

# Comparing Effects of Complex Mixtures versus Single PCBs: Functional Outcomes of Exposure in Field and Lab Studies

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# Assessing Potential Adverse Effects

- Understand the dynamics of the ecology and specifically the habitat and needs of selected species—use sentinel species if possible
- Comparative laboratory trials under controlled conditions to reveal mechanisms and impact
- study the interactions of mixtures, basis for species differences in sensitivities, and vulnerability with life stage

# Dimensions

- **Fitness**
  - **Survival and lifespan**
  - **Reproduction/viability of young**
  - **Health**
- **Reproductive axis and other mechanisms**
  - **Neuroendocrine and molecular endocrine regulators**
  - **Behavior**
  - **Gonadal steroids**
  - **Immune**
  - **physiological function (thyroid, adrenal)**
  - **Neuroendocrine/regulatory**
  - **Organ systems and pathology**
- **“Footprint” of an EDC or mixture?**
  - **Life stage effects and timing of exposure**

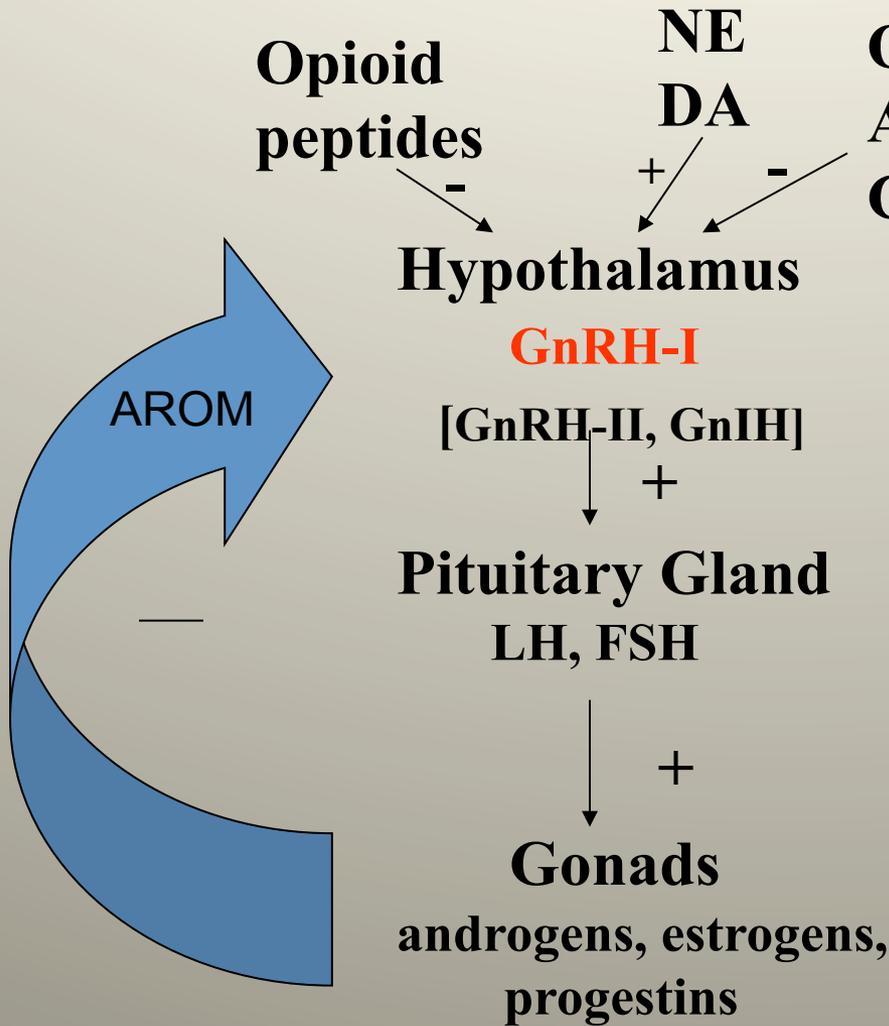
# Field and Lab Studies: PCBs



Photo; PWRC website

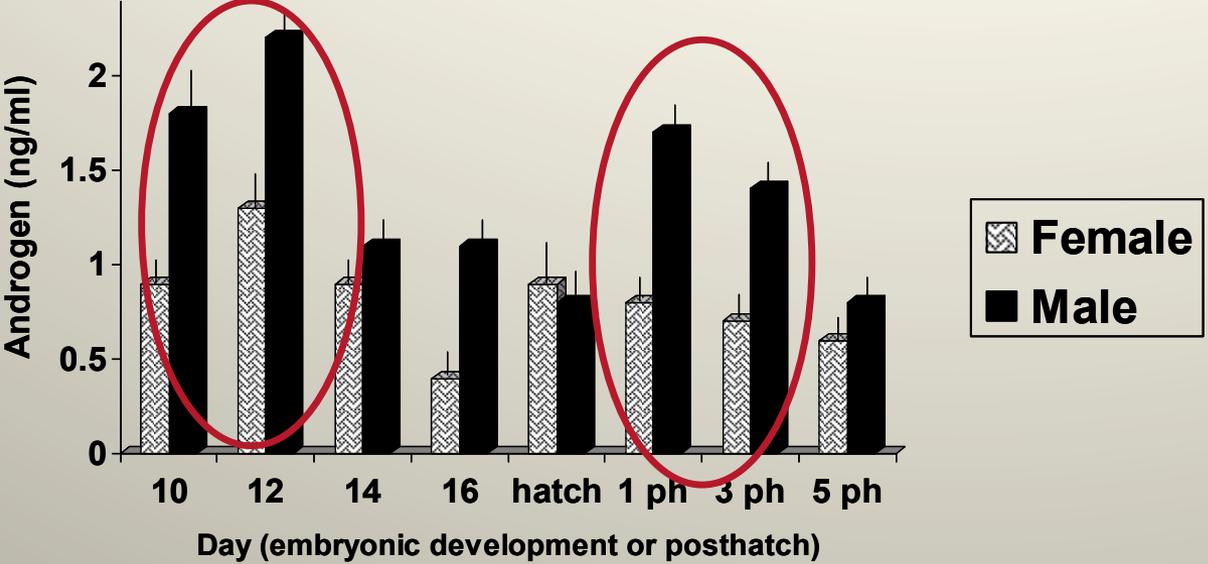
- Japanese Quail—University of Maryland colony
- Chicken – White leghorn eggs from CBT Farms, MD
- Japanese quail – Eggs from University of Maryland random bred colony
- American kestrel —Patuxent Wildlife Research Center
- Screech Owl—Patuxent Wildlife Research Center
- Tree swallow – nest boxes at Patuxent Research Refuge, Great Sacandaga Lake, NY, and Upper Hudson River
- Eastern Bluebird—nest boxes at Patuxent Research Refuge and Upper Hudson River

