

Epigenetic and Transcriptomic Changes Resulting from Long-term Exposure to Contaminant Mixture Associated with Agricultural Land Use.

Mary Jean See*, Weichun Huang*, Nicholas Cipoletti[†], Heiko Schoenfuss[†], Daniel Sullivan*, Adam Biales*.

*Great Lakes Toxicology and Ecology Division, Cincinnati, OH

[†]Aquatic Toxicology Laboratory, St. Cloud State University, St. Cloud, MN

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Agricultural Mixture Identification

Collaborative survey of Great Lakes tributaries reported in Elliot et. al. 2017 and Elliot et. al. 2018

Common name		Use
atrazine		Herbicide
bisphenol-A (BPA)		Plasticizer
bromacil		Herbicide
Diethyltoluamide (DEET)		Insect repellent
estrone		Estrogen (E1)
metolachlor		Herbicide
tributoxyethyl phosphate (TBEP)		Plasticizer and flame retardant
Alkylphenols		Surfactants (cleaning products, personal care products, etc.)
	4-Nonylphenol	
	4-Nonylphenol diethoxylate	
	4-Nonylphenol monoethoxylate	
	4-tert-Octylphenol diethoxylate	
	4-tert-Octylphenol monoethoxylate (Triton X-100)	



Agricultural Mixture Exposure Concentration

Chemical	High (ng/L)	Highest Detected Environmental Concentration	Low (ng/L)
		Medium (ng/L)	
alkylphenols	1880	188	18.8
atrazine	4000	400	40
bisphenol-A (BPA)	600	60	6
bromacil	1200	120	12
Diethyltoluamide (DEET)	2000	200	20
estrone	240	24	2.4
metolachlor	1700	170	17
tributoxyethyl phosphate (TBEP)	21000	2100	210



Laboratory flow-through exposure

Day 60

- Biological End Points
- Tissues frozen

F₀ Fathead Minnows Exposed

Days 21 – 60
Breeding

Day 0 Day 30 Day 60 Day 90 Day 120 Day 150 Day 180 Day 210 Day 240 Day 270 Day 300 Day 330

F₁ Fathead Minnows Exposed

Biological End Points

- Condition Factor (body (g) / length)
- **Secondary Sex Characteristics (SSC, males only)**
- Blood glucose
- Hematocrit
- **Vitellogenin (VTG, plasma)**
- Hepatosomatic index (HSI, liver (g) / body (g))
- Gonadosomatic index (GSI, gonad (g) / body (g))

