Development of an alternative testing strategy for the FELS test using the AOP network "thyroperoxidase and deiodinase inhibition leading to impaired swim bladder inflation"

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Background

Objectives

Develop non-animal testing strategy for prediction of chronic aquatic toxicity, based on the adverse outcome pathway (AOP) framework:

1. Develop thyroid > swim bladder AOP network
2. Select and optimize assays to measure relevant key events (KEs):
   a) *In vitro* assays
   b) Acute *in vivo* exposure
   c) Chronic *in vivo* exposure
3. Validate delineated AOP

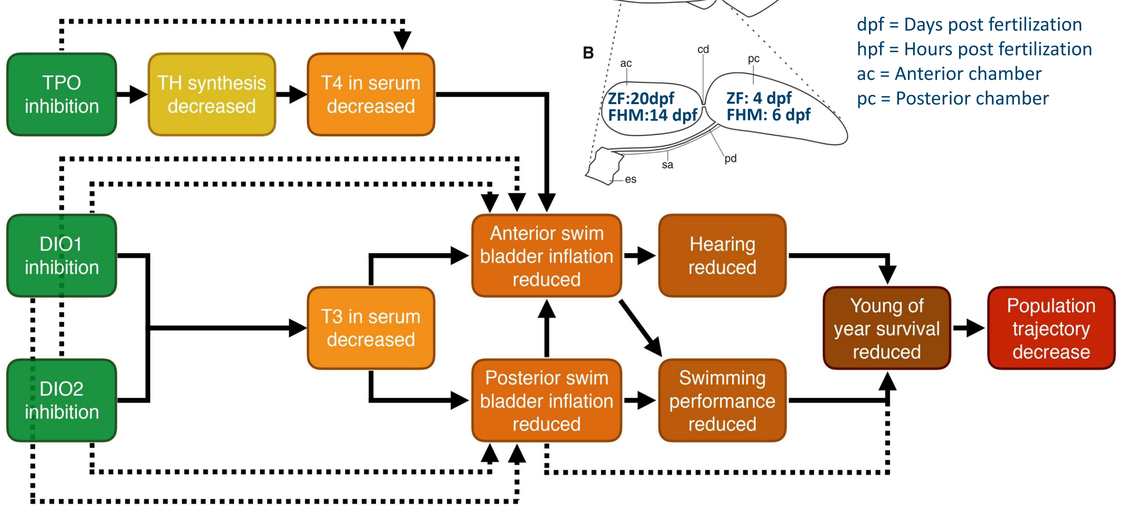
Materials and Methods

	2a. *In vitro* assay	2b. Acute exposure	2c. Chronic exposure
Toxicity test	*In vitro* assays to measure molecular initiating events (MIEs)	Adjusted Fish Embryo Acute Toxicity test (FET, OECD TG 236)	Adjusted Fish Early-life Stage Toxicity test (FELS, OECD TG 210)
Exposure time	30-60 minutes	Zebrafish (ZF): 120/168 hpf Fathead minnow (FHM): 144 hpf	ZF: 32 dpf FHM: 21 dpf
Endpoints	Thyroperoxidase (TPO) and deiodinase (DIO) inhibition	Posterior swim bladder chamber inflation (KE)	Anterior swim bladder chamber inflation (KE)
Tested compounds	51 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

