Modes of Action of Thyroid Disruption: Insights from Zebrafish Transcriptomics
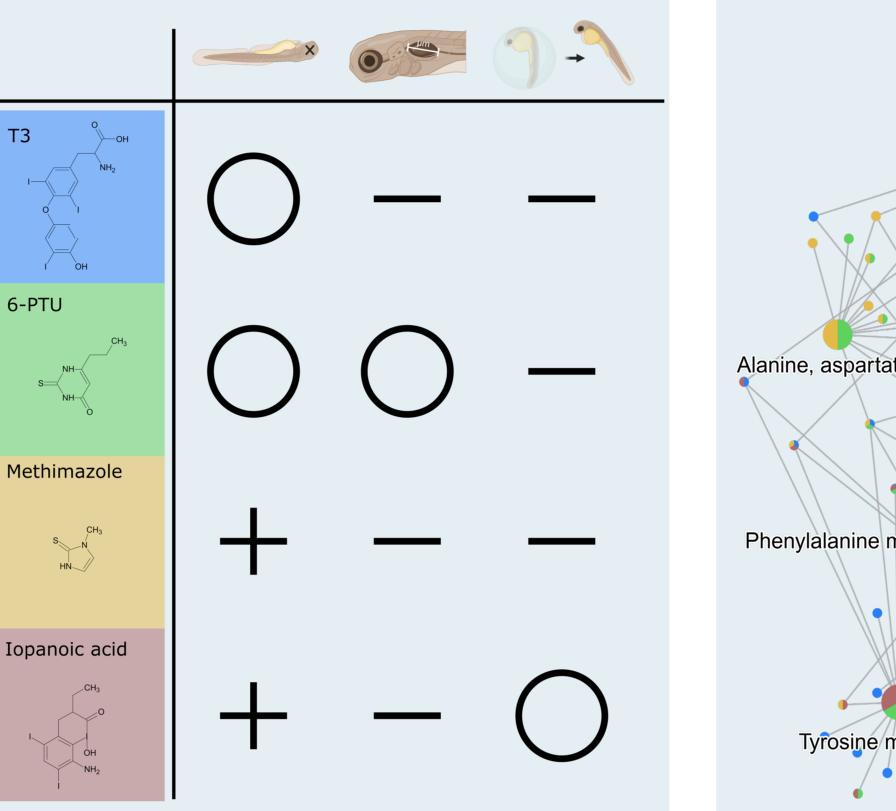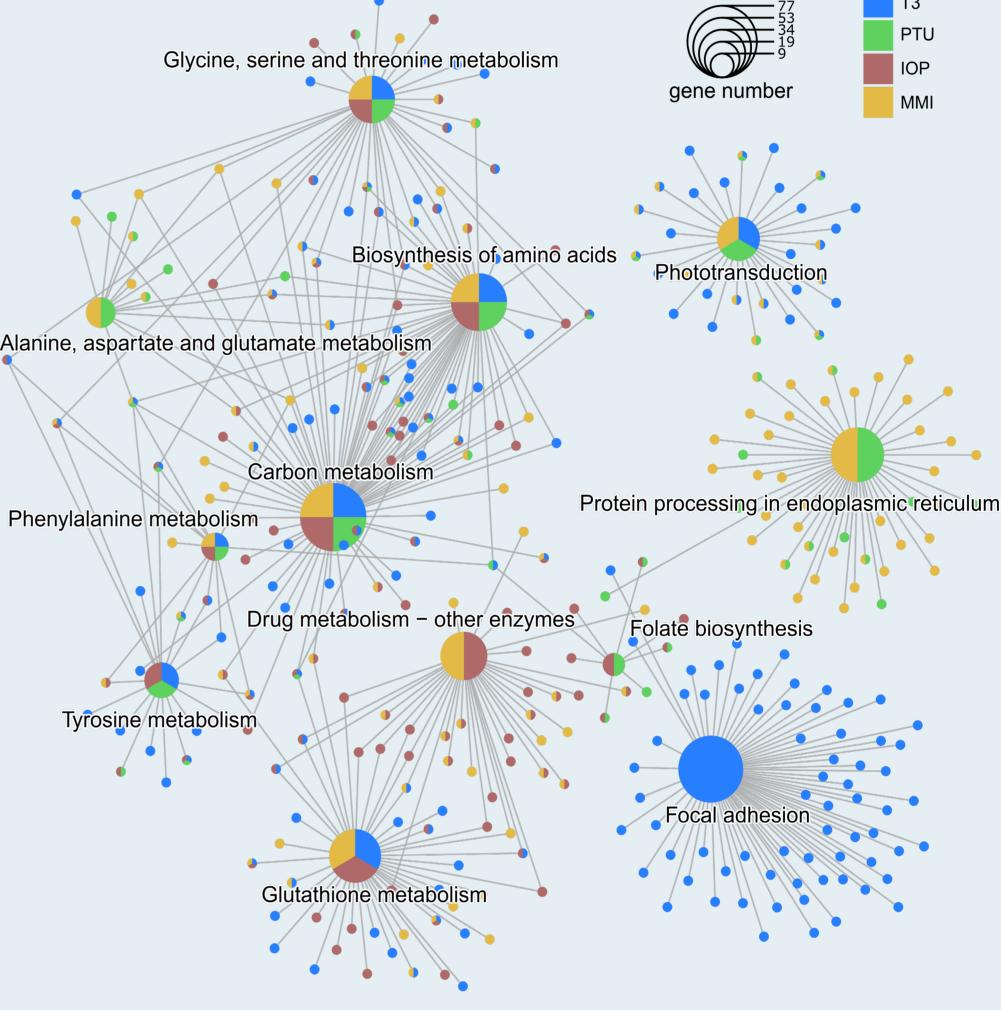
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