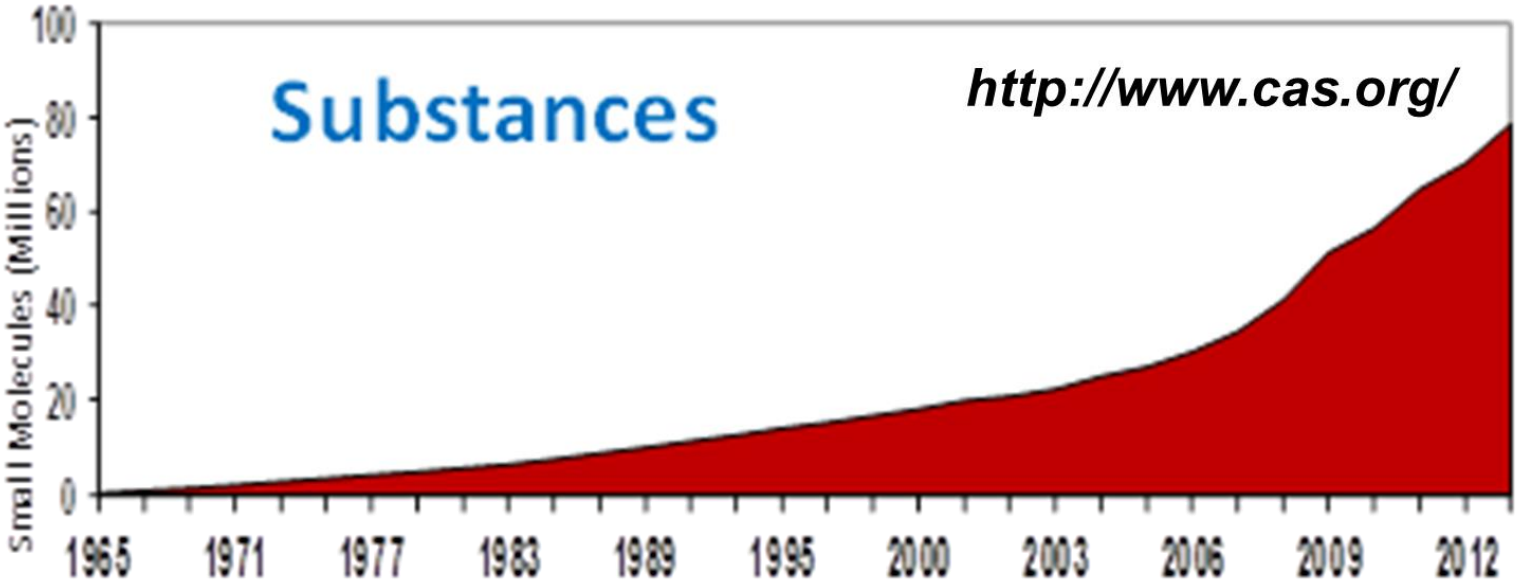
Nearly All Substances are Detectable in Water



(slide from Shane Snyder)