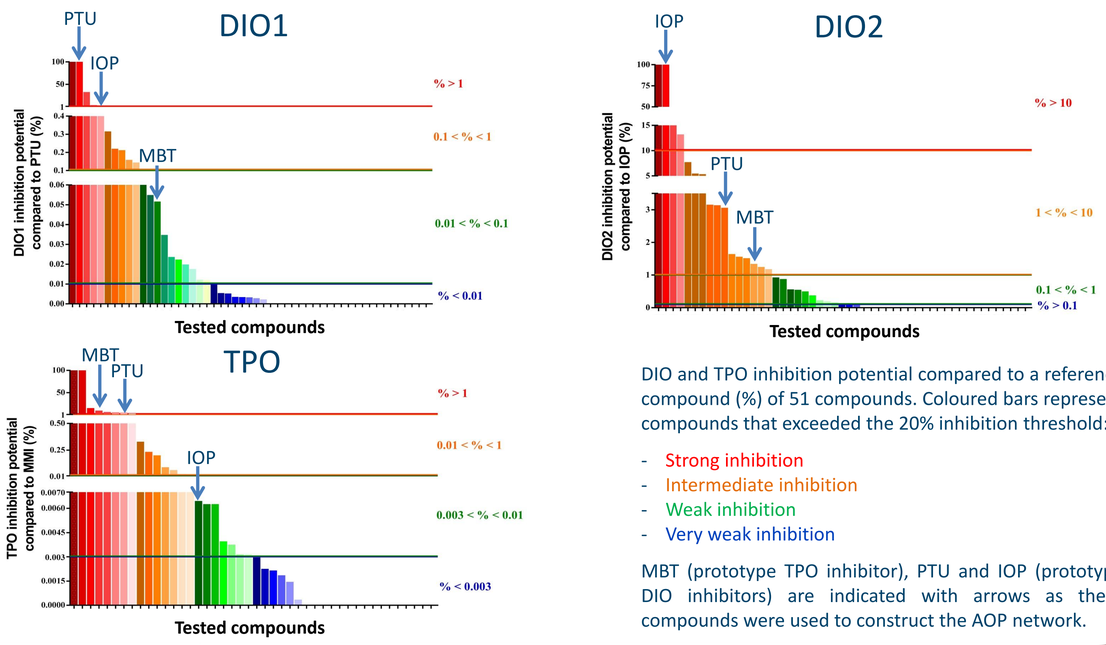
1.

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

2a.

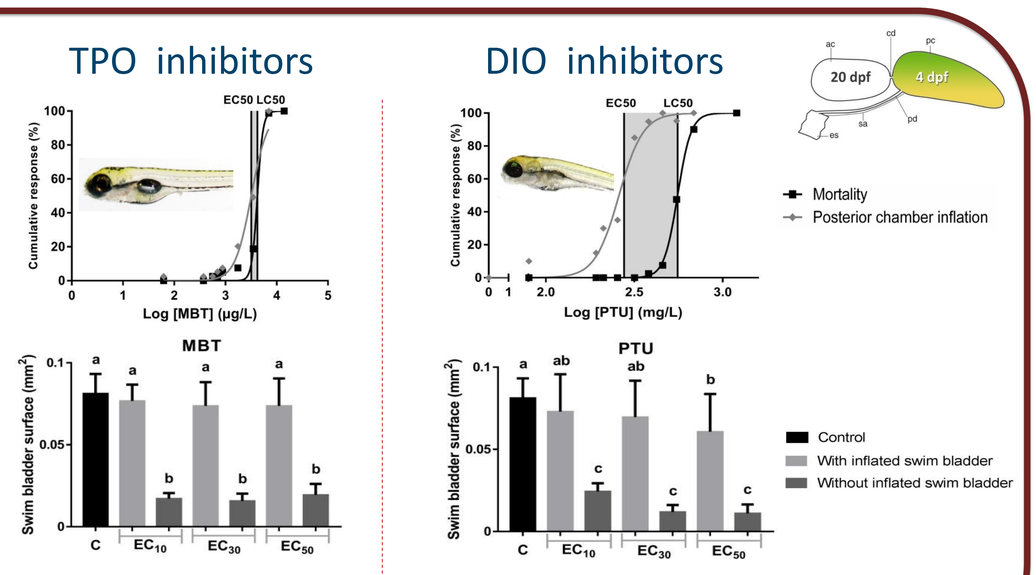


DIO and TPO inhibition potential compared to a reference compound (%) of 51 compounds. Coloured bars represent compounds that exceeded the 20% inhibition threshold:

- Strong inhibition
- Intermediate inhibition
- Weak inhibition
- Very weak inhibition

MBT (prototype TPO inhibitor), PTU and IOP (prototype DIO inhibitors) are indicated with arrows as these compounds were used to construct the AOP network.