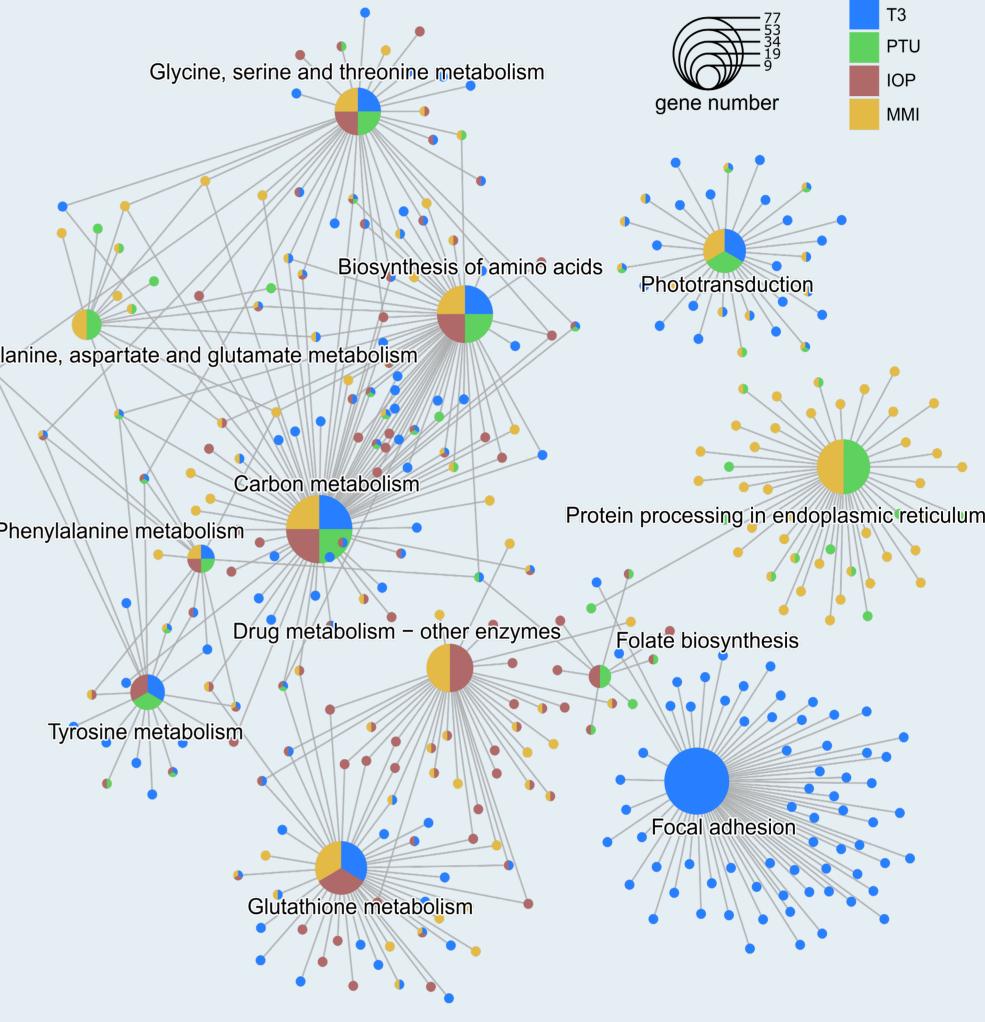
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Why are thyroid disrupting compounds so concerning for environmental health?

