The Effects of Triclosan on Growth and Development in the Pickerel Frog, *Rana palustris*

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Triclosan

- Antimicrobial agent
  - PCPs (shampoo, soap, etc)
    - 0.1-0.3% (w/w) (Sabaliunas et al. 2003)
- US Geological Survey studies (Kolpin et al. 2002)
  - 139 surface waters (1999-2000)
    - Triclosan found in 57.6% (RL=0.05ug/L)
    - Maximum = 2.3 ug/L
    - Median = 0.14 ug/L
- Municipal wastewater effluents
  - 0.24-2.7 ug/L (Reiss et al. 2002)
  - Sparingly water soluble- 12 mg/L at 20°C
Toxicity

- Orvos et al. 2002
  - *Daphnia magna* - 48h LC$_{50}$=390 ug/L
  - Fathead minnow - 96h LC$_{50}$=260 ug/L
- Fraker and Smith 2004
  - *Rana pipiens* tadpoles - reduced survival at 230 ug/L
- Smith and Burgett 2005
  - *Bufo americanus* - reduced survival at 23 ug/L, increased at 230 ug/L
- Fraker and Smith 2005
  - *Xenopus laevis* - no effect on survival - maximum concentration of 230 ug/L
Preliminary Research

*R. pipiens* Time to Metamorphosis

Days

% Wastewater
Preliminary Research

- Delayed metamorphosis
- Thyroid Disruption??

Crofton 2008
Structure

Thyroxine (T4)

Triclosan

Structural similarity - Interfering activity??
Thyroid Disruption

- Crofton et al. 2007
- Rats injected with triclosan

Hypothesized to be a result of Pregnane-X-Receptor (PXR) activation
Thyroid Disruption

Triclosan

Binds with

PXR

Activates metabolic enzymes

Breaks down thyroid hormones

Delays development
Thyroid Disruption

Crofton 2008
BXR

- Amphibians – No PXR
- *Xenopus*- Benzoate-X-Receptor (BXR)
  - Poorly Studied
  - Xenobiotics??
Previous Research  (Veldhoen et al. 2006)

- *Rana catesbeiana* tadpoles- immersed in triclosan, treated with T3
  - 0.15 ug/L triclosan, observed increased hindlimb development, decreased body weight
  - Decreased TRβ mRNA expression in tail fin
  - Increased PCNA transcript in brain

- *Xenopus XTC-2* cells
  - 0.03 ug/L triclosan, altered thyroid hormone receptor expression
Hypotheses

Triclosan exposure → Metabolic pathways → Metamorphosis

Triclosan exposure → Thyroid receptors → Metamorphosis
Consideration of Exposure and Species Sensitivity of Triclosan in the Freshwater Environment

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ABSTRACT

Triclosan (TCS) is a broad-spectrum antimicrobial used in consumer products including toothpaste and hand soap. After being used, TCS is washed or rinsed off and residuals that are not biodegraded or otherwise removed during wastewater treatment can enter the aquatic environment in wastewater effluents and sludges. The environmental exposure and toxicity of TCS has been the subject of various scientific and regulatory discussions in recent years. There have been a number of publications in the past 5 y reporting toxicity, fate and transport, and in-stream monitoring data as well as predictions from aquatic risk assessments. State-of-the-science probabilistic exposure models, including Geography-referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER) for European surface waters and Pharmaceutical Assessment and Transport Evaluation (PhATE™) for US surface waters, have been used to predict in-stream concentrations (PECs). These models take into account spatial and temporal variability in river flows and wastewater emissions based on empirically
Research Goal

To determine the risk posed by triclosan to the development of the pickerel frog, *Rana palustris*
Objectives

Determine the effects of triclosan on the metamorphic process by conducting a chronic exposure experiment

Examine the effects of triclosan on thyroid gland morphology through the use of histological techniques
Methods

- Two clutches of *R. palustris* eggs
- Free-swimming larvae (Gosner stage 25) stocked into test containers
- 8 treatments
  - Control- moderately-hard water
  - 0.25, 1, 10, and 30 ug/L triclosan
  - 5 mg/L benzyl benzoate
  - 3 ug/L T3
  - 3 ug/L T3 + 30 ug/L triclosan
- 3 organisms/replicate
- 750 mL test solution
- Day 30 3 L test solution
- 9 replicates/treatment
Methods

Nominal concentrations confirmed using magnetic particle based ELISA kit (Abraxis)
Methods

- Static renewal
  - 48 hr

- Fed a ground mixture of tetramin:reptomin:rabbit chow

- Time to forelimb emergence recorded
Forelimb Emergence

Figure 7-1. Hypothetical relative levels of pituitary and thyroid hormones in *Rana catesbeiana* during metamorphosis.