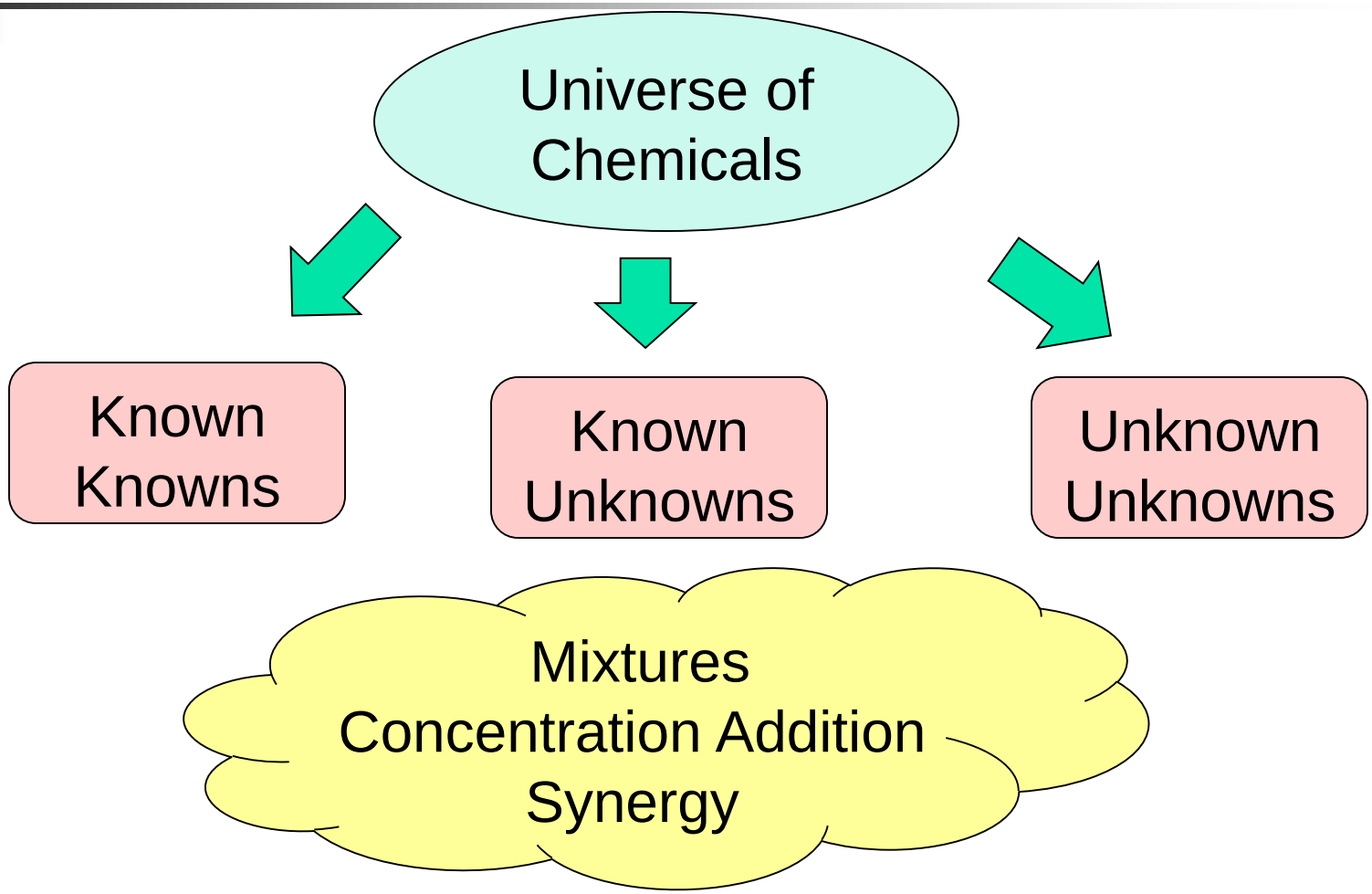
TACKLING THE CHEMICAL UNIVERSE

CHEMCATS **65,768,974** Commercially available chemicals



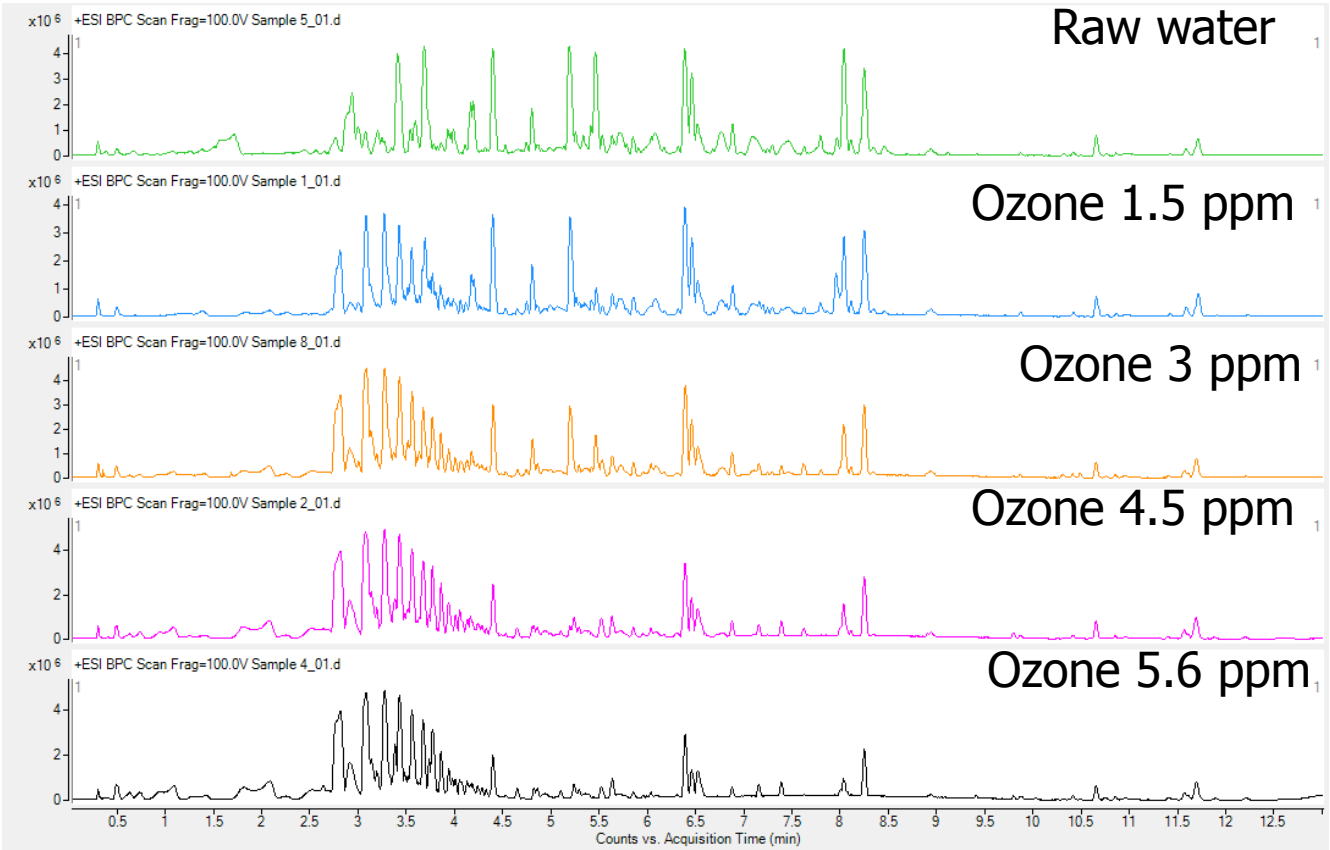
Snyder, S. A., Emerging Chemical Contaminants: Looking for Better Harmony. *J. Am. Water Works Assoc.* **2014,** *106* (8), 38-52.

Contaminants of emerging concern



Transformation Products[H]

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)

