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**Identifying thyroid hormone disruptors by establishing qAOPs
integrating cross-species extrapolations and thresholds**

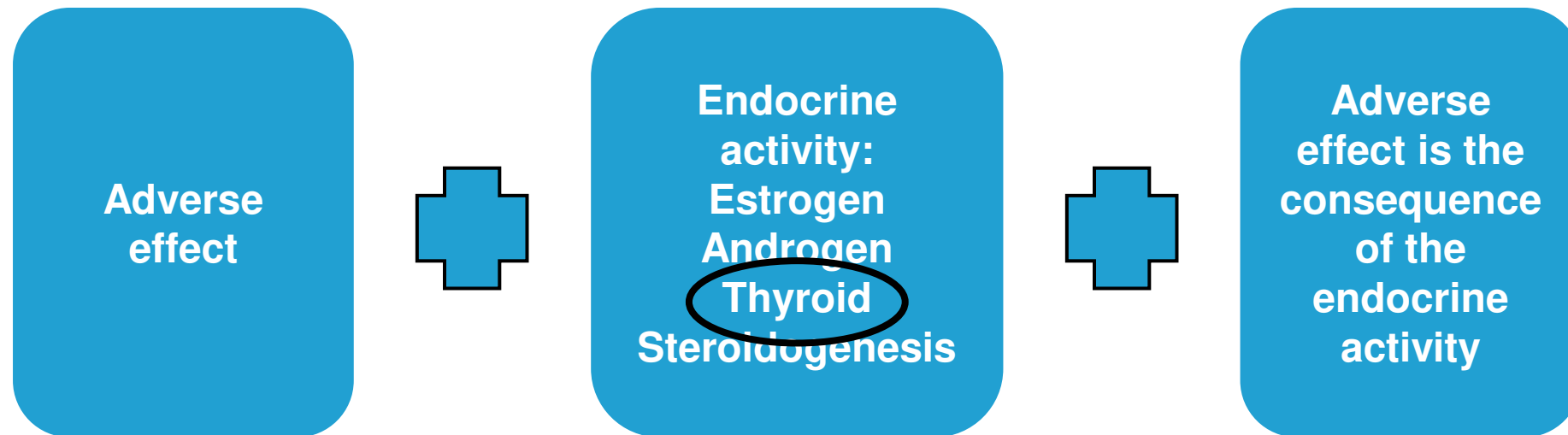
ECETOC Workshop on Quantitative Response-Response
Relationships (qAOPs) – 18–19 October 2022

Stephanie Melching-Kollmuss, BASF SE

Thyroid (hormone) Disruptor – regulatory background in EU

- Endocrine Disruption Criteria are established since 2017/2018: Pesticides and Biocides: **Yes** or No
- Endocrine Disruption Criteria will be implemented under EU CLP in Q1 2023: **ED Cat 1** & ED Cat 2

Hazard-based
regulation



Thyroid toxicity assessment according to Criteria and Guidance

COMMISSION REGULATION (EU) 2018/605

of 19 April 2018



...
 28-day study
 90-day study
 Prenatal toxicity study
 2-Generation study
 Extended-1-
 Generation study
 Chronic/cancer study
 ...

