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# Chemical Inhibition of the Iodide Recycling Enzyme Iodotyrosine Deiodinase, with Human and *Xenopus* Comparison

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# Conflict of Interest Statement

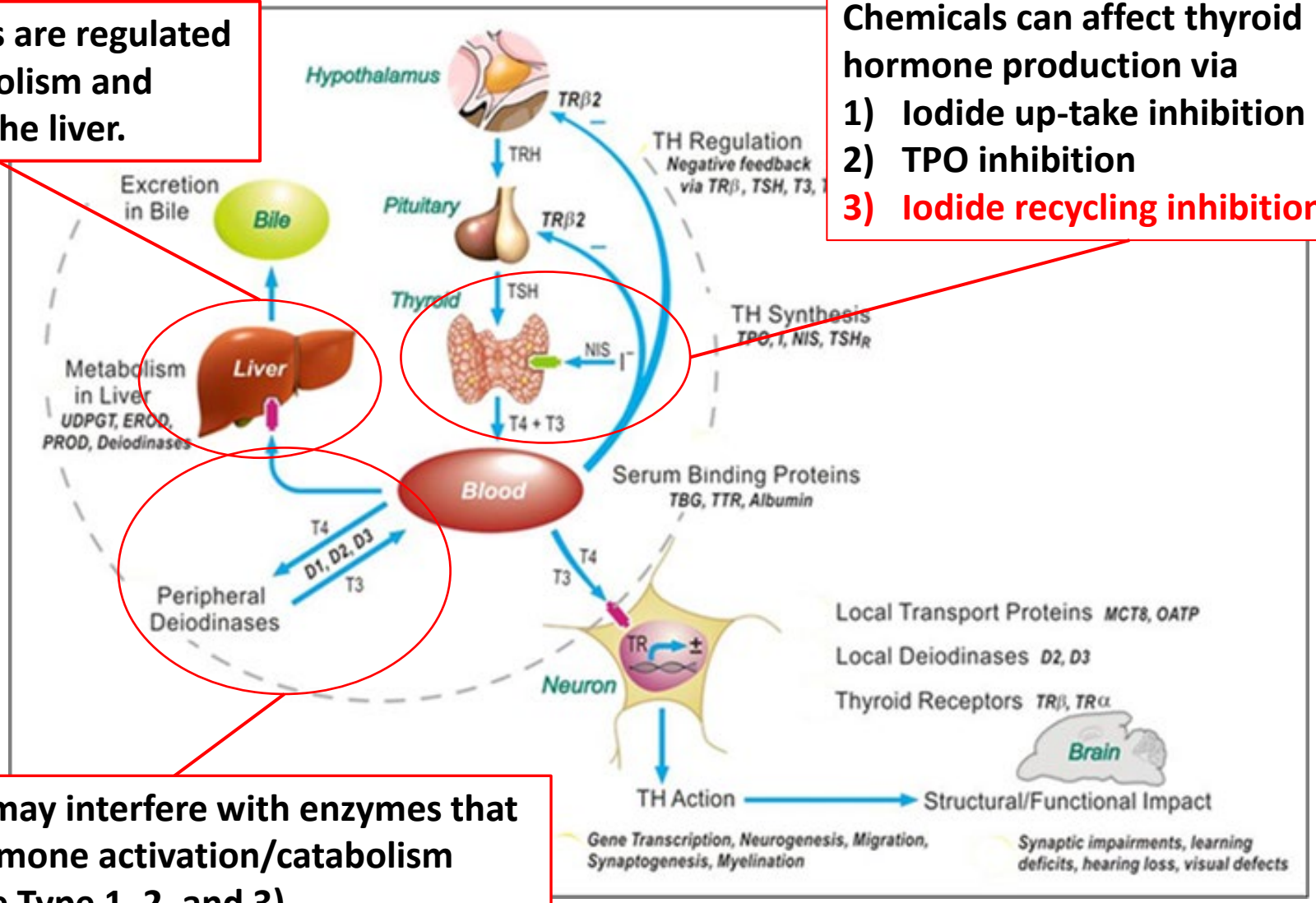
- I have no conflicts of interest to declare.

# Multiple mechanisms by which environmental contaminants can disrupt thyroid function

**Hormone levels are regulated through metabolism and elimination in the liver.**

**Chemicals can affect thyroid hormone production via**

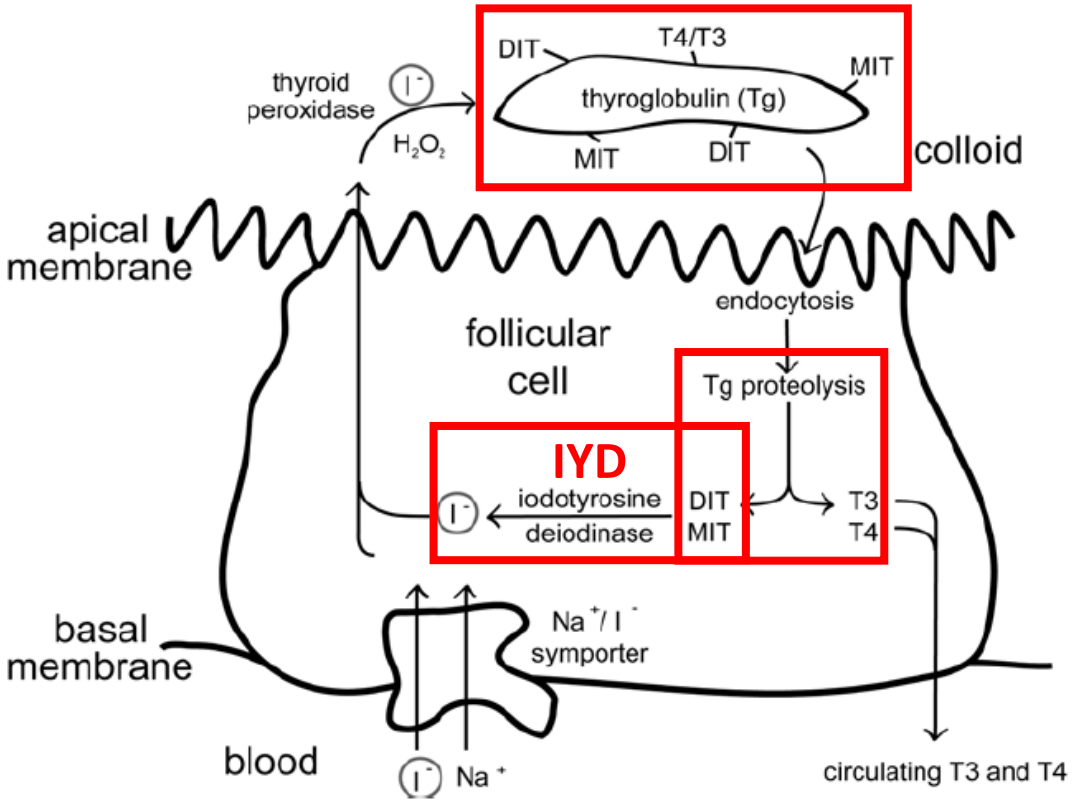
1) **Iodide up-take inhibition (NIS)**
2) **TPO inhibition**
3) **Iodide recycling inhibition (IYD)**




