Screening for Chemical Inhibition of the Iodide Recycling Enzyme, a Novel Molecular Target for Thyroid Axis Disruption

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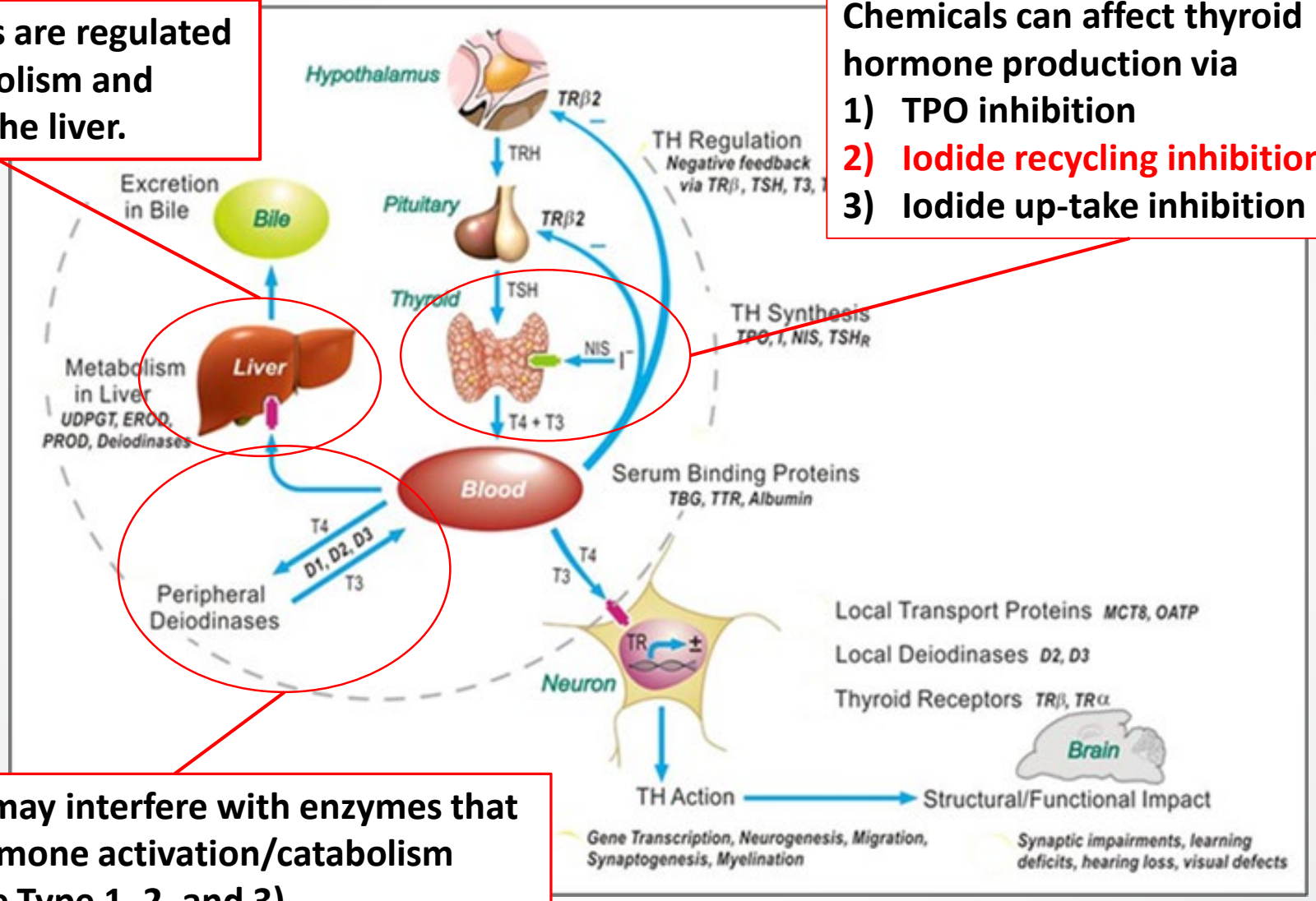
Multiple mechanisms by which environmental contaminants can disrupt thyroid function

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

Chemicals can affect thyroid hormone production via

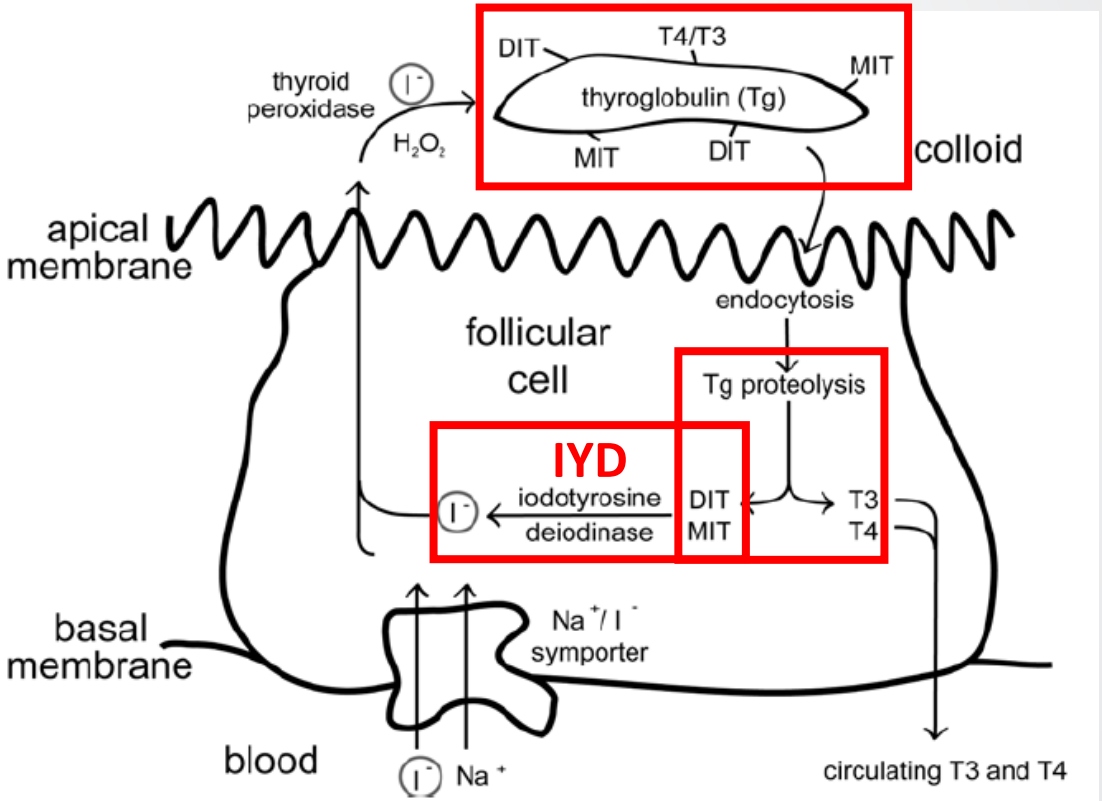
1) **TPO inhibition**
2) **Iodide recycling inhibition (IYD)**
3) **Iodide up-take inhibition (NIS)**



