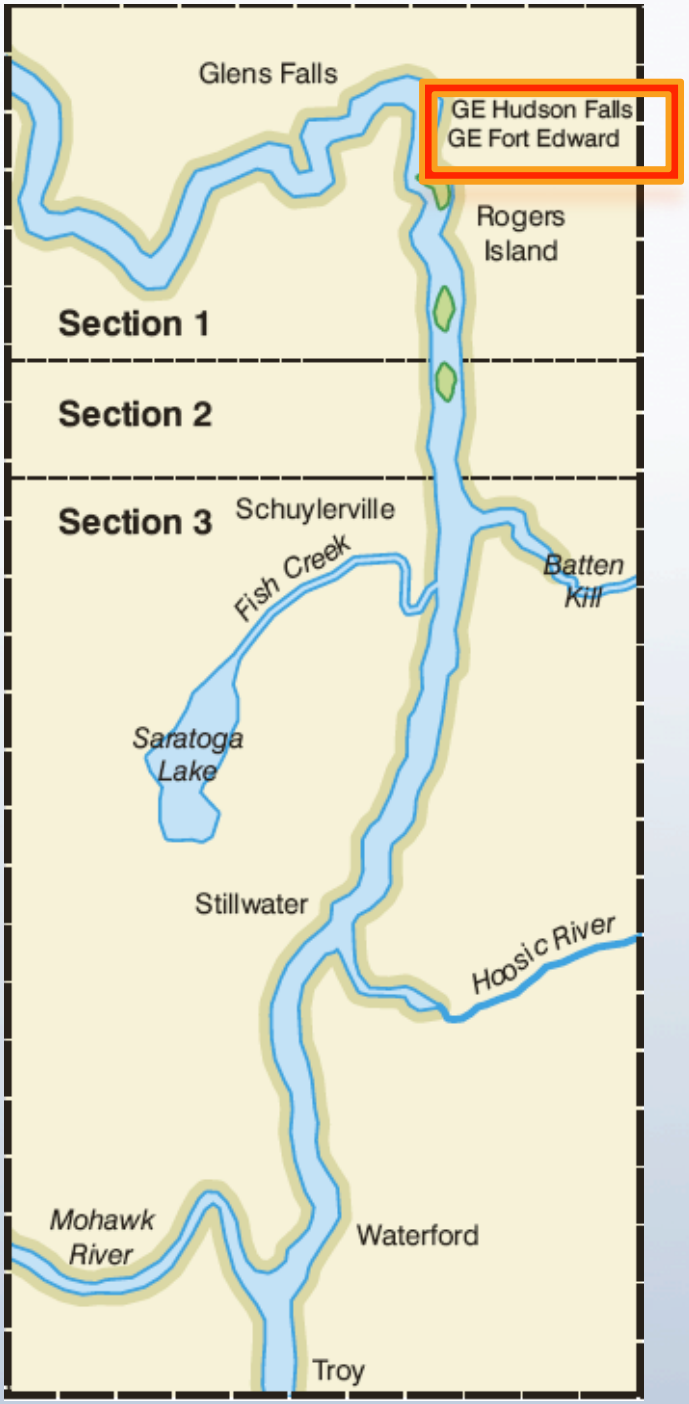
Characterization of Polychlorinated Biphenyl Action on Novel Biochemical Pathways in Japanese Quail (*Coturnix japonica*)

34th Annual SETAC North America Meeting

Meredith Bohannon, Tom Porter, and Mary Ann Ottinger

The opinions expressed in this presentation are those of the authors; the research does not represent the official position of the funding agency.



Background

- General Electric plants on Hudson River (HR)
- PCBs deposited into HR from 1940s until 1977 (PCBs banned) – 209K – 1.3M lbs dumped (EPA estimate)
- Superfund site
- PCB congener profiles found in bird eggs
  - Spotted sandpiper (SPSA, Custer et al., 2004)
  - Tree Swallow (TRES, Echols et al., 2004)