**Chemicals may interfere with enzymes that control hormone activation/catabolism (Deiodinase Type 1, 2, and 3)**

# Iodotyrosine Deiodinase (Dehalogenase, IYD)

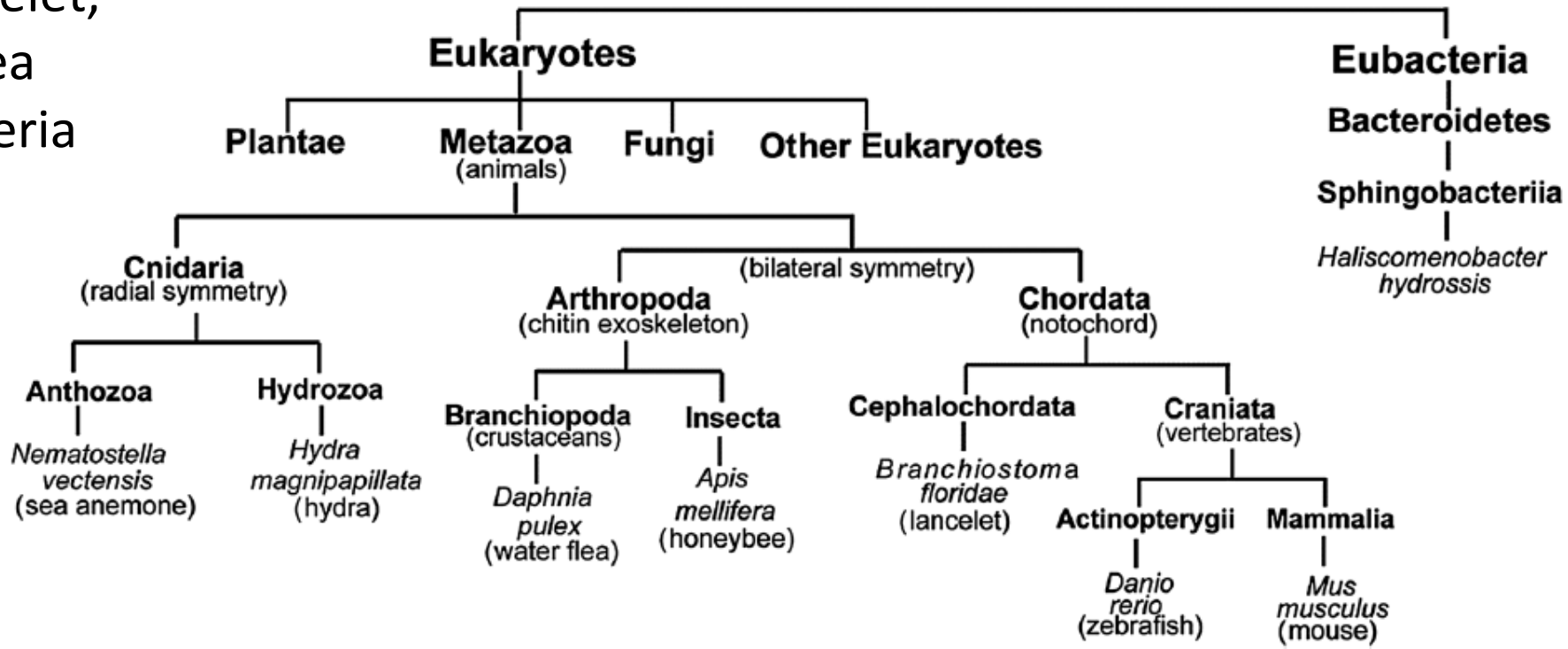
- Catalyzes iodide recycling from the byproducts of thyroid hormone synthesis: monoiodotyrosine (MIT) and diiodotyrosine (DIT)
- Maintains sufficient iodine for thyroid hormone synthesis
- IYD mRNA also detected in liver and kidneys (Gnidehou et al. 2006, Sun et al. 2015, Olker et al. 2018)




From Rokita et al. 2010 *Biochimie* 92(9): 1227-1235

# IYD protein is highly conserved across a wide range of multicellular organisms

Functional IYD documented in mouse, zebrafish, lancelet, honeybee, daphnia, sea anemone, hydra, bacteria




Phatarphekar et al. 2014. *Molecular BioSystems* 2014(10):86-92

# IYD mutation or chemical inhibition results in adverse outcomes

Reduced thyroid hormone synthesis leading to insufficiency in tissues and subsequent detrimental developmental consequences

