Effects of plasticizers on the immune system of juvenile salmon.

Patty Zwollo

With Kelly Martins, Birgit Hagedorn, Chris Pallister, and John Kennish

Department of Biology, The College of William and Mary, Williamsburg VA and Environment and Natural Resources Institute, University of Alaska Anchorage, AK, and Gulf of Alaska Keeper, Anchorage, AK
THE PROBLEM:

Phthalates are plasticizers

Phthalates are non-covalently bound to polymer products

Phthalates slowly migrate into the environment

Biodegradation in aquatic systems is SLOW

Phthalate uptake through gills and intestine

Fish health: dys-regulation of immune function??

Bio-accumulation: during early development
OUR RESEARCH QUESTION:

Do phthalates have detrimental effects on the antibody response of juvenile salmon?
BACKGROUND:

Antibody-producing cells:
secrete antibodies
BACKGROUND:

Antibody-producing cells: secrete **antibodies** needed to neutralize **pathogens**.

Antibody-producing cell (plasma cell) — Antibodies — Pathogens
The “antibody response” in fish

Pathogens:
* viruses
* bacteria
* parasites
* fungi

B cell Proliferation

Differentiation to plasma cells

Antibody production

Antibodies remove pathogens

Cell proliferation expands the pool of B cells with affinity for the pathogen
B cells divide rapidly after exposure to pathogens:
B cells divide rapidly after exposure to pathogens:

Phthalates block the expansion of B cells (which become antibody-secreting cells)
RESULTS
Laboratory studies 1.

Effects of DEHP exposure* on cell numbers in a (continuously dividing) B cell line:

DEHP reduces the number of B cells in a dose-dependent manner

* 48 hours, PD31
§ 4uM=1.56 ppm
Laboratory studies 2.

Effects of DEHP on B cell proliferation in cultured trout immune cells*:

DEHP reduces the abundance of proliferating B cells

* 24 hours, using FLOW CYTOMETRY
§ 4uM=1.56 ppm
CONCLUSIONS FROM LABORATORY STUDY:

DEHP exposure in immune cell cultures results in:

1. Reduction in the number of B cells
2. Reduction in the abundance of proliferating B cells

These changes are Dose-dependent
At Elizabeth Lake, phthalates from marine debris slowly migrate into the water.
### Phthalates in Water and Soil from Elizabeth Island:

<table>
<thead>
<tr>
<th></th>
<th>WATER 2010</th>
<th>WATER 2012</th>
<th>SOIL 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>1.9 - 14.1*</td>
<td>0.6 - 35.3</td>
<td>4.4 - 17.8</td>
</tr>
<tr>
<td>DBP</td>
<td>0.3 - 0.7</td>
<td>0.1 - 6.3</td>
<td>0.0 - 3.3</td>
</tr>
<tr>
<td>BBP</td>
<td>ND</td>
<td>0.0 - 44.3</td>
<td>0.0 - 25.7</td>
</tr>
<tr>
<td>DEP</td>
<td>0.6 - 0.76</td>
<td>0.0 - 1.3</td>
<td>ND</td>
</tr>
<tr>
<td>DnoP</td>
<td>0.0 - 0.2</td>
<td>0.0 - 46.2</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16.1</strong></td>
<td><strong>133.4</strong></td>
<td><strong>46.8</strong></td>
</tr>
</tbody>
</table>

*Risk assessment of just DEHP:
- WHO: < 8 ppb
- EPA ≤ 6 ppb

*IN PARTS PER BILLION
## Phthalates in Tissues from Juvenile Fish:

<table>
<thead>
<tr>
<th></th>
<th>INTESTINE</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>39.8*</td>
<td>91.5</td>
<td>97.0</td>
<td>10.6</td>
<td>15.6</td>
</tr>
<tr>
<td>DMP</td>
<td>ND</td>
<td>9.0</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
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<tr>
<td>DBP</td>
<td>ND</td>
<td>87.7</td>
<td>ND</td>
<td>ND</td>
<td>5.2</td>
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<tr>
<td>BBP</td>
<td>ND</td>
<td>18.7</td>
<td>15.1</td>
<td>ND</td>
<td>1.1</td>
</tr>
<tr>
<td>DEP</td>
<td>ND</td>
<td>7.8</td>
<td>50.4</td>
<td>ND</td>
<td>5.4</td>
</tr>
<tr>
<td>DNoP</td>
<td>ND</td>
<td>24.2</td>
<td>&lt;LOD</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>ND</strong></td>
<td><strong>238.9</strong></td>
<td><strong>162.5</strong></td>
<td><strong>ND</strong></td>
<td><strong>44.7</strong></td>
</tr>
</tbody>
</table>

*IN PARTS PER BILLION*
CORRELATIONS BETWEEN PHTHALATE TISSUE LEVELS AND IMMUNE GENE EXPRESSION

The *Higher* the Phthalate Levels in a fish, the *Lower* its Expression Of Antibody Genes

More Phthalates ➔ Fewer Antibodies produced!