# Hypothalamic-Pituitary-Gonadal Axis in Avian Species

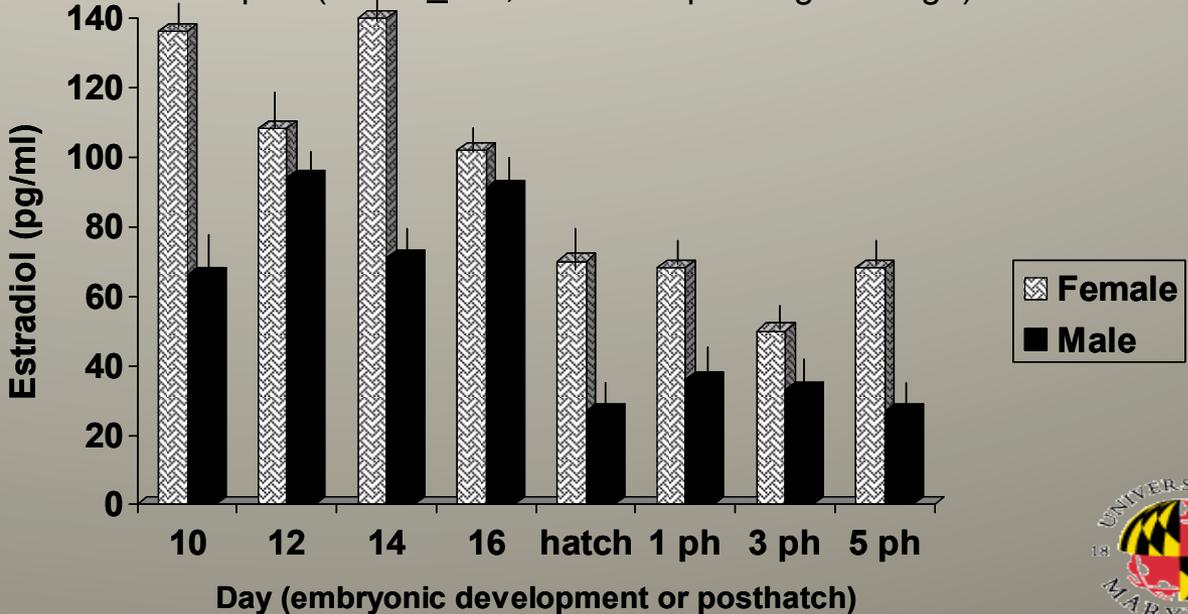


Neuroendocrine systems regulate endocrine and behavioral components of reproduction. Circulating testosterone is aromatized to estradiol in the preoptic region as an essential component in the activation of male courtship and mating behavior as well as in mediating the hormonal signal for negative feedback.

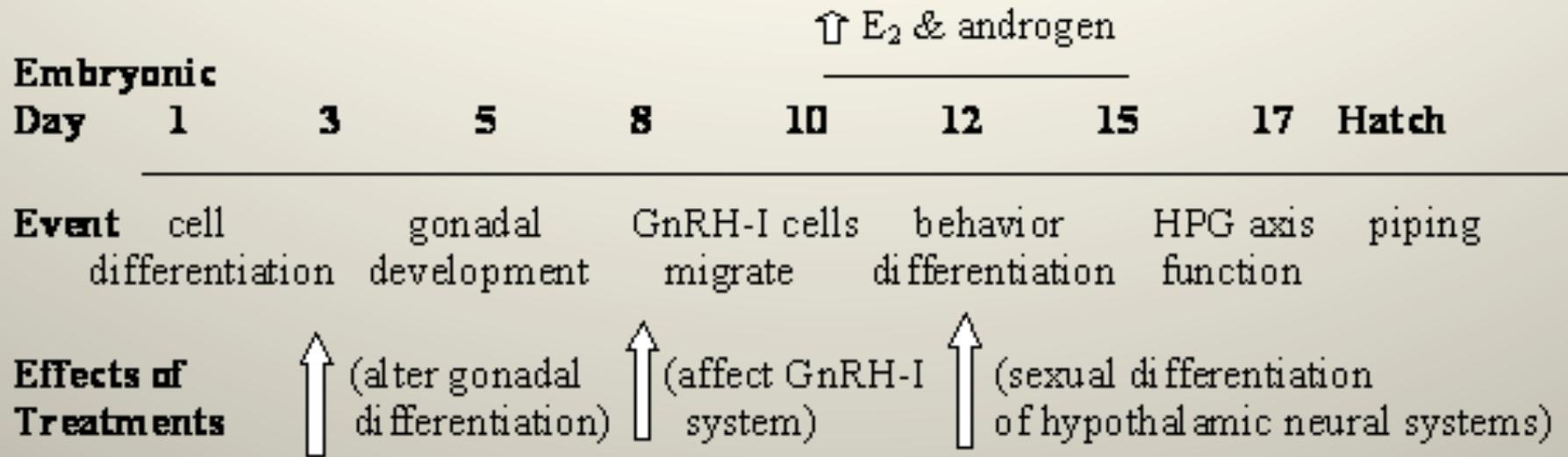
Plasma androgen (ng/ml plasma) in embryonic and post hatch (ph) Japanese quail (Mean  $\pm$  SE; n =5-22 depending on stage)



Plasma estradiol (pg/ml plasma) in embryonic and posthatch (ph) Japanese quail (Mean  $\pm$  SE; n=5-22 depending on stage).



# The Japanese Quail Laboratory Model



**Precocial Species** undergo sexual differentiation during embryonic development and are relatively independent at hatch;

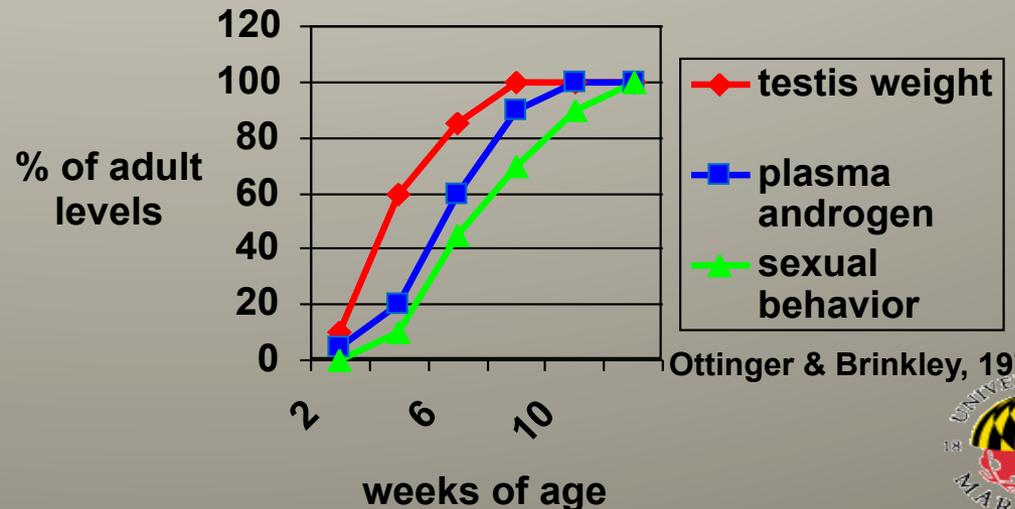
**Altricial species** undergo sexual differentiation later with:

—HPG axis depends on exposure to testosterone/estradiol

—song system depends on exposure to testosterone and other factors

## Sexual Maturation:

Gradual increase in testis function with rising androgen levels followed by onset of courtship and mating behavior and parental behavior.



# Summary of Behavioral Effects of EDCs in Quail:

Egg injections (between embryonic day 0-4) of either estrogenic or androgenic compounds all impaired male sexual behavior in adult males. Subsequent fertility of pairs exposed to low dose EDCs recovered fertility and productivity over time, supporting the insensitivity of fertility and productivity as measures of embryonic EDC exposure.

steroid hormone or EDC Tested	male sexual behavior	Effective dose(s)	references
estradiol	↓	0.5 - 50 - µg/egg	Abdelnabi and Ottinger, 2003; Viglietti-Panzica et al., 2005; 2007
ethinyl estradiol	↓	25-75 ng/egg	Halldin et al., 1999
atrazine	↓	0.5 - 5 - µg/egg	Ottinger et al., unpublished data
methoxychlor	↓	150 - 300 µg/egg	Ottinger et al., 2001
DES	↓	75-700 ng/egg 700 ng/egg	Halldin et al., 1999; Viglietti-Panzica et al., 2005
genistein	↓	100 or 1000 µg/egg	Viglietti-Panzica et al., 2007
PCB-126	↓	0.060-0.5 µg/egg	Ottinger and Lavoie, unpublished data
vinclozolin	↓	25 - 100 µg/egg	McGary et al., 2001
<i>p,p'</i> -DDE	↓	20 - 40 µg/egg	Quinn et al., 2005
trenbolone acetate	↓	0.05 - 50 µg/egg	Quinn et al., 2006

