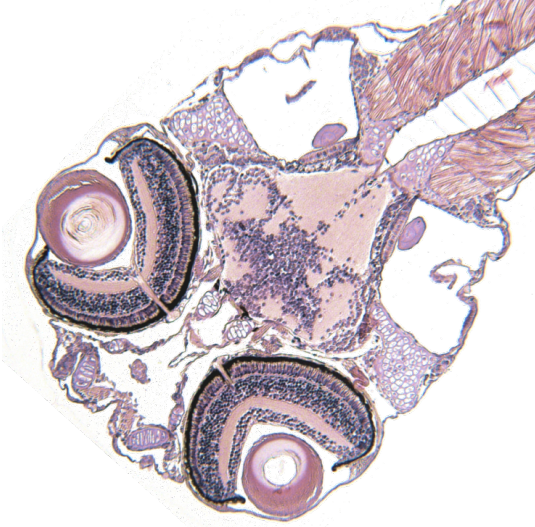
Zebrafish embryos as NAMs for endocrine disruptor testing

–

Insights from the ERGO project



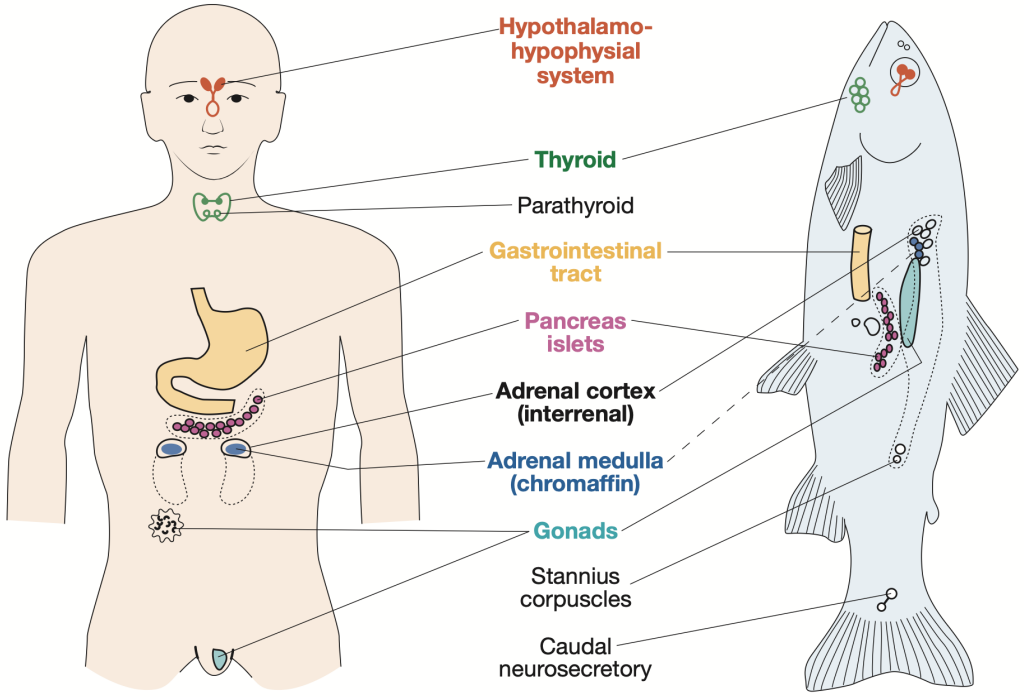
Lisa Baumann

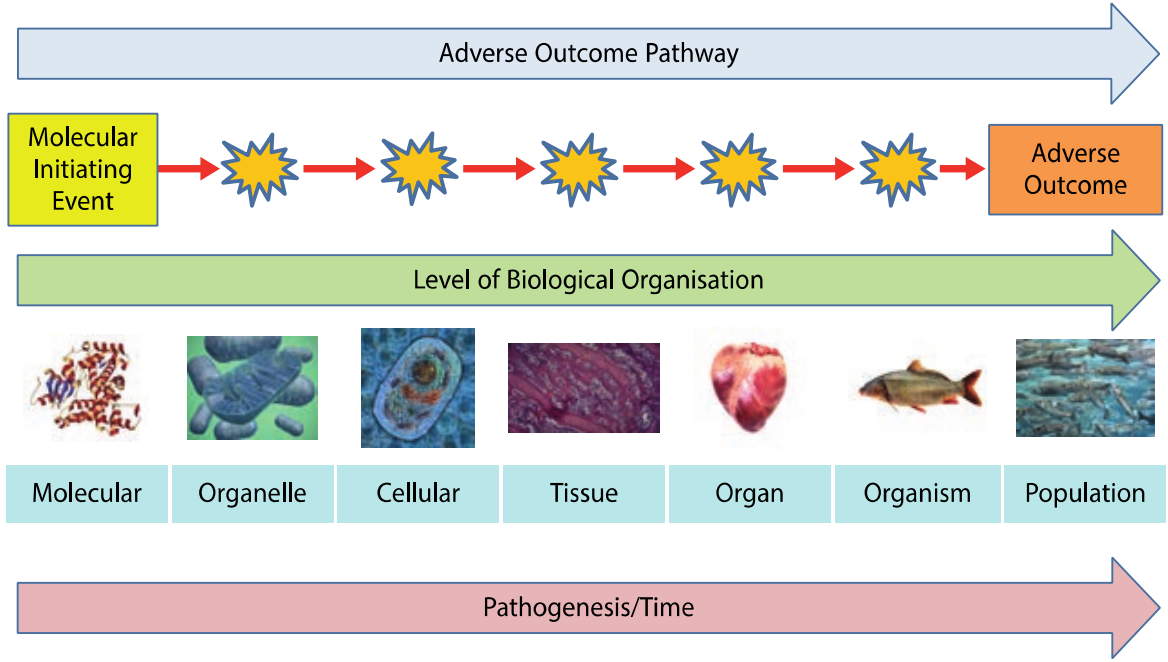
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EndocRine Guideline Optimisation