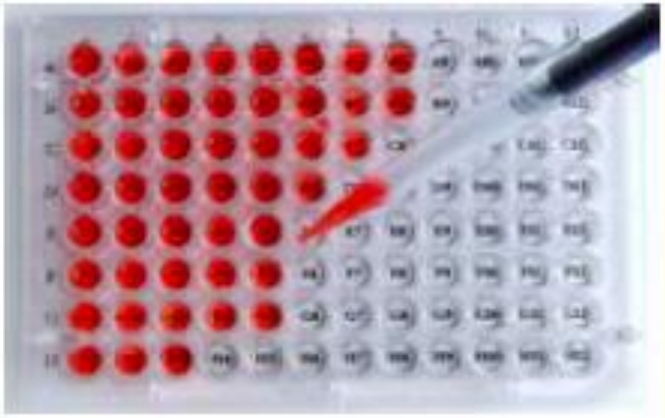
Analytical Chemistry VS Bioassay

Targeted Analytical

Known compounds
Quantitative
Individual compounds

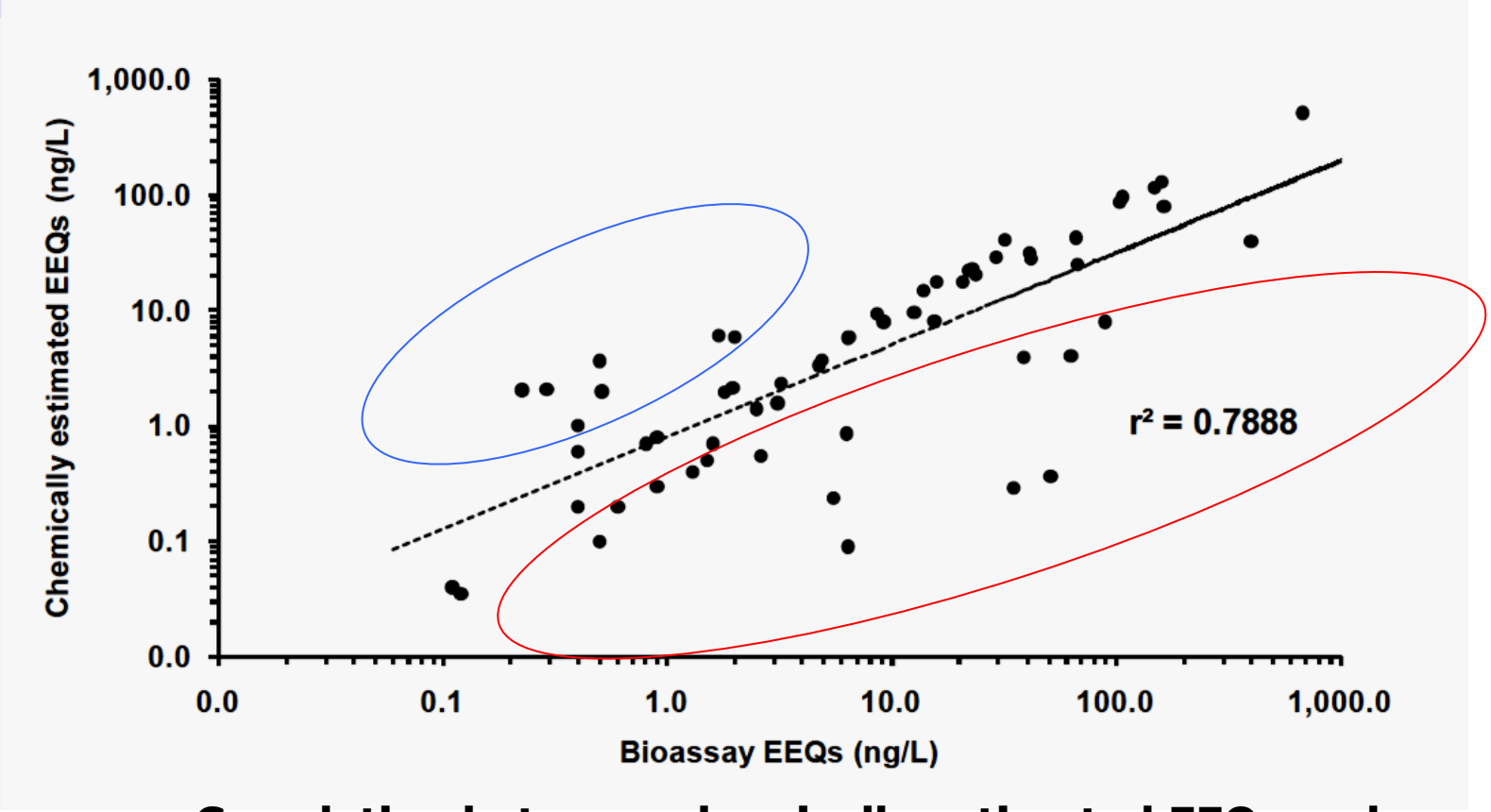
Mechanistic Bioassay

Knowns/unknowns
Semi-quantitative
Synergism/Antagonism



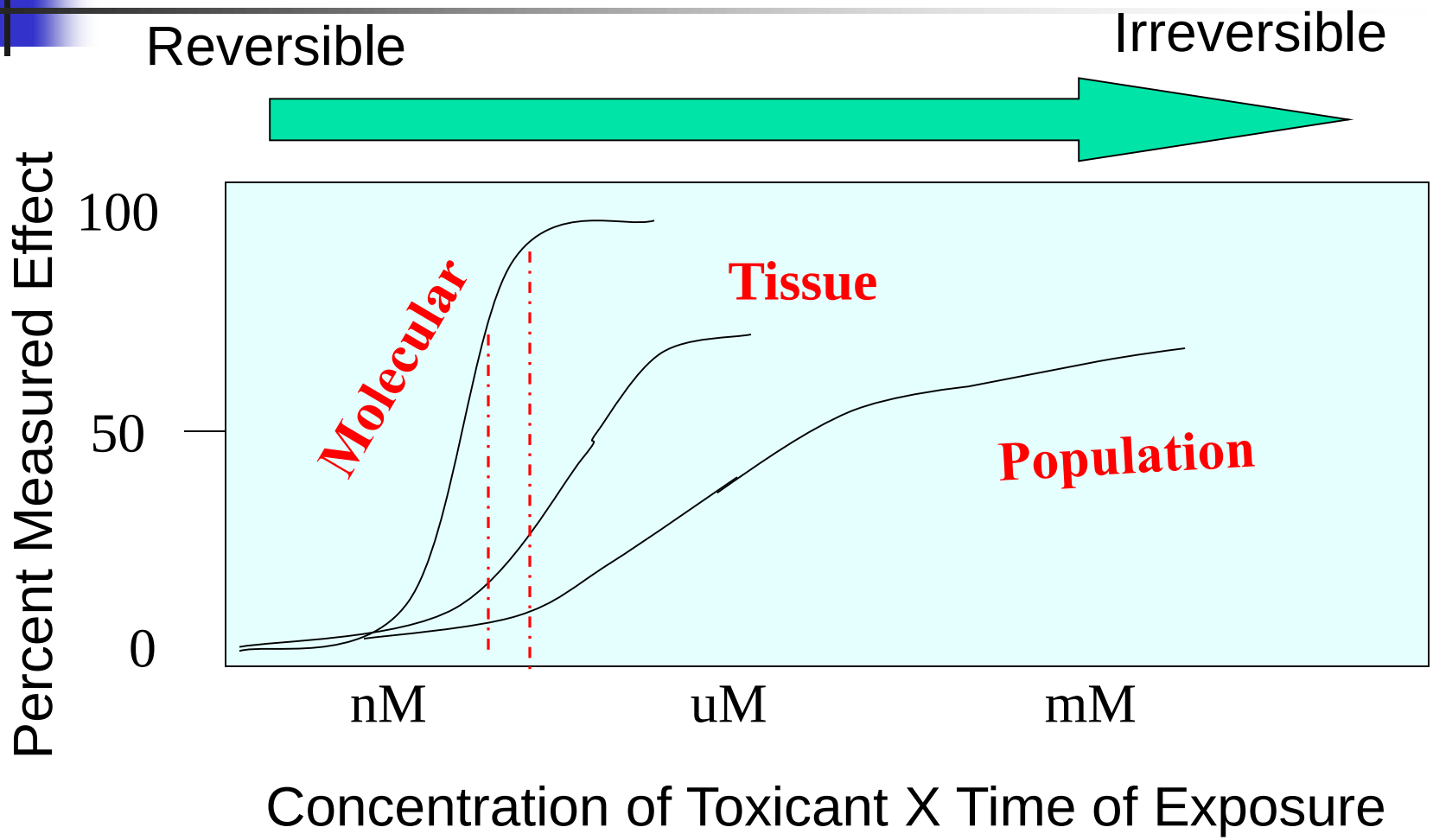
(Slide from Shane Snyder)

Estrogen equivalency

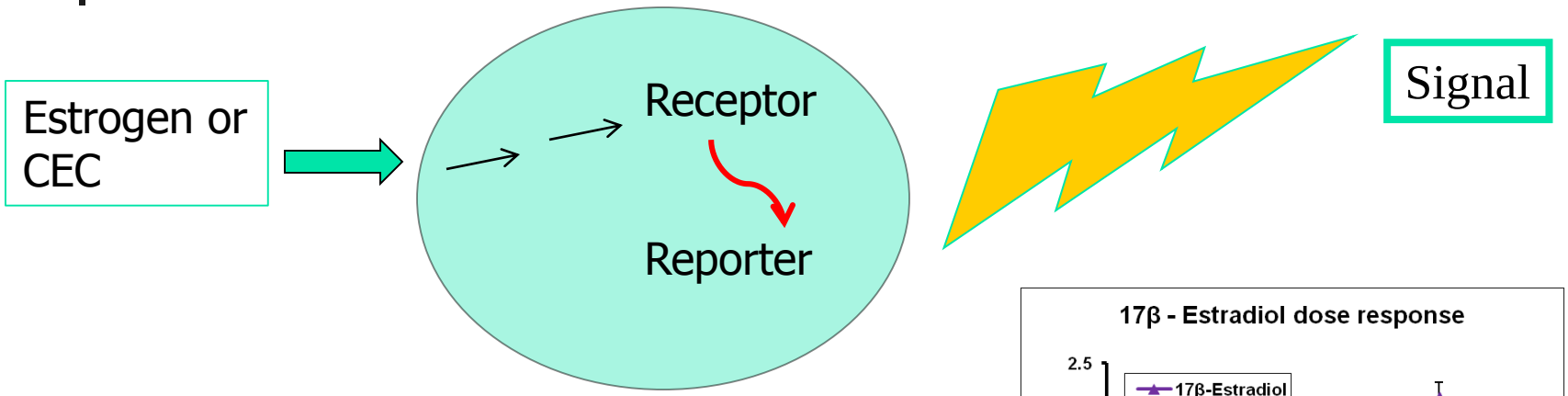


Correlation between chemically estimated EEQs and bioassay EEQs (Bulloch *et al.*, 2010).