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

Iodine Recycling Enzyme: Iodotyrosine Deiodinase (Dehalogenase, IYD)

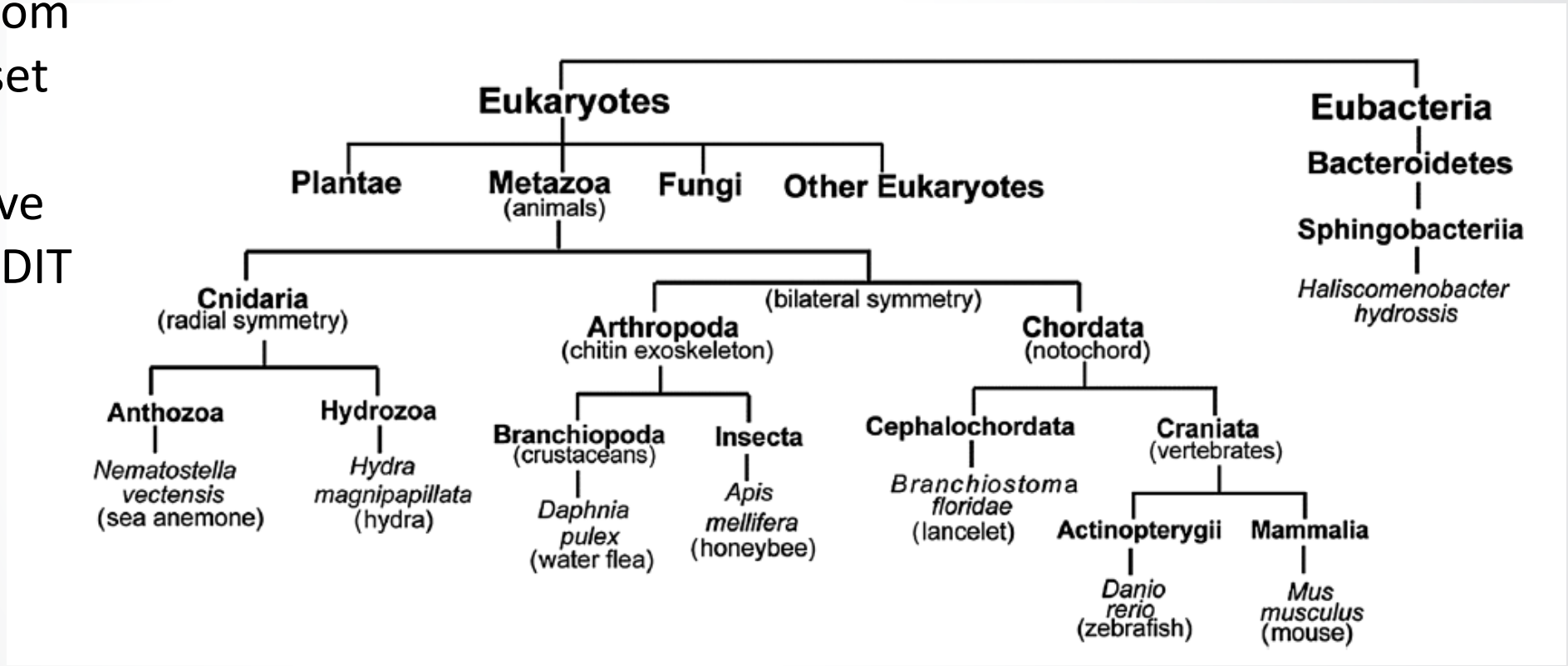
- Catalyzes iodide recycling from the byproducts of thyroid hormone synthesis: monoiodotyrosine (MIT) and diiodotyrosine (DIT)
- Maintains sufficient iodine for thyroid hormone synthesis
- IYD mRNA also detected in liver and kidneys (Gnidehou et al. 2006, Sun et al. 2015, Olker et al. 2018)



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

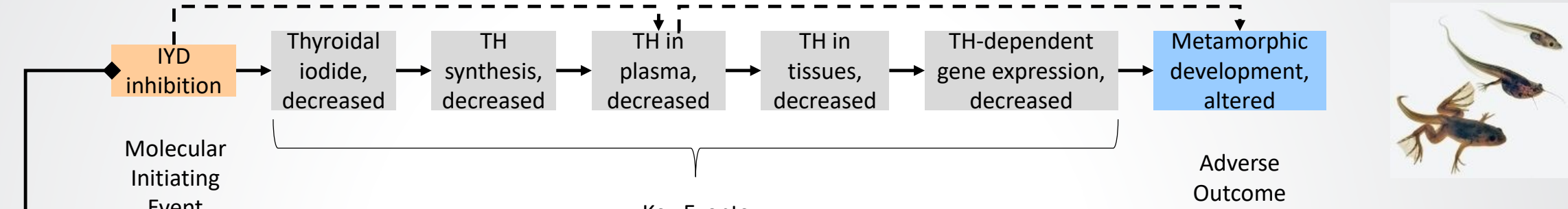
IYD protein is highly conserved across a wide range of multicellular organisms:

- IYD homologs from representative set of organisms all catalyze reductive deiodination of DIT



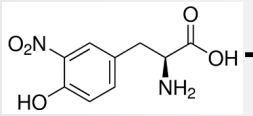
From Phatarphekar et al. 2014. *Molecular BioSystems* 2014(10):86-92