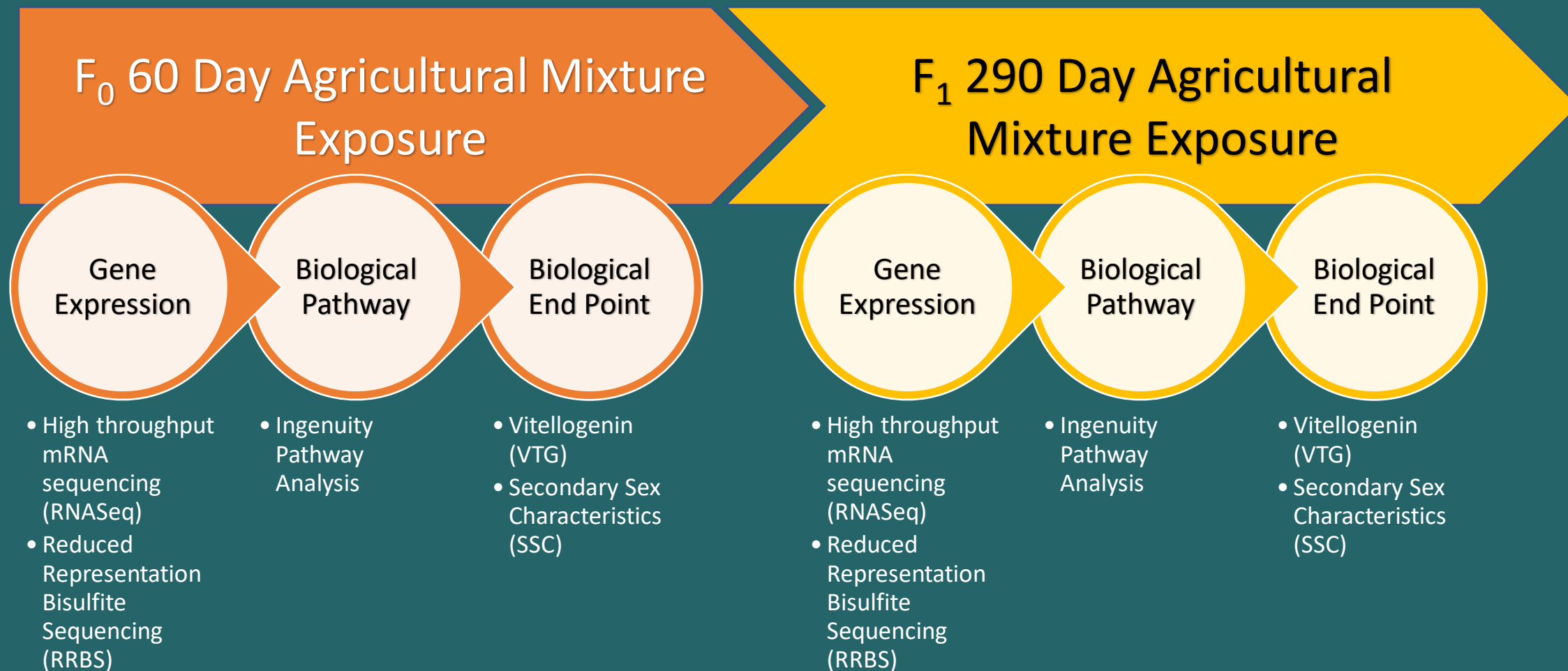
Tissues frozen

- **Liver**
- Gonad

Days 251 – 290
Breeding

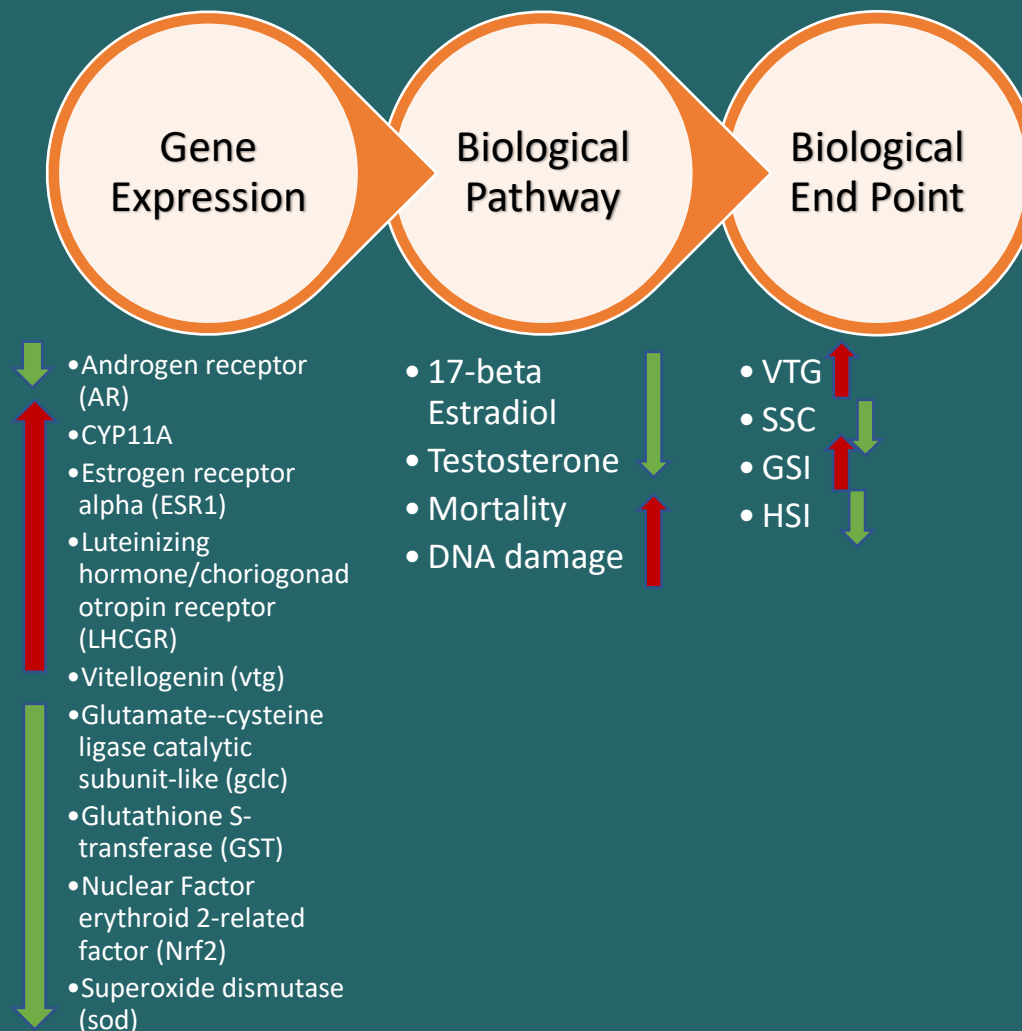
Day 291

- Biological End Points