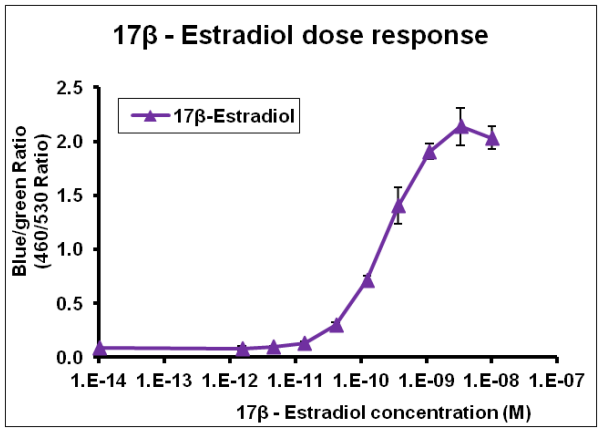
Biomarker sensitivity

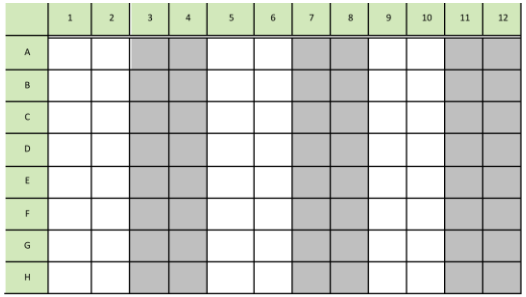


High throughput assays –InVitrogen

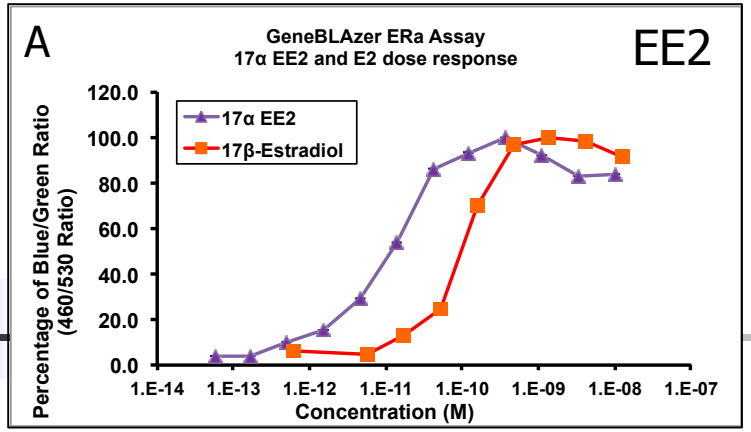


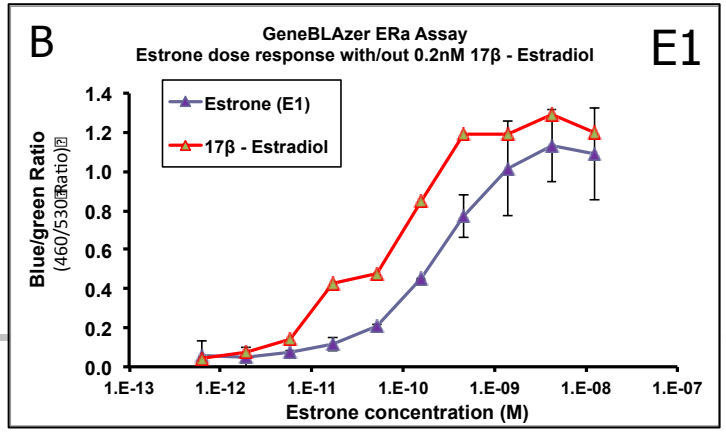
Human Cell Line

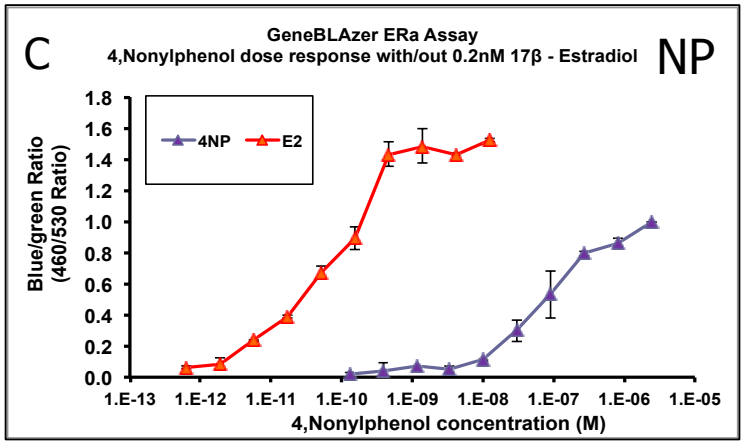


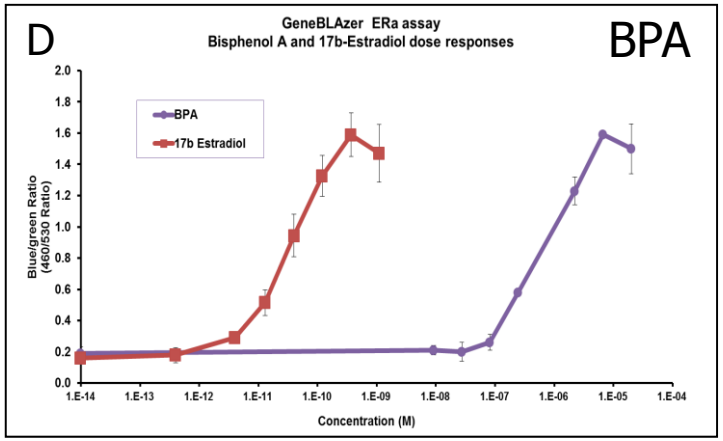


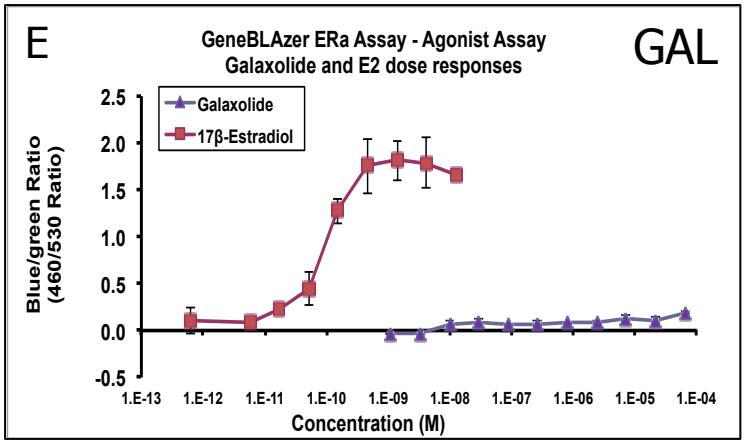
96-well assay

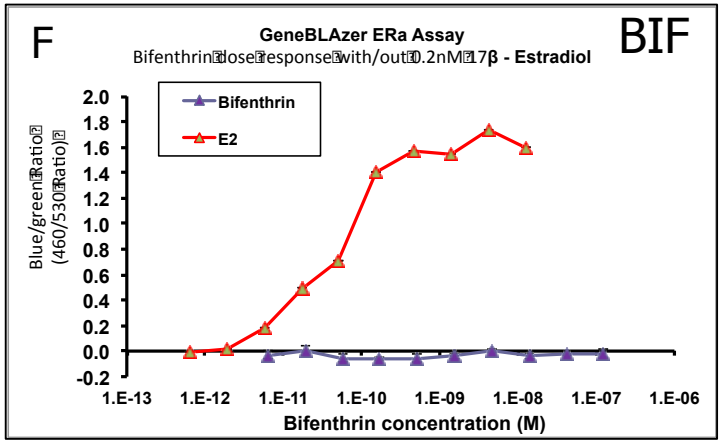

A
GeneBLAzer ERa Assay
17α EE2 and E2 dose response
EE2
17α EE2
17β-Estradiol
Percentage of Blue/Green Ratio (460/530 Ratio)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
1.E-14
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
Concentration (M)


B
GeneBLAzer ERa Assay
Estrone dose response with/out 0.2nM 17β - Estradiol
E1
Estrone (E1)
17β - Estradiol
Blue/green Ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
Estrone concentration (M)

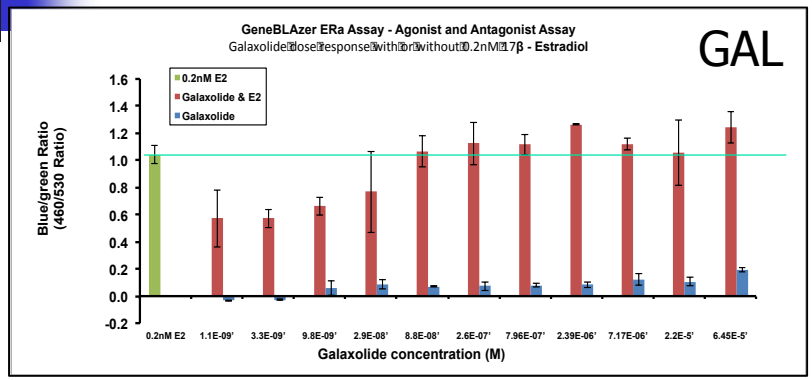

C
GeneBLAzer ERa Assay
4,Nonylphenol dose response with/out 0.2nM 17β - Estradiol
NP
4NP
E2
Blue/green Ratio (460/530 Ratio)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
1.E-06
1.E-05
4,Nonylphenol concentration (M)

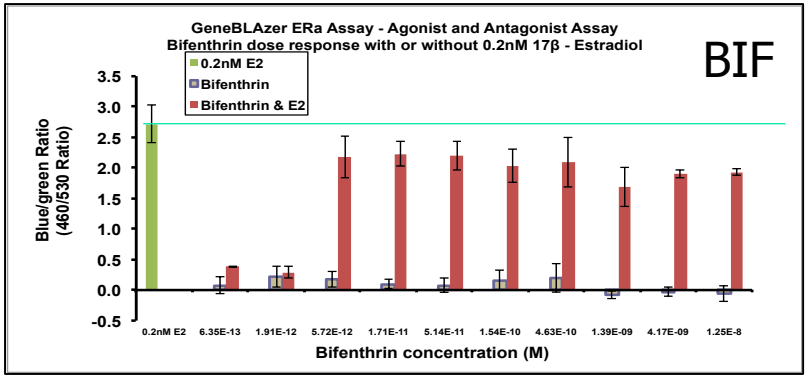

D
GeneBLAzer ERa assay
Bisphenol A and 17b-Estradiol dose responses
BPA
BPA
17b Estradiol
Blue/green Ratio (460/530 Ratio)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.E-14
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
1.E-06
1.E-05
1.E-04
Concentration (M)

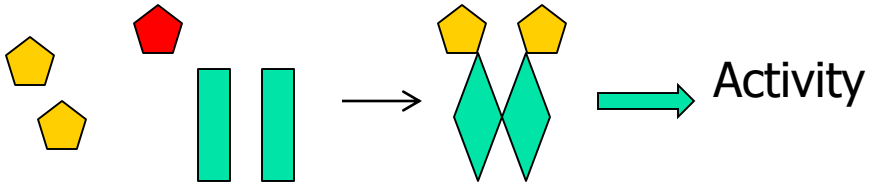

E
GeneBLAzer ERa Assay - Agonist Assay
Galaxolide and E2 dose responses
GAL
Galaxolide
17β-Estradiol
Blue/green Ratio (460/530 Ratio)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
1.E-06
1.E-05
1.E-04
Concentration (M)