Inhibition of IYD causes arrested or delayed metamorphosis

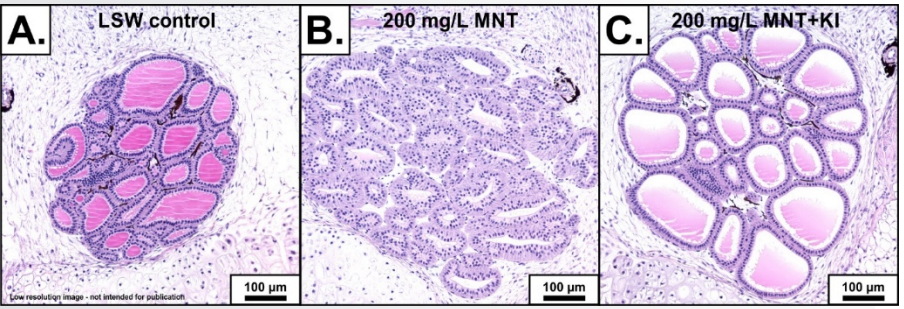


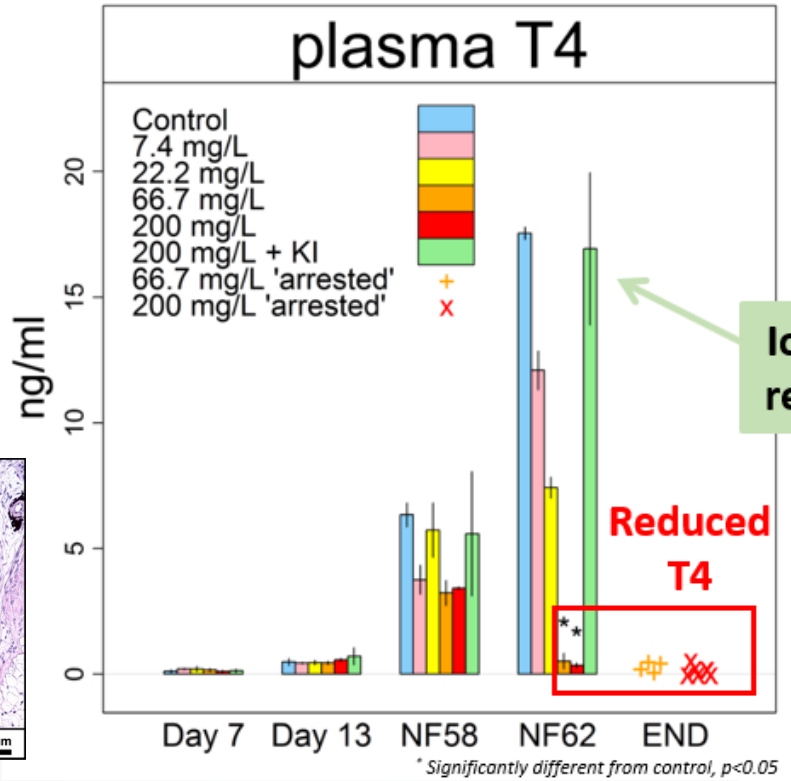
IYD Inhibitor:

3-nitro-L-tyrosine (MNT)

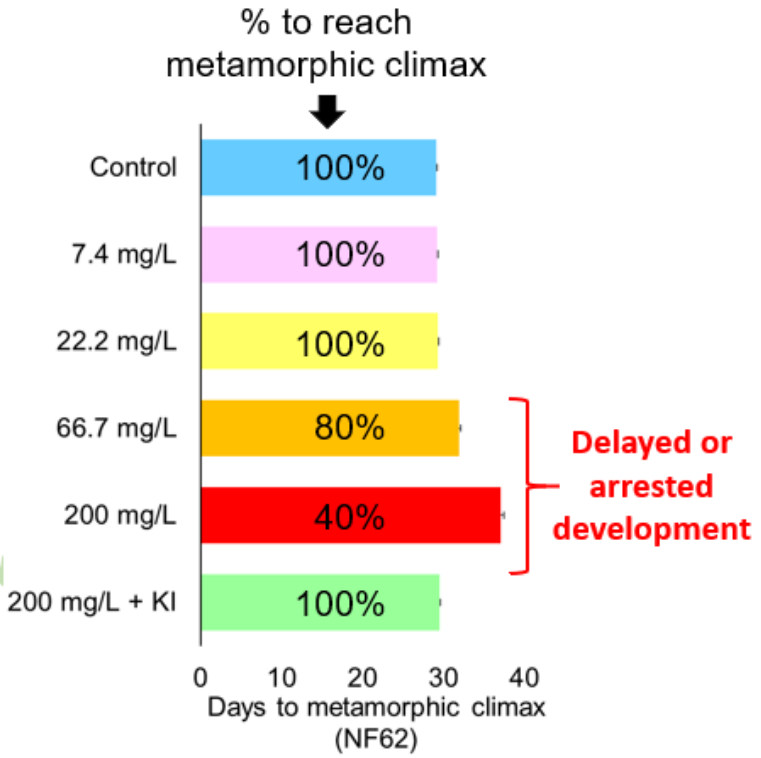


Thyroid gland histopathology





Iodide rescue



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

Objectives

- Develop assays to screen chemicals for inhibition of iodotyrosine deiodinase (IYD).
- Screen a large set of chemicals to identify chemical inhibitors of IYD.
- Compare chemical inhibition of IYD between human and amphibian IYD enzyme.

Developed in vitro screening assays

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

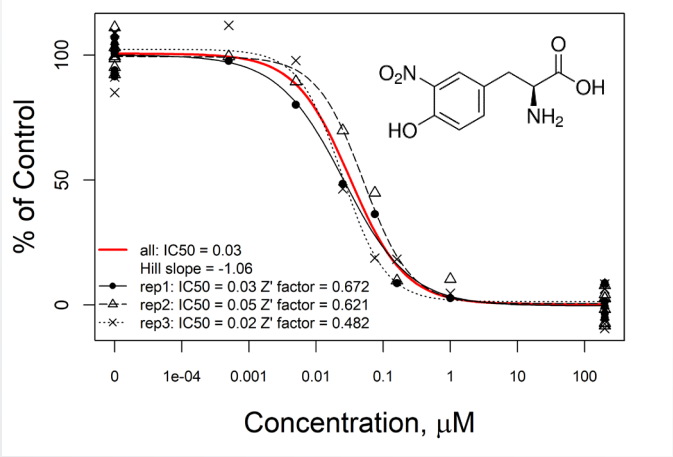
IYD Enzyme Assay

In 96-well plates, 3 h incubation

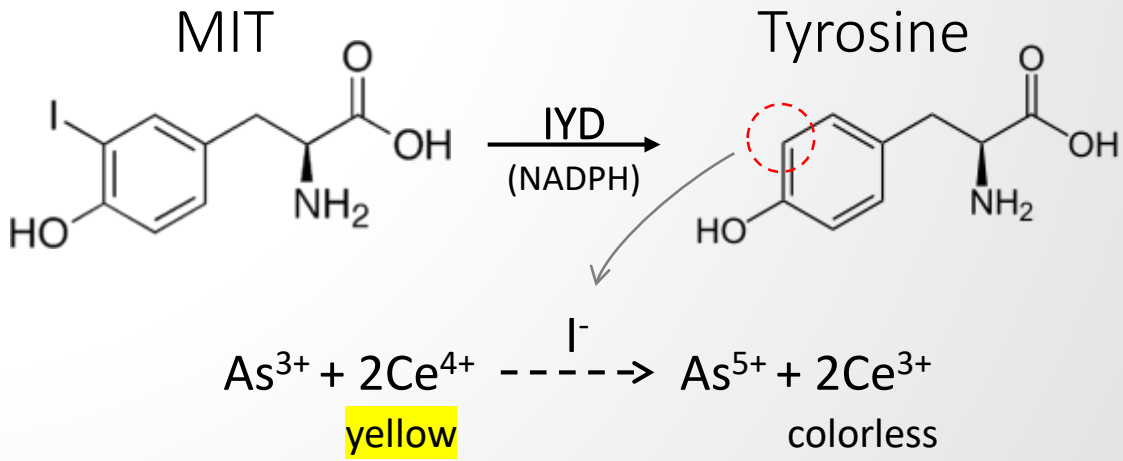
Measure Detection Signal

Sandell-Kolthoff (SK) reaction to detect free iodide

3-Nitro-L-tyrosine (MNT)



