Low-Dose Effects of Thyroid Toxicants on Neurodevelopment

R. T. Zoeller
Thyroid Hormone is Essential for Normal Brain Development

The “critical period” of thyroid hormone action in brain development was defined as that period after birth when TH therapy must be initiated to rescue the infant from cretinism.

Conclusions

Environmental factors that interfere with TH signaling may interfere with brain development

*How do we identify thyroid toxicants and how to we assess the risk?*
**Regulation of TH levels in the blood**

**Hypothalamus**

Is dominant over negative feedback

Elimination from the body

UDP-glucuronosyltransferases

Liver

Blood

T4 binding proteins

T4 binding proteins

Target Tissues

**Hypothalamus**

TRH

Pit

TSH

Thyroid

T3/T4

Liver

Blood

Two receptor subtypes - alpha and beta. The TRb is critical for negative feedback

T4 → T3

5'-deiodinases

5'-deiodinases

Regulation of TH levels in the blood

Hypothalamus is dominant over negative feedback.
Working Hypothesis

• 1) Thyroid hormone produces non-linear dose-dependent effects on endpoints within the developing brain, heart and liver; and some endpoints are more sensitive than others to thyroid hormone insufficiency

• 2) known thyroid toxicants that act at different sites within the HPT axis will produce different dose-response curves on these endpoints

• 3) changes in tissue metabolism of thyroid hormones can account for the differences in dose-responses.
## Both PCB Exposure and Methimazole Reduce Serum T4

### Table 1. Serum hormone levels in pups on P15 [24]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_4$ (µg/dL)</th>
<th>GH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.851 ± 0.38</td>
<td>8.230 ± 2.8</td>
</tr>
<tr>
<td>HTx</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCB</td>
<td>1.365 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.65 ± 5.4</td>
</tr>
<tr>
<td>HTx+PCB</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values below detection limit  
<sup>b</sup> Significantly different from control
PCB Exposure and MMI Do Not Produce the Same Effect on TH Signaling in the Brain
To identify specific PCB congeners that may act as TH agonists, we developed a mixture of 6 PCBs that represent three classes.

**Mono-ortho chlorinated PCBs**
- PCB 105
- PCB 118

**Di-ortho chlorinated PCBs**
- PCB 138
- PCB 153

**Non-ortho chlorinated PCBs**
- PCB 77
- PCB 126
Mixture of 6 PCB Congeners Activates The TR in GH3 Cells
None of the individual PCB congeners exerts a TH-like effect on TRE-driven relative luciferase activity.
PCB 126 is required for the mono-ortho PCBs to increase TRE-driven relative luciferase activity.
BPA antagonizes TR-β mediated negative feedback in vivo

Negative feedback in the rat is not functional for the first week.

Elevated levels of TH increase RC3/Neurogranin expression on P15

• But a TRβ-selective agonist would not affect tissues/processes regulated by the TRα.
Toxicants

• PTU -- used to produce a graded series of groups characterized by different T4 levels (Mary Gilbert).

• Perchlorate -- Acts by a different mechanism of action (NIS inhibitor rather than TPO inhibitor (PTU)) (with Jeff Fisher).

• PBDE -- PHAH that is predicted to act in a manner similar to that of PCBs (with Kevin Crofton).
Specific Aims

1. To determine the relationship between dose of thyroid hormone and response of several developmentally important endpoints in brain, liver, and heart.
   - RIA of total serum $T_4$ and $T_3$, free $T_4$ and $T_3$-index, TSH, circulating transthyretin, thyroxine-binding globulin and thyroglobulin.
   - Endpoints of thyroid hormone action will include the expression of genes known to be thyroid hormone responsive in the developing brain, heart and liver.
   - Endpoints of developmental events will include specific measurements within the cerebral cortex, hippocampus and cerebellum in the brain, and size and weight of heart and liver.
Specific Aims

- RIA of total serum T\textsubscript{4} and T\textsubscript{3}, free T\textsubscript{4} and T\textsubscript{3}-index, TSH, circulating transthyretin, thyroxine-binding globulin and thyroglobulin and tissue-levels of T\textsubscript{3}/T\textsubscript{4}.
Tissue T4/T3

There is a very strong correlation between the amount of tissue extracted and the amount of T4 measured. We should validate using HPLC.
Experimental Design (Mary Gilbert)

PTU Treatment of Pregnant Rats Initiated on G6

Control 1ppm PTU 2ppm PTU 3ppm PTU

Animals Sacrificed on P15, trunk blood collected for T₄ analysis
Brains Harvested for ISH and IHC

Developmental PTU exposure reduces T₄ levels but does not alter body weight
TH increases the number of oligodendrocytes
TH decreases the number of astrocytes
TH controls the ratio of oligodendrocytes to astrocytes
Effect of PTU on MAG mRNA Levels in the Corpus Callosum on P15

Corrected Density (arbitrary units)

0 1 2 3

** ***
Experimental Design (Mary Gilbert)

PTU Treatment of Pregnant Rats Initiated on G6

- Control
- 1ppm PTU
- 2ppm PTU
- 3ppm PTU

Animals Sacrificed on P15, trunk blood collected for T4 analysis
Brains Harvested for ISH and IHC

The dose of PTU required to reduce T4 is lower than the dose of PTU required to affect white matter
Site-Specific Compensation?

- D2
- MCT8
- RC3
In Progress

• Gene expression in brain and heart
  – These studies will define the shape of the dose-response using a compound that produces an “idealized” thyroidal response.
Perchlorate inhibits iodide uptake at the Sodium/Iodide Symporter
Effects of Perchlorate Exposure

• Timed pregnant Sprague-Dawley Rats
  – Exposure in Drinking Water
  – Doses: 0, 10, 100, 250, 500, 5000 ppb
  – Duration: G7 - P21 (weaning)
Dilution of normal P8 rat serum shows a linear relationship between film density and TBG content by Western blot.
Thyroxine Binding Globulin

Deglycosylation of Serum TBG

Normal P15 rat serum was deglycosylated using PNGase F to remove all N-linked glycans. The expected size shift of native TBG (54kD) to deglycosylated TBG (44kD) is observed.
Effect of Perchlorate on serum TBG in P15 Pups
Deiodinase Activity in Cortex of Male and Female Pups on P21

Deiodinase Activity

Enzyme Activity

D1

D2

Series 1
Series 2
Series 3
Series 4
Series 5
Series 6

0
10
100
250
500
5000

0
10
100
250
500
5000
Deiodinase Activity in P21 Males

- Enzyme Activity

- D1

- D2

- Series 1

- Series 2

- Series 3

- Series 4

- Series 5

- Series 6

- 0

- 10

- 100

- 250

- 500

- 5000

* *
Deiodinase Expression in P21 Females

Deiodinase Expression

Enzyme Activity

D1
D2

0
10
100
250
500
5000
In Progress

• Complete serum and tissue hormone analysis in PTU and Perchlorate Experiments
• Complete tissue analysis of TH endpoints
• PBDEs
Acknowledgements

- Ruby Bansal
- Dave Sharlin
- Laurelis Santiago
- Dan Tiege
- Mike DeVito
- Mary Gilbert
- Kevin Crofton
- Jeff Fisher
- Duncan Ferguson

This research is funded by
U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD83213701