Parameters
 assessed in the
 tox studies

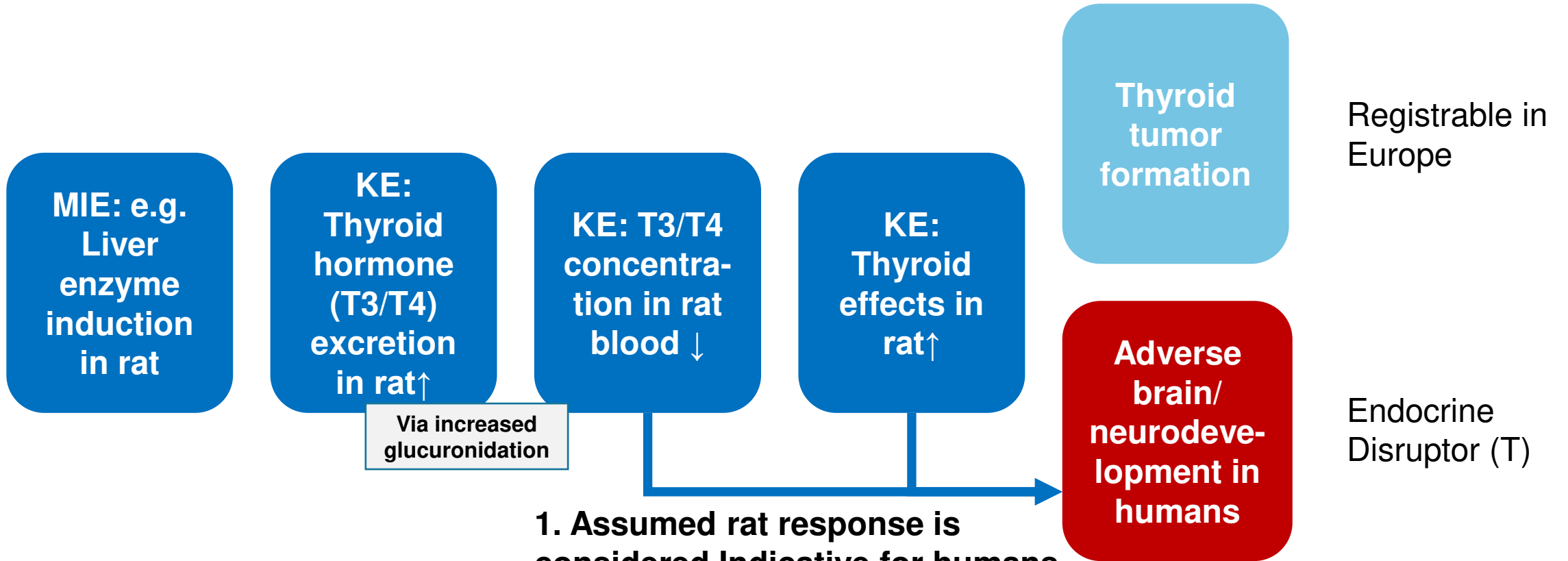
Endocrine disruption criteria		Effects in rats
1.	Adverse effect	Thyroid weight/histopathological changes
2.	Thyroid activity	Thyroid hormone changes (T4, TSH)
3.	Plausible link between 1. & 2.	Established

Considered indicative for adverse neurodevelopment

If not occurring at systemically toxic doses

On these grounds EFSA has identified thyroid (hormone) disruptors

Current default for thyroid endocrine disruption assessment:



1. Assumed rat response is considered Indicative for humans
2. No threshold assumed
3. Potentially relevant key events / key event relationships are missing

Guiding questions for the ECETOC Thyroxine (T4) Task Force - formed in 2018



- How (qualitatively and quantitatively) correlate thyroid hormone levels with neurodevelopmental effects (in humans / in rats)?
- Which neurodevelopmental effects in rodents should be considered indicative for human neurodevelopment?
- Is there a threshold for thyroid hormone changes (in mothers / in offspring), below which no neurodevelopmental change is to be expected?
- How should rodent toxicants be investigated to exclude a concern for human neurodevelopment?

Selection of thyroid-related AOPs with brain-related adverse outcomes (from Marty et al., 2021)

Table 1: Overview of thyroid-related AOPs including neurodevelopmental outcomes in mammals in the AOP Wiki (as per 16 October 2020)