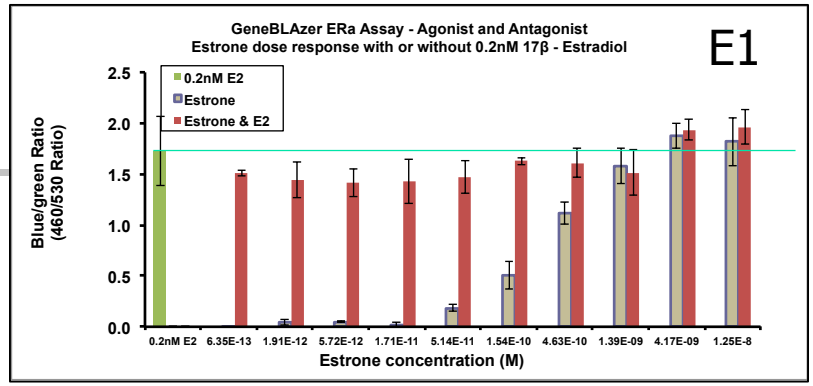

F
GeneBLAzer ERa Assay
Bifenthrin dose response with/out 0.2nM 17β - Estradiol
BIF
Bifenthrin
E2
Blue/green Ratio (460/530 Ratio)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
1.E-07
1.E-06
Bifenthrin concentration (M)