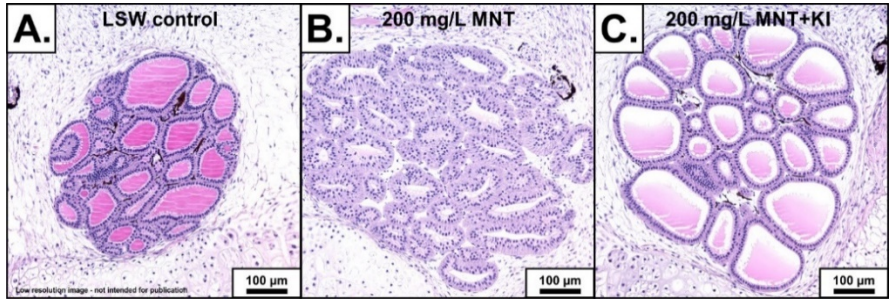
- **Humans -** hypothyroidism, goiter, and mental retardation (Moreno and Visser 2010)
- **Rodents –** decreased serum T4 and T3, increased thyroid gland size and TSH levels (Green 1968, Green1971, Meinhold and Buchholz 1983)

# IYD mutation or chemical inhibition results in adverse outcomes

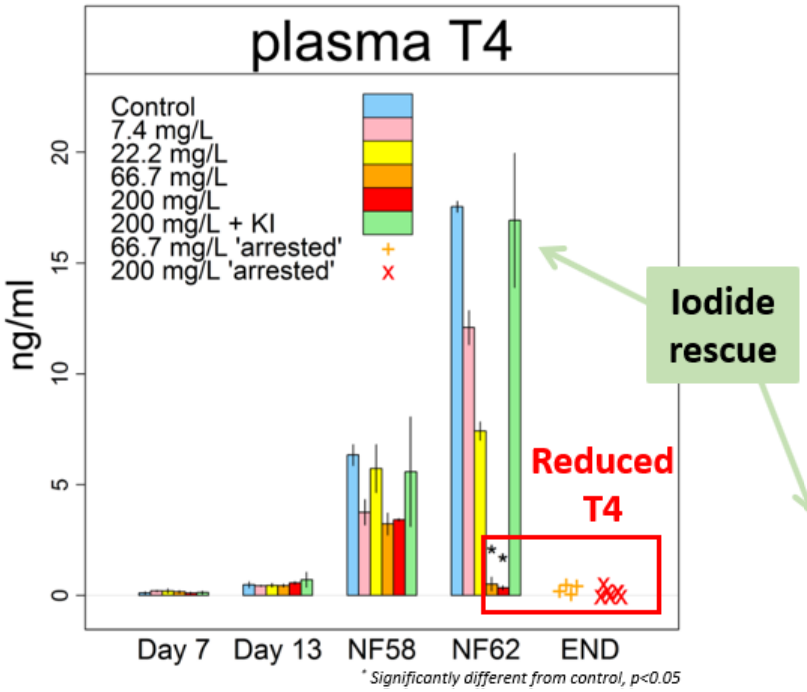
Reduced thyroid hormone synthesis leading to insufficiency in tissues and subsequent detrimental developmental consequences

- ***Xenopus* –**

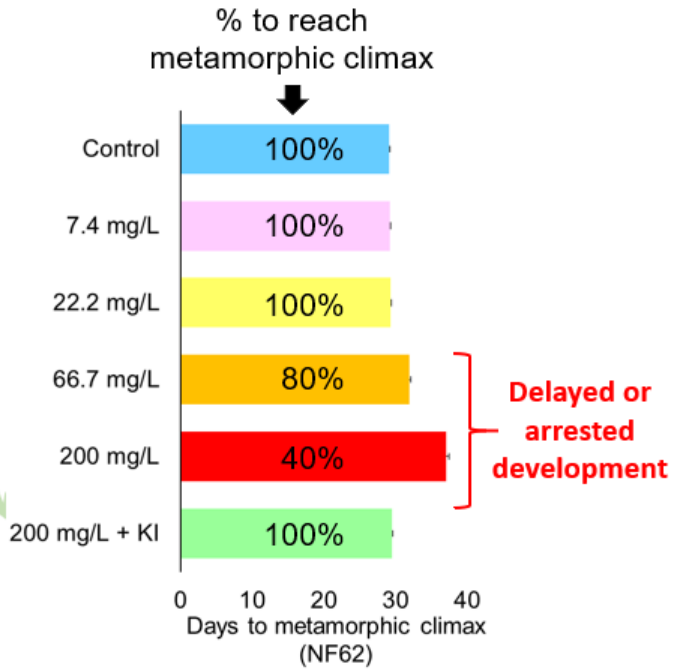
Follicular cell hypertrophy and hyperplasia