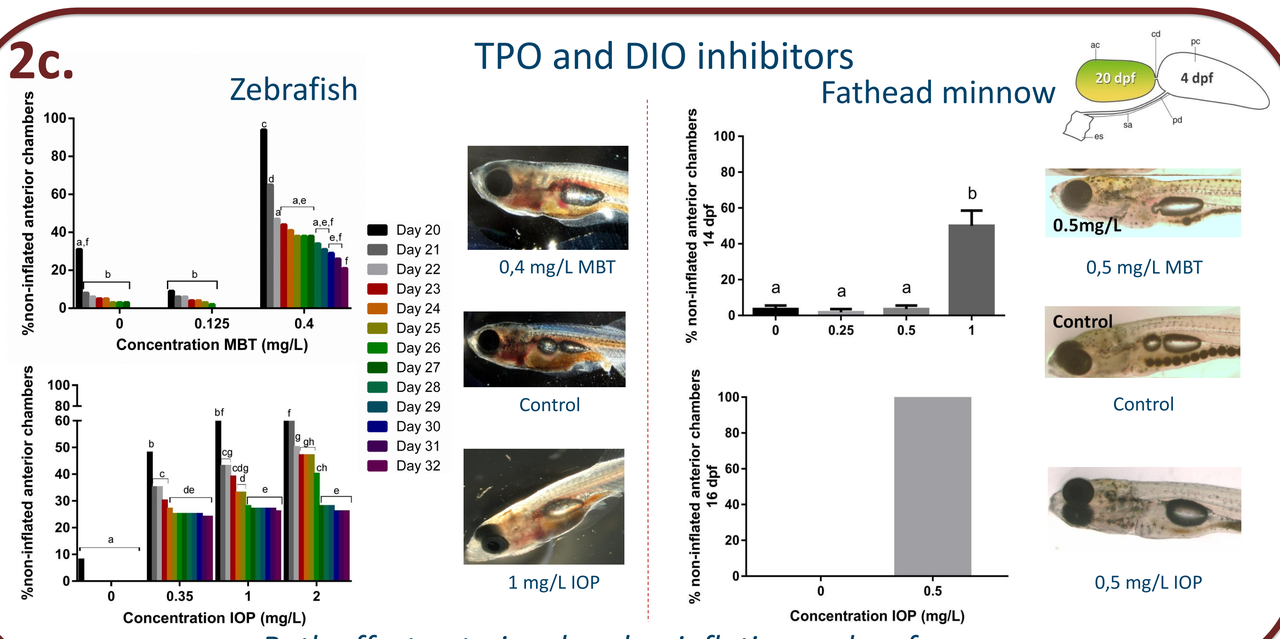
2b.



No impact on posterior inflation or surface area

Impact on posterior inflation and surface area

2c.

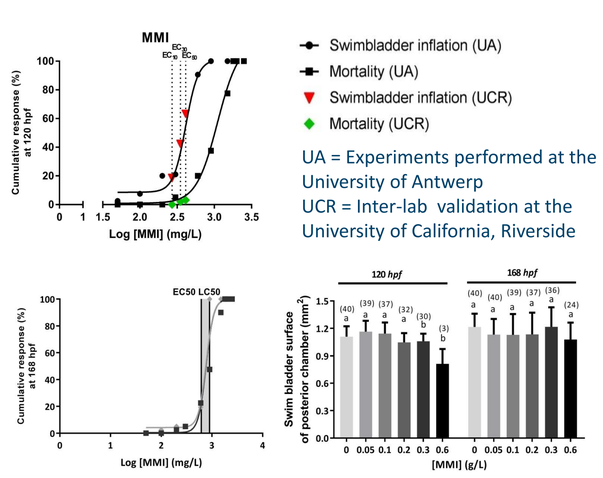


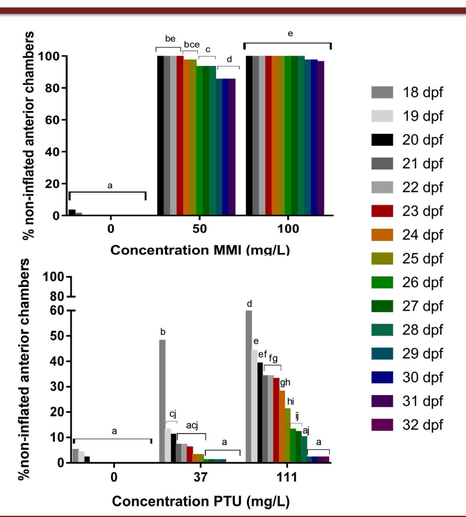
Both affect anterior chamber inflation and surface area

3.

Acute validation		Predicted effect (*in vitro*)	
		NO	YES
Observed effect (FET)	NO	*MBT* MMI PFBS NaPER	TCBPA
	YES	PFOS	*PTU* BPA *IOP* SA BP2 2,4,6-TBP PFOA 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)





Chronic validation		Predicted effect (*in vitro*)	
		NO	YES
Observed effect (FELS)	NO		
	YES		*MBT (Zebrafish, Fathead minnow)* *IOP (Zebrafish, 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)

Conclusion

Our results are in line with, and increase confidence in, our AOP network and we successfully used an AOP-based approach to select KEs, develop assays, and correctly predict acute and chronic toxicity.

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