Organotin compounds are potent inducers of adipogenesis in vertebrates

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Organotins uses and prevalence

- Marine ship paints
  - Trialkytins are potent biocidal antifouling agents for molluscs
    - Widely used 1960-1970s
    - Regulated but not completely phased out (date varies by country)

- Fungicide on high value food crops (potatoes, rice, celery, pecans)
  - e.g. Brestan (Triphenyltin acetate)

- Wood preservative

- Catalysts for organic synthesis

- Heat stabilizers in manufacture of polyolefin plastics (PVC)

- Bioaccumulative and NOT biodegradable
Endocrine disrupting properties of organotins

- **Invertebrates:**
  - Imposex in molluscs (female to male sex characteristics)
  - Direct inhibitory effect on aromatase (CYP19) enzymatic activity
    - Also appears to block storage of testosterone as esters
  - Alters shell development in bivalve molluscs
Endocrine disrupting properties of organotins

- Prevalence of imposex can be quite high
  - Closely associated with shipping and boating in most locations
Endocrine disrupting properties of organotins

- **Vertebrates**
  - **Spermatotoxic**
    - Sperm lack flagella or have impaired motility (fish, rat)
  - **Sex reversal in fish** (*Danio rerio* and Japanese flounder)
    - Increased % males
    - Masculinization of genetically female flounder (~25-30%)
  - **Mild effects on mammalian sex characteristics**
    - Inhibits aromatase in cultured granulosa cells
    - Reduced seminal vesicle weights in male rats
  - **Immunotoxic**
    - induces neutrophil apoptosis
    - Inhibit cytotoxic function of NK cells
  - **Hepatotoxic**
  - **Neurotoxic**
    - Trimethyl and triethyltins are potent specific neurotoxins
  - Effects of early exposure can persist in adulthood
Organotin mechanisms of action

- Direct inhibition of aromatase (CYP19) enzymatic activity
  - Inhibition of testosterone storage
  - Requires µM concentrations

- Transcriptional effects on aromatase expression
  - CYP19 in human ovarian granulosa cells is sensitive to inhibition by TBT, RXR- and PPARγ-specific ligands

- Mitochondrial toxicity
  - Increases mitochondrial permeability
  - Release of cytochrome c -> caspase activation and apoptosis

- Cytoskeletal disruption

- Modification of specific cellular proteins e.g. stannin
How do organotins modulate transcription?

- Effects are seen at nM doses and below
  - Alter activity of transcription factors?
  - Possible ligands for nuclear receptors?

- Test a panel of nuclear receptors for activation or inhibition by TBT
  - Steroid receptors
  - RXR and its partners
    - RAR, TR, VDR, LXR, FXR, CAR, SXR, PPARs, EcR/USP
  - Selected other nuclear receptors
    - NGFI-B family
    - SF-1/AD4BP
## Nuclear receptor LBD activation by TBT

<table>
<thead>
<tr>
<th>Construct</th>
<th>Fold activation (60 nM TBT)</th>
<th>Permissive RXR heterodimer?</th>
</tr>
</thead>
<tbody>
<tr>
<td>RXRα (human)</td>
<td>60</td>
<td>Yes</td>
</tr>
<tr>
<td>RXRα (xenopus)</td>
<td>25</td>
<td>Yes</td>
</tr>
<tr>
<td>RXRγ (xenopus)</td>
<td>7.0</td>
<td>Yes</td>
</tr>
<tr>
<td>NURR1 (human)</td>
<td>7.0</td>
<td>Yes</td>
</tr>
<tr>
<td>LXRα (human)</td>
<td>2.1</td>
<td>Yes</td>
</tr>
<tr>
<td>PPARα (mouse)</td>
<td>0.7</td>
<td>Yes</td>
</tr>
<tr>
<td>PPARγ (human)</td>
<td>5.3</td>
<td>Yes</td>
</tr>
<tr>
<td>PPARδ (human)</td>
<td>1.7</td>
<td>Yes</td>
</tr>
<tr>
<td>RARα (human)</td>
<td>0.7</td>
<td>No</td>
</tr>
<tr>
<td>TRβ (human)</td>
<td>0.4</td>
<td>No</td>
</tr>
<tr>
<td>VDR (human)</td>
<td>0.5</td>
<td>No</td>
</tr>
<tr>
<td>SXR (human)</td>
<td>1.0</td>
<td>No</td>
</tr>
</tbody>
</table>
RXR - A key partner for many pathways