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A-LIFE Amsterdam Institute for Life and Environment

Background:
Conservation of the vertebrate endocrine system





- All vertebrate classes (reptiles, amphibians, birds, fish, mammals) share most endocrine glands
- Strong structural similarity of hormones and receptors, functionality can partially differ
- Read-across species is biologically highly plausible
- Consequence: conservation of adverse outcome pathways (AOPs)

Tata, J.R. One hundred years of hormones. *EMBO Rep* **6**, 2 (2005).
OECD Series on Testing and Assessment, No. 260, 2017

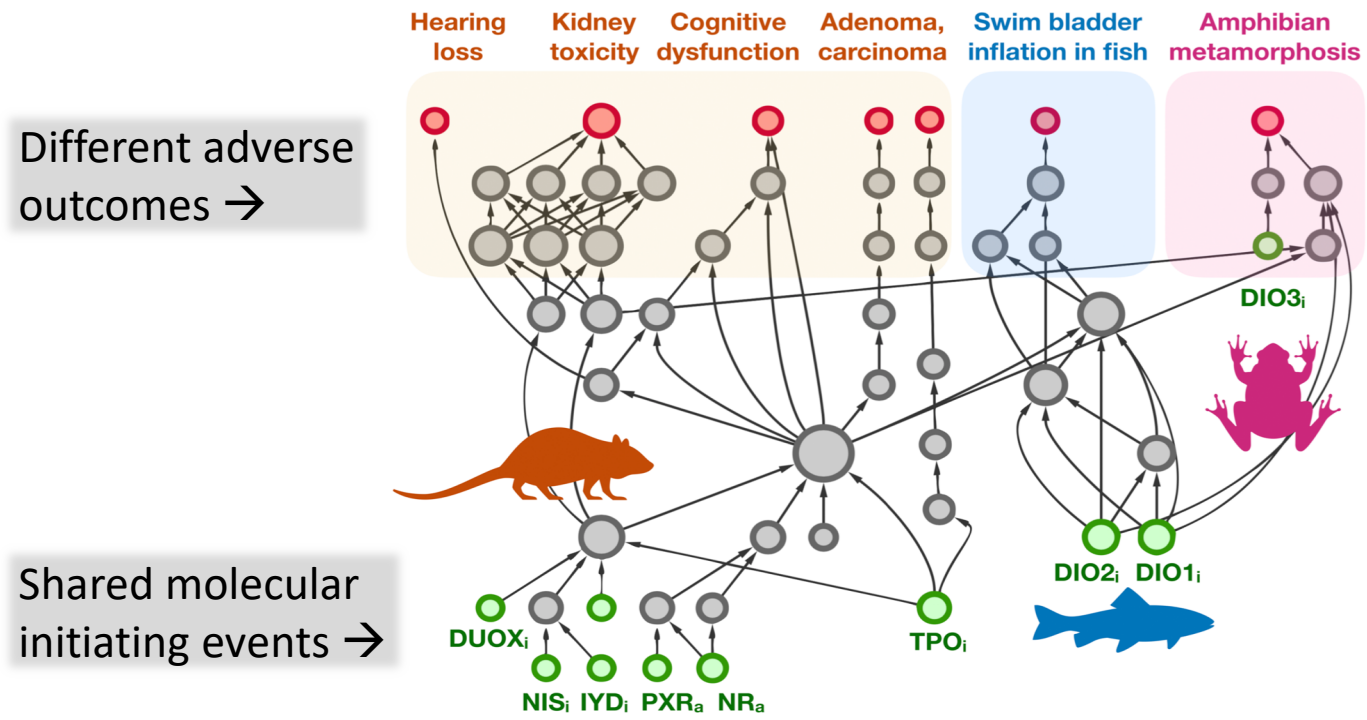
Breaking down the wall between human health and environmental testing of endocrine disrupters



