Development of battery of in vitro bioassays for screening of thyroid hormone disruptive potential of chemicals

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Introduction and aims

Chemicals disrupting thyroid hormone (TH) system interfere with TH signaling in human, which can be linked with many human health problems such as neurodevelopmental disorders in children, thyroid neoplasms, thyroid autoimmune disorders and increased cardiovascular risk due to altered lipid metabolism in adults.

Aims: In the EU H2020 ERGO project, we are designing a battery of in vitro assays for evaluating chemicals disrupting thyroid hormone signaling. The project is involved in studies concerned with:

(i) the development and pre-validation of a battery of in vitro methods, which are based on adverse outcome pathway network concept (AOP, Fig.1) addressing priority molecular initiation events (MIEs) in the thyroid hormone regulation

(ii) characterization of the potential of selected compounds relevant to human exposure to disrupt the thyroid hormone system at different levels

(iii) linking in vitro mechanisms to adverse affects observed in vivo

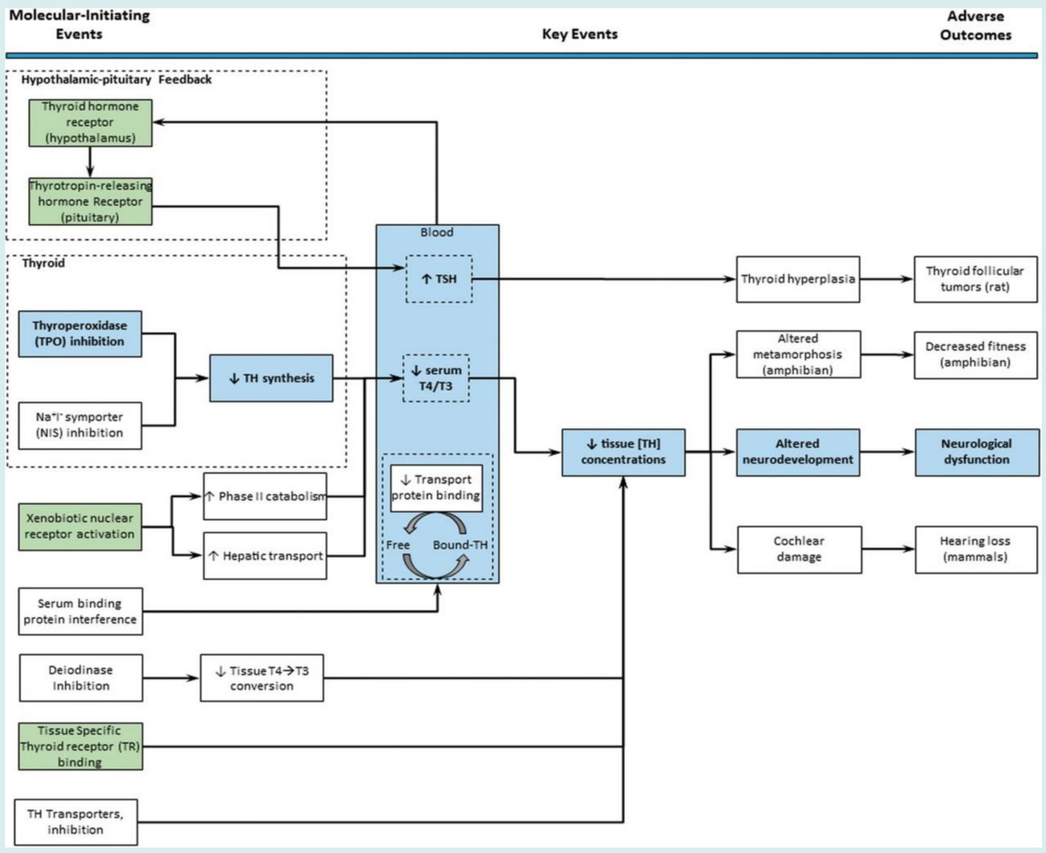

Figure 1 Combined adverse outcome pathway (AOP) network integrated from several putative adverse outcome pathways (AOPs) for thyroid hormone (TH) disruption. A Molecular Initiating Event (MIE) is the initial interaction between a compound and a biomolecule or biosystem that can be causally linked to an outcome via a pathway. MIEs proceed to key events, and then to adverse outcomes. (Noyes et al. 2019)

Methods

Several in vitro models are used in assays for the assessment of MIEs for thyroid hormone signaling disruption:

TH metabolization: AZ-AhR Luciferase reporter cell line is used for the assessment of interaction of samples with aryl hydrocarbon receptor (AhR) that is a ligand-activated transcriptional factor that regulates genes implicated in chemical metabolism.

TH synthesis: Enzyme inhibition assays for Thyroid peroxidase (TPO) is performed to evaluate the interaction of samples with the activity of TPO enzyme that is crucial for TH synthesis. Nthy-ori 3-1 (human) and FRTL-5 (rat) cell lines are used to build thyroid in vitro models.

TH signaling: PZ-TR Luciferase reporter cell line is used to assess interaction of chemicals with TH receptor.

TH transport: thyroxine-transthyretin (T4-TTR) binding assay is performed to examine the effects on TH transport.

Future

Identification and optimization of novel endpoints and biomarkers.

Developing high-throughput assessment assays of further prioritized MIEs in the in vitro models based on the results of gene and proteins expression.

Cross-species comparison for sensitivity of effects on prioritized molecular initiating events in the thyroid disruption.

Modelling/testing the effects of detected exposure mixtures.