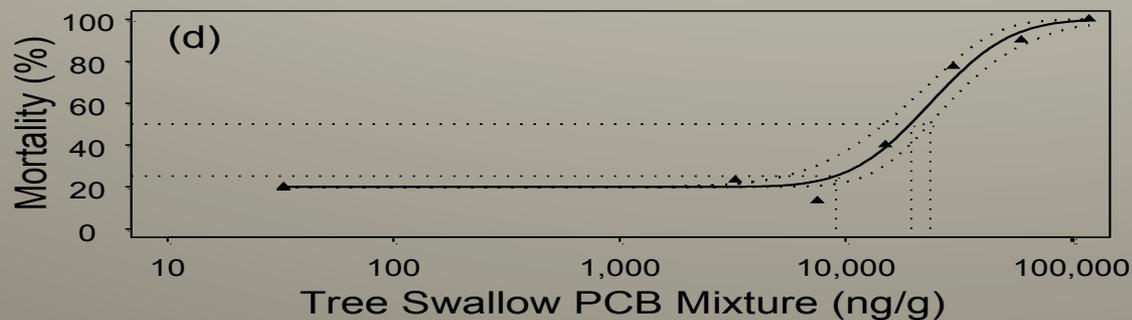
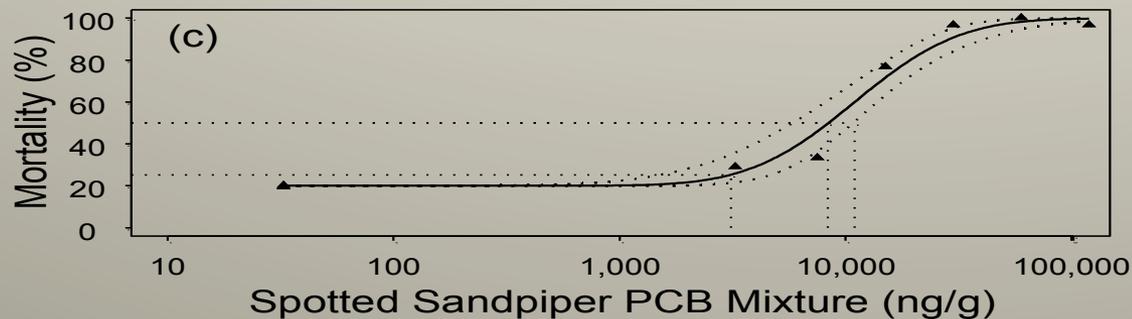
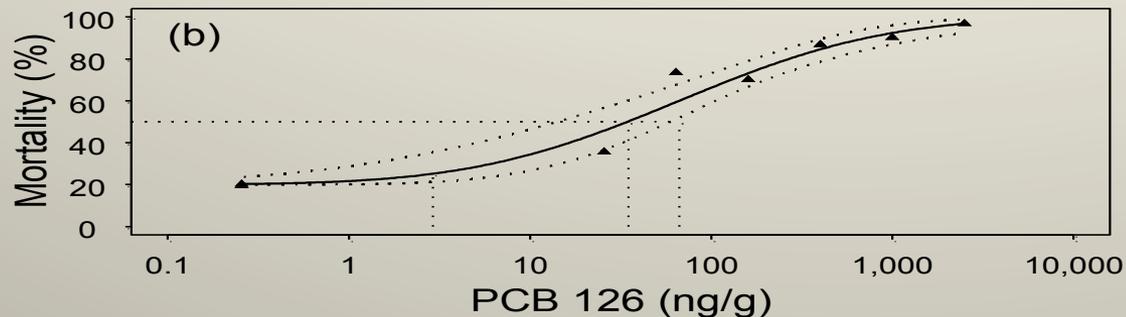
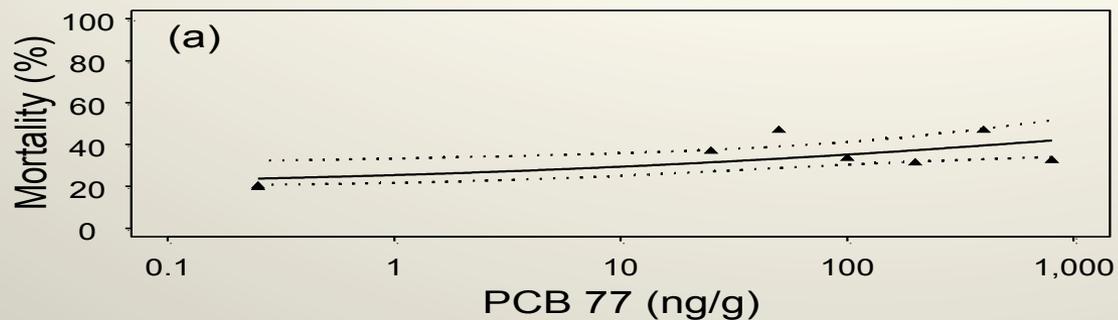
# PCB mixtures:

- Based on profile of PCBs measured in spotted sandpiper or tree swallow eggs collected at the Upper Hudson River, NY in 2004
- 58 or 66 different congeners in injection mixture : 49 of highest ranked congeners on a mass basis; represented 95% of the total PCB content in the sandpiper eggs on a mass basis; for details, see [http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/Trustee\\_USGS\\_Avian\\_Egg\\_Injection\\_Studies\\_Dosing\\_Solutions\\_final.pdf](http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/Trustee_USGS_Avian_Egg_Injection_Studies_Dosing_Solutions_final.pdf)  
<http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/58CongenerReportREVISED.pdf>
- 9 dioxin-like PCBs added because of toxicological importance
  - All congeners in mixture at relative proportion to levels in sandpiper egg sample
  - Excluded non dioxin-like congeners that cumulatively constituted <5% of the mass