Iodotyrosine deiodinase (IYD) reaction scheme

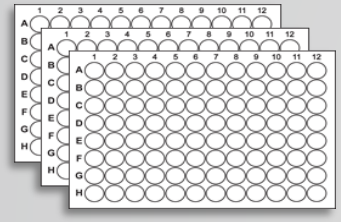


Screened >1,800 chemicals to identify chemical inhibitors of IYD

Chemical Set	No. of Chemicals
Test set – identified from the literature	10 4 known or suspected inhibitors and 6 non-inhibitors
ToxCast Phase 1_v2	~300 Primarily pesticides
ToxCast Phase 2	~750 Industrial/consumer products, food additives
ToxCast e1k	~750 Potential endocrine related activity

Screening Approach

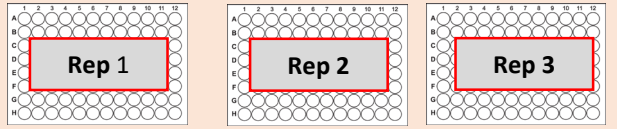
Chemical Source Plates



- ToxCast chemical libraries
- 1,828 unique chemicals provided in 96-well plates with identities masked
- Target concentration of 20 mM in DMSO

Single-Concentration Screening

Target Test Concentration: **200 µM**



Is enzyme activity inhibited compared to control?

no

Set aside as inactive

yes

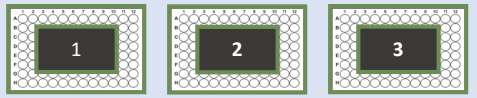
Inhibition >20%:
Potential IYD inhibitors

≥ 50% inhibition

Concentration-Response Testing

Serial Dilutions:

200, 100, 20, 4.0, 0.8, 0.16, 0.032 µM



- Concentration-response modeling
- Determine IC50, rank chemicals

Human IYD single-concentration screening results

Single-Concentration Screening

1,828 Chemicals
Target conc. = 200 µM

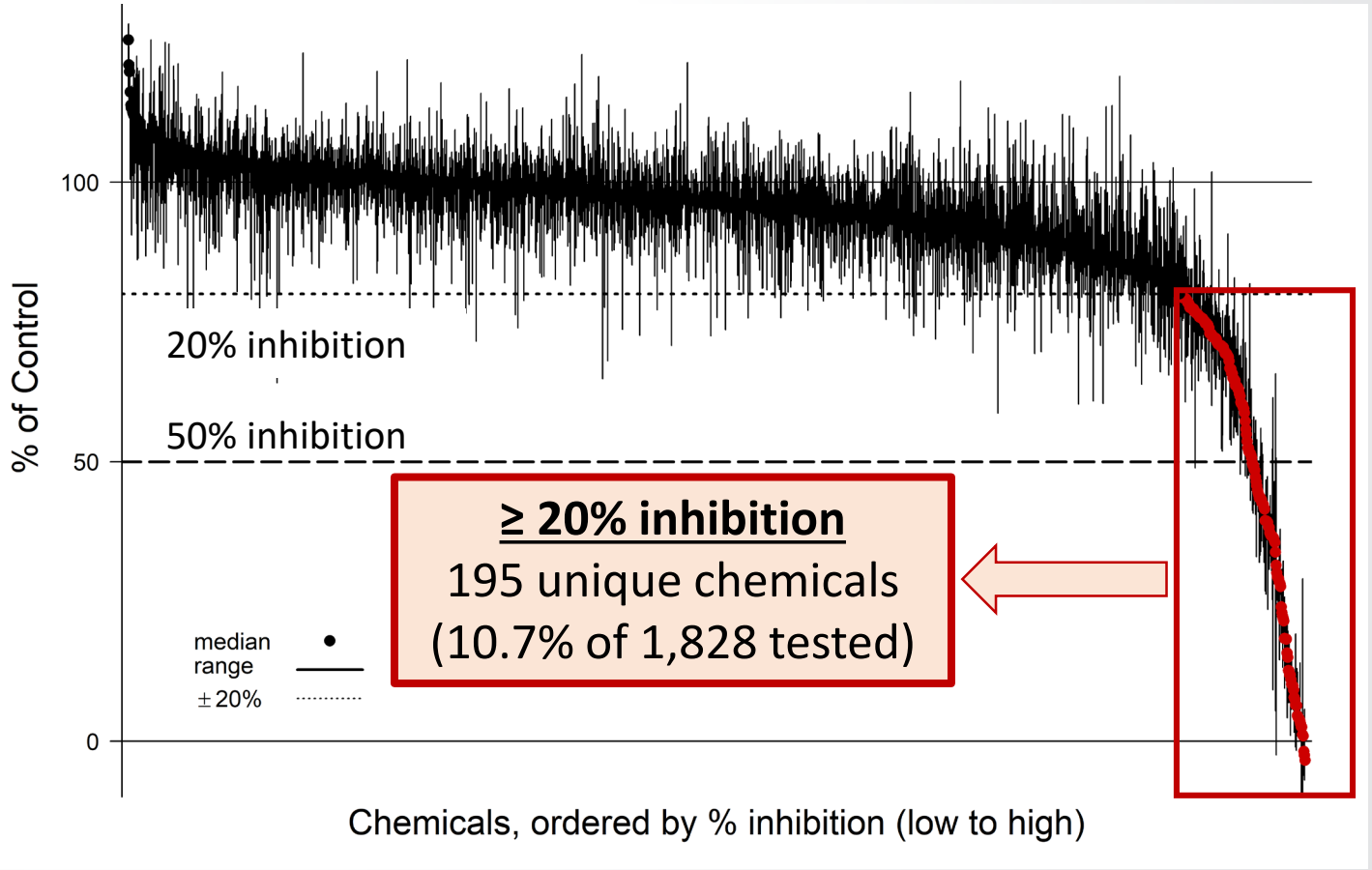
Potential IYD inhibitors

> 20% inhibition
195 Chemicals

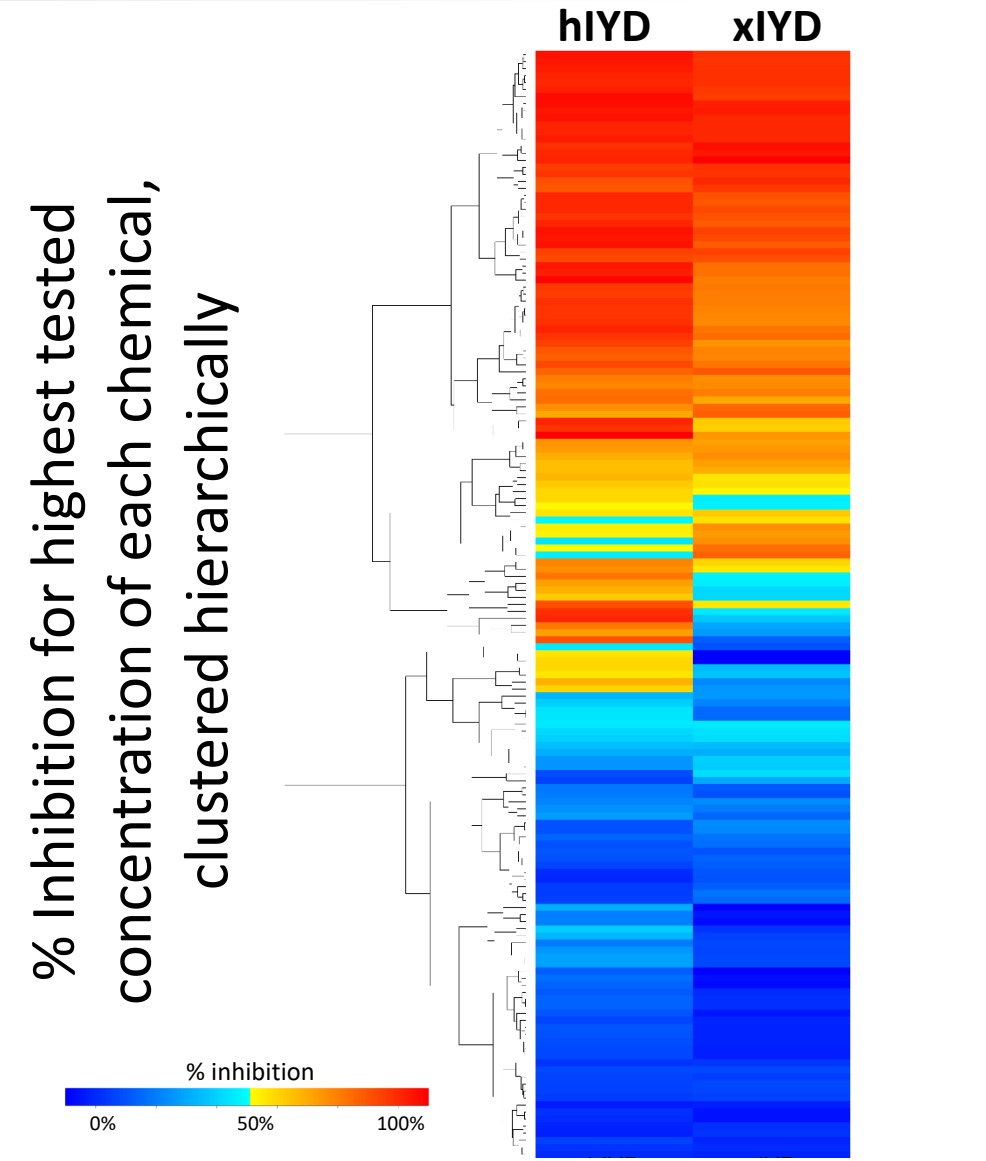
Concentration-Response Screening

≥ 50% inhibition
83 Chemicals
(0.032 - 200 µM)