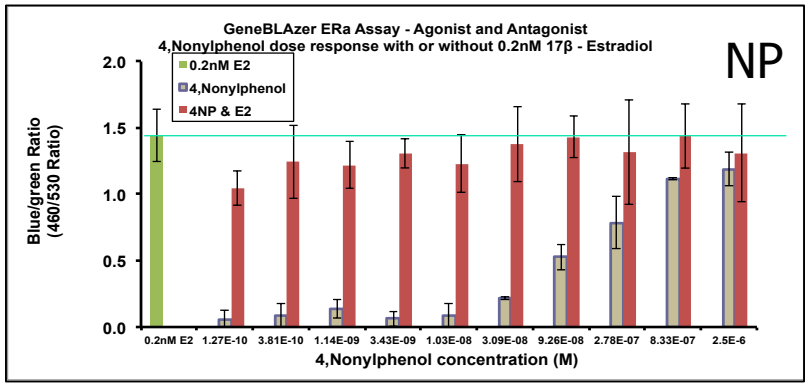
Antagonist activity

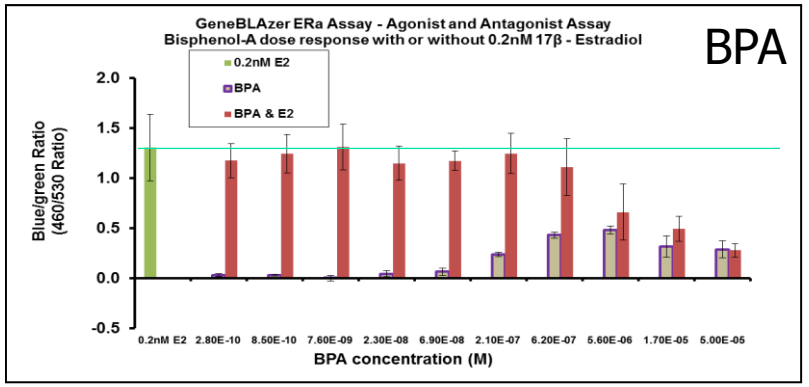




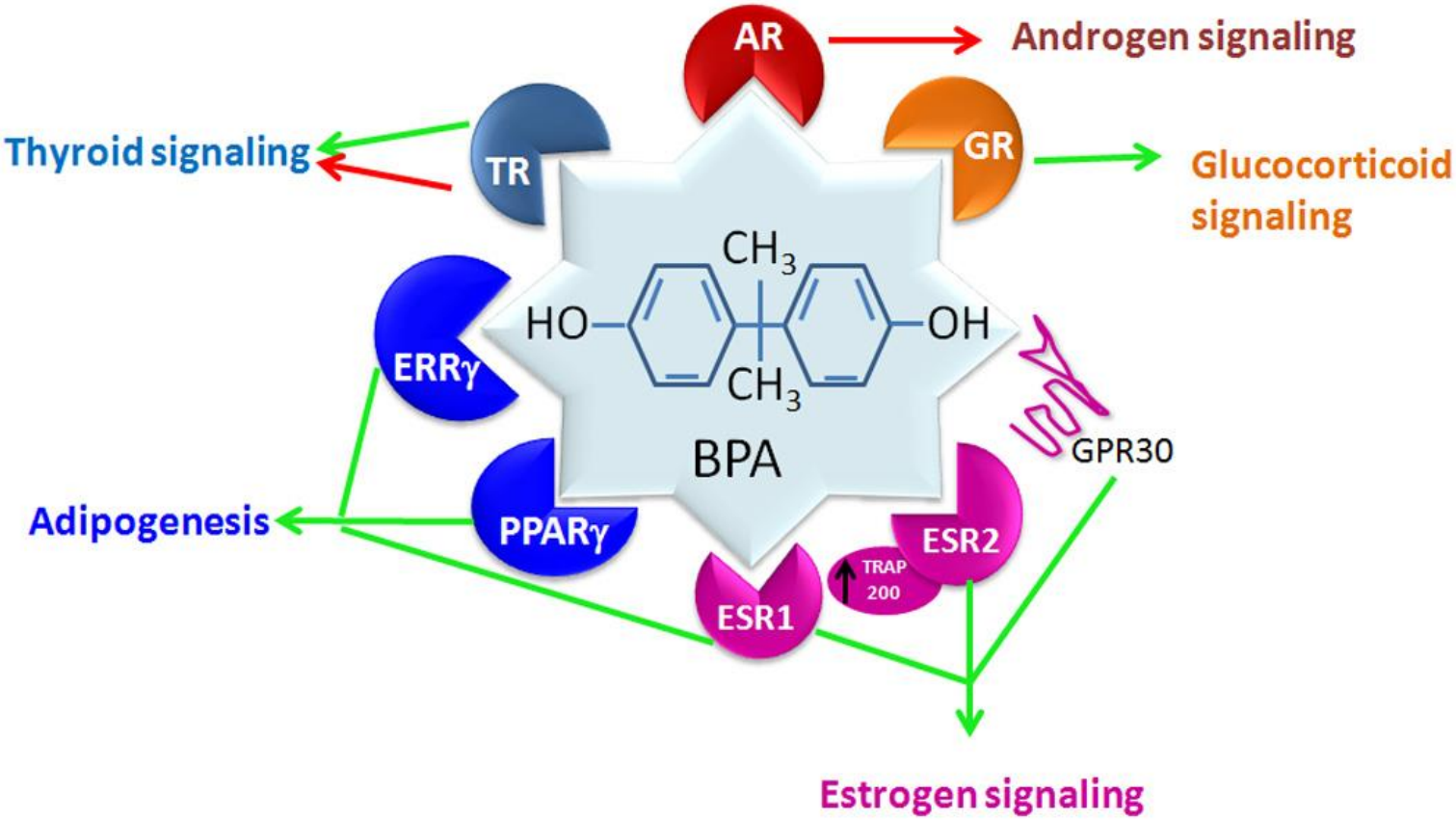






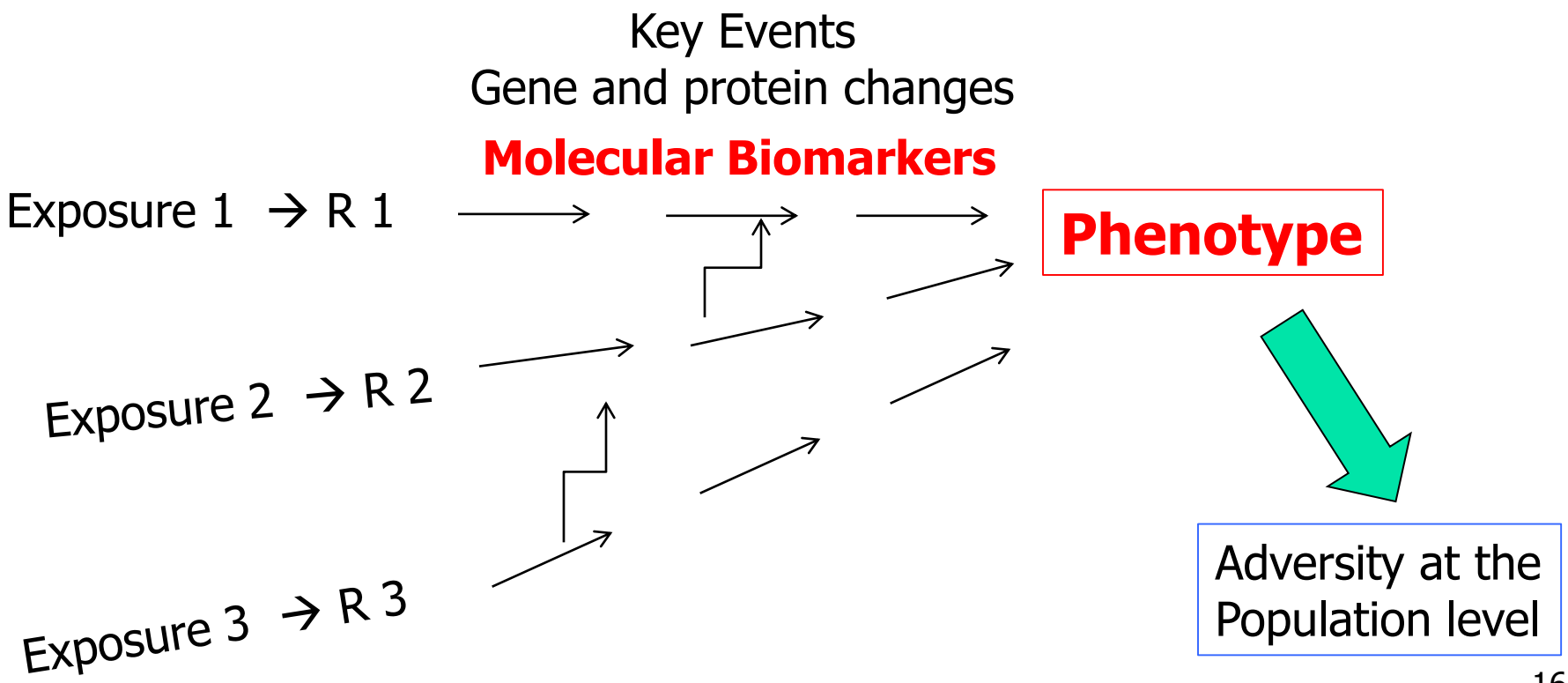


Signaling systems affected by BPA



Linkage from bioassays to higher endpoints

- Development of Adverse Outcome Pathways

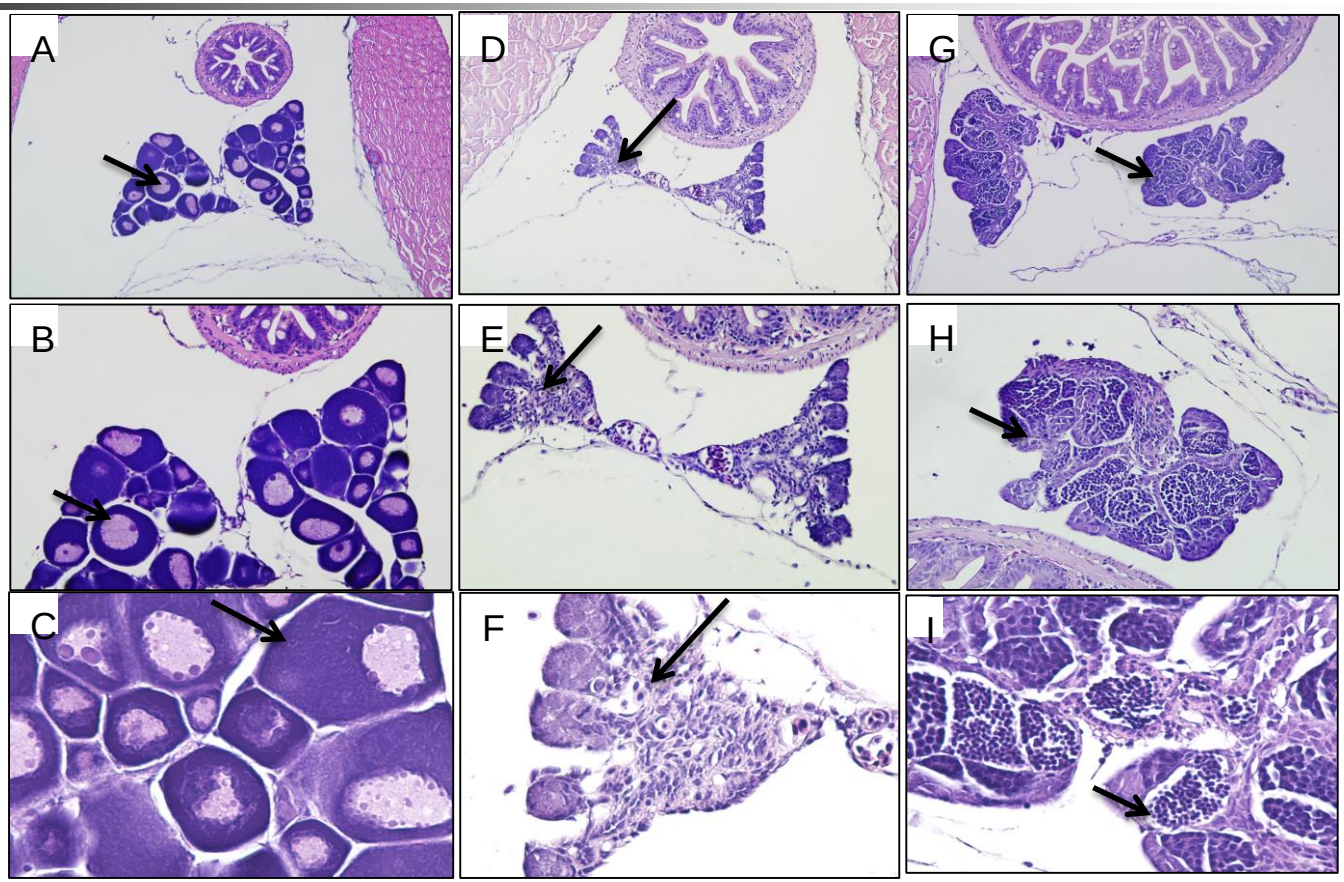