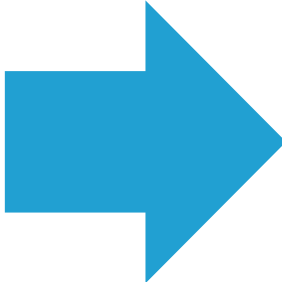
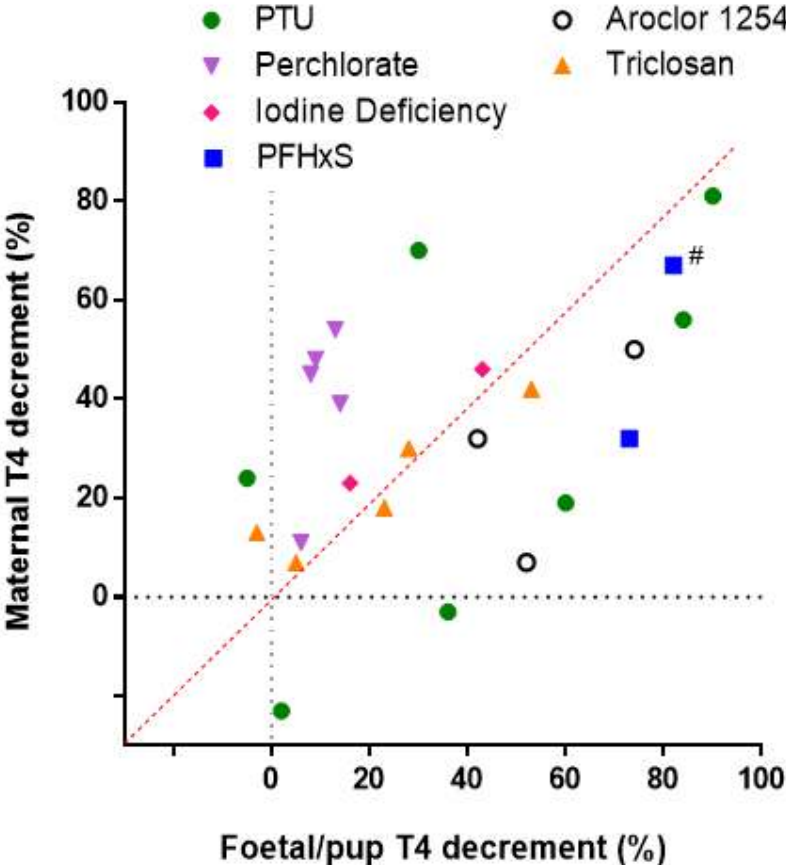
AOP Wiki	AOP 8	AOP 42	AOP 54	AOP 134	AOP 152
AOP title	Activation of hepatic nuclear receptors, & subsequent neurodev. AOs in mammals	Inhibition of TPO & subsequent neurodev. AOs in mammals	Inhibition of NIS leads to learning and memory impairment	NIS inhibition & subsequent neurodev. AOs in mammals	Interference with TTR & subsequent human neurodev. toxicity
1st Author	Katie Paul Friedman	Crofton et al. (2019)	Rolaki et al. (2019)	Mary Gilbert	Erik Janus
Status	Under development; in OECD workplan	Endorsed; in OECD Workplan	Endorsed; in OECD Workplan	Under development	Open for adoption; under development; in OECD workplan
MIE	PXR activation	TPO inhibition	NIS inhibition	NIS inhibition	Binding, TTR in serum
KE1	Upregulation of UGT activity; induction	Thyroid hormone synthesis, decreased	Thyroidal iodide, decrease	Thyroidal iodide, decrease	Displacement, serum T4 from TTR
KE2	Biliary excretion of thyroid hormone glucuronide; increase	T4 in serum; decrease	Thyroid hormone synthesis, decrease	Thyroid hormone synthesis, decrease	Serum fT4, increase
KE3	T4 in serum; decrease	T4 in neuronal tissue; decrease	T4 in serum; decrease	T4 in serum; decrease	Uptake of T4 into tissue, increase
KE4	T4 in neuronal tissue; decrease	Hippocampal gene expression, altered	T4 in neuronal tissue; decrease	T4 in neuronal tissue; decrease	Clearance of T4 from tissue, increase
KE5	Hippocampal gene expression, altered	Hippocampal anatomy, altered	Brain-derived neurotrophic factor, reduced	Hippocampal gene expression, altered	T4 in serum; decrease
KE6	Hippocampal anatomy, altered	Hippocampal, physiology decreased	GABAergic interneurons, decreased	Hippocampal anatomy, altered	T4 in neuronal tissue; decrease
KE7	Hippocampal physiology, decreased		Synaptogenesis, decreased	Hippocampal physiology, altered	Hippocampal gene expression, altered
KE8			Neuronal network function, decreased		Hippocampal anatomy, altered
KE9					Hippocampal physiology decreased
AO	Cochlear function, loss	Cochlear function, decreased / loss // Cognitive function, decreased [a]	Impairment, learning and memory	Cochlear function, decreased // Cognitive function, decreased [a]	Cochlear function, decreased // Cognitive function, decreased [a]