## Injection methods and concentration range

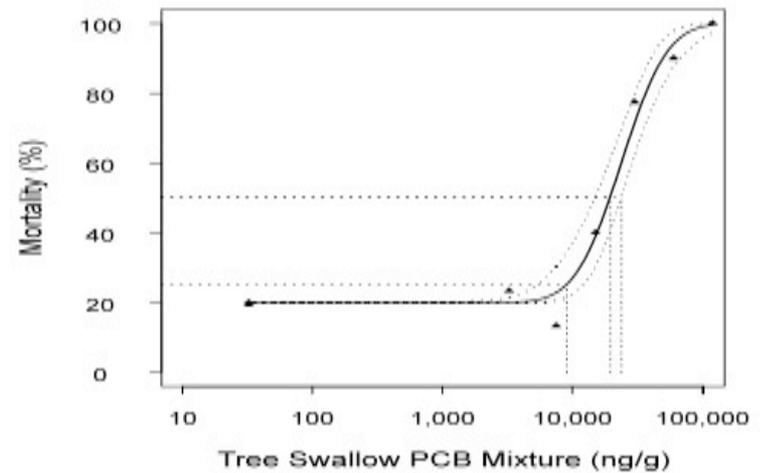
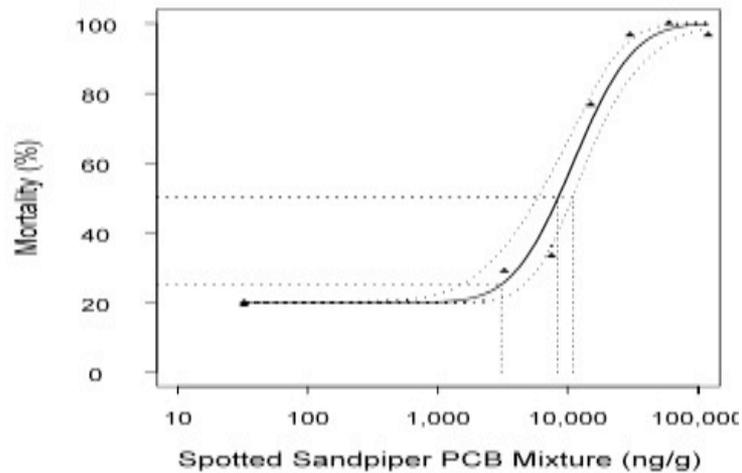
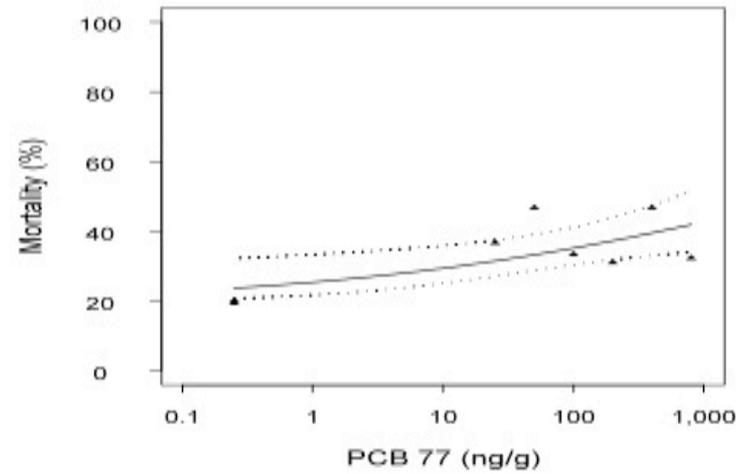
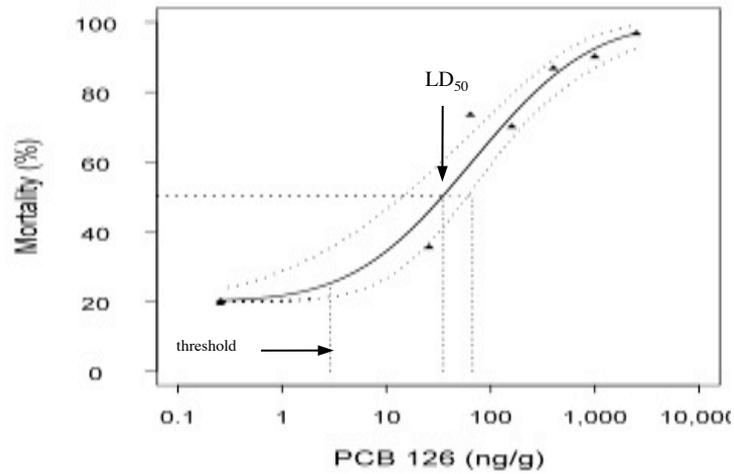
PCB 126	PCB 77	SPSA PCB mix	TRES PCB mix
0.1µl/g egg			
charcoal stripped corn oil			
injection prior to incubation			
dioxin-like congener	dioxin-like congener	58 congeners	66 congeners
2500 ng/g egg	800 ng/g egg	120 µg/g egg	120 µg/g egg
1000	400	60	60
400	200	30	30
160	100	15	15
64	50	7.5	7.5
25.6	25	3.75	3.75

➤ controls: untreated, sham injected and vehicle injected



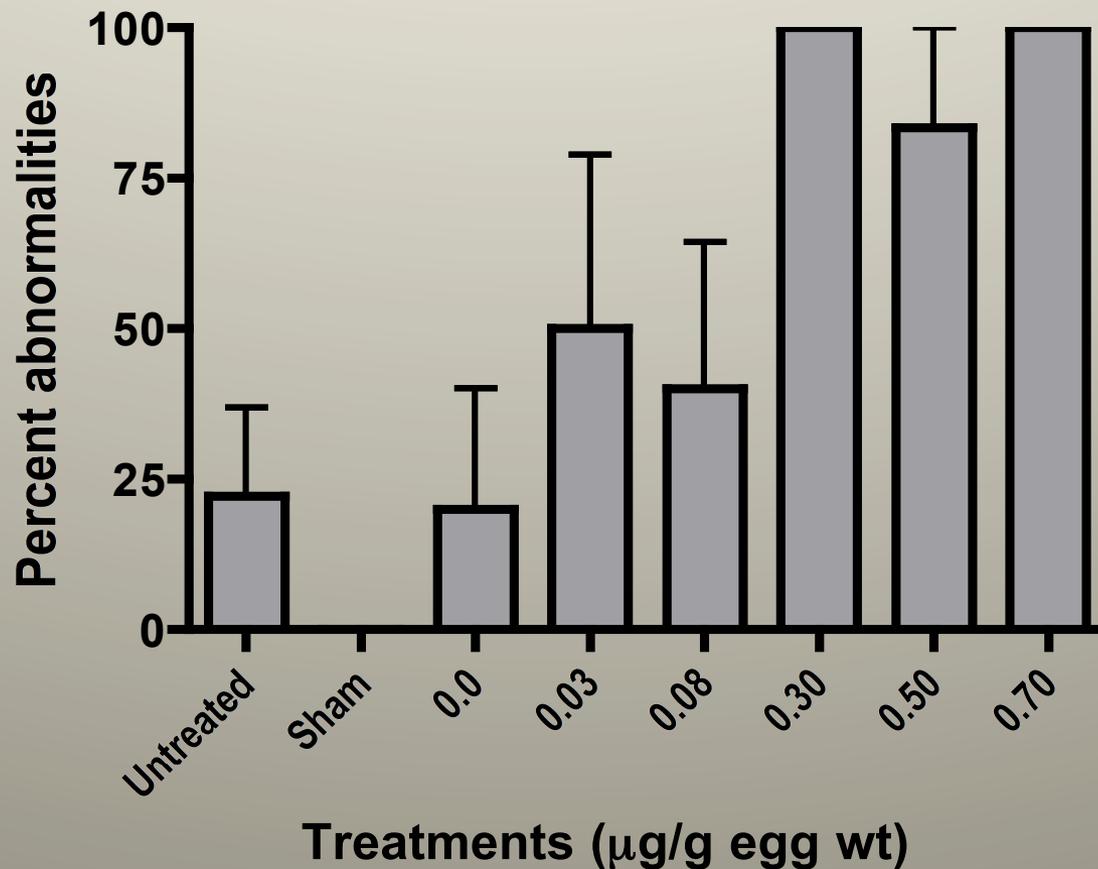
Lethality curves:  
Probit Analysis  
provide comparisons  
of embryonic effects  
of the PCBs, either  
separately or as  
mixes.