- Tissues frozen



Known Individual Chemical Responses

Fathead Minnow Response to Agricultural Mixture Chemicals





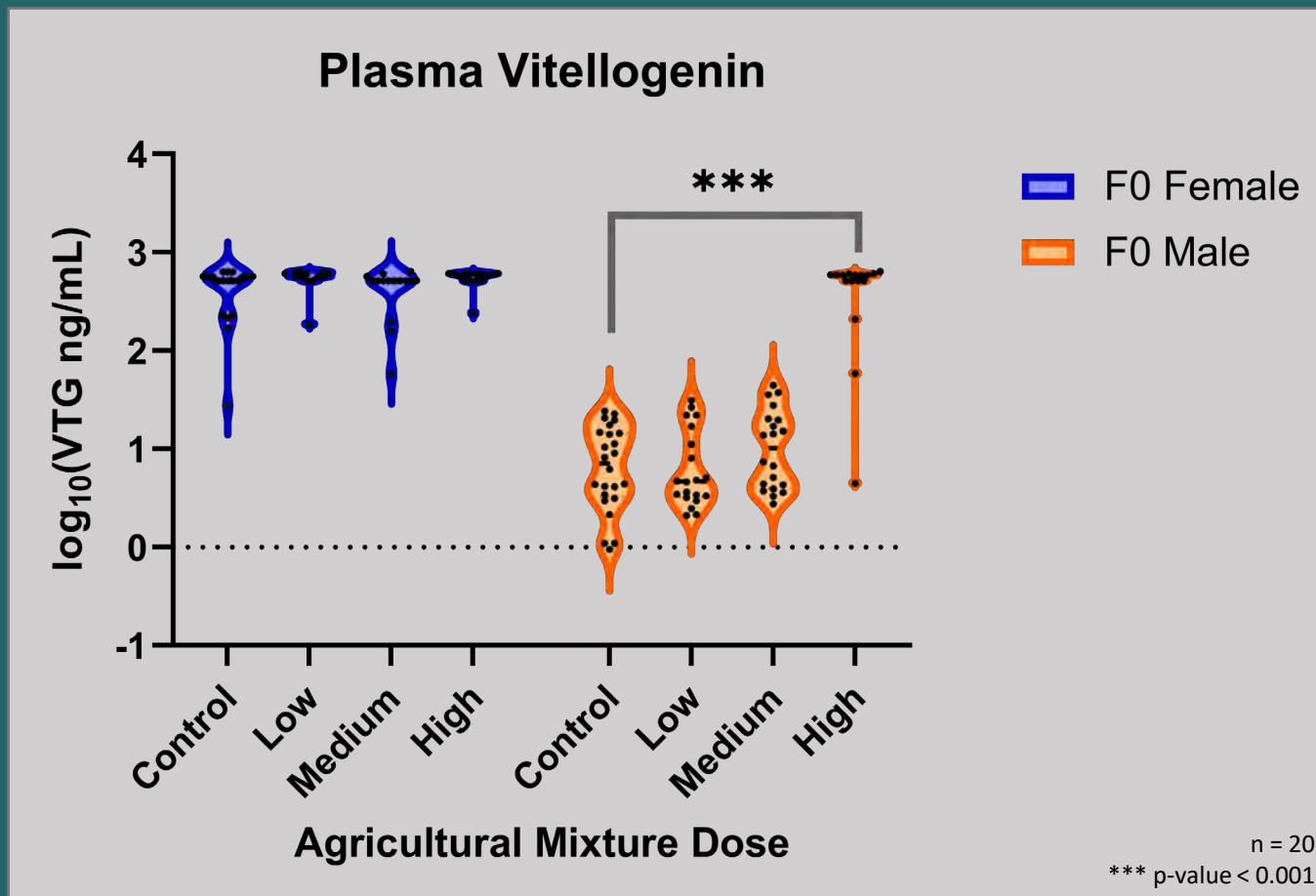
Ag Mix Exposure Increases VTG