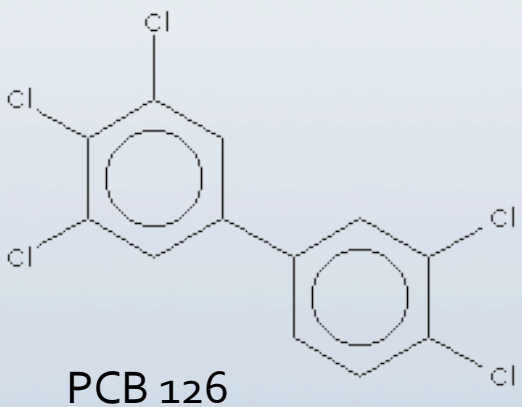
Previous Work

- Microarray - ~19K genes
  - Japanese quail – laboratory model
  - *In ovo* dosing
  - Spotted Sandpiper mixture
  - Hepatic genes
- Pathways affected:
  - Endocrine disruption
  - Oxidative damage
  - Xenobiotic metabolism
  - Energy balance

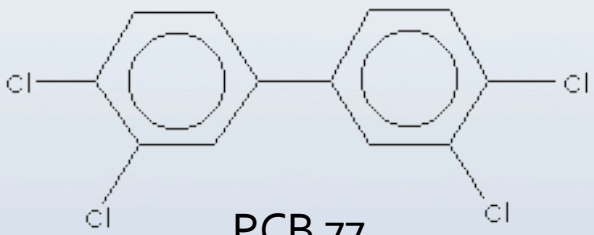
This Study

- Mixtures Relevant to UHR
  - Spotted sandpiper (SPSA) mixture
  - Tree swallow (TRES) mixture
  - PCB 126
  - PCB 77

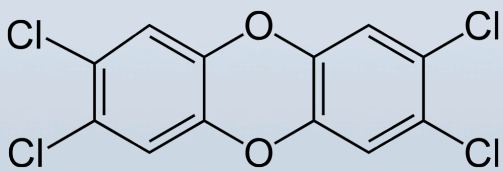
Non-ortho-substituted congeners → AhR Activation



PCB 126



PCB 77



TCDD

This Study

Pathway/System	Gene Name	Abbreviation
Xenobiotic Metabolism	Cytochrome P450 1A4	CYP1A4
Xenobiotic Metabolism	Cytochrome P450 1A5	CYP1A5
Xenobiotic Metabolism	Cytochrome b5	CYB5
Xenobiotic Metabolism	Aryl Hydrocarbon Receptor	AHR1
Oxidative Damage	Gluthathione S-Transferase	GSTA
Oxidative Damage	Glutathione Peroxidase	GPX
Oxidative Damage	Glutathione Reductase	GR
Oxidative Damage	Catalase	CAT
Oxidative Damage	Thioredoxin	TXN
Endocrine Disruption/Energy Balance	HMG-CoA Synthase	HCS
Energy Balance	Fructose Bisphosphate Aldolase B	ALDOB
Endocrine Disruption	Deiodinase Type 2	DIO2

Hypotheses

- Xenobiotic metabolism
  - H_a: increase → direct regulation of CYP450 genes by AhR
- Oxidative Damage
  - H_a1: increase → increased oxidative load
  - H_a2: decrease → system crash
- Energy Balance
  - H_a1: increase → metabolic need
  - H_a2: decrease → system crash
- Endocrine Disruption
  - H_a1: increase → hormone mimicking
  - H_a2: decrease → hormone antagonism or system crash

Study Design and Methods

- Egg Collection, Injection, and Incubation
  - Injected on ED0
- 4 Compounds of Interest
  - Six doses - equivalent TEQs across compounds
  - Three control groups:
    - Untreated
    - Sham-injected
    - Vehicle: sterol-stripped corn oil
- Tissue Collection
  - Livers collected at hatch, snap-frozen in liquid N2, and stored at -80°C
- RNA isolation and quantification → cDNA synthesis, primer design/validation → qPCR