Reduced circulating T4 and T3




Delayed metamorphosis



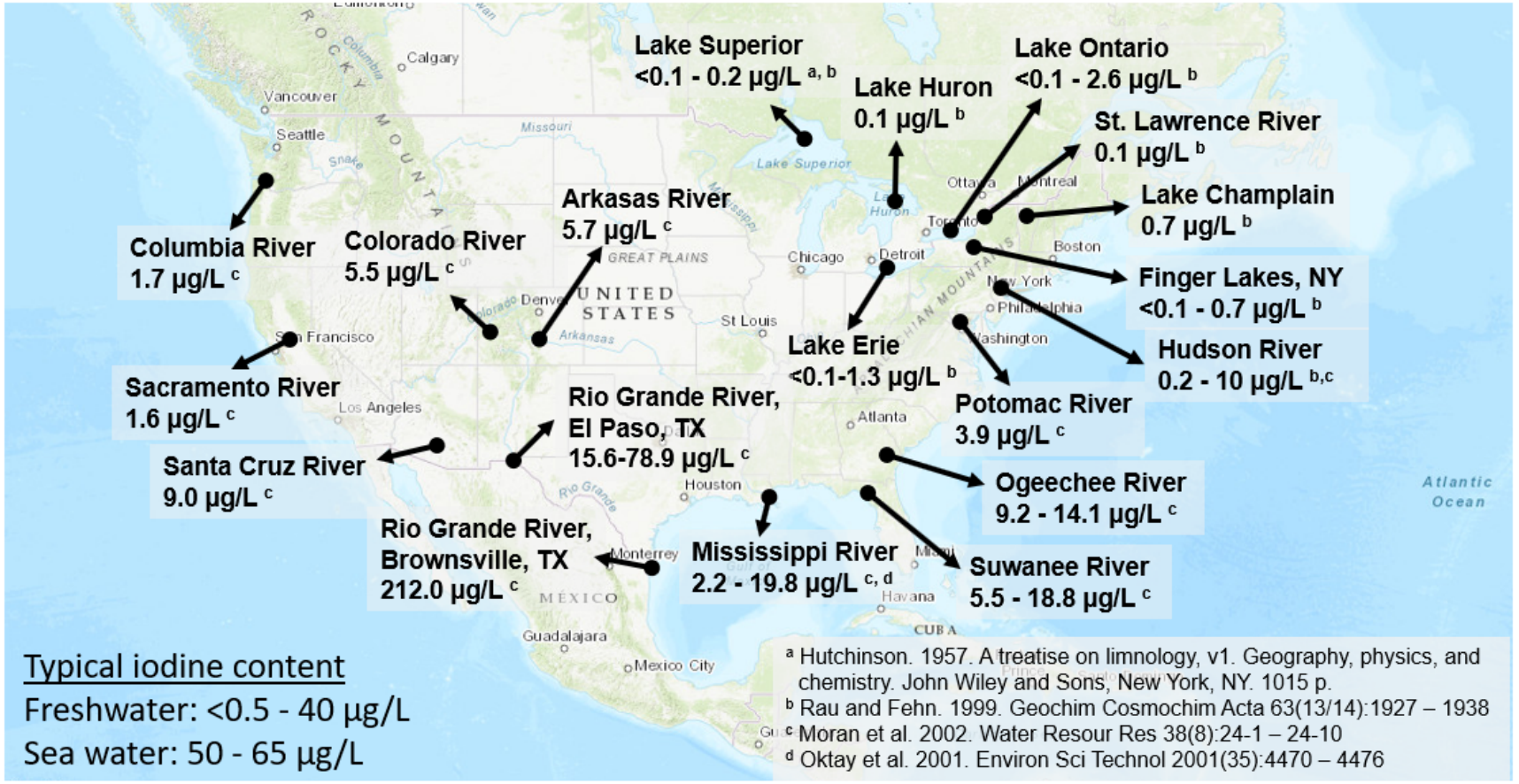

Olker et al. 2018. *Toxicological Sciences*, *166*(2): 318-331

# Iodine recyling critical in multiple environments

Especially for:

- Low iodine diets
- Low iodine environments, including most freshwater ecosystems

*Iodine content in U.S. freshwater lakes and rivers*



# Objectives

- Develop assays to screen chemicals for inhibition of iodotyrosine deiodinase (IYD).
- Screen a large set of compounds to expand evaluation of chemical inhibition of IYD.
- Compare cross-species sensitivity to chemical inhibition of IYD.

# *In vitro* screening assay: inhibition of Human IYD

- Developed and optimized assay

**Recombinant human IYD (hIYD) Enzyme Production** → **IYD Enzyme Assay** → **Measure Detection Signal**

Produced with baculovirus system in insect cells

In 96-well plates, 3 h incubation

Sandell-Kolthoff (SK) reaction to detect free iodide

- Tiered screening approach

**Single-Concentration Screening** — **1,825 Chemicals** Target conc. = 200 µM

↓

**Potential IYD inhibitors** — **≥ 20% inhibition 194 Chemicals**

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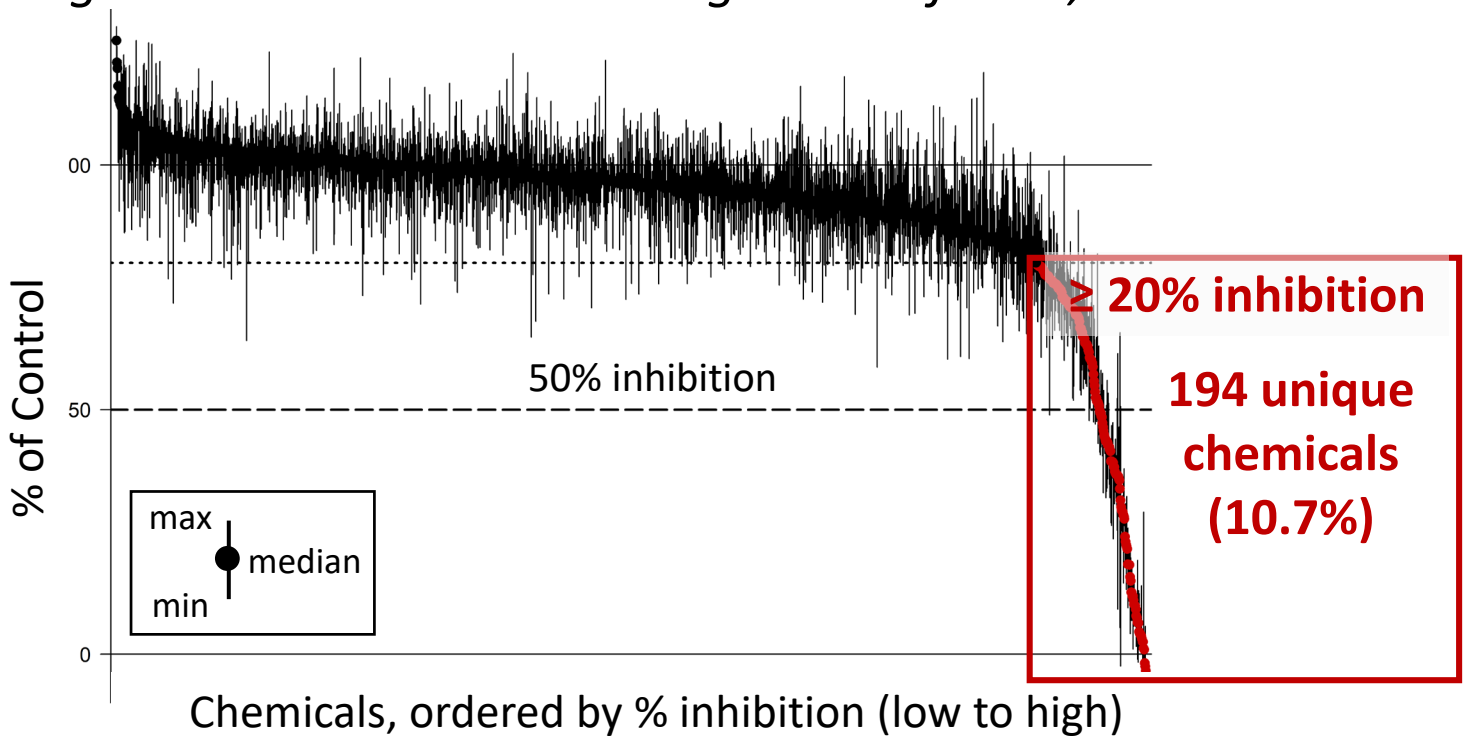
**Concentration-Response Screening** — **≥ 50% inhibition 84 Chemicals** (0.032 - 200 µM)