F₀

Gene
Expression

Biological
Pathway

Biological
End Point





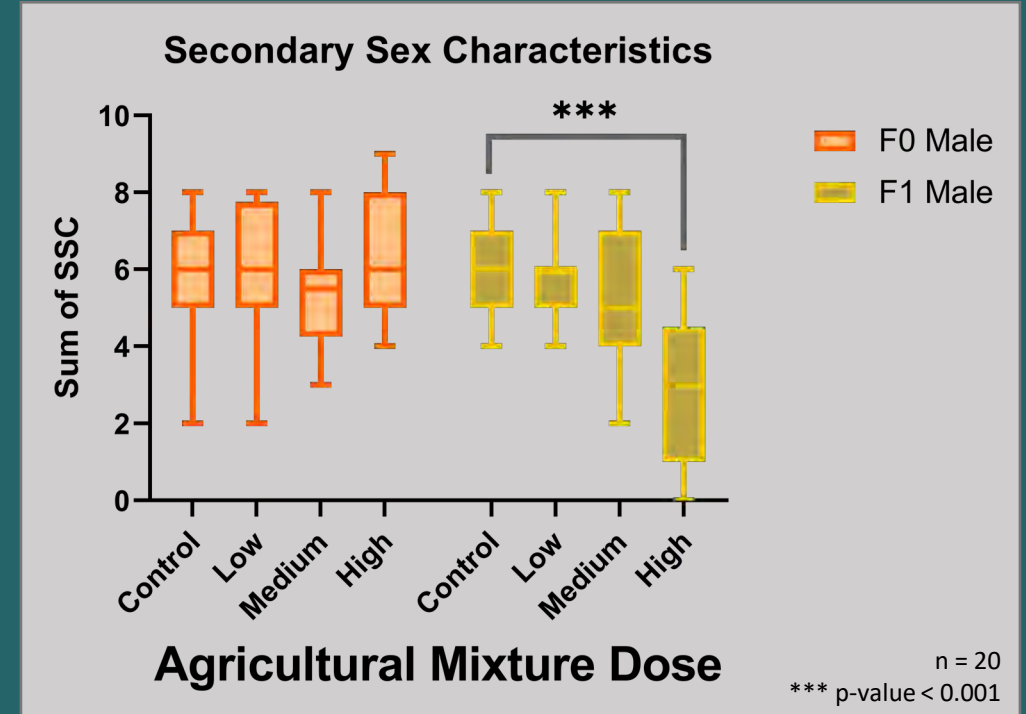
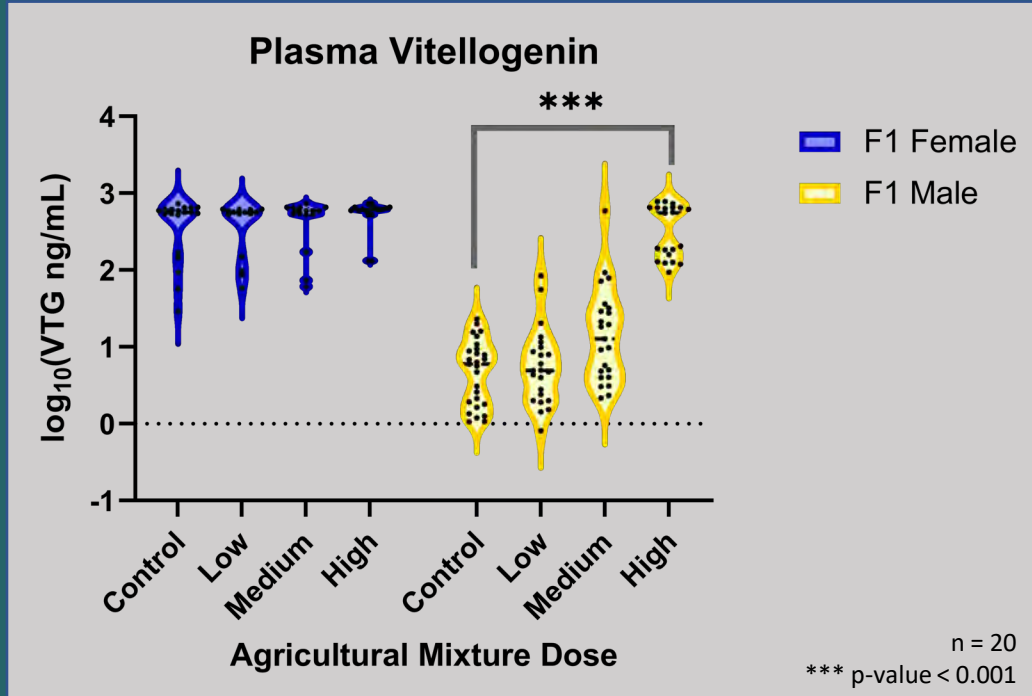
Ag Mix Exposure Decreases SSC