Martins et al, 2016
### Tissue phthalate levels:

<table>
<thead>
<tr>
<th></th>
<th>ANTERIOR KIDNEY</th>
<th>POSTERIOR KIDNEY</th>
<th>SPLEEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI SEC MEM BLIMP PAX5</td>
<td>SEC MEM BLIMP</td>
<td>SEC MEM BLIMP</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.02 0.45 P</td>
<td>0.06 0.32 0.02 0.40 &gt;0.1</td>
<td>0.04 &gt;0.1 0.01 &gt;0.1 0.09 &gt;0.1 0.02</td>
</tr>
<tr>
<td>DMP</td>
<td>0.00 0.76 P</td>
<td>0.01 0.74 0.02 0.46 0.01 0.54</td>
<td>0.08 0.30 &gt;0.1 0.11 &gt;0.1 0.01</td>
</tr>
<tr>
<td>DBP</td>
<td>0.06 0.28 P</td>
<td>0.01 0.46 0.01 0.42 &gt;0.1 0.10</td>
<td>0.03 0.40 &gt;0.1 0.06 &gt;0.1 0.00</td>
</tr>
<tr>
<td>BBP</td>
<td>&gt;0.1 0.11 P</td>
<td>&gt;0.1 0.29 0.03 0.35 &gt;0.1</td>
<td>&gt;0.1 0.07 &gt;0.1 0.01 &gt;0.1 0.00</td>
</tr>
<tr>
<td>DEP</td>
<td>&gt;0.1 0.02 P</td>
<td>&gt;0.1 0.17 0.09 0.22 &gt;0.1</td>
<td>&gt;0.1 0.13 &gt;0.1 0.12 &gt;0.1 0.14</td>
</tr>
<tr>
<td>DnoP</td>
<td>&gt;0.1 0.21 P</td>
<td>&gt;0.1 0.25 0.06 0.34 &gt;0.1</td>
<td>&gt;0.1 0.01 &gt;0.1 0.15 &gt;0.1 0.08</td>
</tr>
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<tr>
<td></td>
<td>MUSCLE SEC MEM BLIMP PAX5</td>
<td>SEC MEM BLIMP</td>
<td>SEC MEM BLIMP</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.10 0.23 P</td>
<td>0.02 0.42 0.00 0.56 &gt;0.1 0.11</td>
<td>&gt;0.1 0.19 0.05 0.35 &gt;0.1 0.07</td>
</tr>
<tr>
<td>DMP</td>
<td>ND ND ND ND ND ND ND ND ND</td>
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<td>&gt;0.1 0.13</td>
</tr>
<tr>
<td>BBP</td>
<td>&gt;0.1 0.04 P</td>
<td>&gt;0.1 0.11 &gt;0.1 0.00 &gt;0.1 0.06</td>
<td>&gt;0.1 0.05 &gt;0.1 0.10 &gt;0.1 0.03</td>
</tr>
<tr>
<td>DEP</td>
<td>&gt;0.1 0.04 P</td>
<td>&gt;0.1 0.12 &gt;0.1 0.08 &gt;0.1 0.06</td>
<td>&gt;0.1 0.03 &gt;0.1 0.03 &gt;0.1 0.00</td>
</tr>
<tr>
<td>DnoP</td>
<td>&gt;0.1 0.00 P</td>
<td>&gt;0.1 0.11 &gt;0.1 0.07 &gt;0.1 0.19</td>
<td>&gt;0.1 0.23 &gt;0.1 0.11 0.00 0.70</td>
</tr>
</tbody>
</table>

The hematopoietic organ of juvenile fish is highly sensitive to phthalates DEHP, DMP, DBP and BBP.

Levels of phthalates in gastrointestinal tissue correlate more strongly to immune gene expression than levels in muscle.
CONCLUSIONS FROM FIELD STUDY:

1. Phthalate levels in water, soil, and fish tissues from EI are much higher than the WHO/EPA “safe” levels.

2. Phthalate concentrations in juvenile fish tissues correlate with reduced expression of essential antibody genes.

3. The hematopoietic organ of juvenile fish is highly sensitive to phthalate exposure.

4. The levels of phthalates in Gastrointestinal tissue are a good indicator for predictive effects on the immune system.
Phthalates **MIMIC** Natural Ligands for PPAR\(_\gamma\)*

**Natural ligand**: Prostaglandin J\(_2\), CLA

***Phthalates:***

- DEHP
- DMP
- DBP

* Sarath Josh et al., 2013.
**prostaglandin J\(_2\), CLA
... and can compete for binding*

Pristine environment:

- PPARγ + Natural ligand = Natural complex

Phthalate pollution:

- PPARγ + Phthalates = agonist complex

Many phthalates have higher binding affinity for PPARγ than the natural ligand
In a pristine environment:

- Normal B cell division
- Optimal Antibody-producing cells
- Sufficient protective antibody

HEALTHY FISH!

In presence of phthalates:

- Limited B cell division
- Insufficient Antibody-producing cells
- Insufficient protective antibody

INFECTIONS!
FINAL CONCLUSIONS

1. Phthalates From Marine Debris Likely Affect the ANTIBODY RESPONSE in Juvenile Salmon.

2. Phthalates May Cause LONG-TERM IMMUNE DYSFUNCTION in salmonids.

3. Phthalates likely Increase the Risk For Infection And Disease In Wild Salmon Populations.
Publications:


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Chemical Analysis: Applied Science Engineering and Technology Laboratory, UAA.

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Questions? Patty Zwollo pxzwol@wm.edu