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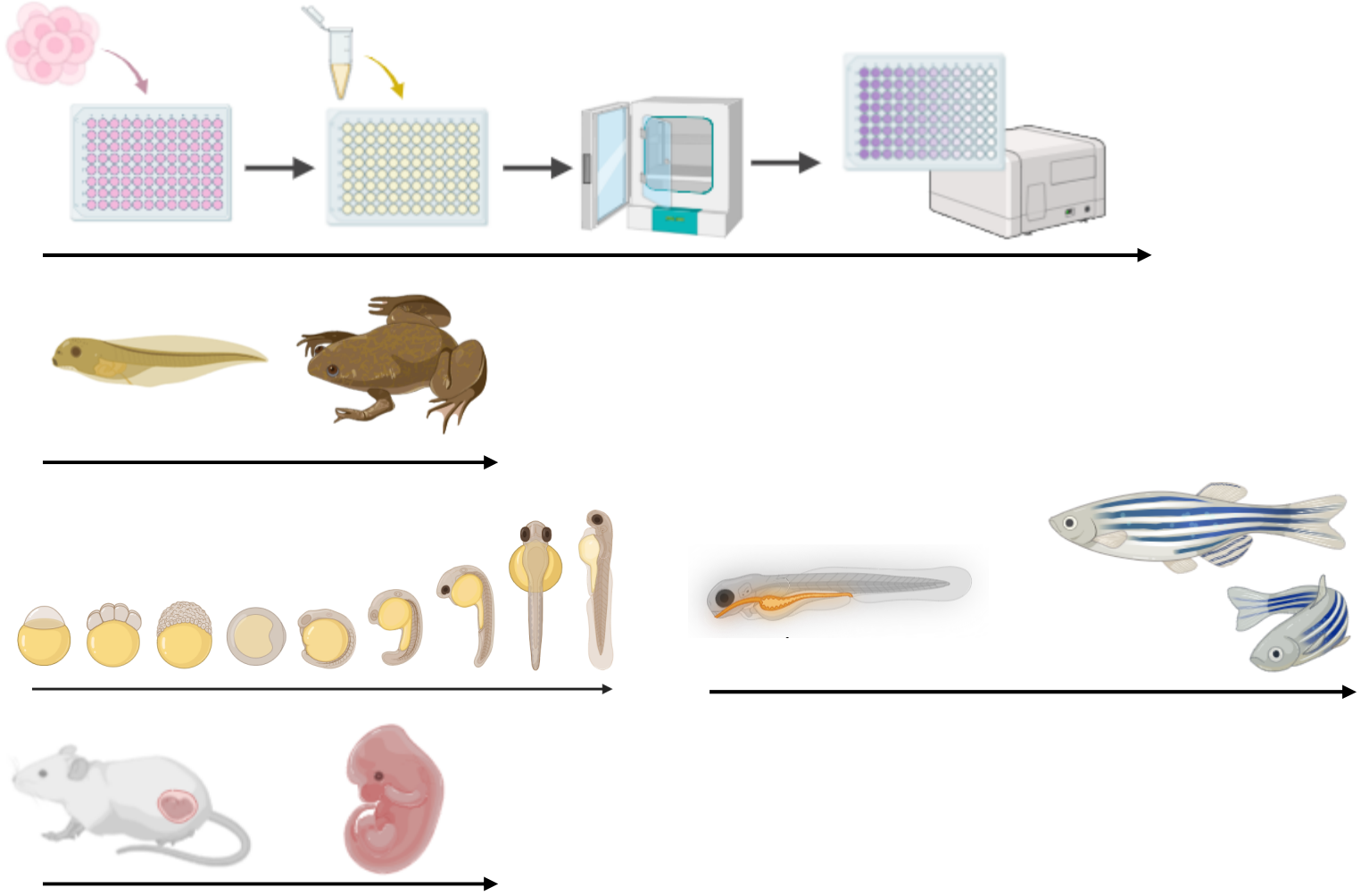
EURION European Cluster to Improve Identification of Endocrine Disruptors

- To demonstrate feasibility to extrapolate effects of endocrine disruptors **across vertebrate classes**
- i.e. an adverse effect on an endocrine-specific endpoint observed in a fish or amphibian study will also raise concern about an adverse effect in humans
- **The cross-vertebrate class approach** is relevant for all conserved endocrine axes/systems such as the Retinoid X Receptor (RXR), the Hypothalamus Pituitary Gonadal (HPG) and the Hypothalamus Pituitary Thyroid (HPT) axes