F₁

Gene
Expression

Biological
Pathway

Biological
End Point





Ag Mix Exposure Damages Liver

F₀

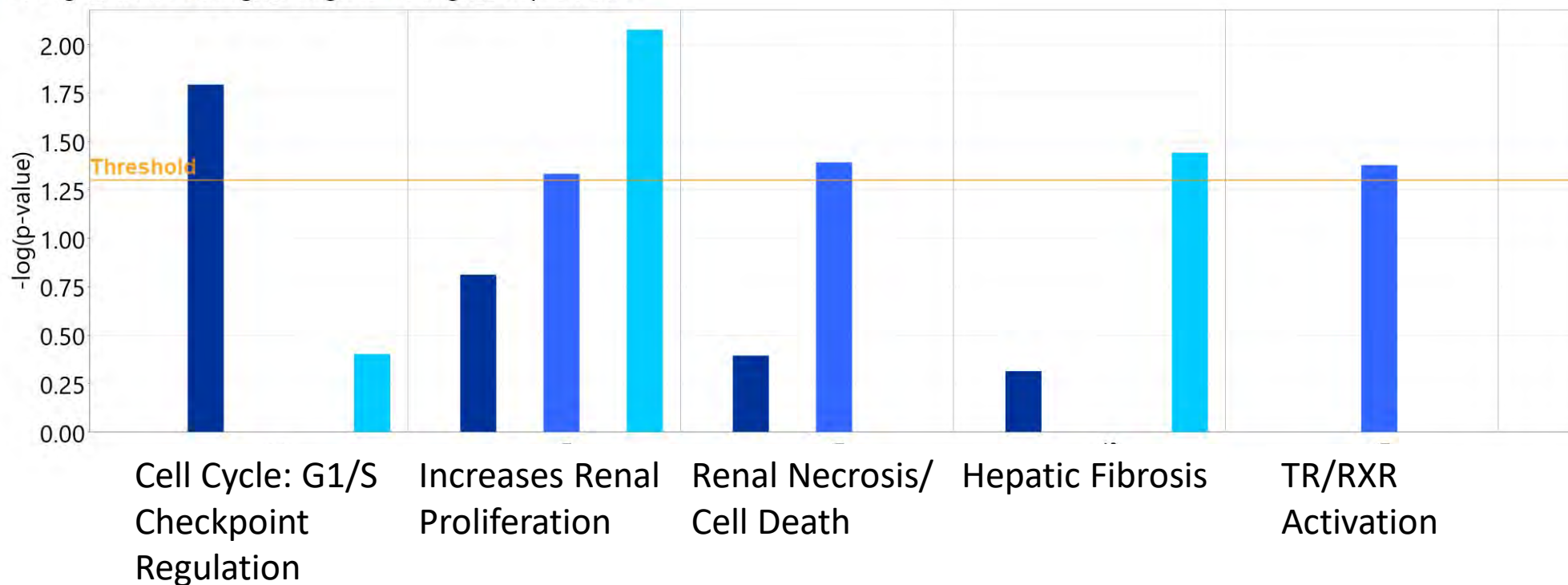
Gene
Expression

Biological
Pathway

Biological
End Point

Analysis: F0 High_DM promoter

■ F0 High_mRNA ■ F0 High_DM gene ■ F0 High_DM promoter



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Ag Mix Exposure Damages Liver