Duellman and Trueb 1994
Methods

- **Endpoints**
  - Snout-vent length (SVL)
  - Total body weight
  - Hind limb length

- Heads processed for histological examination
Methods

- Thyroid Histology
  - Assessed following guidelines laid forth in the US EPA’s Guidance Document on Amphibian Thyroid Histology
    - Thyroid gland hypertrophy/atrophy
    - Follicular cell hypertrophy
    - Follicular cell hyperplasia
Thyroid Disruption

Crofton 2008
RESULTS
Results

- 3 ug/L T3 treatment - **100% mortality**
  - Observed T3 mediated processes

- 3 ug/L T3 + 30 ug/L triclosan treatment - **100% mortality**
  - **Co-treatment with triclosan did not delay T3 mediated mortality**
Results

![Graph showing survival rates for different treatments.

- Control
- 0.25 ug/L
- 1 ug/L
- 10 ug/L
- 30 ug/L
- BB

Percent Survival

- Survival for each treatment is indicated by different letters:
  - Control: A
  - 0.25 ug/L: AB
  - 1 ug/L: AB
  - 10 ug/L: AB
  - 30 ug/L: A
  - BB: B

The graph illustrates the survival rates for each treatment level, with 30 ug/L and BB showing significantly lower survival rates compared to the control and other treatments.]
Results

![Graph showing Metamorph Weight](image)

- **Control**: 0.25 ug/L
- **Treatment**:
  - 0.25 ug/L: A
  - 1.0 ug/L: A
  - 10 ug/L: A
  - 30 ug/L: A
  - BB: B

The graph indicates the weight (g) of Metamorphs under different treatments.
Results

The graph shows the Metamorph SVL (Snout-vent length) of treatment groups compared to the control. The treatment groups include:

- Control
- 0.25 ug/L
- 1.0 ug/L
- 10 ug/L
- 30 ug/L

The graph indicates that the length of the Treatment groups is significantly higher than the Control group, with the 30 ug/L group showing the highest length, denoted by 'A'. The BB group is also highlighted, indicating a different result.

The treatment of 30 ug/L shows the maximum length, suggesting a potential effect at this concentration level.
Results

Hind limb Length

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
</tr>
<tr>
<td>0.25 µg/L</td>
<td>32</td>
</tr>
<tr>
<td>1.0 µg/L</td>
<td>31</td>
</tr>
<tr>
<td>10 µg/L</td>
<td>31</td>
</tr>
<tr>
<td>30 µg/L</td>
<td>30</td>
</tr>
<tr>
<td>BB</td>
<td>34</td>
</tr>
</tbody>
</table>

The data shows significant differences in hind limb length across different concentrations of treatment.
Results

![Graph showing percent reaching forelimb emergence for different treatments. The graph compares control and various doses of BB.](image-url)
Results

![Graph showing time to forelimb emergence for different treatments. The x-axis represents the treatments (Control, 0.25 ug/L, 1 ug/L, 10 ug/L, 30 ug/L, BB), and the y-axis represents the days to forelimb emergence. Bars are labeled with letters (A, B) indicating statistical significance. Control and 0.25 ug/L show similar emergence times, while 1 ug/L, 10 ug/L, 30 ug/L, and BB show increasing emergence times. The graph includes error bars to indicate variability.]
Results - Thyroid Histology

- Control
Results - Thyroid Histology

- 0.25 ug/L
Results - Thyroid Histology

- 1 ug/L
Results-Thyroid Histology

- 10 ug/L
Results-Thyroid Histology

- 30 ug/L
Results-Thyroid Histology

- Benzyl benzoate – 5 mg/L
Summary

- **Survival**- No effect
- **Size**- Increased length and weight in benzyl benzoate treatment
  - No triclosan effect
- **Time to forelimb emergence**
  - Greater proportion reaching forelimb emergence fastest at intermediate triclosan concentrations (1 ug/L)
  - Decreasing trend at higher concentrations of triclosan and in benzyl benzoate treatment
- **Thyroid histology**
  - No observed effects of any treatment
Summary

At 10 and 30 ug/L triclosan:

Triclosan exposure → Metabolic pathways → Metamorphosis

At 0.25 and 1.0 ug/L triclosan:

Triclosan exposure → Thyroid receptors → Metamorphosis
At environmentally relevant concentrations triclosan does not appear to pose a threat to the successful growth and development of the pickerel frog.

Possible enzymatic induction at higher concentrations??

Better understanding of xenobiotic metabolic systems in amphibians

Further Work- Examine the response of Xenopus laevis using a similar exposure scenario.
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