Knapen et al. Environ. Sci. Technol. 2020, 54, 8491−8499

The ERGO test battery



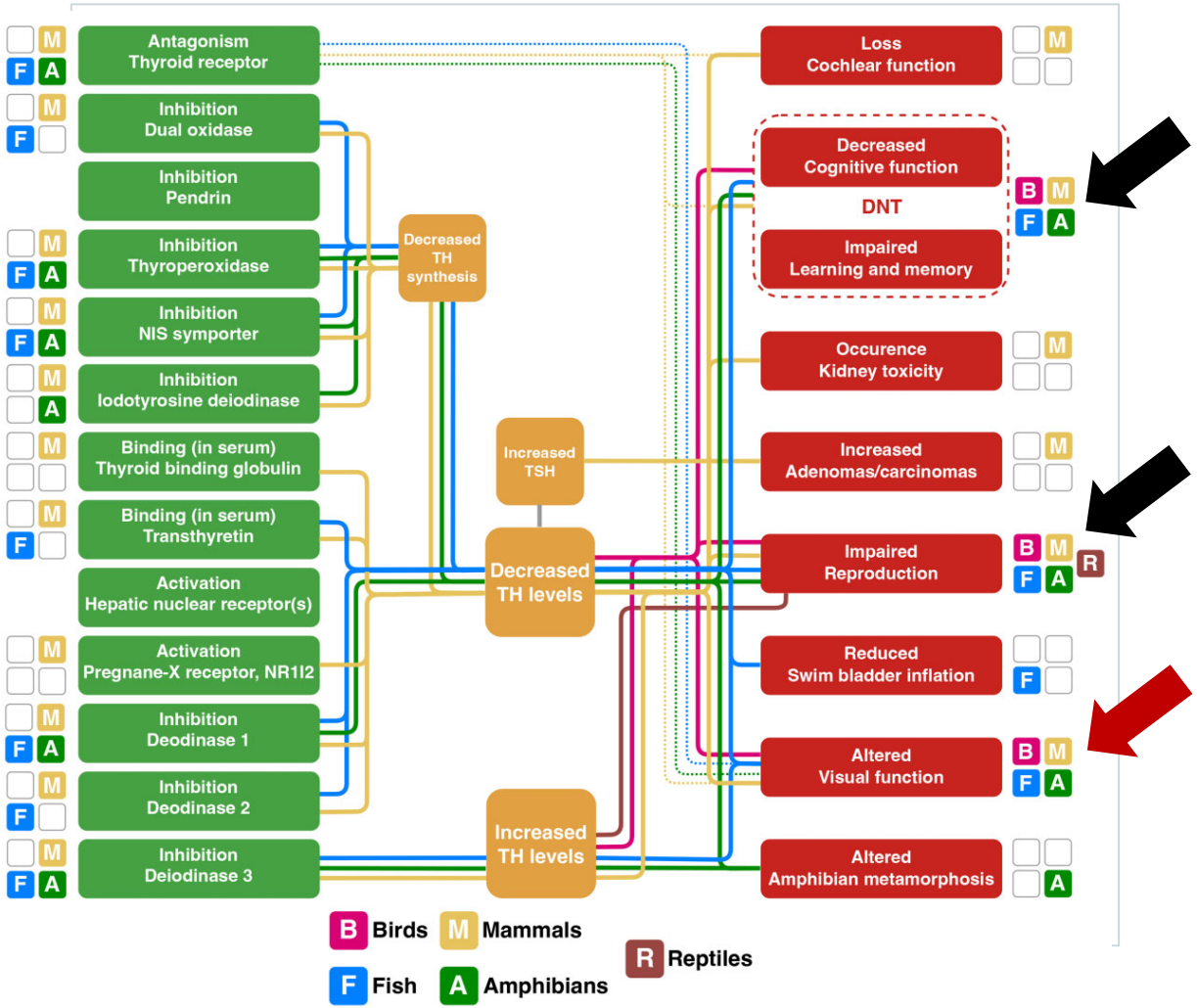
> 20 EDCs
tested in total

6 model compounds
tested across all assays

Complemented with **literature data**

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Breaking down the wall: a cross-species THSD AOP network



Systematic taxonomic domain of applicability analysis

50 AOPs

50 THSD-specific AOPs from the AOP-Wiki combined into one network.
Plus 439 AOPs with adjacent KERs

Before ERGO

no AOP in the network covered more than one vertebrate class

After ERGO

13 MIEs converge on altered TH levels and diverge to 9 shared adverse outcomes across multiple vertebrate classes

Conclusion

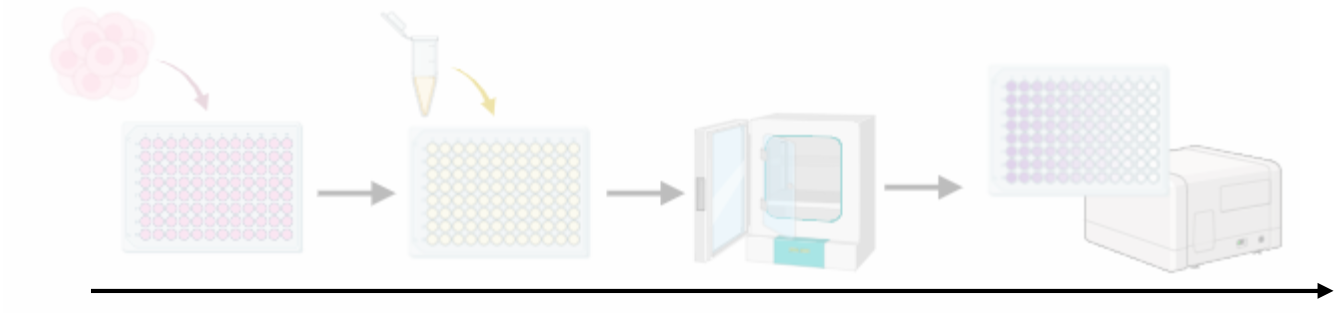
evidence-based foundation for extrapolating THSD data across vertebrate species.
For example: **fish can inform about mammals and vice versa**

THSD: thyroid hormone system disruption

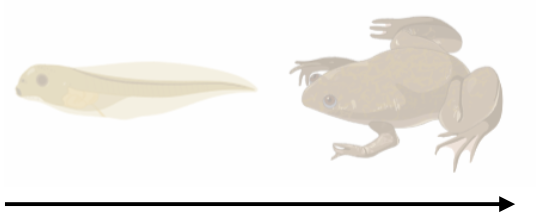
Haigis, et al. 2023, Toxicol Sci 195:1, Fig. 3 (doi:10.1093/toxsci/kfad063).