Also:
AOP 300, 402
<http://aopwiki.org>

Relevant results from literature evaluations (variety of MIEs assessed)



Figure 1: Relationship between maternal serum T4 decrements measured on GD 20 – GD 21 and foetal / pup serum T4 decrements measured on GD 20 – PND 0



Offspring serum thyroid hormone levels are more decisive compared to maternal THs

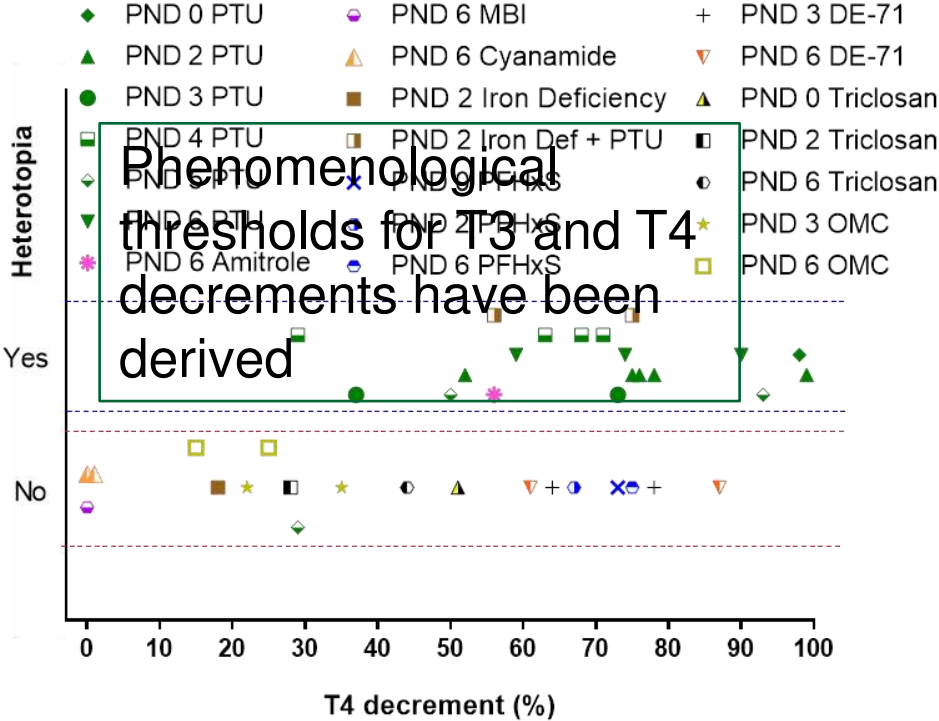
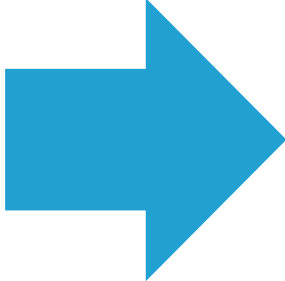
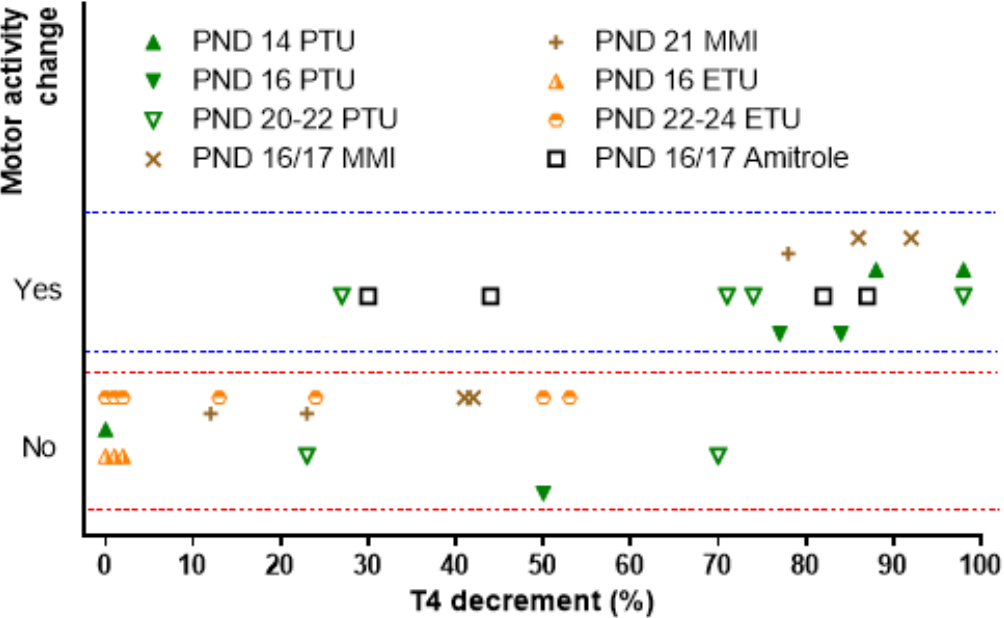
Relevant results from literature evaluations (variety of MIEs assessed)