Data Analysis

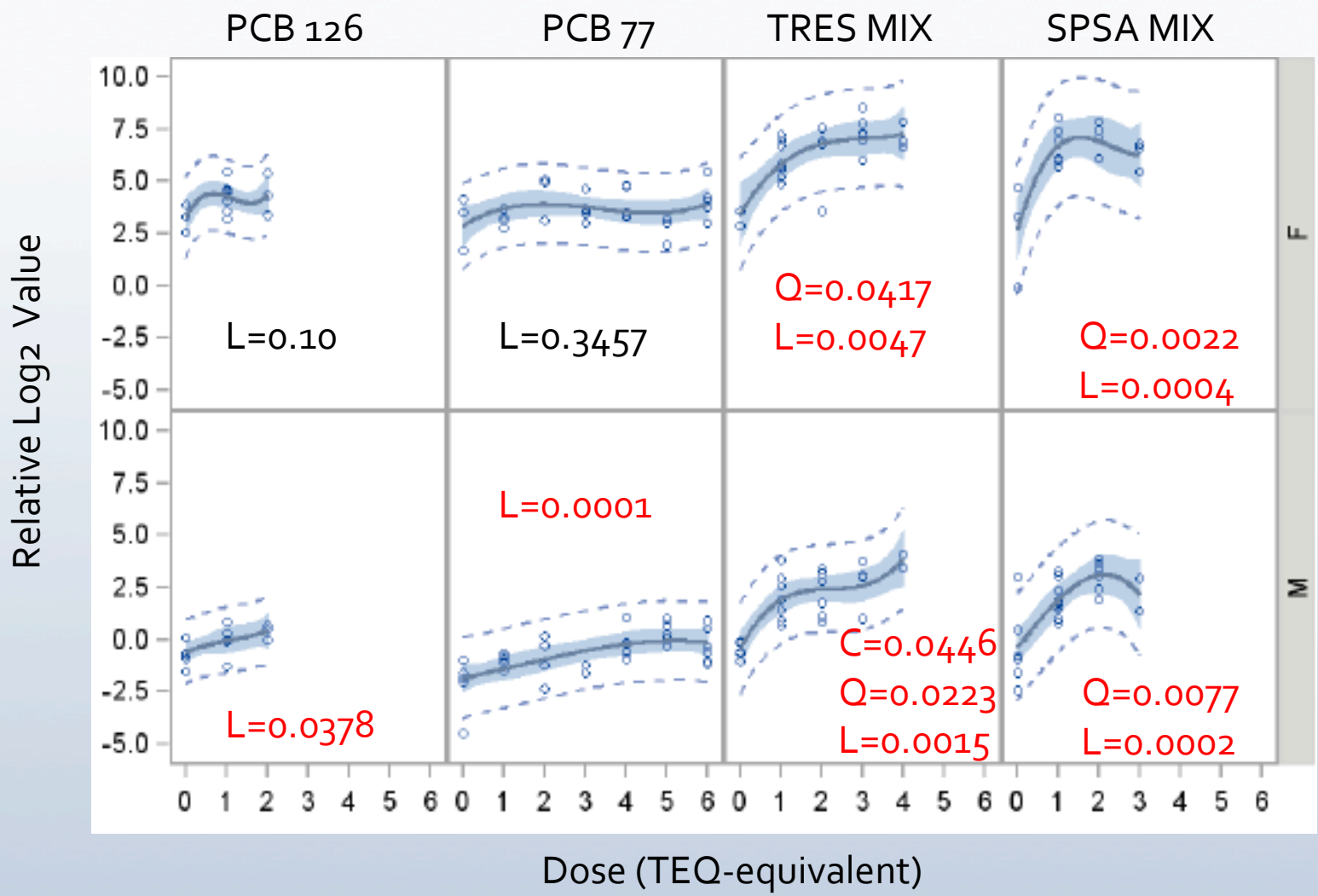
- GENMOD procedure
  - Test the nature of the dose response curve
  - Tests cubic, quadratic, and linear components of the dose-response curves