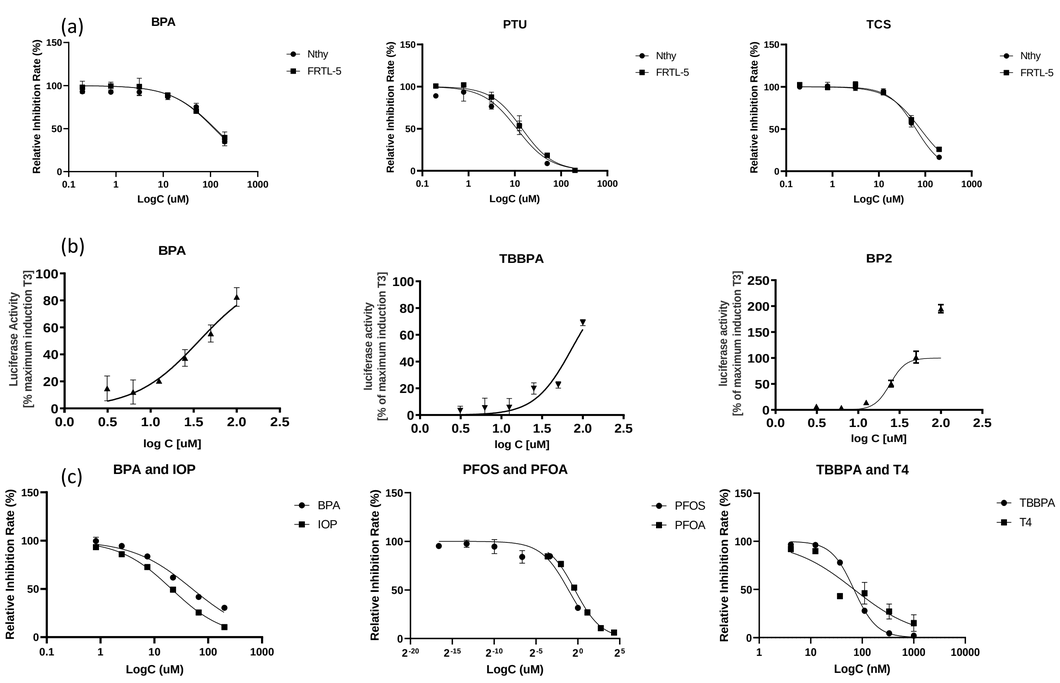

Figure 2 (a) TPO inhibition assay results in Nthy-ori (human) and FRTL-5 (rat) cell lines; (b) PZ-TR assay results; (c) TTR assay results.

Results

ERGO prioritized chemicals were tested in the PZ-TR assay, AZ-AhR assay, TTR assay and TPO assay. Inhibition could be found for several chemicals in TPO, and namely in TTR assay, while some compounds significantly activated TH receptor in PZ-TR assay. Moreover BPA and TCS showed effects in all assays except AZ-AhR assay. This indicates that some widespread environmental pollutants can disrupt TH signaling via multiple MIEs.

Table 1 EC50 values of prioritized chemicals in AZ-AhR assay, PZ-TR assay, TPO assay and T4-TTR assay. NA means no EC50 could be derived due no low or no effect.

Substance		AZ-AhR	PZ-TR	TTR	hTPO	rTPO
		EC50 (μM)	EC50 (μM)	IC50 (μM)	IC50 (μM)	IC50 (μM)
BPA	2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A)	n/a	38.7	57.6	120.4	142.9
PTU	6-propylthiouracil	n/a	n/a	n/a	11.7	13.2
DBP	Dibutylphthalate	n/a	203	241.1	n/a	n/a
PFOS	Perfluorooctane sulfonate	n/a	n/a	0.47	n/a	n/a
IOP	Iopanoic acid	n/a	125.8*	19.1	n/a	n/a
PFOA	Perfluorooctanoic acid	n/a	n/a	0.83	n/a	n/a
PCL	Perchlorate	n/a	n/a	n/a	n/a	n/a
TPP	Triphenyl phosphate	n/a	61.9	403.4	n/a	n/a
TDCPP	Tris(1,3-dichloro-2-propyl) phosphate	n/a	n/a	n/a	n/a	n/a
CBZ	Carbamazepine	n/a	n/a	n/a	n/a	n/a
T3	3,3',5-Triiodo-L-thyronine	n/a	1.1	0.195	n/a	n/a
T4	3,3',5,5''-Tetraiodo-L-thyronine	n/a	0.077	0.077	n/a	n/a
DON	Deoxynivalenol	n/a	n/a	n/a	n/a	n/a
TCS	Triclosan	n/a	15.1	2.71	62.5	77.5
BP2	2,2'-4,4'-tetrahydroxy benzophenone	n/a	21.5	0.24	38.5	29.6
AMP	Ampicilin	n/a	n/a	n/a	n/a	n/a
RSC	Resorcinol	n/a	N/T	n/a	13.84	15.73
SA	Salicylic acid	n/a	N/T	n/a	n/a	n/a
MMI	Methimazole	n/a	N/T	n/a	3.906	3.689
ETU	Ethylene thiourea	n/a	N/T	n/a	n/a	n/a
SMX	Sulfamethoxazol	n/a	N/T	n/a	n/a	n/a

Ref: Noyes, P. D. et al. (2019). Evaluating chemicals for thyroid disruption: Opportunities and challenges with in vitro testing and adverse outcome pathway approaches. Environmental health perspectives, 127(9), 095001.

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