ToxCast ph1_v2, ph2, and e1k chemicals across all plates



Concentration-Response Testing

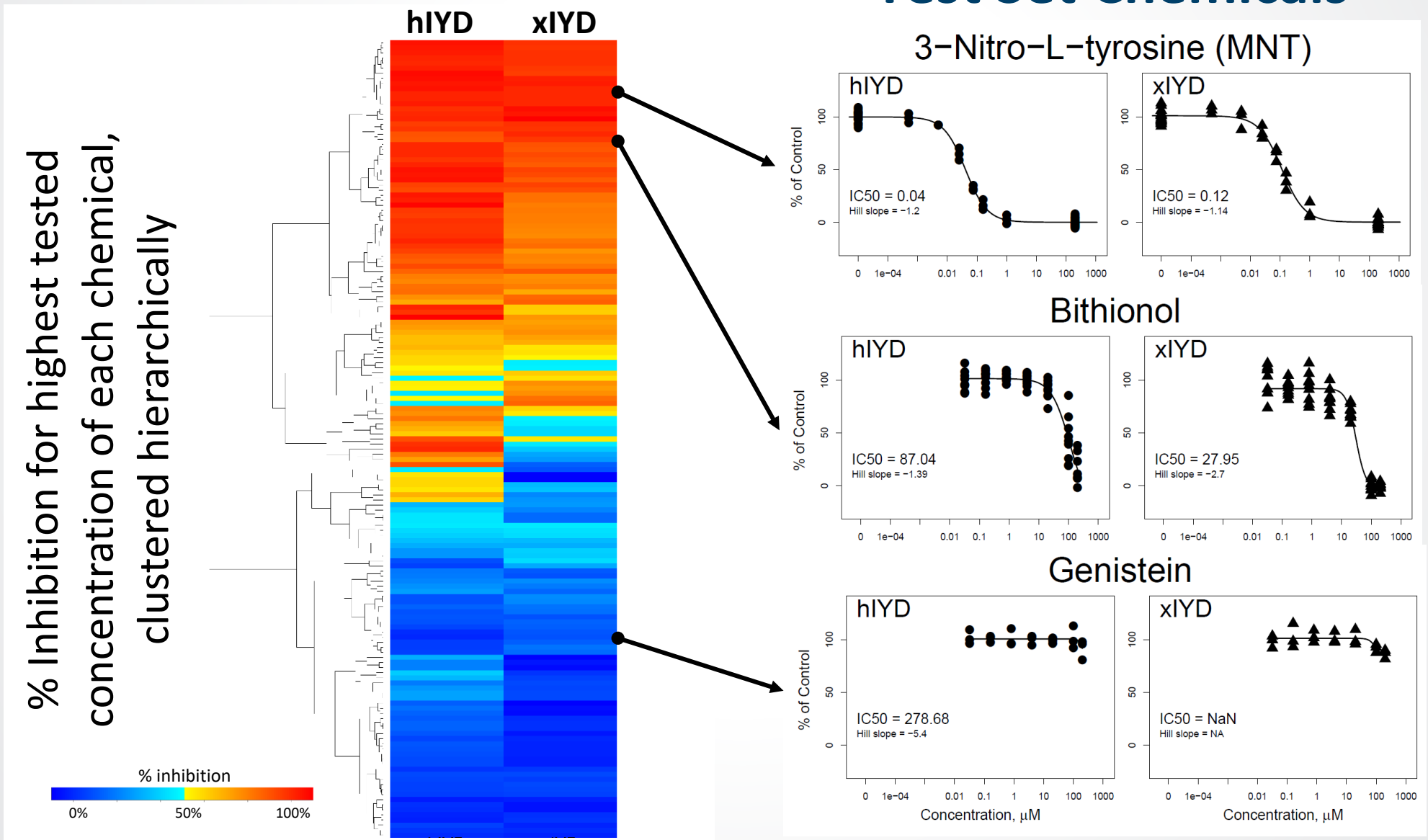


154 unique chemicals tested in both species with 7 concentrations

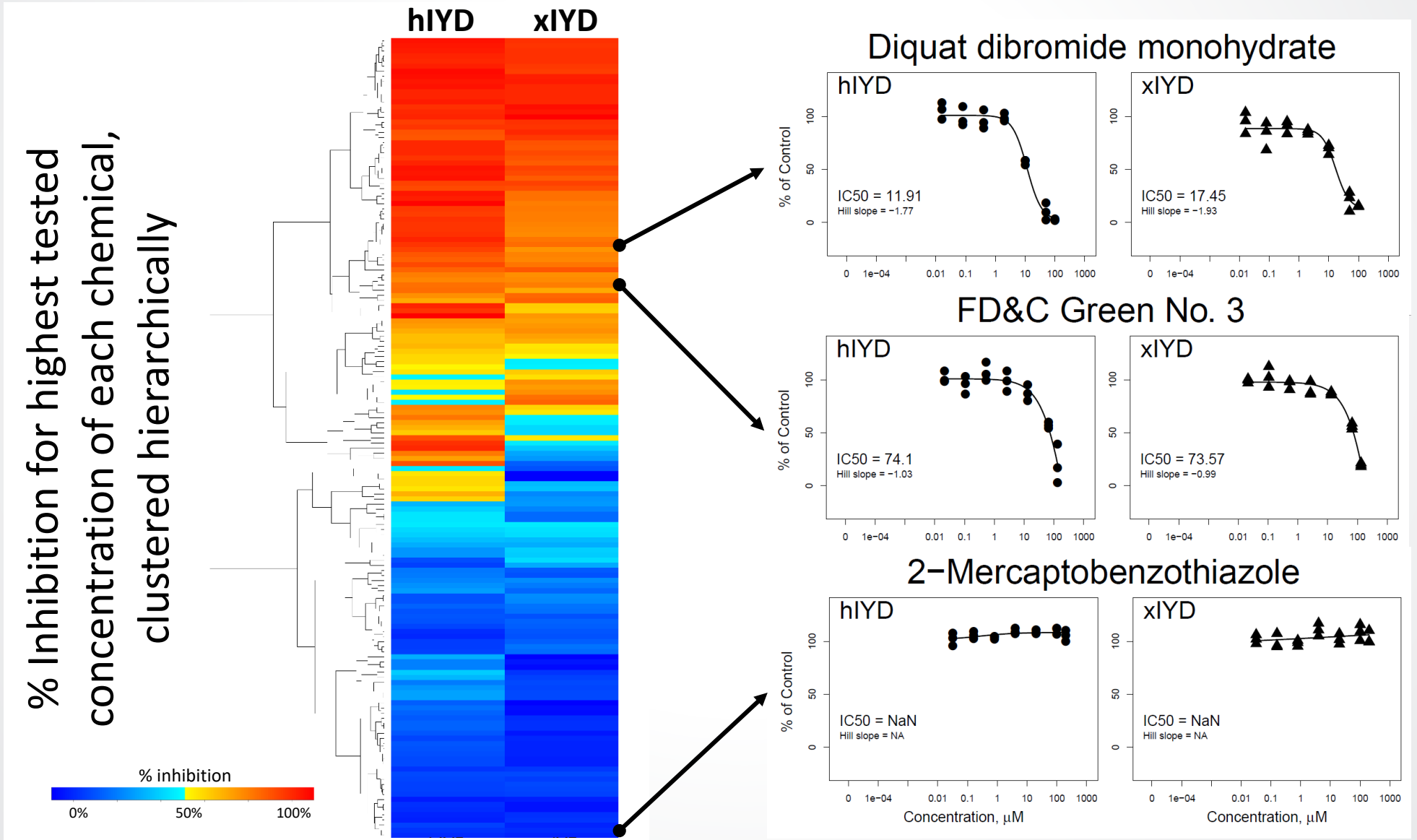
- 83 that produced >50% inhibition in hIYD
- 71 others, including:
  - Test set from literature
  - Non-inhibitors for hIYD
  - DIO and TPO inhibitors

