In Vivo and In Vitro Screening for Thyroid Hormone Disruptors

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Outline

• Thyroid Biology 101
• What do we know about pathways and adverse outcomes
• Recent advances in screening for thyroid hormone disruptors
• Challenges for use of HTS in hazard assessment
**Thyroid Hormones**

**Thyroxin (T4)**

**Triiodothyronine (T3)**

- Development - Critical for differentiation and growth
  - Transient disruption = permanent effects (eg., cretinism)

- Adult – Important for energy and thermoregulation
  - Transient disruption = transient effects
Regulation of Thyroid Hormones

Hypothalamus

TRH

Pituitary (Pit)

TRβ

Thyroid

TRβ

Liver

Elimination from the body

Catabolic Enzymes

Blood

Target Tissues

T4 → T3

5′-deiodinases

Thyroid hormone binding proteins

T3/T4

Acts as a ligand for nuclear thyroid hormone receptors (TRs)

Office of Research and Development
National Health and Environmental Effects Research Laboratory
Linking Disruption of Up-Stream Targets to Adverse Outcome

Adverse Outcome Pathway

Toxicity Pathway

Thyroidal

Exposure

Thyroperoxidase
Iodine Symporter

Extra-Thyroidal

Hepatic UDPGTs

Deiodinases

Cellular Transporters

↓ T4–TTR Binding

Thyroid Receptors

Serum T3 & T4 Changes

↓ TSH

Tissue T3 Changes

Altered Development

Thyroid Hyperplasia

Thyroid Tumors

Birth Defects
Screening Methods for Thyroid Disruptors

1. Link early key events to adverse outcomes
   • Qualitative – Yes
   • Quantitative – no yet

2. Build efficient screens for each “target”
   • High Throughput Screens (HTS)
     • Very efficient, lack integrated systems
   • Low-Medium Throughput Screens
     • Less efficient, but integrated systems
Ongoing Activities - Examples

1) Receptor based screening at EPA’s NCCT, & NIH NTP/NCGC
   - e.g. TR-beta, hepatic targets PXR, AhR, PPAR
   - recently completed screening of 320 pesticidal chemicals, now working on over 1000 additional chemicals

2) TPO screening
   - 96 well medium throughput assay for inhibition of porcine TPO
     (Mike Hornung at ORD’s Duluth Lab)

3) TH signaling in transgenic X. laevis

4) OECD/IPCS Advisory Group on Molecular Screening and Toxicogenomics”.
   1) Critical summary of available assays,
   2) Collating list of chemicals with evidence of thyroid disruption
      (update to Brucker-Davis list)
GeneBLAzer TRβ-UAS-bla HEK293 Cell Line

Cell line contains a beta-lactamase reporter gene under the control of an UAS response element stably integrated in Hek293 cells. This line also stably expresses a fusion protein consisting of the GAL4 DNA binding domain and the TRβ ligand binding domain.

Courtesy of Keith Houck, NCCT
Human TR\(\beta\) Reporter Gene Assay

Heat Map of AC50’s
1456 chemicals; 14 concentrations; agonist and antagonist modes

human TR\(\beta\) Agonist: Selectivity of Actives

human TR\(\beta\) Antagonist: Selectivity of Actives

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Courtesy of Keith Houck, NCCT
L-MTS Example - WatchFrog®

- Fluorescent transgenic *X. laevis* tadpoles bearing a TH/bZIP-eGFP construct
- Can detect receptor agonists & antagonists, and synthesis inhibitors

96 well plates with automatic quantification of the activation of TR

Slide courtesy of B. Demeniux
WatchFrog – Positive Controls

![Graph showing RFU values for different treatments]

Fini et al. (2007)
M-LTP Example
Thyroid Peroxidase Inhibition Assay

- Use porcine thyroid microsomes
  - Chemical + Substrate
  - Color readout
- 96 well plates
- Currently screened over 100 chemicals
  - One example – MBT (2-Mecaptobenozothiazole)

- Positives are followed by ex vivo thyroid explant assay
- Then in vivo testing in X. laevis
Challenges in Use of Data from Screening Methods

• Can we use HTS data for risk assessment right now? NO
  – Links between target and outcomes are not all there
  – Issues with relating testing concentration (media) to target tissue dose
  – Lack of integrated developing systems
  – Statistical and biological models for data interpretation
  – Many targets are enzymes

• What can we do now?
  – Screening for Prioritization (rather than prediction)
Accumulation of PBDE-47 in Primary Cell Cultures of Rat Cortical Neurons

1 uM in media = 100 uM in cells after 60 min

Mundy et al 2005
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Application of Research to Levels of Organization Based on Source to Outcome

Key Event

Toxicity Pathway

Mode of Action

Adverse Outcome Pathway

Source to Outcome Pathway