The ERGO test battery

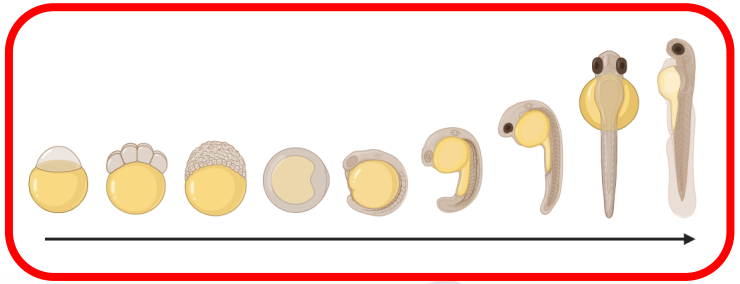
Cell-based



Amphibians



Fish



Mammals



→ Zebrafish embryos up to 5 dpf are (still) non-protected life stages (NAMs)

→ Can be used to study ED activity and adversity

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Regulatory relevance of embryo models



Endocrine Mode of Action

Causal linkage

Adverse effect



- **Endocrine activity** can be assessed with ***in vitro*** cell-based NAMs, which represent crucial tools to understand the endocrine mode of action of a compound
- **Endocrine adversity** such as growth, development or reproduction can only be assessed using ***in vivo*** models that possess an endocrine system and its complex regulatory functions
- ECHA's Key Areas of Regulatory Challenge name NAMs that can conclude on adversity at organism level as a priority
- Embryo-based tests are now under scrutiny with the EU roadmap for phasing out animal experiments

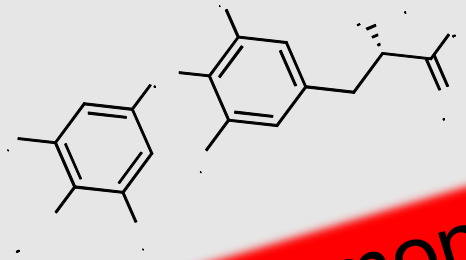
WHO/UNEP 2012; OECD GD 150 (2018); EC C(2023) 5041; ECHA 2025 (KARC, ECHA-25-R-04-EN); Directive 2010/63/EU.

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Implementation of thyroid-related endpoints into the fish embryo toxicity test (FET, OECD test guideline 236)



Thyroid follicle morphology
Transgenic line (tg:mCherry)
or antibody stain



Thyr

First embryo-based test system that demonstrates ED adversity
→ highly valuable NAM to investigate complexity of ED effects
→ Currently under OECD validation, project 2.64

→ **tFET**

Eye development
Histopathology

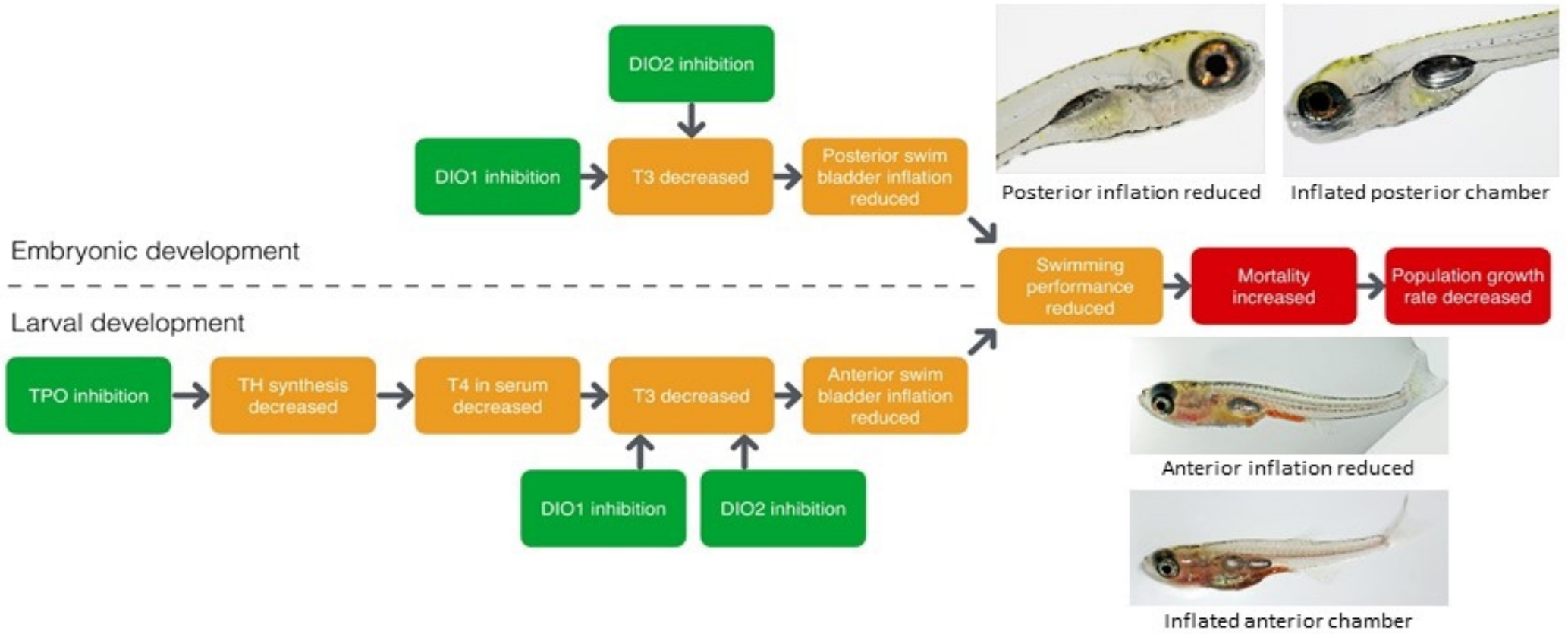


Swim bladder inflation
Binary assessment

→ T-mediated
ED **adversity**
(if combined with positive follicle / TH effect)