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Associations between T4 decrement in offspring and motor activity / heterotopia

Case study 1



T4 levels measured in offspring between PND 14 and 21

Quantitative modelling of T4 concentrations in blood of adults in rats vs humans

Quality Scientific Solutions, LLC. 

Main species differences (rat vs humans): nature and binding capacity of TH binding proteins → T4 half life

Figure 2: Structure of the Thyroid Hormone Model (THM)

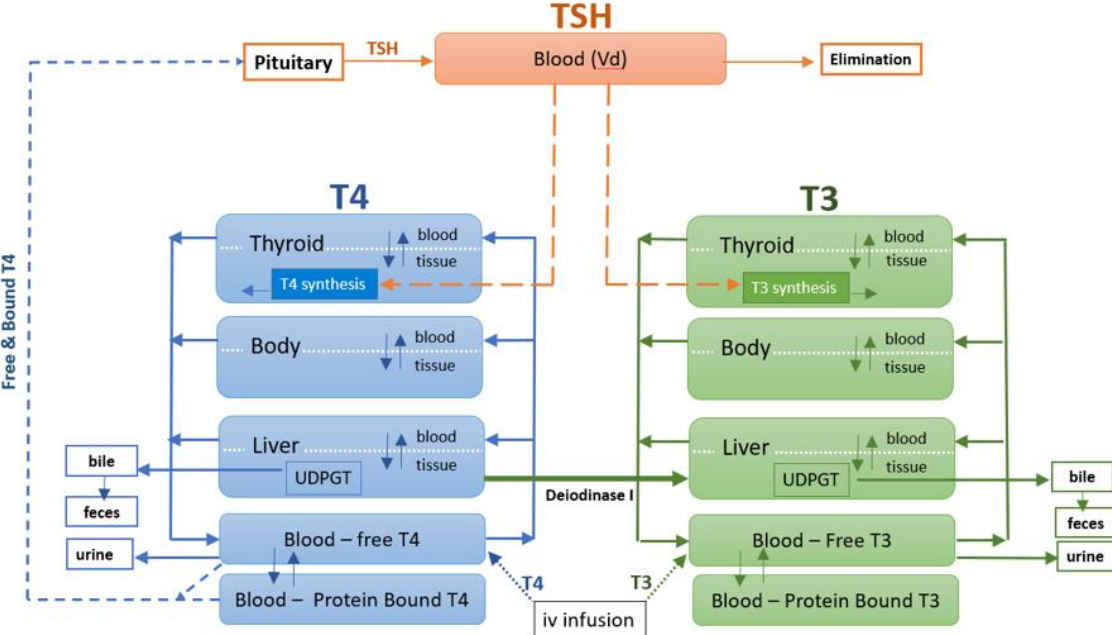
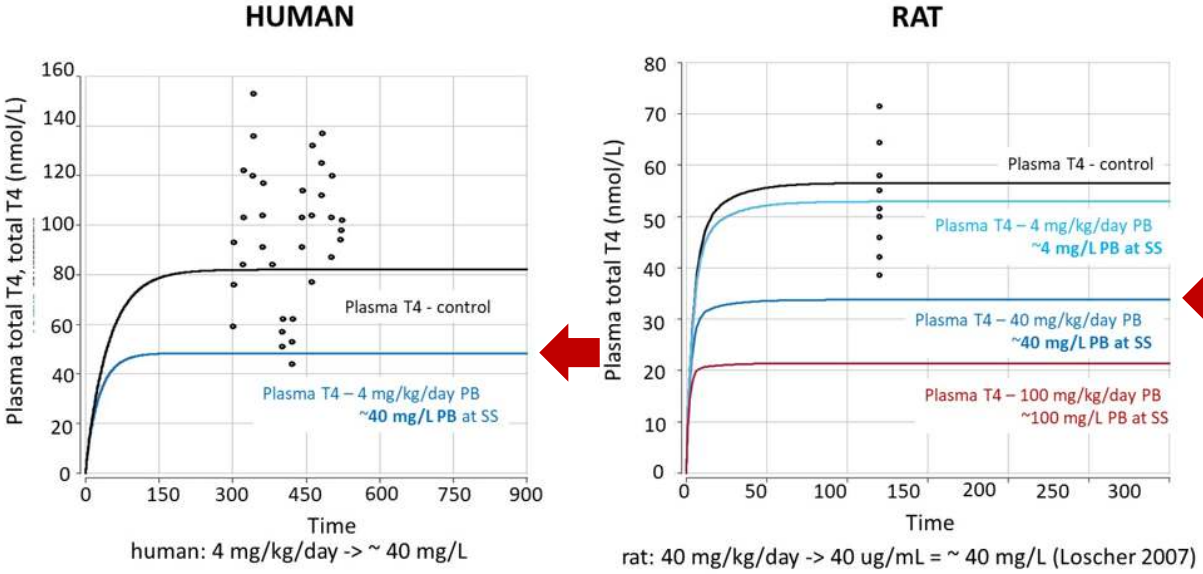