- Disruption of thyroid function during development poses significant risks to growth, development, and metabolic balance. These key molecular events can be the initiators of far-reaching effects on environmental populations. This link is described by the Adverse Outcome Pathway (AOP) framework.[1]

What was our aim?

- Our study aimed to reduce reliance on extensive animal testing by contributing to the development of New Approach Methods (NAMs) and the AOP framework for thyroid disruption assessment[1]
- Specifically, we sought to identify transcript biomarkers for thyroid disruption using RNA-seq analysis on zebrafish embryos treated with four well-known thyroid-active compounds: triiodothyronine (T3), 6-propyl-2-thiouracil (PTU), methimazole (MMI), and iopanoic acid (IOP)
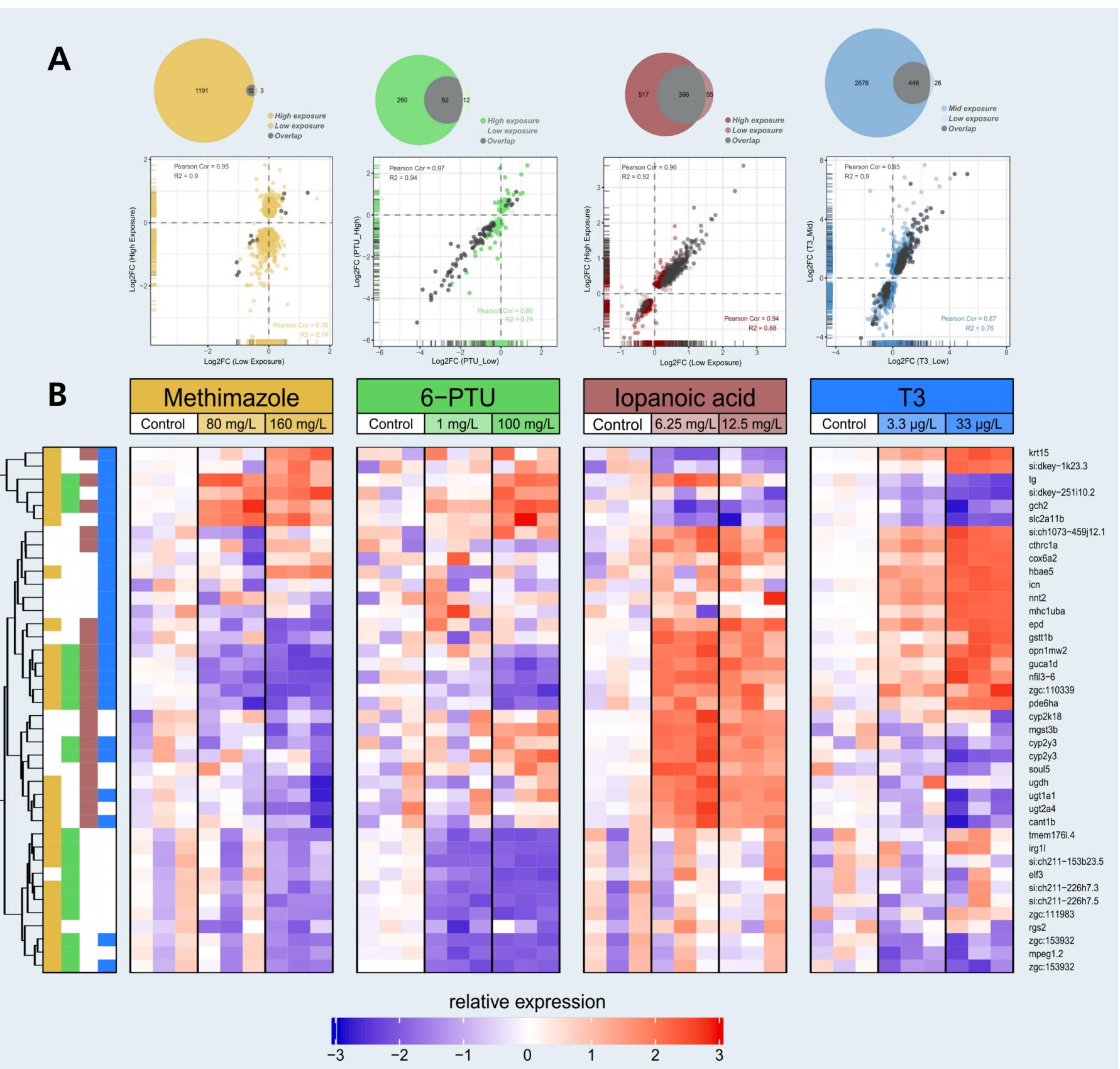
How did we proceed?