*Single-Concentration Screening Results for >1,800 Chemicals*

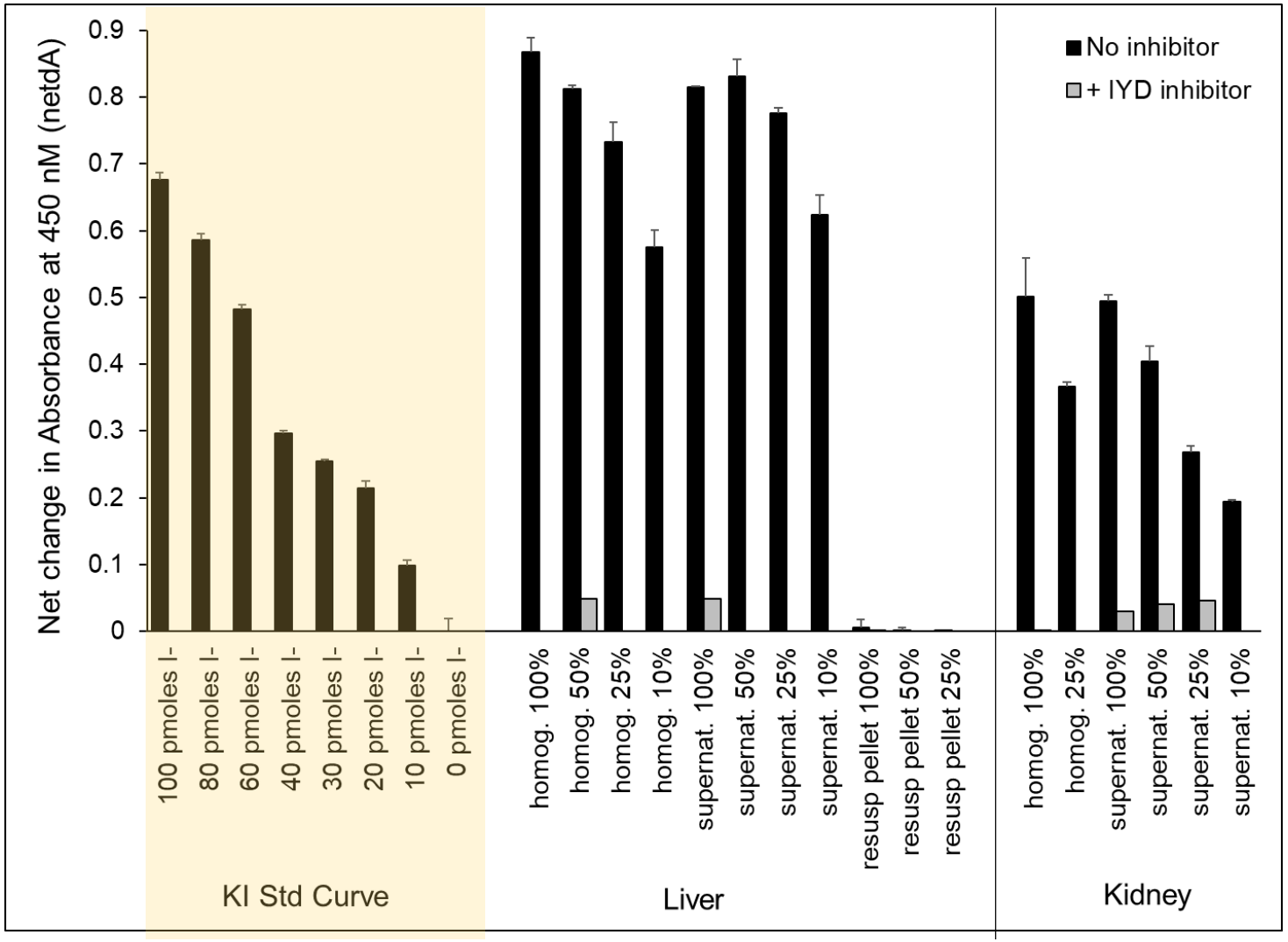



Olker et al. *in Review.*

# *In vitro* screening assay: inhibition of *Xenopus* IYD

- Identified source of amphibian IYD enzyme
- Based on IYD ontogeny data (Olker et al. 2018)
- NF59 *Xenopus laevis* tadpoles
- Liver microsomal fractions

