Cross-species assay validation using the AOP "deiodinase inhibition leading to impaired posterior chamber inflation"

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Background

Objectives

Assessment of the cross-species applicability of the AOP-based deiodinase (DIO) assay:

1. Develop DIO> swim bladder AOP network
2. Perform DIO *in vitro* assay using porcine liver homogenates
3. Perform acute and chronic *in vivo exposure* experiments in fish
4. Perform DIO *in vitro* assay using rat and fish liver homogenates
5. Assess cross-species applicability

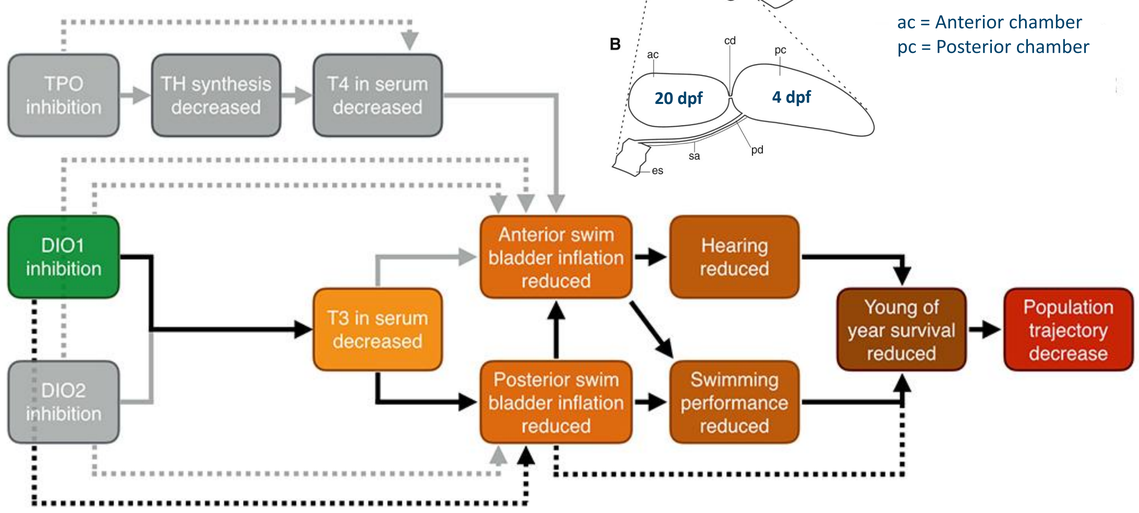
Materials and Methods

	2. *In vitro* assay	3a. Acute exposure	3b. Chronic exposure
Toxicity test	*In vitro* assays to measure molecular initiating event (MIE)	Adjusted Zebrafish Embryo Acute Toxicity test (ZFET, OECD TG 236)	Adjusted Fish Early-life Stage Toxicity test (FELS, OECD TG 210)
Exposure time	30-60 minutes	5/7 days post fertilization (dpf)	32 dpf
Endpoints	Deiodinase (DIO) inhibition	Posterior swim bladder chamber inflation (KE)	Anterior swim bladder chamber inflation (KE)
Tested compounds	22 compounds with suspected thyroid disrupting activity	MBT, MMI, PTU, 2-TU, IOP, PFOS, PFBS, PFOA, BPA, TCBPA, BP2, 2,4,6-TBP, ANS, SA, NaPER	MBT, MMI, IOP, PTU

Results and Discussion

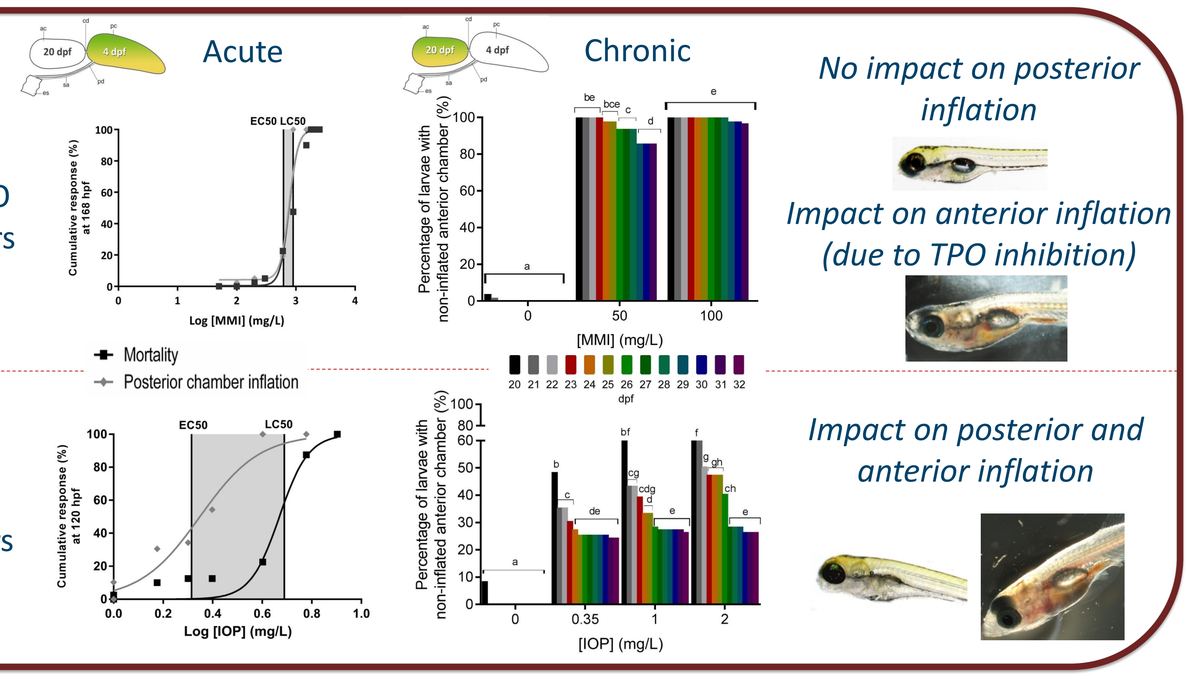
1.