# Lethality Curves for Japanese Quail at Hatch



# Heart Effects of PCB Mix

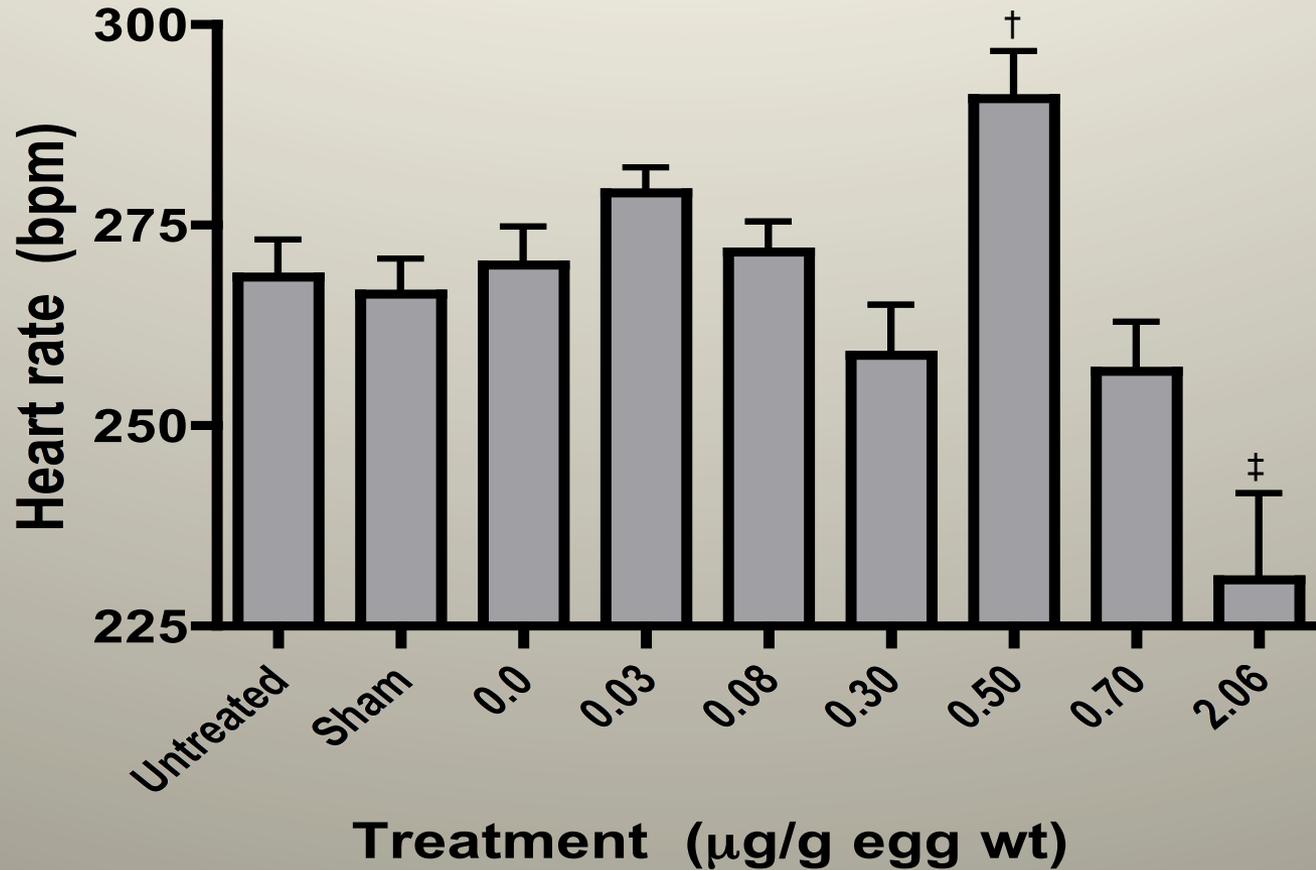
Incidence of Cardiomyopathies Increased in PCB Treatments Compared to Controls



From Carro et al., 2012 dissertation

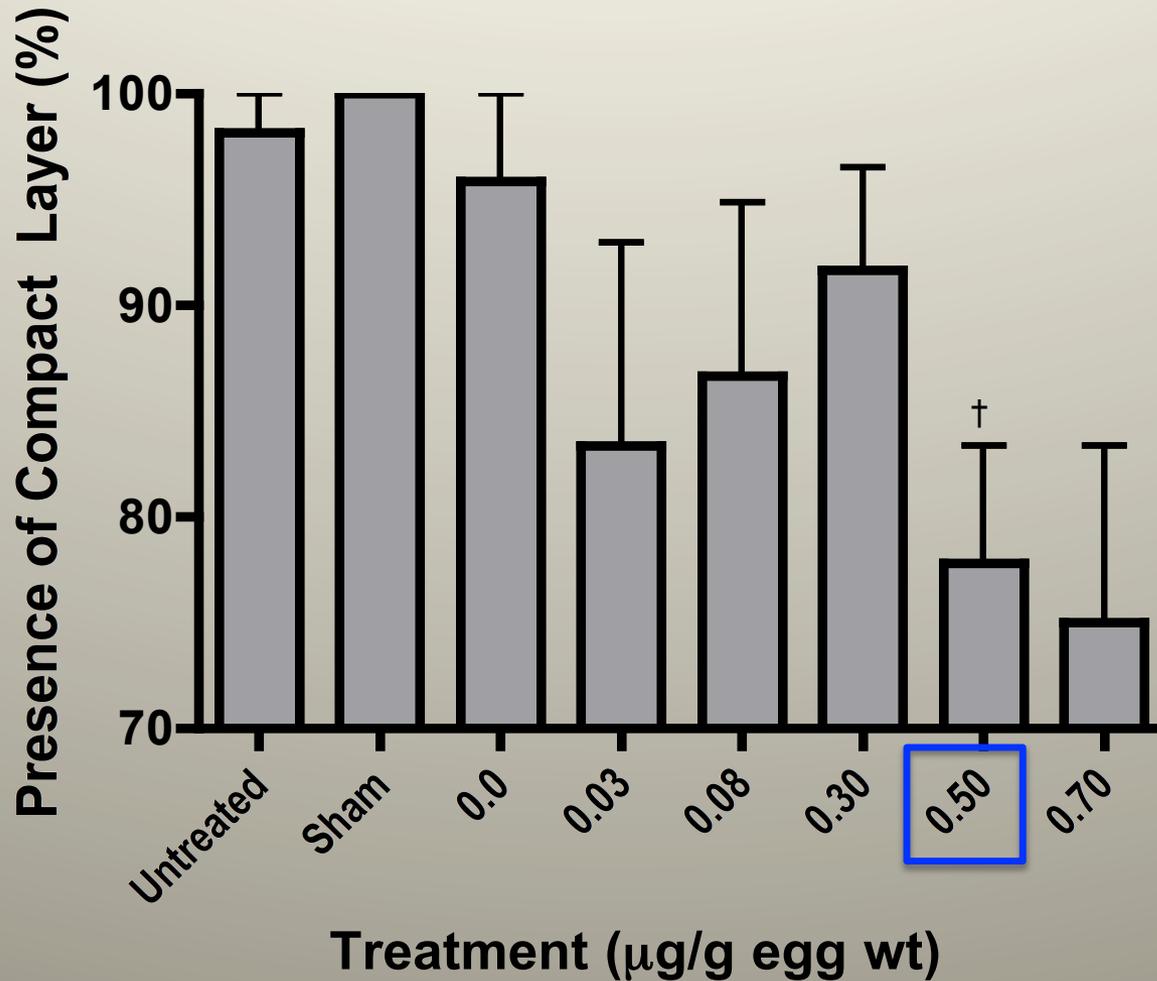


# Embryos Exposed to PCB Mix Exhibited Cardiac Arrhythmia at Embryonic Day 14



Tukey's post-hoc criteria:  $p < 0.05$   
(† & ‡ separately compared to controls)