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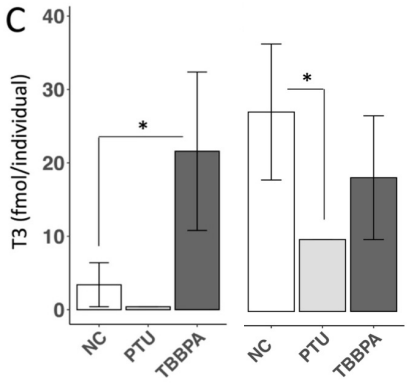
AOPs for disrupted swim bladder inflation



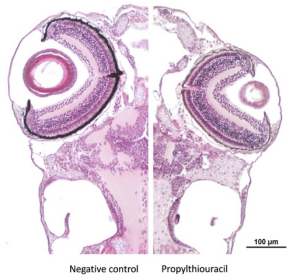
Stinckens et al. 2020, AOPs 155-159

AOP for disrupted eye development

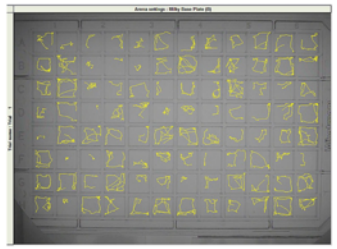
TPO inhibition → TH synthesis decreased → T4 in serum decreased → T3 in serum decreased → Retinal layer structure altered → Visual function altered → Increased mortality → Population trajectory decreased



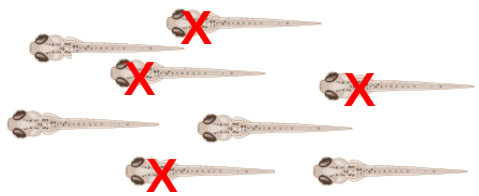
TH levels



Eye histopathology



Vision → Behavior

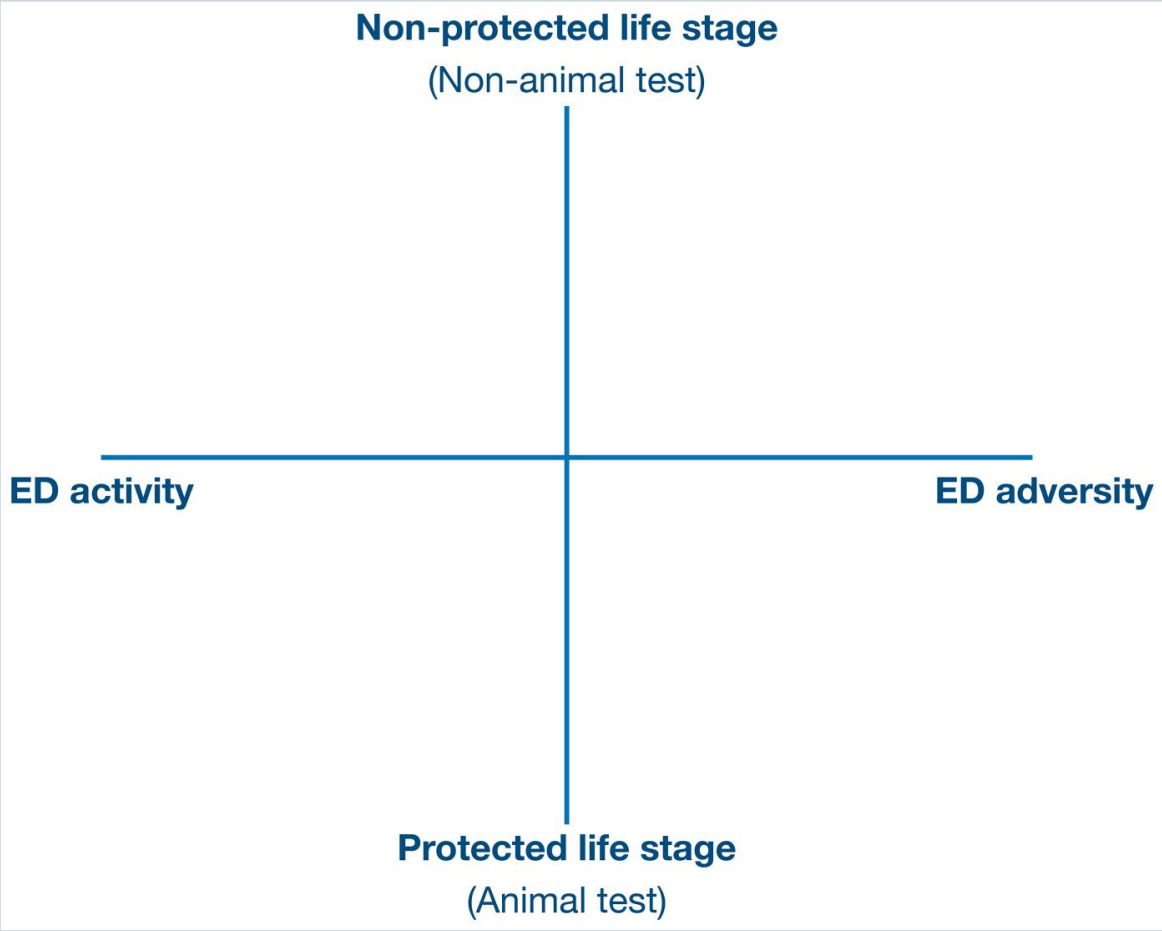


Mortality

Gölz et al. 2022. AOP report. https://aopwiki.org/aops/363

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Where embryo tests fit in the ED testing framework