F₁

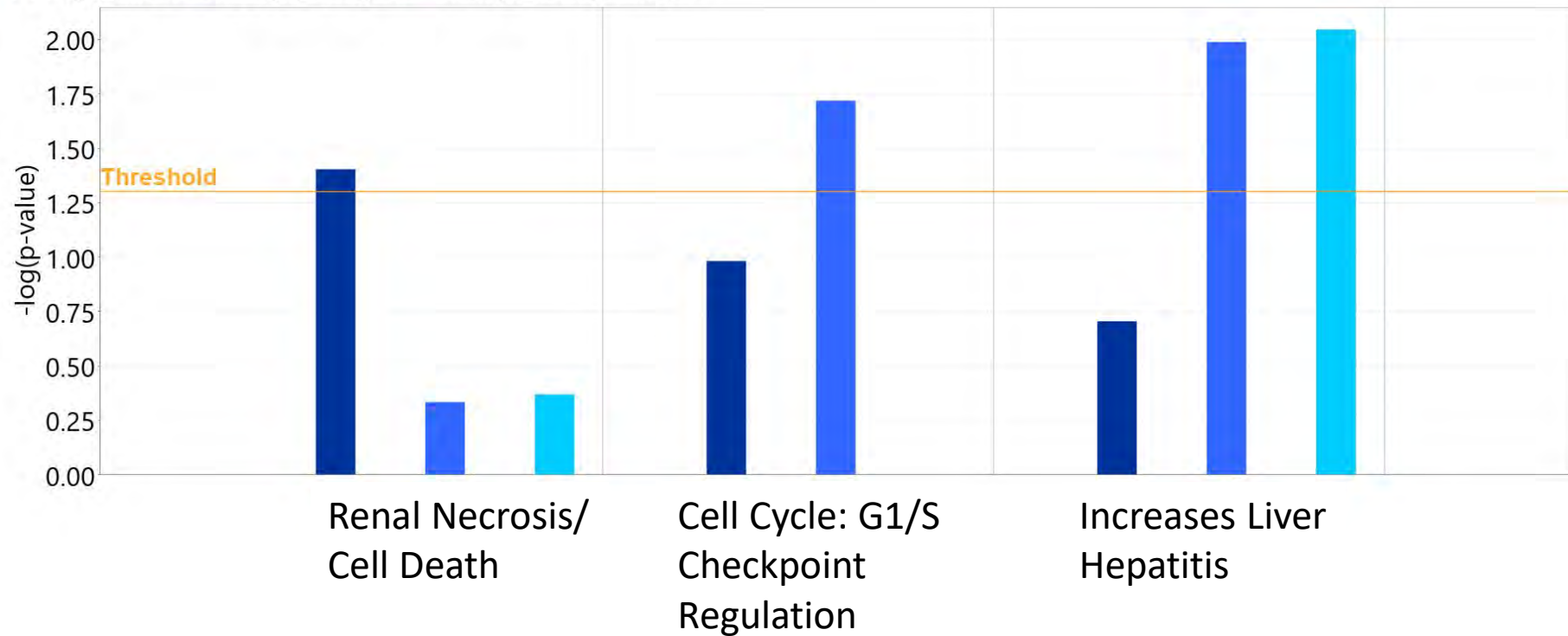
Gene
Expression

Biological
Pathway

Biological
End Point

Analysis: F1 High_mRNA

■ F1 High_mRNA ■ F1 High_DM gene ■ F1 High_DM promoter



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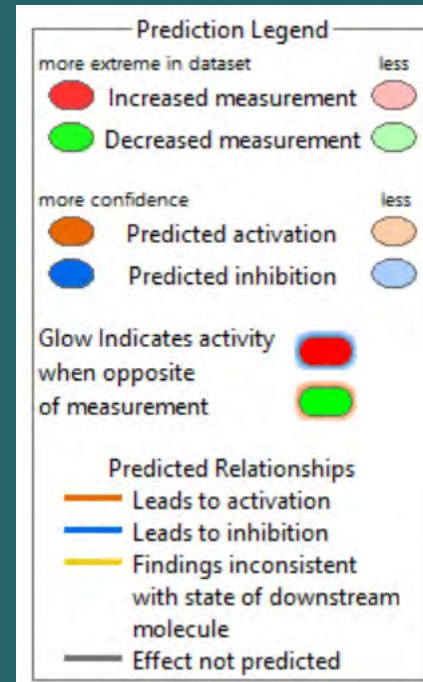
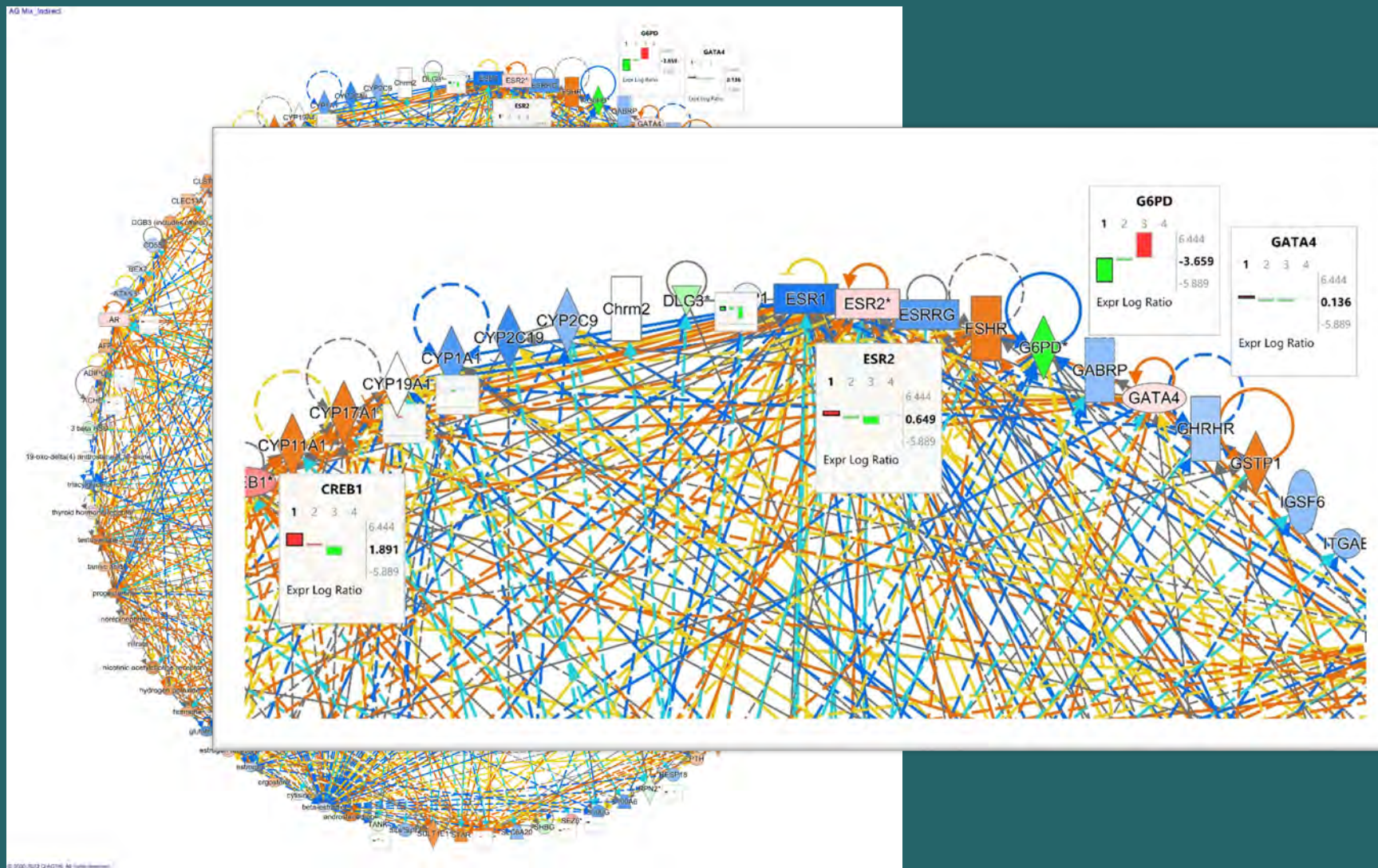
Ag Mix Exposure Alters Liver DEGs and DMRs