# Ventricular Wall Compact Layer was Absent in Embryos Exposed to the PCB Mix



†: Tukey's post-hoc  
(compared to controls)  
 $p < 0.05$

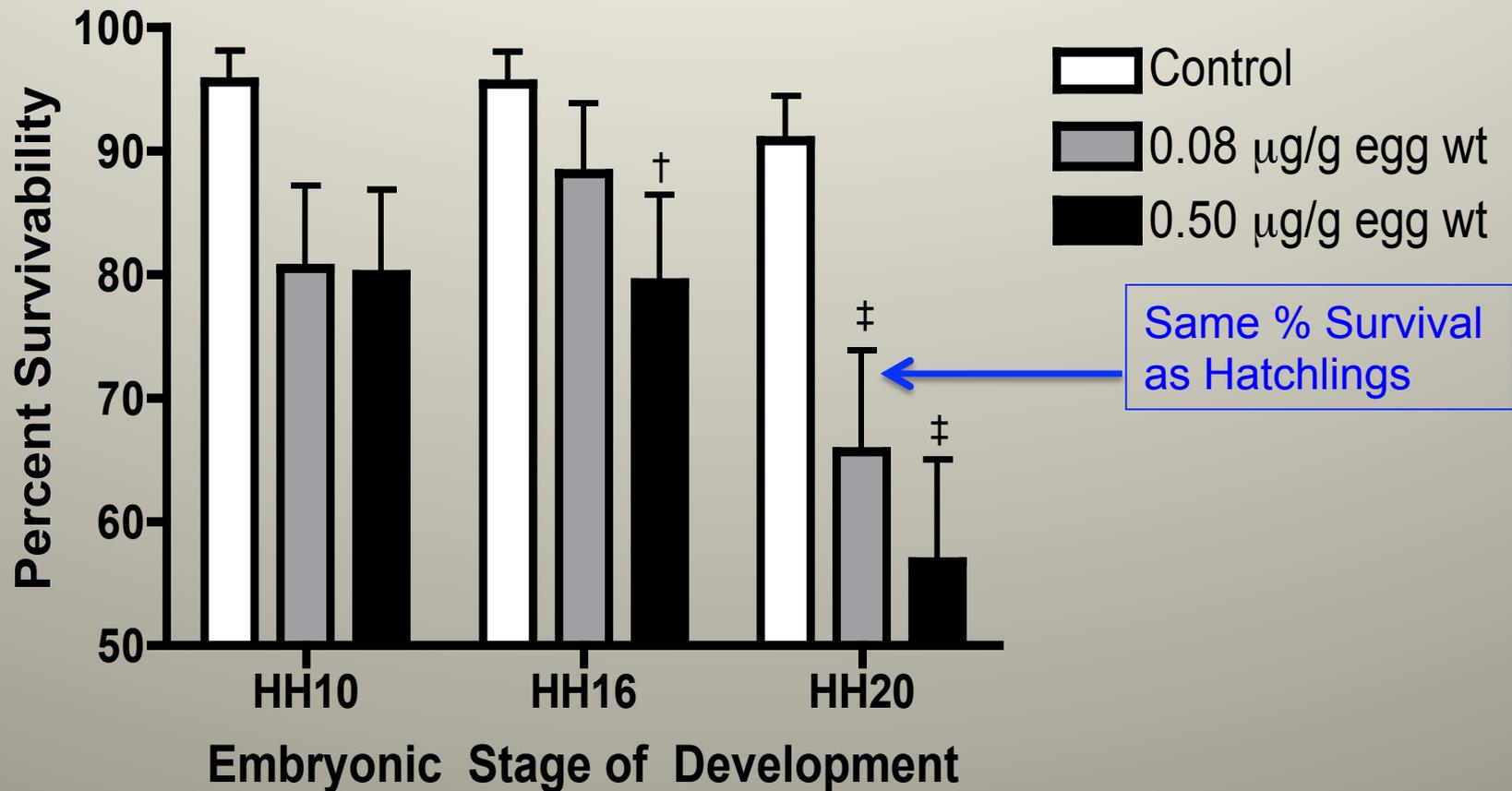
# Percent of Tree swallow hatchling hearts with Compact Layer absences following *in ovo* exposure to PCB 77.



Treatment	(n)	# hearts w/ intact CL	% hearts with CL deformities
Untreated	15	13	13.33
Vehicle	11	10	9.09
100 ng/g*	14	8	42.86
1000 ng/g*	15	4	73.33
UHR e. e.	8	8	0.00

Chi square ( $p < 0.001$ )