The tFET and the 3Rs

Replace protected vertebrate THSD tests, such as amphibians

Reduce higher-tier animal tests — in a tiered approach with *in vitro* assays, EDs are flagged earlier

Refine: embryos are less sentient and exposures are much shorter

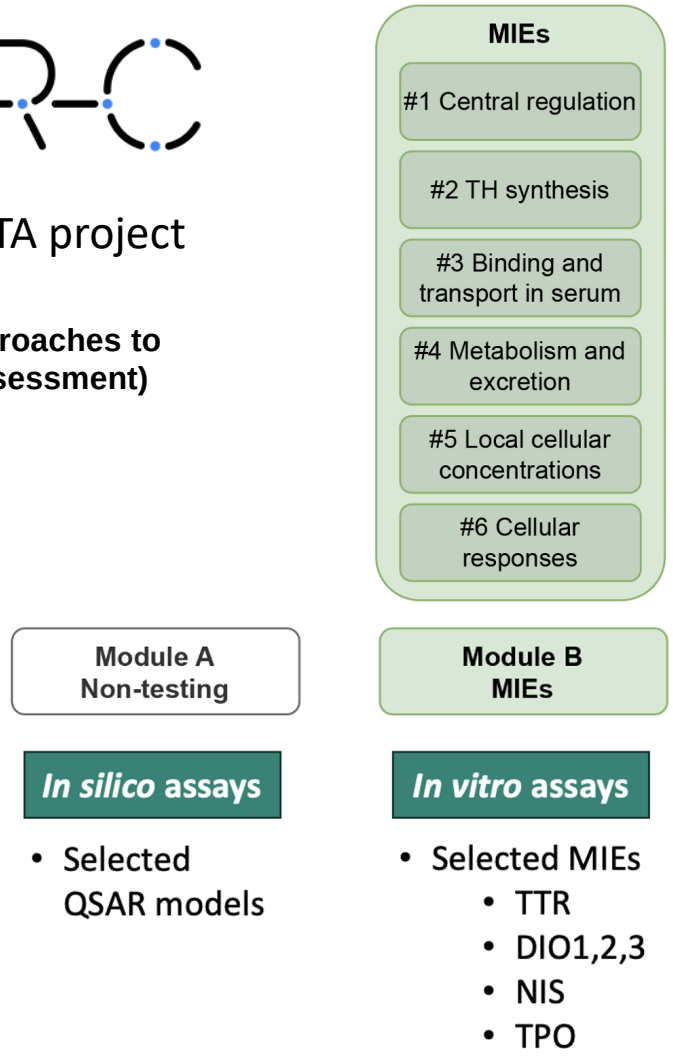
Knapen, Vergauwen, Baumann & Holbech 2026, Environ Toxicol Chem 45:289, Fig. 1 (doi:10.1093/etojnl/vgaf258).

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From AOP network to regulatory application

FISH-THSD IATA project

(Integrated Approaches to Testing and Assessment)

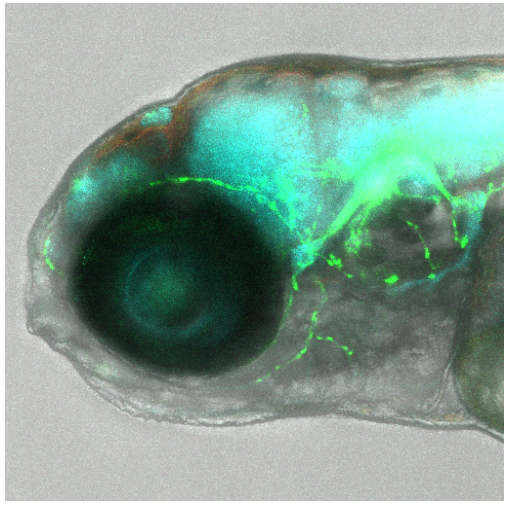


Vergauwen et al., 2026, DOI: 10.1016/j.envint.2026.110451

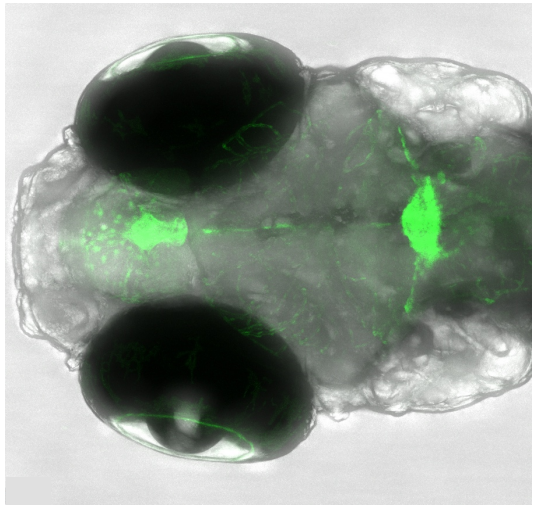
Ongoing projects using zebrafish embryos to assess adverse effects of endocrine disruptors

- ✓ Only **full organisms** allow for detailed and relevant assessment of chemical exposure on **complex organ systems**
- ✓ Fish embryos represent a **crucial bridge** between cell-based *in vitro* systems and higher-level *in vivo* test
- ✓ Fish embryos contribute to the **3Rs without weakening the protection goals** for human and environmental health