F₀

Gene
Expression

Biological
Pathway

Biological
End Point





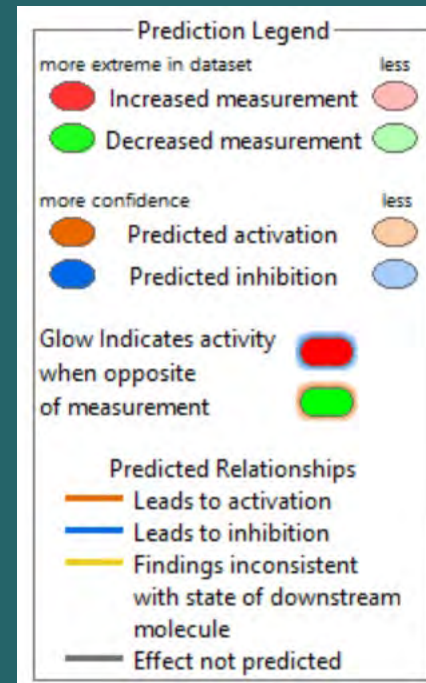
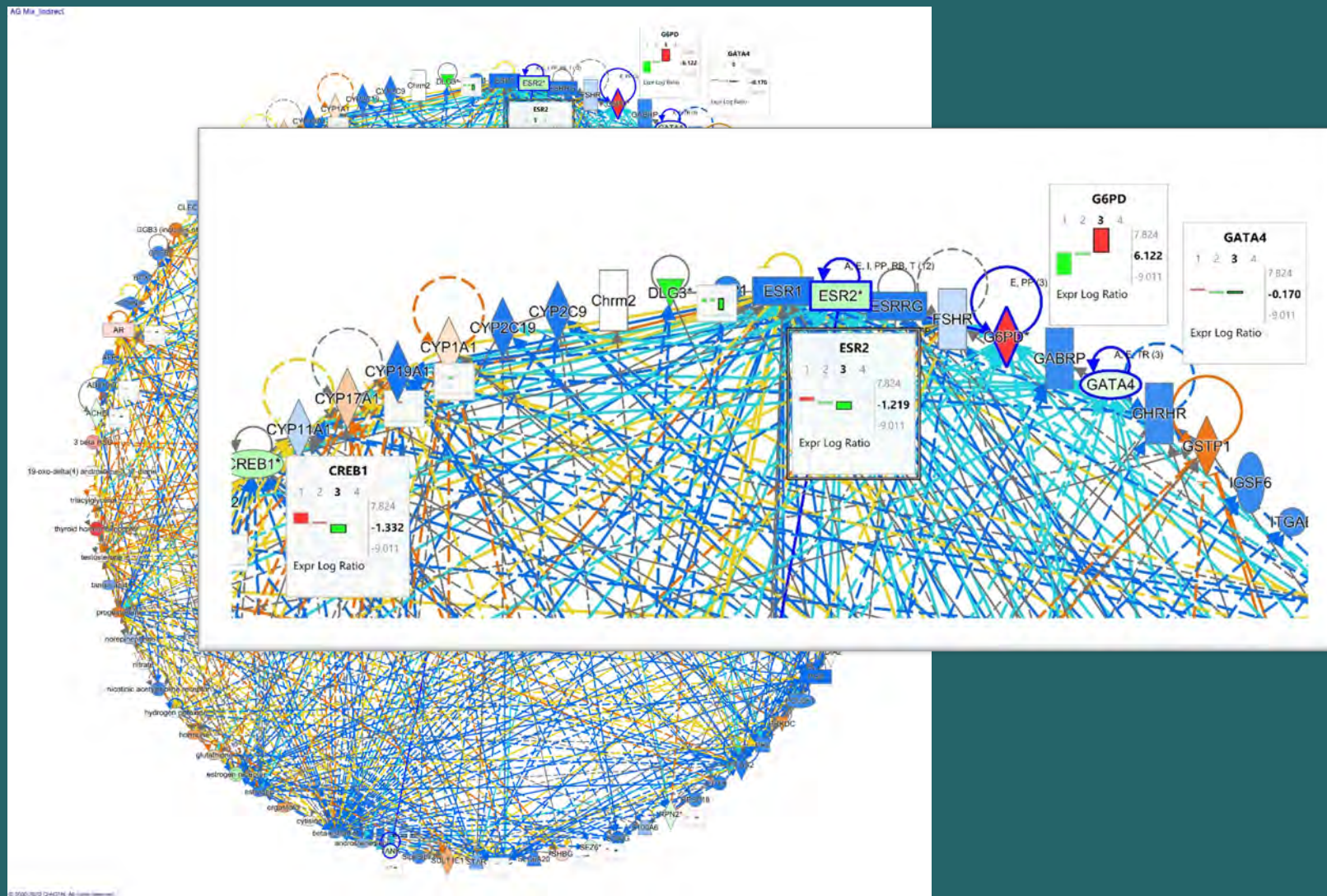
Ag Mix Exposure Alters Liver DEGs and DMRs