Concentration-Response Testing

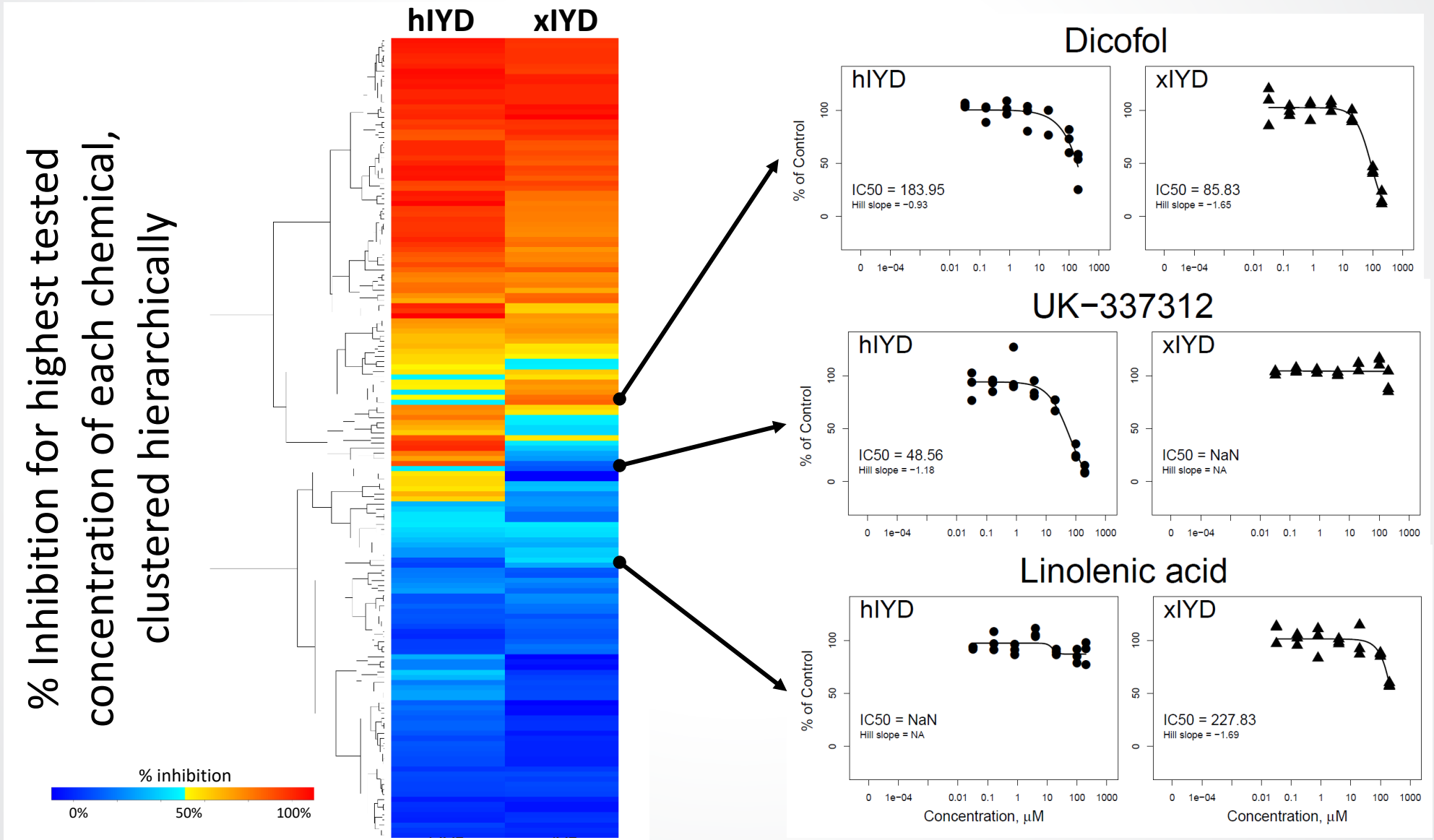
Test Set Chemicals



Concentration-Response Testing

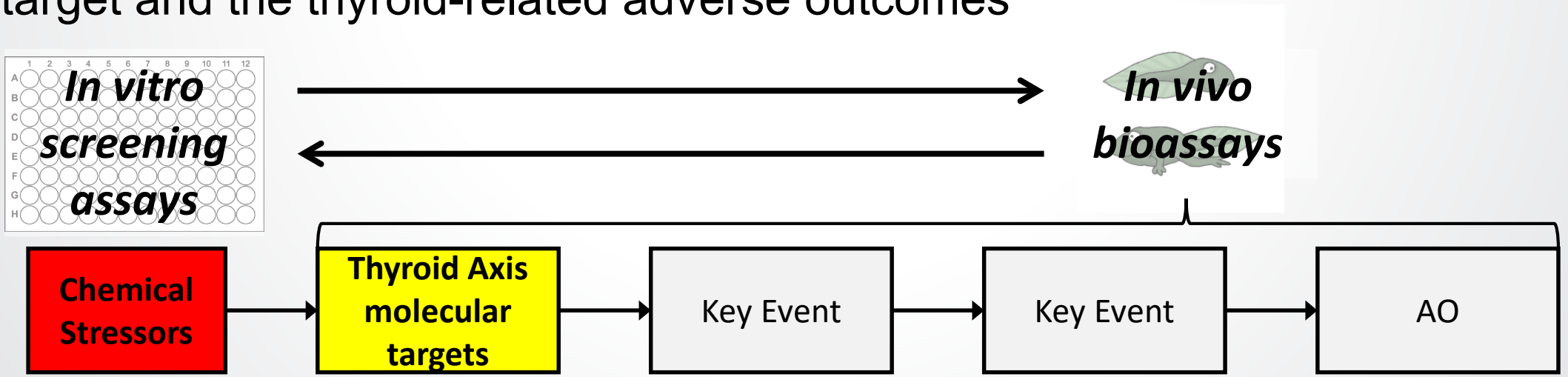


Concentration-Response Testing



Conclusions

- IYD inhibition assays provide an additional screening tool for thyroid hormone disruption
  - Screening >1,800 chemicals greatly expands compounds tested for inhibition of IYD
  - Most chemicals tested (1,633/1,828) produced little to no inhibitory activity
- Response of IYD activity to potential chemical inhibitors, in general, is conserved across human and *Xenopus*
- Iterative approach for causal linkages between chemical inhibition of a molecular target and the thyroid-related adverse outcomes



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Human and Xenopus laevis DIO3

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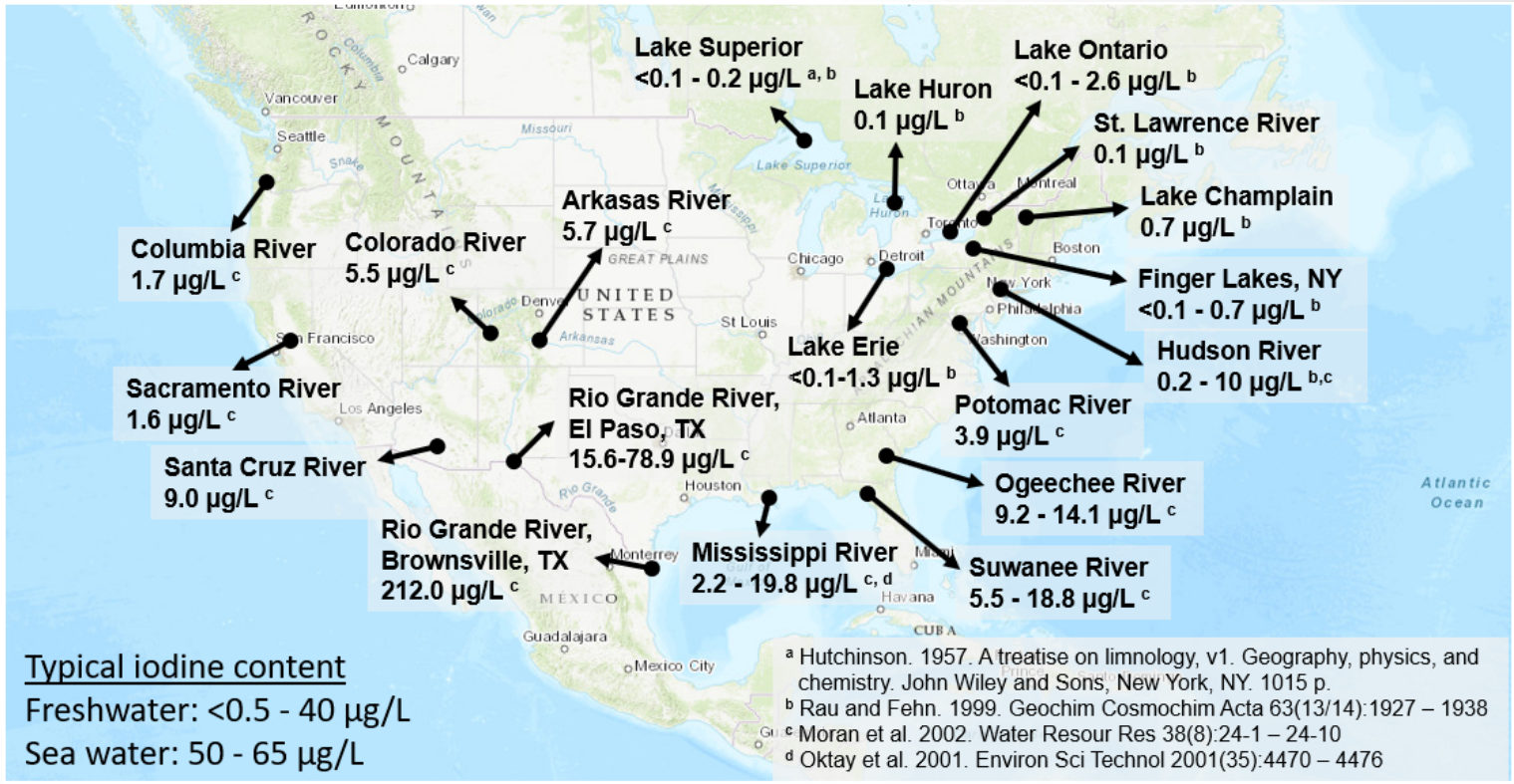
EXTRA SLIDES