Concentration*Concentration*Concentration = Cubic

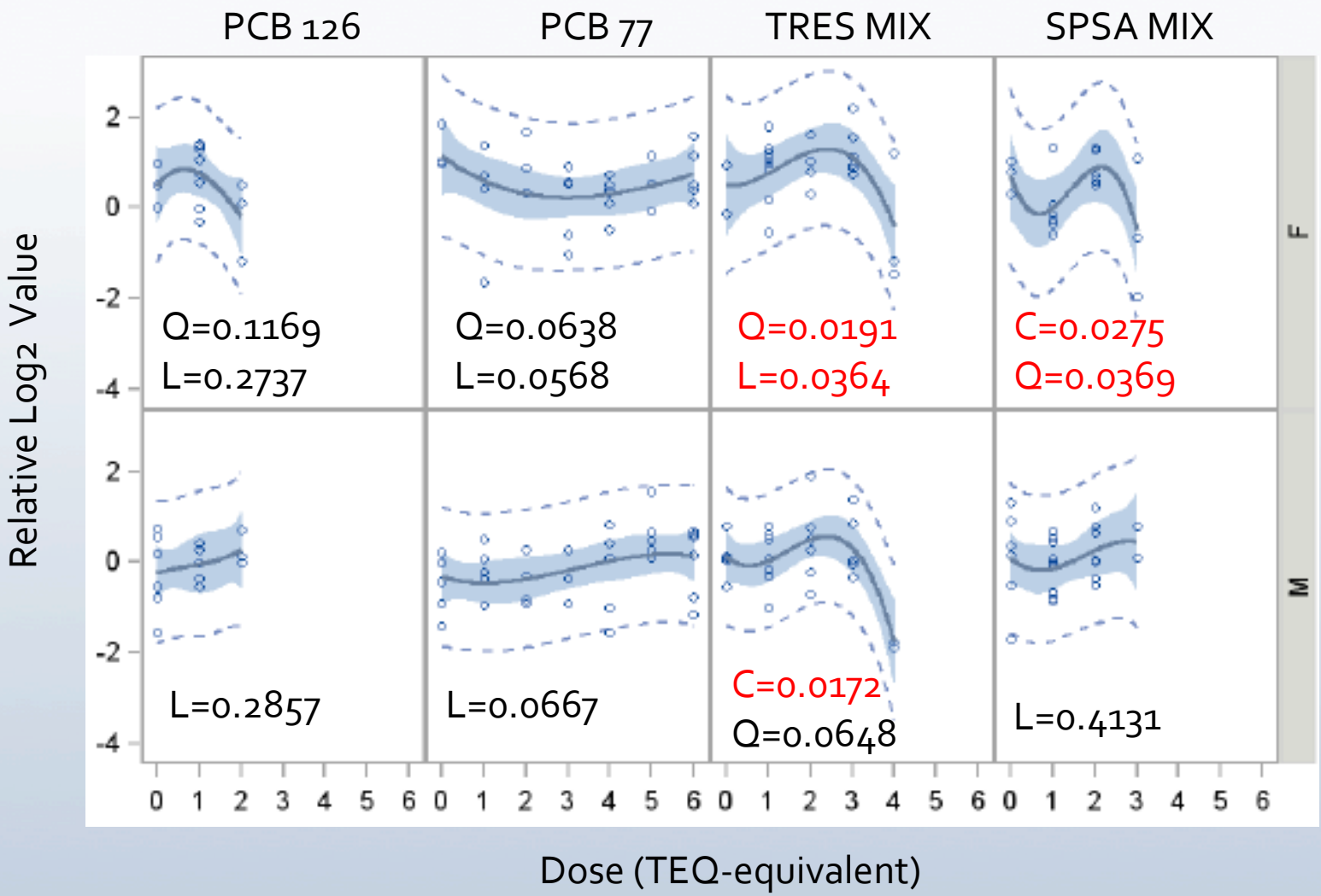
Concentration*Concentration = Quadratic

Concentration = Linear

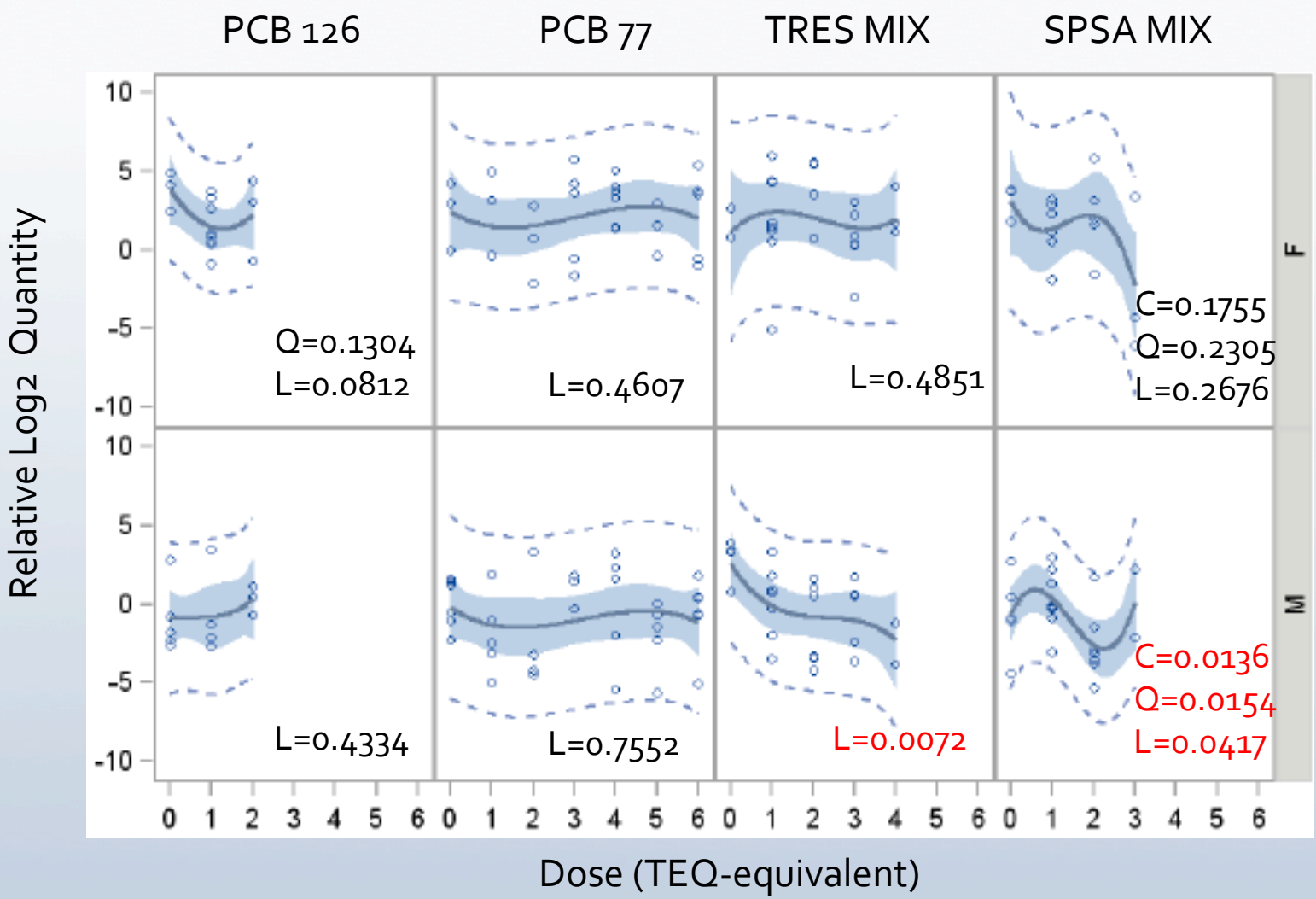
CYP1A4


PCB 126
PCB 77
TRES MIX
SPSA MIX
Relative Log2 Value
Dose (TEQ-equivalent)
F
M
L=0.10
L=0.3457
Q=0.0417
L=0.0047
Q=0.0022
L=0.0004
L=0.0001
L=0.0378
C=0.0446
Q=0.0223
L=0.0015
Q=0.0077
L=0.0002

Catalase


PCB 126
PCB 77
TRES MIX
SPSA MIX
F
M
Q=0.1169
L=0.2737
Q=0.0638
L=0.0568
Q=0.0191
L=0.0364
C=0.0275
Q=0.0369
L=0.2857
L=0.0667
C=0.0172
Q=0.0648
L=0.4131
Relative Log2 Value
Dose (TEQ-equivalent)

DIO2


PCB 126
PCB 77
TRES MIX
SPSA MIX
Relative Log2 Quantity
Dose (TEQ-equivalent)
F
M
Q=0.1304
L=0.0812
L=0.4607
L=0.4851
C=0.1755
Q=0.2305
L=0.2676
L=0.4334
L=0.7552
L=0.0072
C=0.0136
Q=0.0154
L=0.0417

Conclusions

- Unique dose-response curves
  - Gene specific responses
  - Compound specific responses
  - Sex specific responses
  - PCB 126 and 77 cannot account for responses of mixtures
- PCB 77 does not elicit huge responses
- PCB 126 not good for sublethal measures
- CYP1A4 survivor effect
- Several-fold difference of response
- What is "adverse"? → hormesis

Acknowledgements

- Mary Ann Ottinger Lab, UMD
  - Karen Dean
- Tom Porter Lab, UMD
- Natalie Karouna-Renier – qPCR
- Franca Barton - statistics
- Funding Agency - USFWS

The opinions expressed in this presentation are those of the authors; the research does not represent the official position of the funding agency.