*Enzyme Activity Tests with Multiple Tissue Types*



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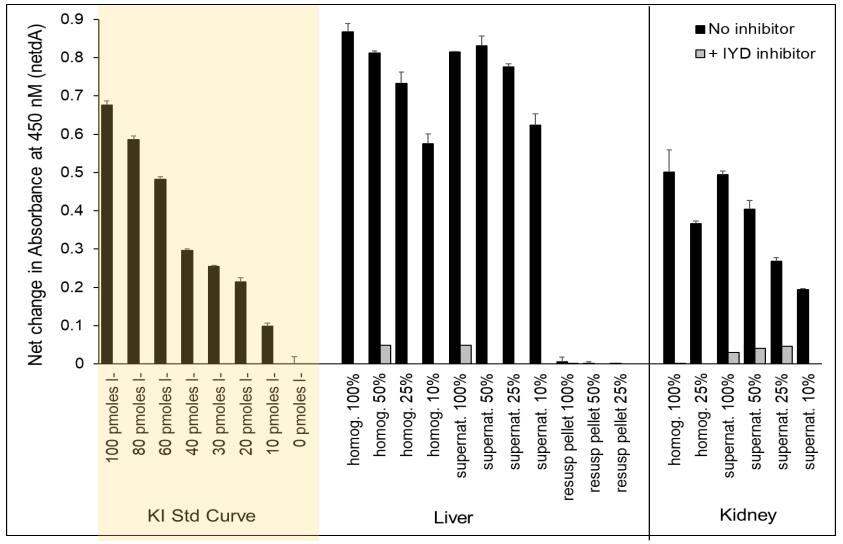



- Developed and optimized *Xenopus* IYD assay parallel to Human IYD assay

Recombinant human IYD (hIYD) Enzyme Production

Produced with baculovirus system in insect cells

*Xenopus* IYD (xIYD) Enzyme Source

Liver microsomal fractions from *Xenopus laevis* tadpoles

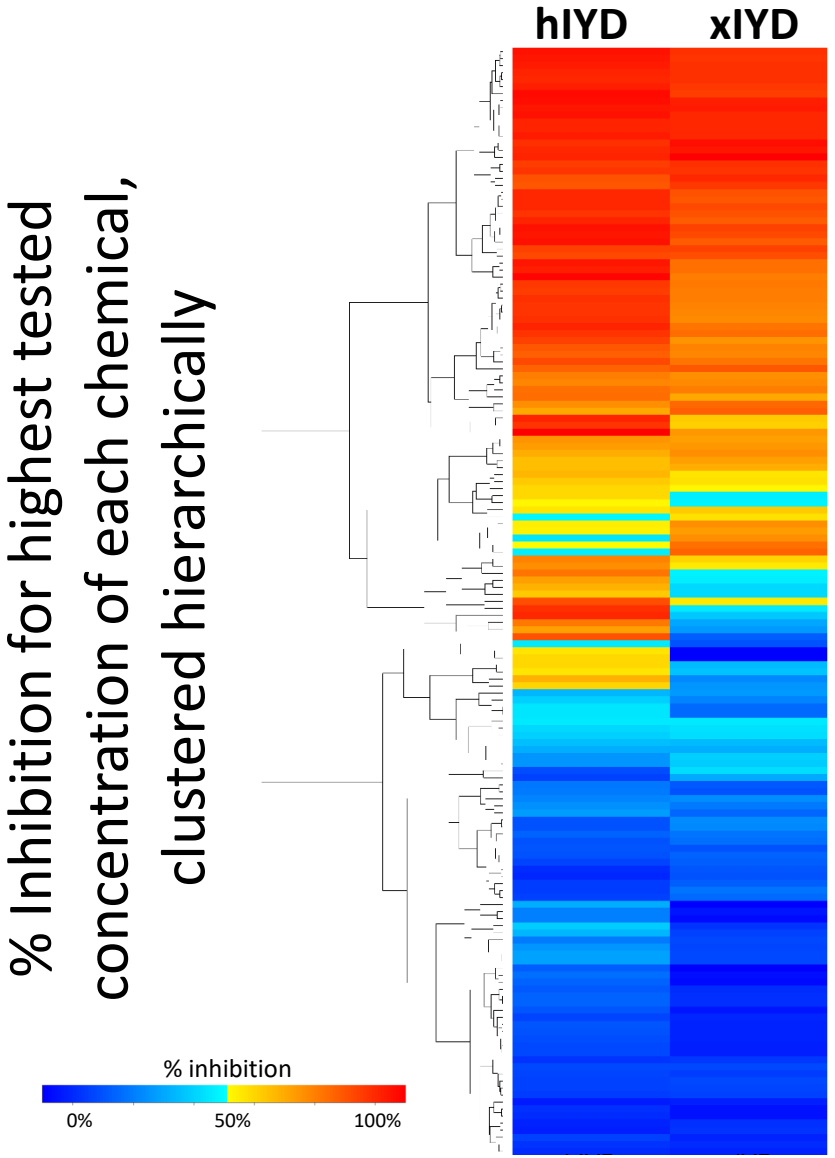
IYD Enzyme Assay

In 96-well plates, 3 h incubation

Measure Detection Signal

Sandell-Kolthoff (SK) reaction to detect free iodide

# Further Testing: Human and *Xenopus* IYD




**154 unique chemicals**
**7 concentrations (0.032 - 200 µM)**

- 83 that produced >50% inhibition in hIYD
- 71 others, including:
  - Test set from literature
  - Non-inhibitors for hIYD
  - DIO and TPO inhibitors
- Consistency across assays:
  - 80% (120/154) of chemicals produced similar maximum inhibition in both assays
  - Similar rank-order potency

