Human and *Xenopus laevis* type 3 iodothyronine deiodinase enzyme cross-species sensitivity to inhibition by ToxCast chemicals

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Introduction and Objectives

Three iodothyronine deiodinase enzymes, DIO1, DIO2 and DIO3, identified in both mammals and amphibians, are selenoproteins anchored in the cell membranes. DIO1 and DIO2 catalyze the removal of an iodine from thyroid hormones to activate the hormone in specific tissues at appropriate times, and the DIO3 inactivates the hormone when it is no longer needed. We produced *Xenopus laevis* type 3 iodothyronine deiodinase enzyme (**Xldio3**) in human HEK293 cell culture to conduct screening assays. The objectives of screening chemicals are to:

- Determine whether a chemical inhibits a specific target component of the thyroid system;
- Compare responses across species;
- Directly identify molecular initiating events for Adverse Outcome Pathways (AOPs);
- Provide screening data (e.g., IC_{50} values) that can be used to prioritize chemicals and support risk assessment decisions;
- Categorize chemicals for further testing *in vivo*.

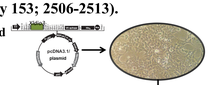
Methods

Concentration-response testing (79 chemicals selected from Xldio3 357-chemical single-concentration screening and previous human type 3 deiodinase [hDIO3] screening results):

- Chemicals inhibiting Xldio3 activity to < 20% of median control activity;
- Chemicals inhibiting Xldio3 activity to < 40% of median control and that inhibited Xldio3 more than hDIO3 by a difference of greater than 15%; and
- Chemicals found in the literature to be of potential thyroid disrupting concern that inhibited hDIO3 by generally more than 50%.

Deiodinase assay closely followed Renko et al. 2012 (Endocrinology 153; 2506-2513).

Transfect plasmid construct into HEK293 cells.

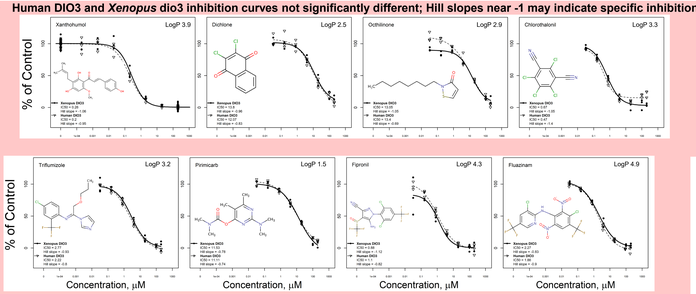


Lyse/sonicate cells. Incubate 3h with T3 substrate + DTT cofactor + chemical or DMSO carrier in HEPES buffer. Extract free iodide through Dowex.

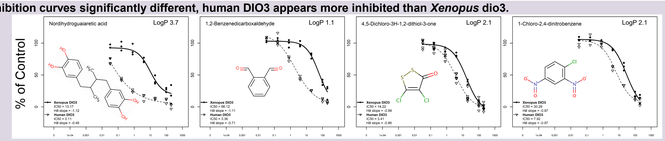
Sandell-Kolthoff assay (reduction of yellow Ce+4 to colorless Ce+3 in presence of As+3) was used to detect free iodide at absorbance of 420 nm in a 96-well plate reader.

Concentration-Response Curve, Hill Slope and IC50 Results and Potential Mechanisms

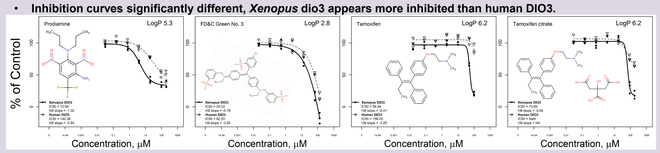
- **Human DIO3 and *Xenopus* dio3 inhibition curves not significantly different; Hill slopes near -1 may indicate specific inhibition.**

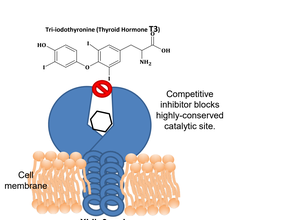


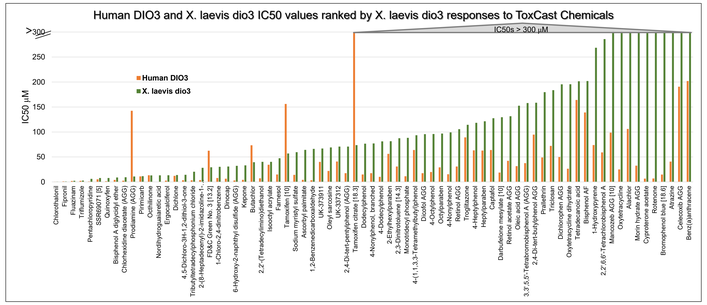
- **Inhibition curves significantly different, human DIO3 appears more inhibited than *Xenopus* dio3.**