Choice of species: *Menidia beryllina (silverside)*

- 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

Oogonia Undifferentiated Spermatogonia



20X

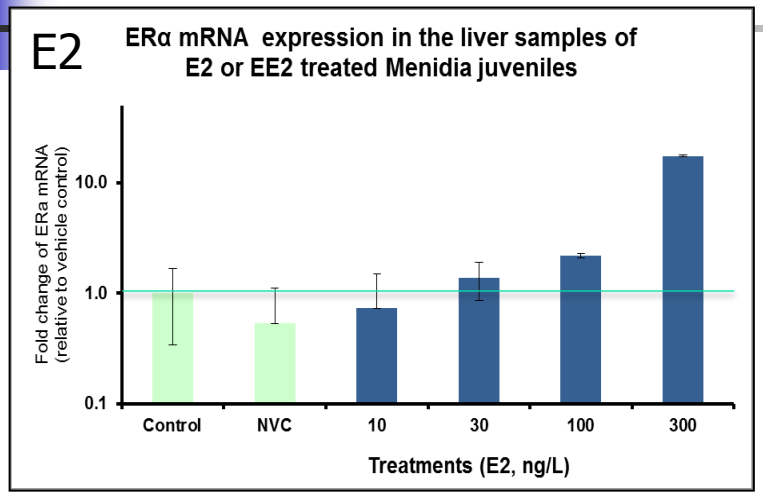
40X

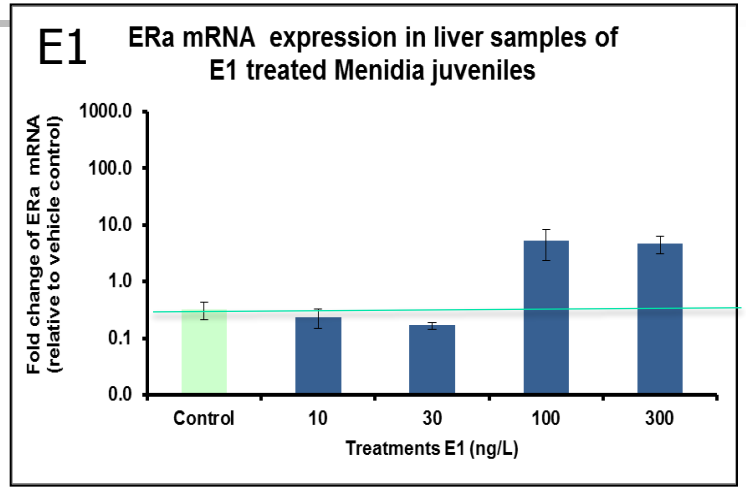
60X

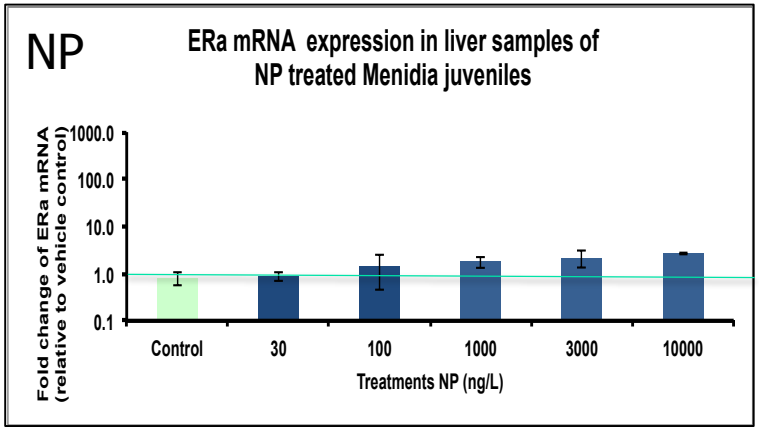
Gonadal differentiation between day 50 -60 post hatch