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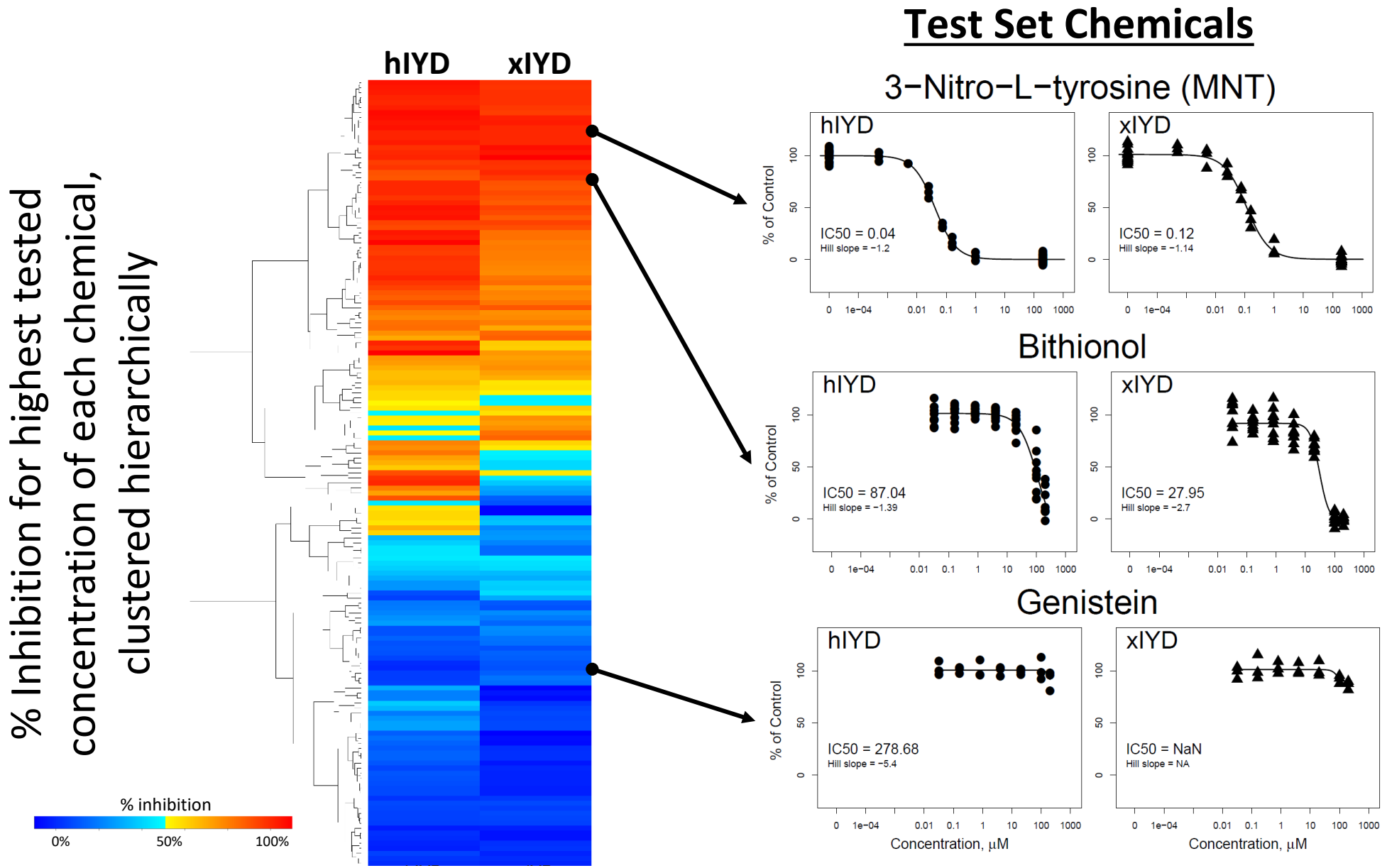

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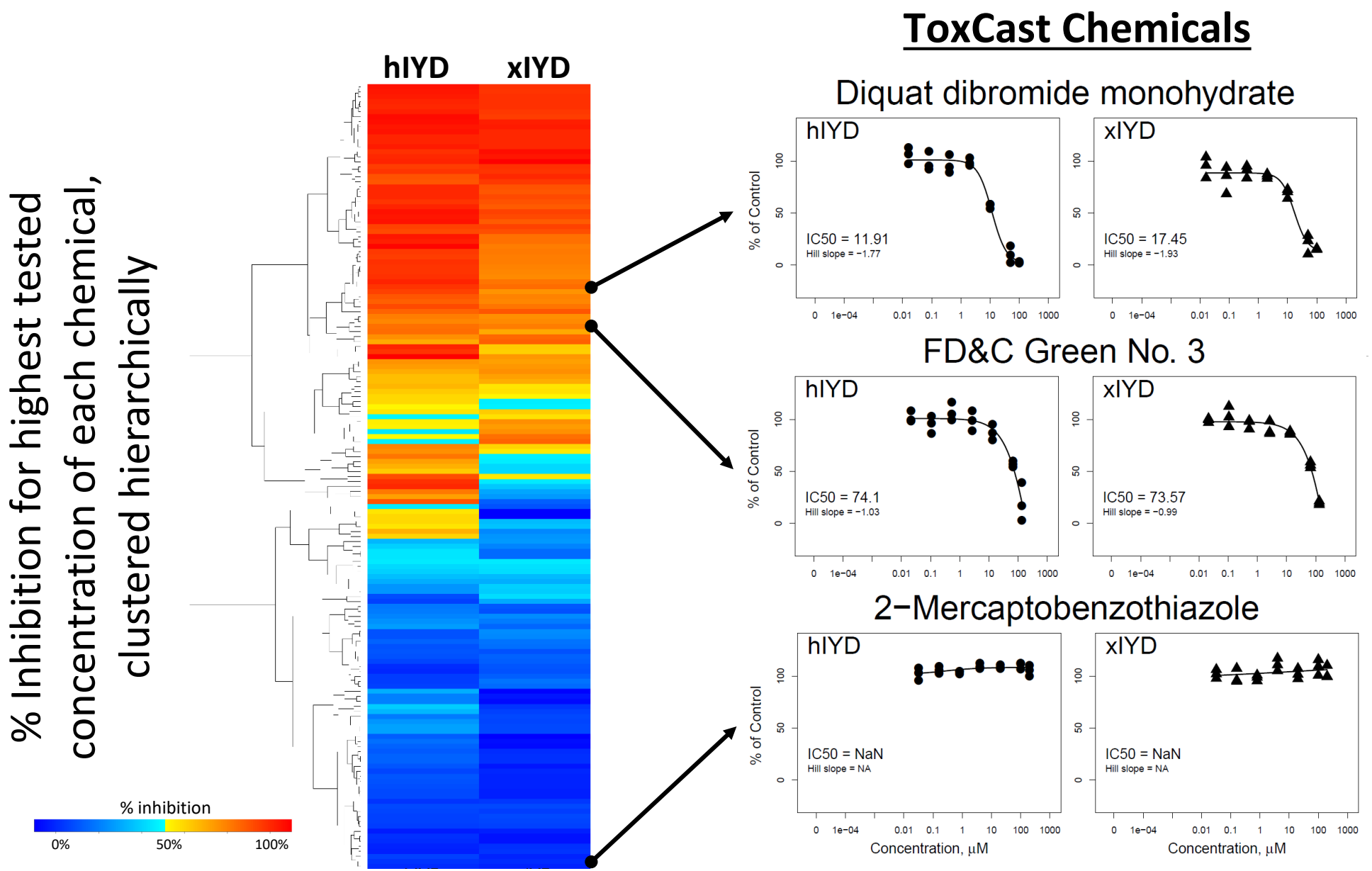