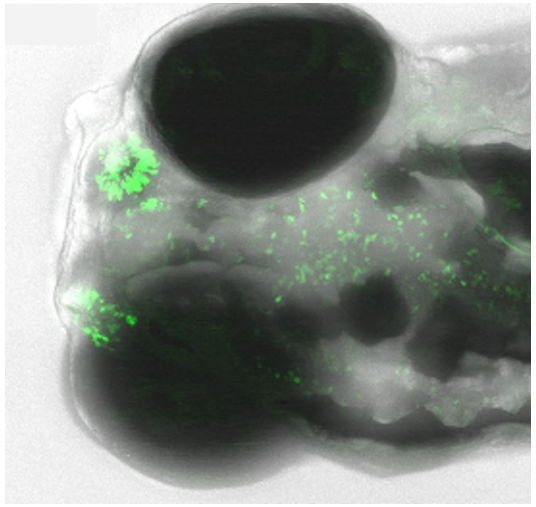
Brain



Blood-brain barrier



Olfactory system

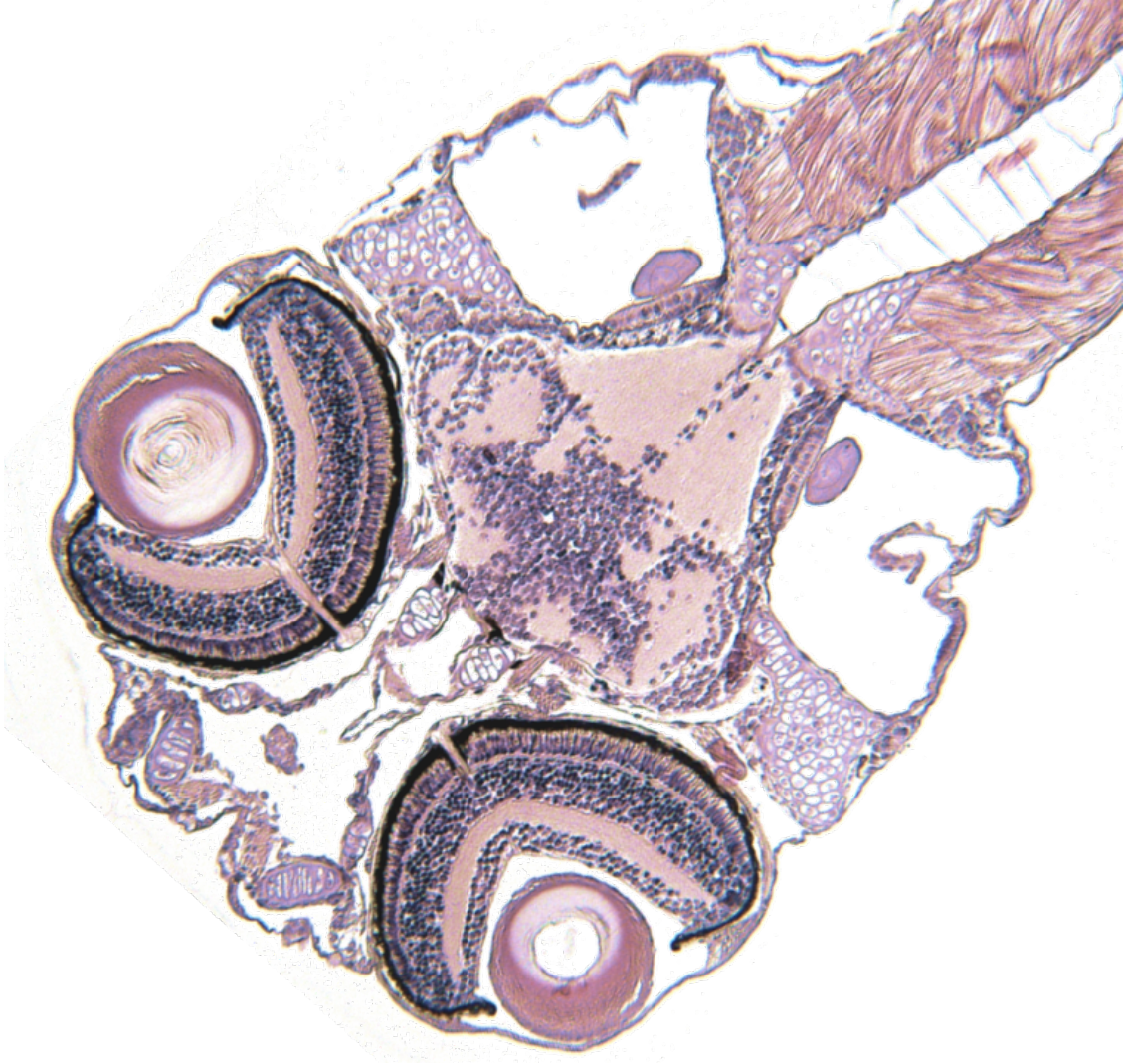


NeXED
Training the next generation of toxicologists

Merlon
Researching the health impacts of endocrine disrupting chemicals

Images:
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Nora Karlsson
Clara Kempkens

Thanks to my collaboration partners:
Henrik Holbech (University of Southern Denmark)
Dries Knapen & Lucia Vergauwen (University of Antwerp)



Thank you for your attention!

Questions? l.a.baumann@vu.nl

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