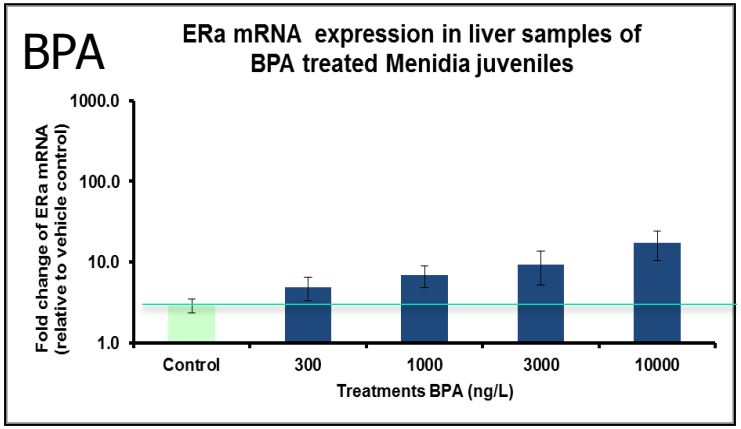
Gene expression in 70 dph fish

ERα



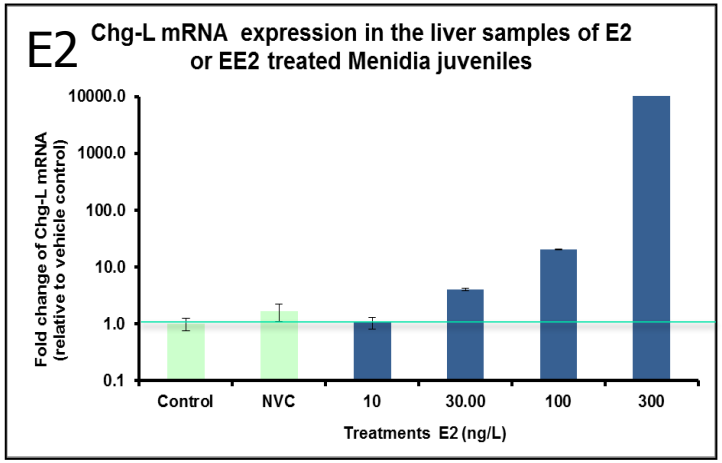


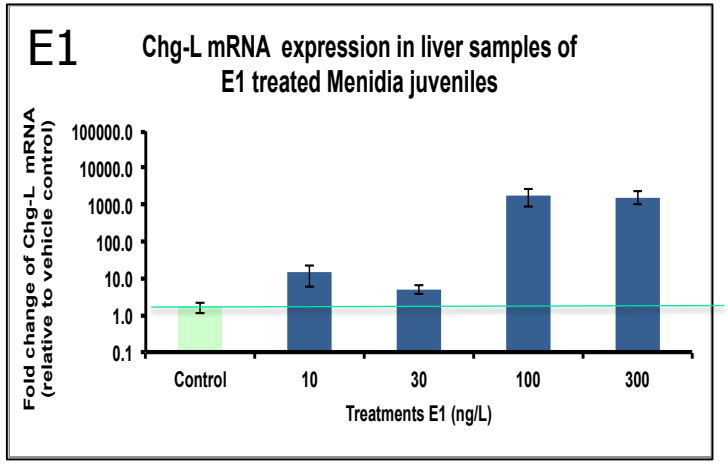


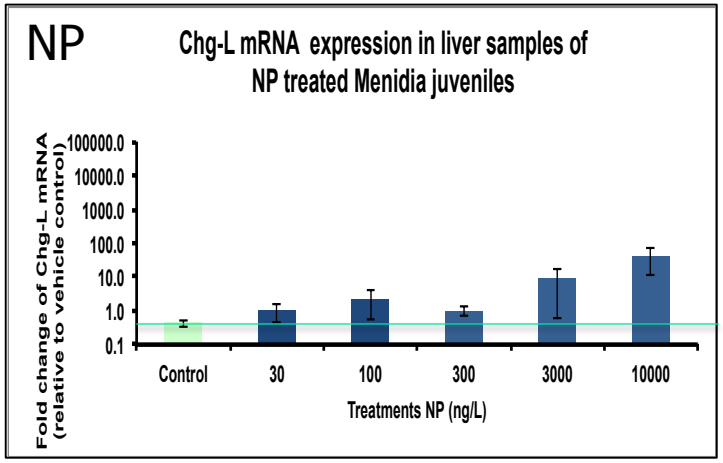


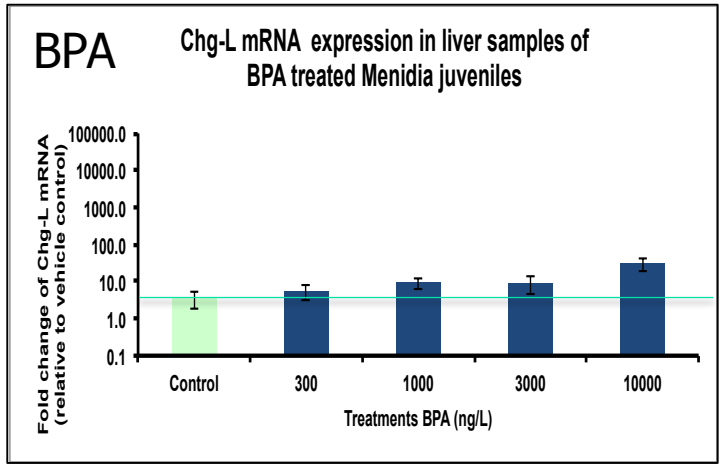
Gene expression in 70 dph fish

Chg



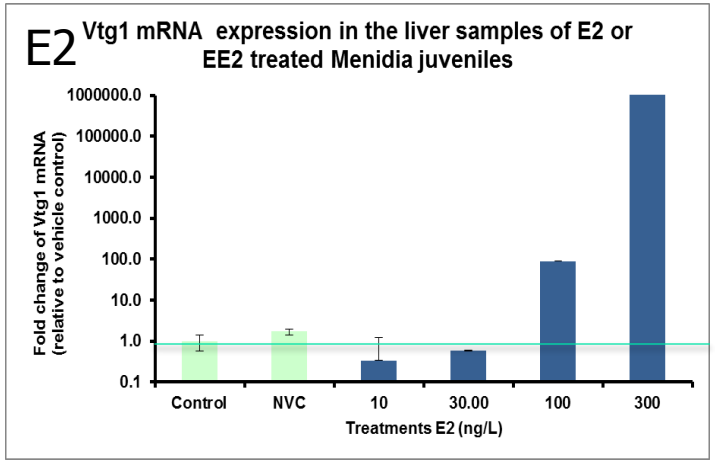


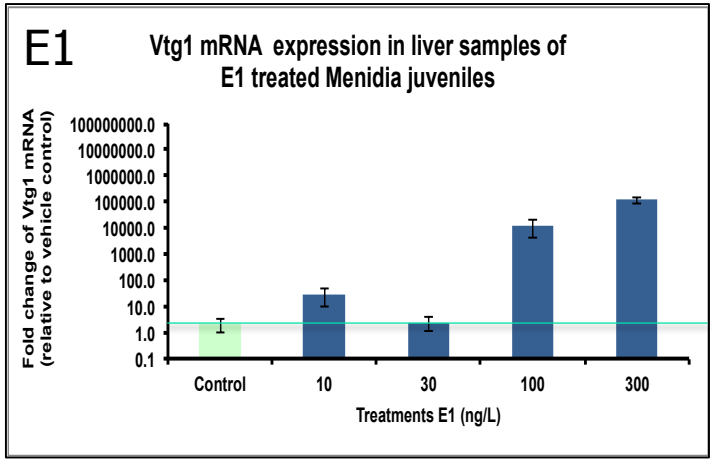


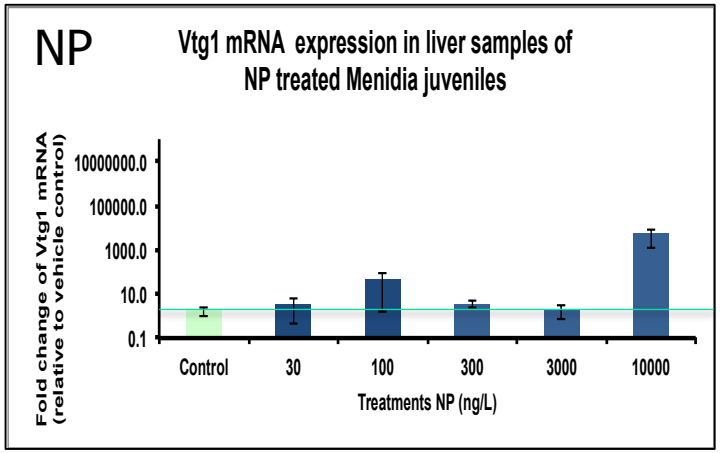


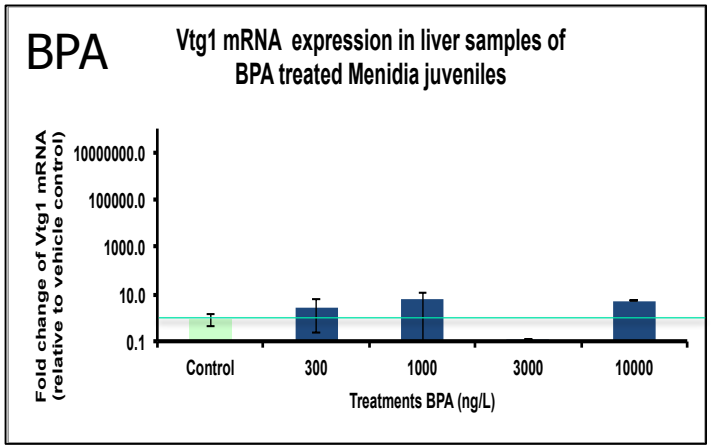
Gene expression in 70 dph fish

Vtg





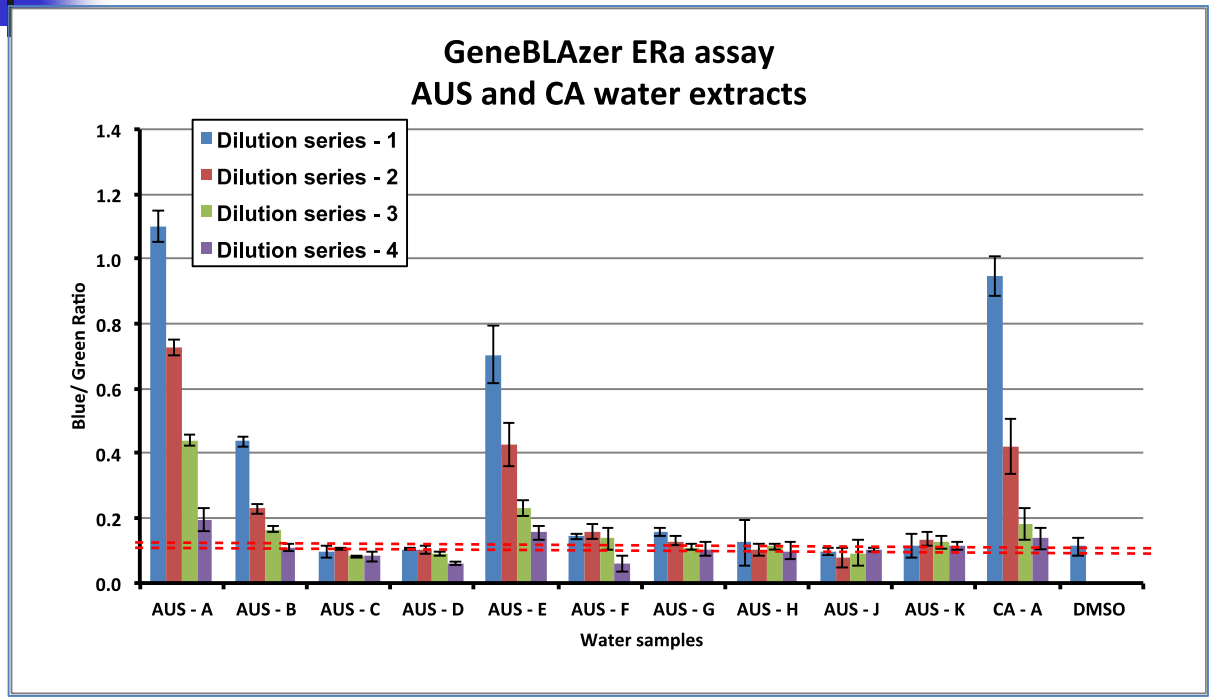




Next Steps in Project

- 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



Water samples

Legend for samples

A= Effluent 2

B= Effluent 1

C= Ozonation

D= Storm water

E= Membrane

F= RO

G= River Water

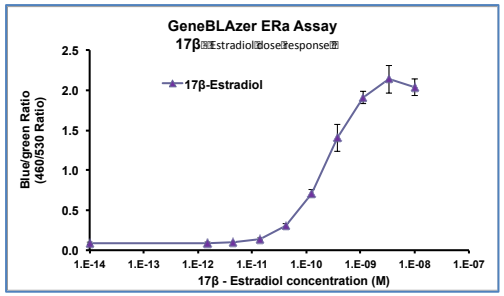
H = AO

J= Blank

K= Drinking water

CA= SCCWRP proj

Standard curve



Activity of nuclear receptors/transcription factors by WWTP effluent 73 receptors/TFs tested

Nuclear Receptors/transcription factors		Level of activity	Nuclear Receptors/transcription factors		Level of activity
AhR	Aryl Hydrocarbon receptor	++++	PPARa	Peroxisome proliferator-activated receptor	++++
AP1	Activator protein 1	+	PPARd1	Peroxisome proliferator-activated receptor	+
AR	Androgen receptor	+	PPARg	Peroxisome proliferator-activated receptor	++
CAR	Constitutive androstane receptor	+++	PXR	Pregnane-X-receptor	+
ERa	Estrogen receptor alpha	++++	RARa	Retinoic Acid receptor, alpha	++++++
ERb	Estrogen receptor beta	+++	RARb	Retinoic Acid receptor, beta	+++++
ERRg	Estrogen receptor related gamma	++	RARg	Retinoic Acid receptor, gamma	++++++
FXR	Farnesoid X Receptr	+	RORb	Retinoid related orphan receptor beta	++++
GR	Glucocorticoid receptor	++	RXRa	Retinoic-X receptor, alpha	+
HNF4a	Hepatocyte Nuclear factor 4 alpha	+	RXRb	Retinoic-X receptor, beta	+++
LXR	Liver X receptor	+	VDR	Vitamin D receptor	+
NRF2	Nuclear factor erythroid 2-related factor 2	+++			

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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