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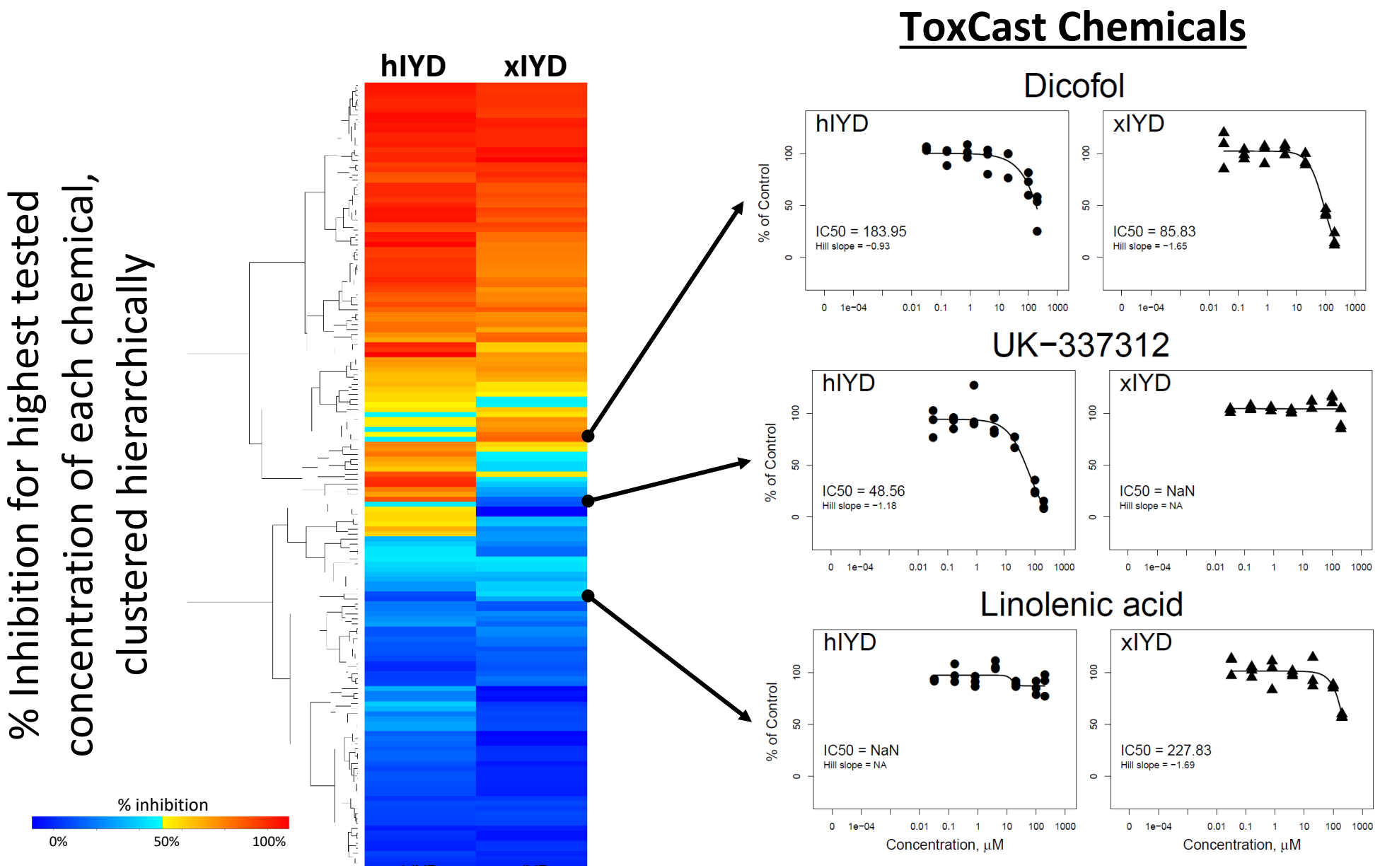

# Conclusions

- IYD inhibition assays expand the coverage of molecular targets for which chemicals can be screened for thyroid disruption.
- Screening >1,800 chemicals greatly expands compounds tested for inhibition of IYD.
- Concordance in response of IYD activity to chemicals in human and *Xenopus* assays.
- In general, mammalian-based assay would be protective of chemical effects on amphibian IYD

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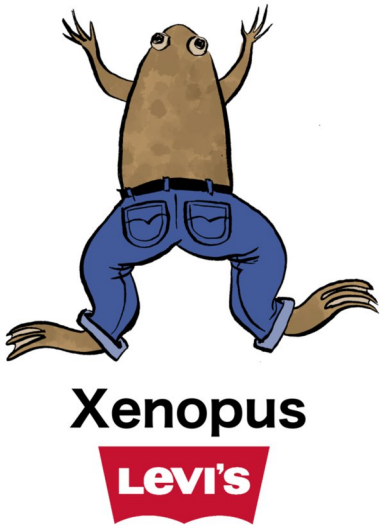
# Thank you!



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