Iodine recycling is especially critical for:

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

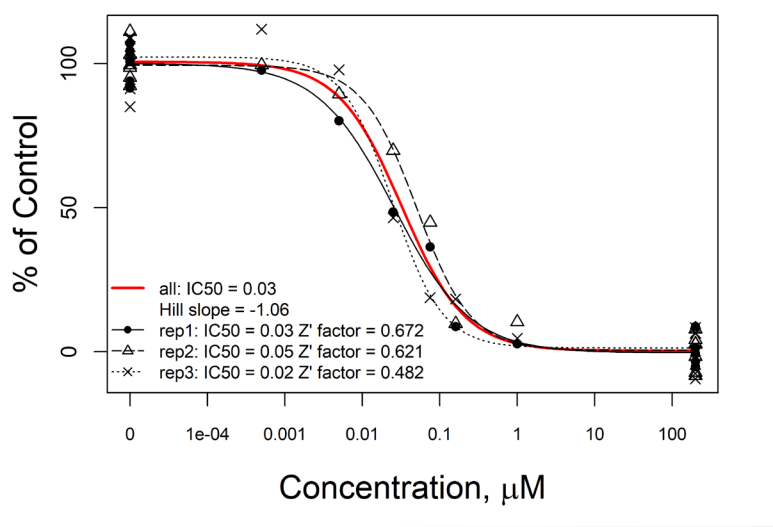
Iodine content in U.S. freshwater lakes and rivers



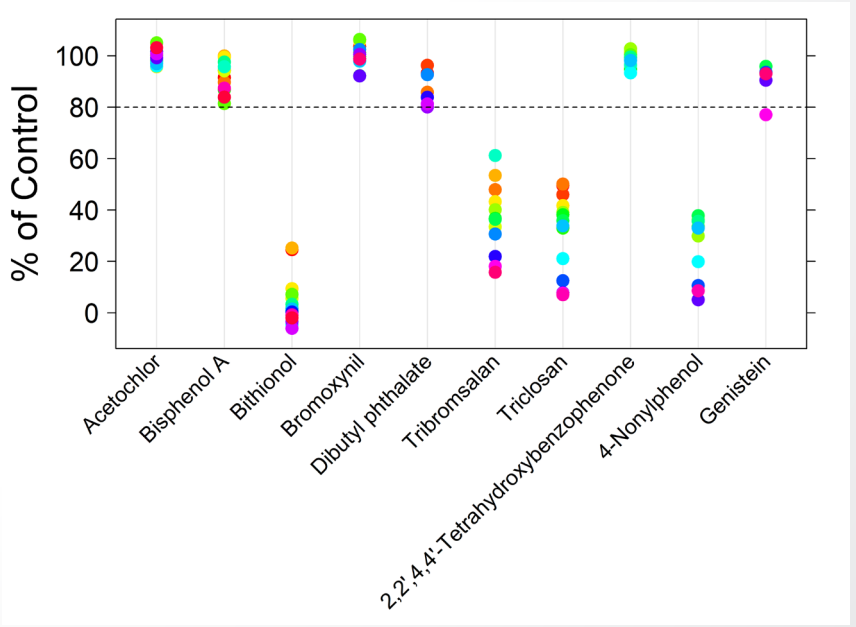
Assay Performance

- Inhibition curves for positive control chemical (MNT) were consistent:
- IC50: 0.01 – 0.05 for hIYD and 0.15 for xIYD
- Hill slopes near -1
- Good dynamic range and acceptable variability in positive control and solvent controls, indicated by Z' factor at or above 0.6.
- Replicated test set of chemicals consistently identified inhibitors of hIYD.

Example MNT Inhibition Curve

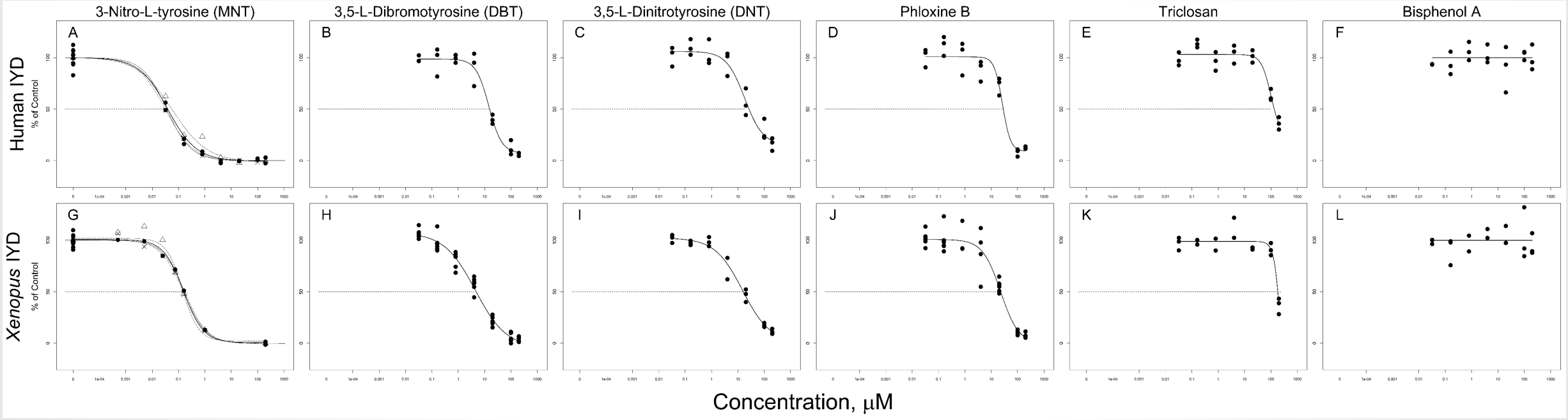


Replicated Test Chemicals



Human versus *Xenopus* IYD in vitro screening species comparison

Results from initial set of 10 chemicals



154 chemicals were tested in concentration response in <u>both</u> **human IYD** and ***Xenopus* IYD**

- 7 concentrations (target: 0.032 – 200 uM)
- 83 of these were selected based on inhibition of human IYD of 50% or greater
- 71 others, including >30 'non-inhibitors' of human IYD, 15 DIO inhibitors, and PFAS