The AOP network is part of Project 1,35 of the OECD AOP development programme workplan



AOP-Wiki numbers: 155, 156, 157, 158 and 159

3.



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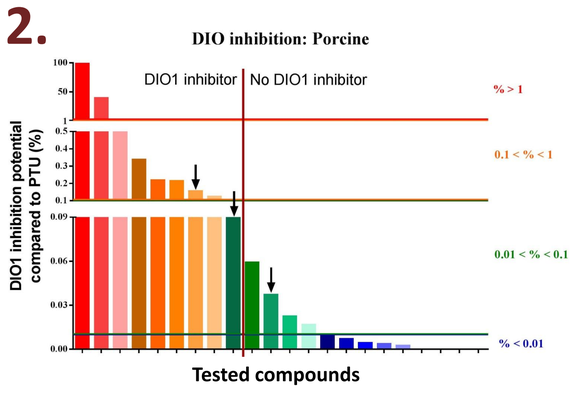
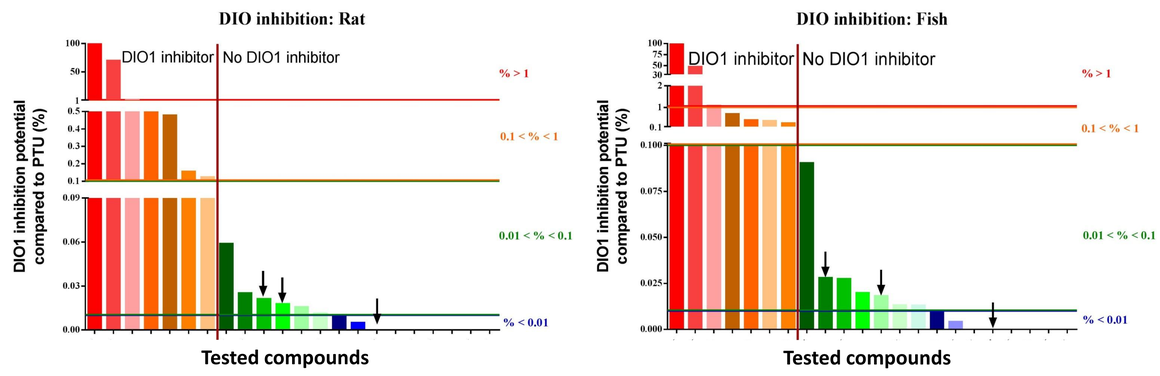


DIO1 inhibition potential compared to PTU (%) of 22 compounds. Coloured bars represent compounds that exceeded the 20% inhibition threshold:

- Strong inhibition
- Intermediate inhibition
- Weak inhibition
- Very weak inhibition
- Black arrows = compounds that shift between species

4.



Black arrows = compounds that shift across species (Bisphenol A and derivatives)

5.

Chronic validation in fish is identical for predictions made using fish, rat and porcine tissue

Chronic validation		Predicted effect based on *in vitro* assays using fish, rat and porcine tissue	
		NO	YES
Observed effect (FELS) in fish	NO		
	YES		*MBT (Zebrafish, Fathead minnow)* *IOP (Fathead minnow)* MMI (Zebrafish) PTU (*Zebrafish*)

Validation of predictions made regarding anterior chamber inflation using FELS experiments (Blue = compounds used to construct AOP network, Green = correct predictions)

Acute validation in fish using porcine tissue in *in vitro* assay

Acute validation		Predicted effect based on *in vitro* assays using porcine tissue		
		NO	YES	
Observed effect (FET) in fish	NO	*MBT* MMI PFBS NaPER	TCBPA	
	YES	PFOS	*PTU* *IOP* BP2 PFOA 2-TU	BPA SA 2,4,6-TBP ANS

Acute validation in fish using fish and rat tissue in *in vitro* assay

Acute validation		Predicted effect based on *in vitro* assays using fish and rat tissue		
		NO	YES	
Observed effect (FET) in fish	NO	*MBT* MMI PFBS NaPER ***TCBPA***		
	YES	PFOS ***BPA***	*PTU* *IOP* BP2 PFOA	2-TU SA 2,4,6-TBP ANS

Validation of predictions made regarding posterior chamber inflation using FET experiments (Blue = compounds used to construct AOP network, Green = correct predictions, Orange = False positive prediction, Red = False negative prediction)

- BPA and derivatives have lower inhibition potential in rat and fish compared to porc
- TCBPA: no longer false positive prediction
- BPA: becomes a false negative prediction
  - affects T3 signaling due to indirect cross talk between the T3 and estrogenic pathways
  - binds to and antagonizes the T3 receptor

Conclusion

Our results are in line with, and increase confidence in, our AOP network. For most compounds, tissue originating from different species can be used in our DIO1 assay to predict apical outcomes in fish.

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