- To establish test concentrations, we conducted rangefinding tests focusing on physiological endpoints such as mortality, swim bladder length, and hatching. Low (~EC5) and high (~EC20) concentrations were defined for each compound (T3: 3.3 and 33 µg/L; PTU: 1 and 100 mg/L; MMI: 80 and 160 mg/L; IOP: 6.25 and 12.5 mg/L).
- Total RNA was extracted from pooled larvae samples (10 larvae/sample) in triplicate at 96 hours post-fertilization. RNA-seq data were analyzed using DESeq2 and clusterProfiler. [2]
- Comparative analysis included yet unpublished data on IOP and MMI with data on T3 and PTU. [2]

What did we find?

- A reduction in hatching rates was observed in response to T3, PTU and MMI. Only PTU did not affect swim bladder size. Mortality was observed to be altered by MMI and IOP (data summarized in Figure 1). Although some of these observations differ from other studies, this may be due in part to different test concentrations.
- Functional analysis using overrepresentation analysis on the KEGG database revealed that pathways involving key thyroid-related amino acids were significantly perturbed. In addition, genes involved in focal adhesion, protein processing, and phototransduction, which are also closely related to the thyroid system, were overrepresented (Figure 2).
- The heatmap (Figure 3) visualizes the 10 most significantly regulated genes taken from every compound specific overlap.
  - Clear substance-specific patterns are visible. MMI and PTU, both thyroperoxidase inhibitors, are particularly similar
  - Seven genes were regulated by all compounds, indicating promising biomarker candidates
  - Several mode of action (MoA) specific genes were identified

What is the conclusion?

- By testing compounds with different thyroid-related MoAs, we were able to not only identify genes specific for thyroid disruptors in general, but also differentiate between thyroid hormone activation, thyroperoxidase inhibition, and deiodinase inhibition.
- Our findings indicate that transcriptome-based thyroid biomarkers offer a valuable contribution to the AOP framework for risk assessment of thyroid-affecting substances.

Figure 1: Graphical summary of physiological endpoints: mortality, swim bladder length, and hatching rate, using symbols: circle (no effect), plus sign (increase), and minus sign (decrease).

Figure 2: Overrepresented KEGG pathways. For each compound the ten most significant terms were selected.

Figure 3: A) Venn and scatter plots comparing the number and effect size of differentially expressed genes in both exposure concentrations of all test compounds. B) Heatmap showing a subset of DEGs selected from the ten most significantly regulated genes for each compound. The colored panel on the left indicates whether a gene is also significantly regulated by the other compounds in at least one of the exposure concentrations.

References

1 Noyes et al. (2019), Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. Environ Health Perspect.

2 Reinwald et al. (2021), Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos. Science of the Total Environment, 760, 143914