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

- 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.

Ottinger et al., 2003
Plasma androgen (ng/ml plasma) in embryonic and posthatch (ph) Japanese quail (Mean ± SE; n = 5-22 depending on stage).

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

<table>
<thead>
<tr>
<th>Steroid Hormone or EDC Tested</th>
<th>Male Sexual Behavior</th>
<th>Effective Dose(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>estradiol</td>
<td>↓</td>
<td>0.5 - 50 µg/egg</td>
<td>Abdelnabi and Ottinger, 2003; Viglietti-Panzica et al., 2005; 2007</td>
</tr>
<tr>
<td>ethinyl estradiol</td>
<td>↓</td>
<td>25-75 ng/egg</td>
<td>Halldin et al., 1999</td>
</tr>
<tr>
<td>atrazine</td>
<td>↓</td>
<td>0.5 - 5 µg/egg</td>
<td>Ottinger et al., unpublished data</td>
</tr>
<tr>
<td>methoxychlor</td>
<td>↓</td>
<td>150 - 300 µg/egg</td>
<td>Ottinger et al., 2001</td>
</tr>
<tr>
<td>DES</td>
<td>↓</td>
<td>75-700 ng/egg</td>
<td>Halldin et al., 1999; Viglietti-Panzica et al., 2005</td>
</tr>
<tr>
<td>genistein</td>
<td>↓</td>
<td>100 or 1000 µg/egg</td>
<td>Viglietti-Panzica et al., 2007</td>
</tr>
<tr>
<td>PCB-126</td>
<td>↓</td>
<td>0.060-0.5 µg/egg</td>
<td>Ottinger and Lavoie, unpublished data</td>
</tr>
<tr>
<td>vinclozolin</td>
<td>↓</td>
<td>25 - 100 µg/egg</td>
<td>McGary et al., 2001</td>
</tr>
<tr>
<td>p,p’-DDE</td>
<td>↓</td>
<td>20 - 40 µg/egg</td>
<td>Quinn et al., 2005</td>
</tr>
<tr>
<td>trenbolone acetate</td>
<td>↓</td>
<td>0.05 - 50 µg/egg</td>
<td>Quinn et al., 2006</td>
</tr>
</tbody>
</table>
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 [link](http://www.fws.gov/contaminants/restorationplans/HudsonRiver/docs/Trustee_USGS_Avian_Egg_Injection_Studies_Dosing_Solutions_final.pdf) [link](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

<table>
<thead>
<tr>
<th>PCB 126</th>
<th>PCB 77</th>
<th>SPSA PCB mix</th>
<th>TRES PCB mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1µl/g egg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>charcoal stripped corn oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>injection prior to incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>dioxin-like congener</th>
<th>dioxin-like congener</th>
<th>58 congeners</th>
<th>66 congeners</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500 ng/g egg</td>
<td>800 ng/g egg</td>
<td>120 µg/g egg</td>
<td>120 µg/g egg</td>
</tr>
<tr>
<td>1000</td>
<td>400</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>400</td>
<td>200</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>160</td>
<td>100</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>64</td>
<td>50</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>25.6</td>
<td>25</td>
<td>3.75</td>
<td>3.75</td>
</tr>
</tbody>
</table>

- 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.

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

Chi square (*p*<0.001)
Percent Survival Decreased Across PCB Treatments at HH16 and HH20

Embryonic Stage of Development

Control

0.08 µg/g egg wt

0.50 µg/g egg wt

†: Tukey’s post-hoc p<0.05
‡: Tukey’s post-hoc p<0.05

Same % Survival as Hatchlings
Exp 2: Results

Incidence of Cardiomyopathies Increased in PCB Treatments

Embryonic Stage of Development

- HH10
- HH16
- HH20

Hearts with normal morphology (%)

- Untreated
- 0.08 µg/g egg wt
- 0.50 µg/g egg wt

Low # of surviving embryos

†: Tukey’s post-hoc p<0.05
‡: Tukey’s post-hoc p<0.05
Exp 2: Results

Rates of Proliferating Cardiomyocytes Decreased Across PCB Treatments

†: Tukey’s post-hoc p<0.05
‡: Tukey’s post-hoc p<0.05
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

Bohannon, Porter and Ottinger, unpublished
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$_{50}$ 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$_{50}$ =
  - 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

LIGANDS

- Dioxins
- Dibenzofurans
- PCBs

AhR
- Ligand binding
- Dimerization
- DNA binding
- Translocation and release
- Increased transcription of CYP

Cytoplasm

Nucleus

ARNT

XRE
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

<table>
<thead>
<tr>
<th>(ng/g egg)</th>
<th>treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCB 77</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt; dose</td>
<td>-------</td>
</tr>
<tr>
<td>TEQ</td>
<td>-------</td>
</tr>
</tbody>
</table>
## Utility of an Endocrine Disruption Index (EDI)?

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Interpretation of observed effect</th>
<th>Relevant for field birds?</th>
<th>Conserved across species?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethality</td>
<td>Relative effects on individuals</td>
<td>At high exposures</td>
<td>Yes, species differences</td>
</tr>
<tr>
<td>Deformities</td>
<td>Impact viability and longevity</td>
<td>Yes if birds are found</td>
<td>Yes, species differences</td>
</tr>
<tr>
<td>Organ weights, morphology</td>
<td>Impaired physiology</td>
<td>Yes if birds are found</td>
<td>Yes-organ and system specific</td>
</tr>
<tr>
<td>Neural/neuroendocrine effects</td>
<td>reduced fitness, altered behavior</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Endocrine impairment</td>
<td>reduced function impaired fitness</td>
<td>variable</td>
<td>yes—survivor effect?</td>
</tr>
<tr>
<td>Delayed maturation</td>
<td>Reduced fitness</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Impaired immune and thyroid system function</td>
<td>Reduced fitness/survival</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Parental, reproductive and migratory behavior</td>
<td>Reduced fitness/survival</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Accelerated aging</td>
<td>Reduced fitness/survival</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
Studies in Birds

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