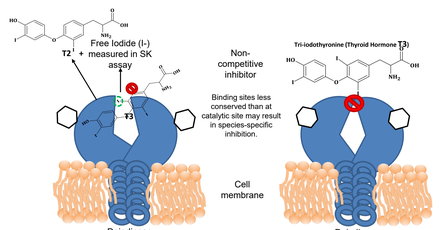


- **Inhibition curves significantly different, *Xenopus* dio3 appears more inhibited than human DIO3.**

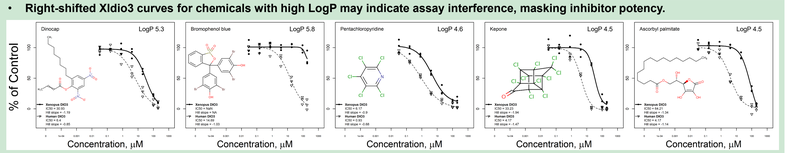




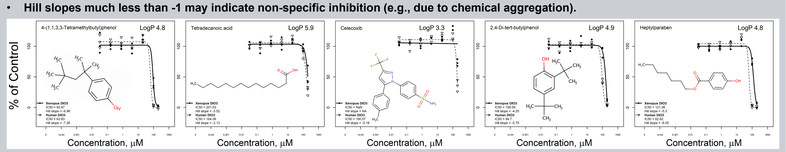


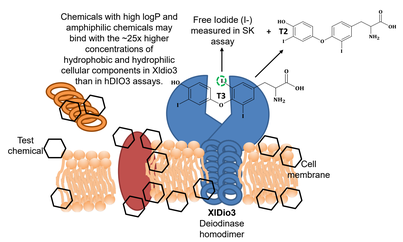


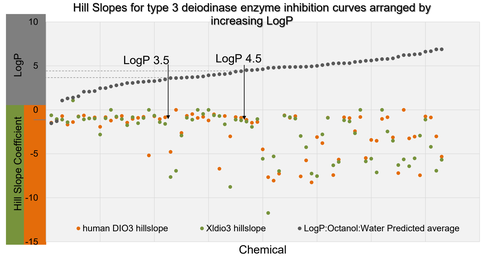
- **Right-shifted Xldio3 curves for chemicals with high LogP may indicate assay interference, masking inhibitor potency.**

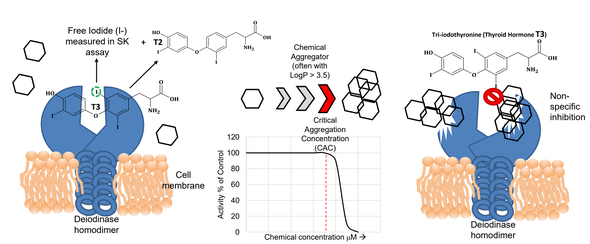


- **Hill slopes much less than -1 may indicate non-specific inhibition (e.g., due to chemical aggregation).**









Key Conclusions

- *X. laevis* dio3 activity improvements were made that allow screening of ToxCast chemicals using the SK assay system.
- Several chemicals appear to be specific, competitive inhibitors of type 3 deiodinase in both species resulting in near-identical concentration-response curves, Hill slopes and IC50 values, likely due to the conserved amino acid residues in the enzyme catalytic site.
- Non-competitive inhibition may be a mechanism in which some chemicals exhibit different levels of inhibition between species by binding at specific, less-conserved sites outside of the catalytic core.
- However, the ~25x larger amount of cell lysate required in the Xldio3 than in human DIO3 assays may bind some chemicals non-specifically, preventing interaction with the enzyme and giving the general appearance of less inhibition of the amphibian *Xenopus laevis* dio3.

High Through-Put Cross-Species Testing

- **Recognition (or elimination) of assay interferences and non-specific inhibition is crucial to identifying actual inter-species differences in chemical sensitivity.**
- This will require increased expression of recombinant non-mammalian proteins in future cross-species high through-put testing through:
  - improved transfection methods,
  - more productive cell lines, and
  - knowledge of accessory proteins and chaperones that aid in protein folding and trafficking within the cell.

Acknowledgements

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