**Known ligands**
- RAR$_{\alpha,\beta,\gamma}$: all-trans RA
- TR$_{\alpha,\beta}$: thyroid hormone (T3)
- VDR: 1,25-(OH)$_2$-Vit D3
- PPAR$_{\alpha,\beta/\delta,\gamma}$: fatty acids, eicosanoids
- LXR$_{\alpha,\beta}$: oxysterols
- FXR: bile acids
- BXR: benzoates
- EcR: 25-OH ecdysone

**Activatable orphans**
- SXR/PXR: steroids, xenobiotics
- CAR$_{\alpha,\beta}$: androstane, xenobiotics
Structures of RXR-specific agonists

9-cis-RA
- $K_d = 1 \text{nM}$
- $EC_{50} = 15 \text{nM}$

LG268
- $K_d = 3 \text{nM}$
- $EC_{50} = 3 \text{nM}$

Phytanic acid
- $K_d = 4 \mu M$
- $EC_{50} > 10 \mu M$

Tributyltin-Cl
- $K_d = 12 \text{nM}$
- $EC_{50} = 5 \text{nM}$

Docosahexaenoic acid
- $K_d = \text{ND}$
- $EC_{50} = 5-10 \mu M$
Organotin activation of RXRα

<table>
<thead>
<tr>
<th>Concentration nM</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBT</td>
<td>&gt; 2800 nM</td>
</tr>
<tr>
<td>TBT</td>
<td>5 nM</td>
</tr>
<tr>
<td>4BT</td>
<td>150 nM</td>
</tr>
</tbody>
</table>

Fold Activation

Concentration nM

- LG268
- Butyltin
- Dibutyltin
- Tributyltin
- Tetrabutyltin
- Butyltin Tris(2-EHA)
TBT is a PPARγ (Partial) Agonist
TBT activates the ligand independent orphan receptor NURR1

Nurr1 is key for dopaminergic neuron development
TBT Activation is Nuclear Receptor Specific

No activation of most other nuclear receptors by TBT
Effect of halide and alkyl chain length on receptor activation

<table>
<thead>
<tr>
<th>Ligand</th>
<th>GAL4-hRXR α EC&lt;sub&gt;50&lt;/sub&gt; nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tributyl tin fluoride</td>
<td>3</td>
</tr>
<tr>
<td>Tributyl tin chloride</td>
<td>3</td>
</tr>
<tr>
<td>Tributyl tin bromide</td>
<td>4</td>
</tr>
<tr>
<td>Tributyl tin iodide</td>
<td>4</td>
</tr>
<tr>
<td>Triethyl tin bromide</td>
<td>2800</td>
</tr>
<tr>
<td>Trimethyl tin chloride</td>
<td>&gt; 100,000</td>
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</table>

EC<sub>50</sub> values were determined from dose-response curves of transiently transfected Cos7 cells after 24 hr exposure to ligands.
## Nuclear receptor activation by organotins

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Nuclear Receptor LBD EC$_{50}$ nM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hRXR $\alpha$</td>
</tr>
<tr>
<td>LG268</td>
<td>2-5</td>
</tr>
<tr>
<td>AGN203</td>
<td>0.5-2</td>
</tr>
<tr>
<td>9-cis RA</td>
<td>15</td>
</tr>
<tr>
<td>all-\textit{trans} RA</td>
<td>na</td>
</tr>
<tr>
<td>Butyltin chloride</td>
<td>na</td>
</tr>
<tr>
<td>Dibutyltin chloride</td>
<td>3000</td>
</tr>
<tr>
<td>Tributyltin chloride</td>
<td>3-8</td>
</tr>
<tr>
<td>Tetrabutyltin chloride</td>
<td>150</td>
</tr>
<tr>
<td>Di(tri phenyltin) oxide</td>
<td>2-10</td>
</tr>
<tr>
<td>Butyltin-tris (2-ethylhexanoate)</td>
<td>na</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>na</td>
</tr>
</tbody>
</table>

\textit{na} - not active; \textit{ND} - not determined

EC$_{50}$ values from dose response curves in transiently transfected COS7 cells after 24 hour ligand exposure
Cos7 cells were transiently transfected, ligands or solvent control (DMSO) were added to ITLB/DMEM media for 24 hrs at the following concentrations: 100 nM TBT, LG268, AGN203 or TTNPB, and 10 µM TROG, WY-14643 and GW-9662.
Conclusion - TBT binds well to both RXRs and PPAR γ