F₁

Gene
Expression

Biological
Pathway

Biological
End Point





Agricultural Mixture Exposure Results

F₀ 60 Day Agricultural Mixture Exposure

Gene Expression

- Increased expression of ESR2, CREB1, and decreased G6PD
- Genes known to respond to Ag Mix are differentially methylated

Biological Pathway

- Liver damage

Biological End Point

- Increased VTG

F₁ 290 Day Agricultural Mixture Exposure

Gene Expression

- Decreased expression of ESR2, CREB1, and increased G6PD
- Genes known to respond to Ag Mix are differentially methylated

Biological Pathway

- Liver damage

Biological End Point

- Increased VTG
- Decreased SSC



Future Directions

- Explore association between VTG expression level and expression / methylation levels of individual genes.
- Identify differential isoform usage.
- RNASeq of gonad.



THANK YOU

Contact

Mary Jean See (*she/her/hers*)

see.maryjean@epa.gov

Linked in www.linkedin.com/in/mary-jean-see-ba7942ba

Researchgate: M.J. See

Molecular biologist

CCTE/GLTED/Molecular Indicators Branch

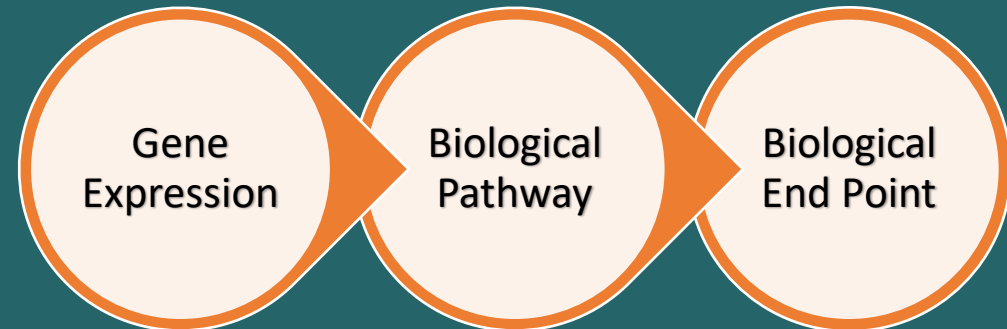
26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Office: 513-569-7629

Known Individual Chemical Responses

Fathead Minnow Response to Agricultural Mixture Chemicals



Gene Expression (mRNA)	Chemicals with Significant Result	Trend
AR	DEET	Decreased
CYP11A	atrazine	<i>Increased</i>
ESR1	atrazine, BPA	<i>Increased</i>
LHCGR	atrazine	<i>Increased</i>
vtg	BPA	<i>Increased</i>
GCLC	BPA	Decreased
GST	BPA	Decreased
Nrf2	BPA	Decreased
SOD	BPA	Decreased

Biological Pathway	Chemicals with Significant Result	Trend
17-b Estradiol	BPA	Dec.
Mortality	TBEP	Inc.
Testosterone	BPA	Dec.
DNA Damage	BPA	Inc.

- Glutathione S-transferase (GST)
- Nuclear Factor erythroid 2-related factor (Nrf2)
- Superoxide dismutase (SOD)

Biological End Point	Chemicals with Significant Result	Trend
VTG	4-nonylphenol, BPA, estrone	Inc.
SSC	BPA, DEET	Dec.
GSI	BPA	Inc.
HSI	DEET	Dec.