## Percent Survival Decreased Across PCB Treatments at HH16 and HH20

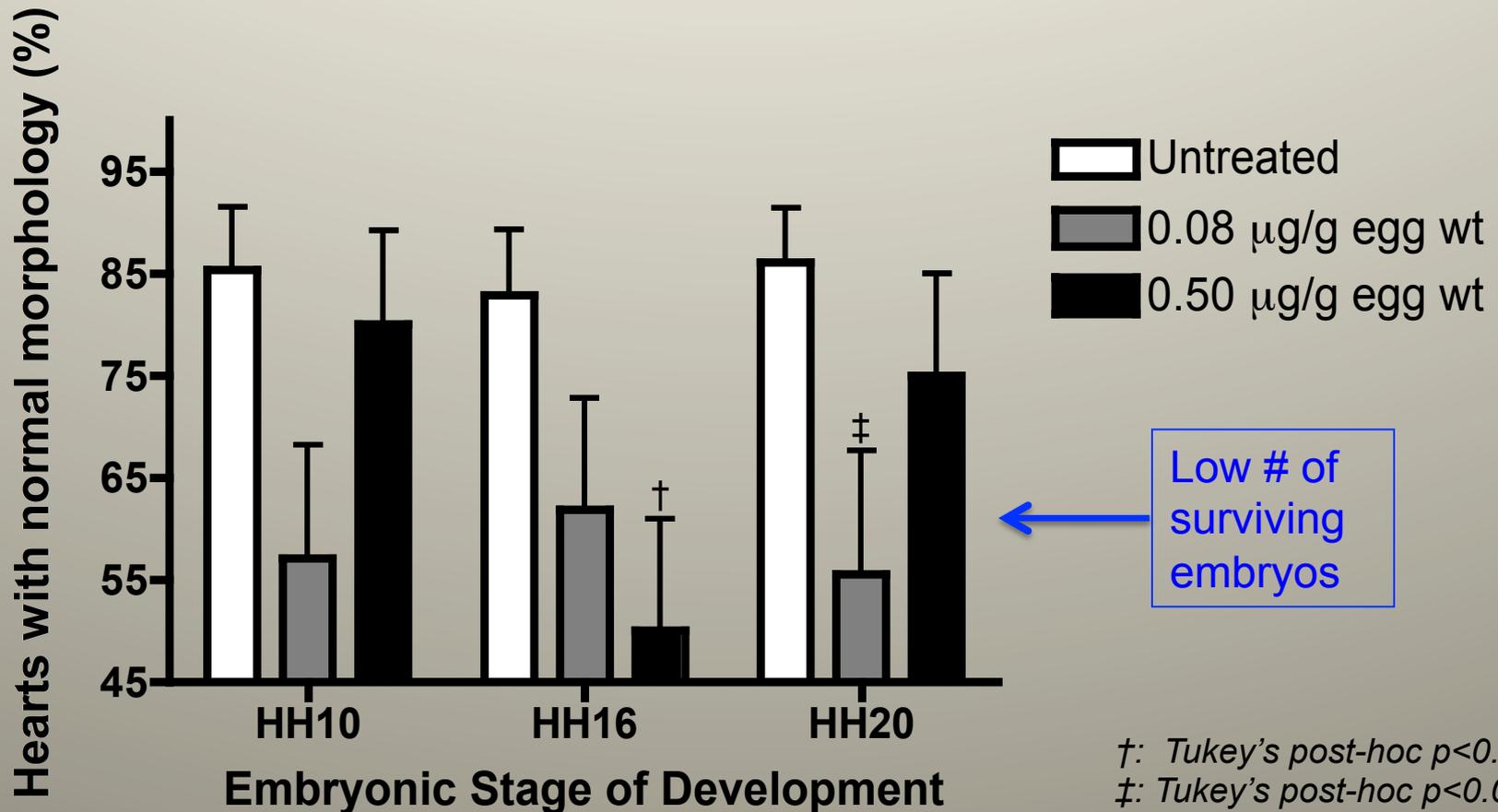


†: Tukey's post-hoc  $p < 0.05$

‡: Tukey's post-hoc  $p < 0.05$

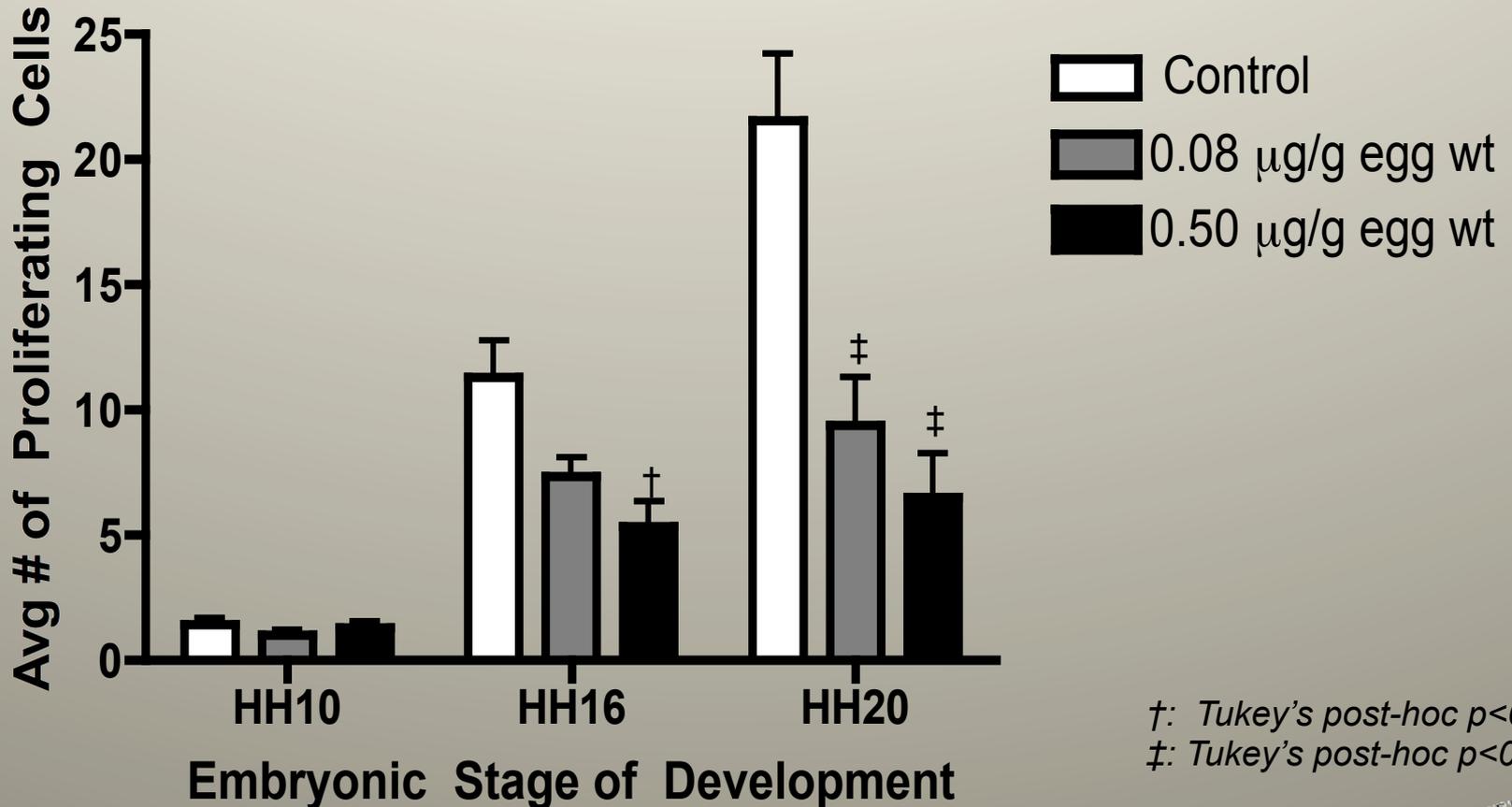
# Exp 2: Results

## Incidence of Cardiomyopathies Increased in PCB Treatments



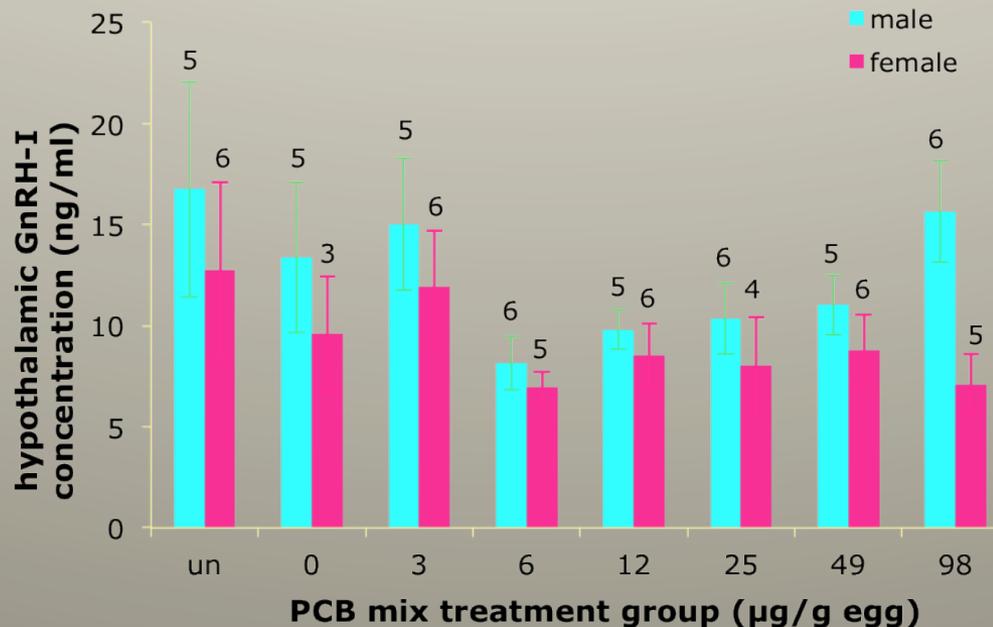
# Exp 2: Results

## Rates of Proliferating Cardiomyocytes Decreased Across PCB Treatments

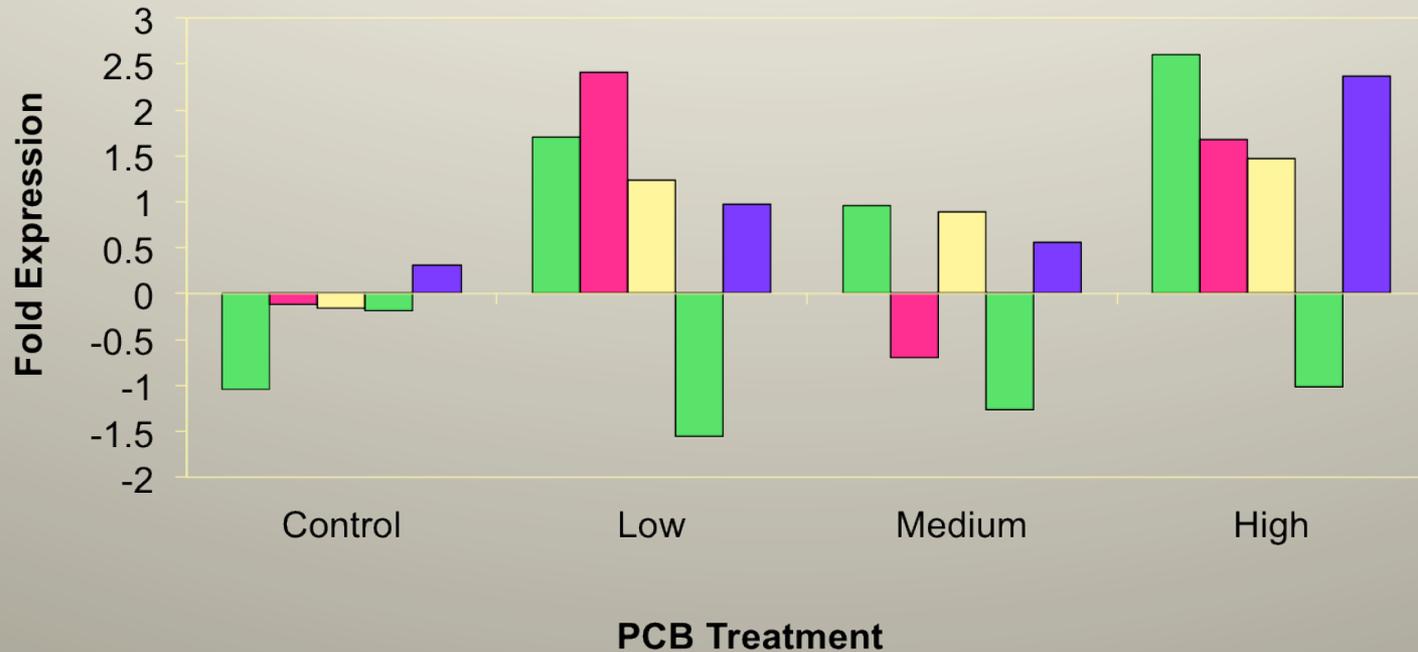


# Neuroendocrine

- No significant differences were observed for any of the monoamines tested in PCB 126 treated Japanese quail eggs
- GnRH concentrations in SPSA treated quail did not show a significant difference in response to dose, but there was a significant sex difference ( $p = 0.01$ )



# Gene Expression: Candidate genes in Japanese quail



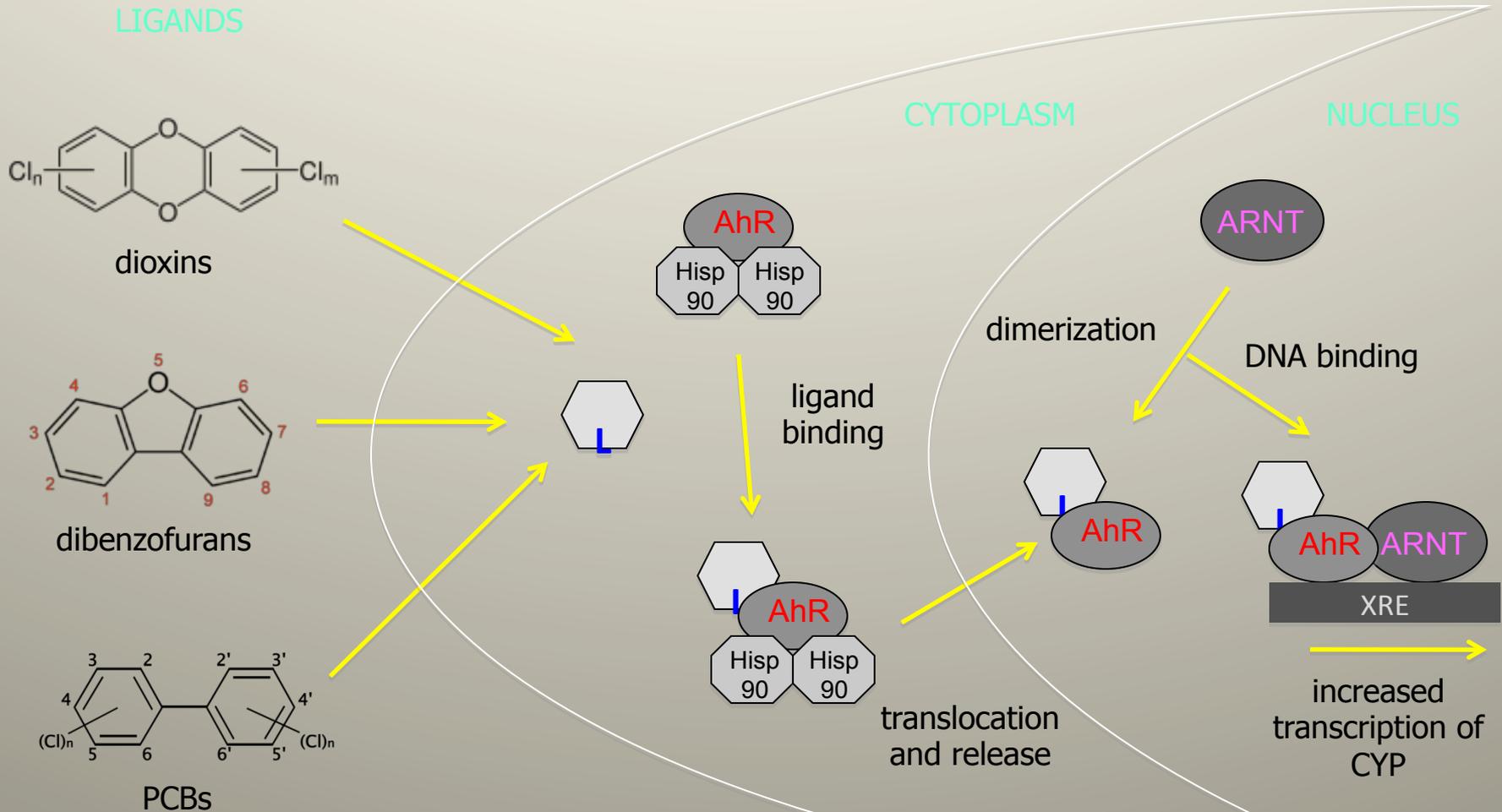
■ CYP4501A5 ■ Ornithine Decarboxylase Antizyme ■ Oxysterol-Binding Protein Isoform ■ Syntaxin 4 Binding Protein ■ STAT 5B or 6

# What does this all mean for measurements of PCBs in eggs collected from the Upper Hudson River?

- PCB egg content of UHR eggs from
  - SPSA: 9128 ng/g (TEQ 0.728; Custer *et al.*, 2009)
  - TRES: 2373.6 ng/g (TEQ 0.125; 2006 UHR QA)
- PCB mixture LD<sub>50</sub> values for Japanese quail:
  - SPSA: 8350 ng/g egg
  - TRES: 19600 ng/g egg
- Estimate that 25% of the applied dose reaches yolk in quail at time of hatch then the actual LD<sub>50</sub> =
  - SPSA: 2087.5 ng/g egg
  - TRES: 4900 ng/g egg

Differences in deposition method as well as differences in measurement techniques must be considered when determining environmental relevance of concentrations in egg injection studies

# The Ah Receptor and Characteristics of Interacting Compounds



# Does TEQ Predict Toxicity?

- TEQ (toxic equivalents) does NOT explain toxicity
- PCB 77 should be highly toxic, based on the TEQ; however, PCB 77 toxicity is very low in quail embryos.
- Conversely, the TRES mix should not be as toxic as observed, based on activation of the Ah receptor
- Further, when the TEQ factor for PCB 77 is removed, both mixes have TEQs well below that for PCB 126

(ng/g egg)	treatment			
	PCB 77	PCB 126	SPSA mix	TRES mix
LD <sub>50</sub> dose	-----	34.9 – 66.6	9210 - 10995	19557 - 23728
TEQ	-----	3.49 – 6.66	1.02 – 1.23	5.32 – 6.52

# Utility of an Endocrine Disruption Index (EDI)?

measurement	Interpretation of observed effect	Relevant for field birds?	Conserved across species?
lethality	Relative effects on individuals	At high exposures	Yes, species differences
deformities	Impact viability and longevity	Yes if birds are found	Yes, species differences
organ weights, morphology	Impaired physiology	Yes if birds are found	yes-organ and system specific
Neural/neuroendocrine effects	reduced fitness, altered behavior	yes	yes
Endocrine impairment	reduced function impaired fitness	variable	yes—survivor effect?
Delayed maturation	Reduced fitness	yes	yes
Impaired immune and thyroid system function	Reduced fitness/survival	yes	yes
Parental, reproductive and migratory behavior	Reduced fitness/survival	yes	yes
accelerated aging	Reduced fitness/survival	yes	yes

# Studies in Birds

**Mary Ann Ottinger, PhD**

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**Emma Lavoie , PhD**

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**Allegra Marcell**

**Nichola Thompson**

**Sean Madden**

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