Figure 29: Plasma concentration of T4 in humans and rats after repeated daily treatment with phenobarbital after adjusting for the 10 fold greater clearance of PB from the blood in rats compared to humans.



PB - Phenobarbital
SS – steady state

Clewell et al., 2022
(SOT Poster)

Quantitative modelling of T4 concentrations in blood of adults in rats vs humans

Table 11: THM-predicted effect of CAR/PXR activators on plasma T4 levels in rat.

Chemical	BMD ¹ (mg/kg/day)	Relative Potency Factor ²	PB Equivalent BMD	THM-Predicted ³	Observed ⁴
			(mg/kg/day)	(% of Control)	
Control	0	--	0	100%	100%
Phenobarbital	5.23	1.0	5.23	92.2%	94.6%
3MC	1.24	4.22	22.03	67.6% *	88.9%
PCN	0.84	6.24	32.63	56.8% *	84.8%
PCB	0.17	30.42	158.96	22.8% *	44.4%
Fluxapyroxad	178.02	0.03	0.15	99.6%	95.8%

*Total T4 levels are expected to be statistically significantly different from control group (Mean = 61.98; SD = 15.85) for euthyroid male rats (Table 6)

¹BMDs were calculated for PB, 3MC, PCN, and PCB based on the dose-response data for T4-UDPGT from Liu et al. (1995) and for fluxapyroxad using data from Buesen et al. (2019).

²Relative Potency Factors (RPF) were calculated as the ratio of the mg/kg/day BMD for the putative CAR/PXR activator to the mg/kg/day BMD for phenobarbital.

³THM-predicted effects on total plasma T4 of PB and the other putative CAR/PXR receptor activators were determined by entering the PB equivalent dose (Column 4) into the model and converting the result to a percentage of the control T4 levels.

⁴THM-predicted effects of PB, 3MC, PCN, PCB, and fluxapyroxad on total T4 in plasma were compared to the observed effect on total T4 in plasma for PB, 3MC, PCN, and PCB based on the study by Liu et al. (1995) and fluxapyroxad in the study by Buesen et al. (2019).

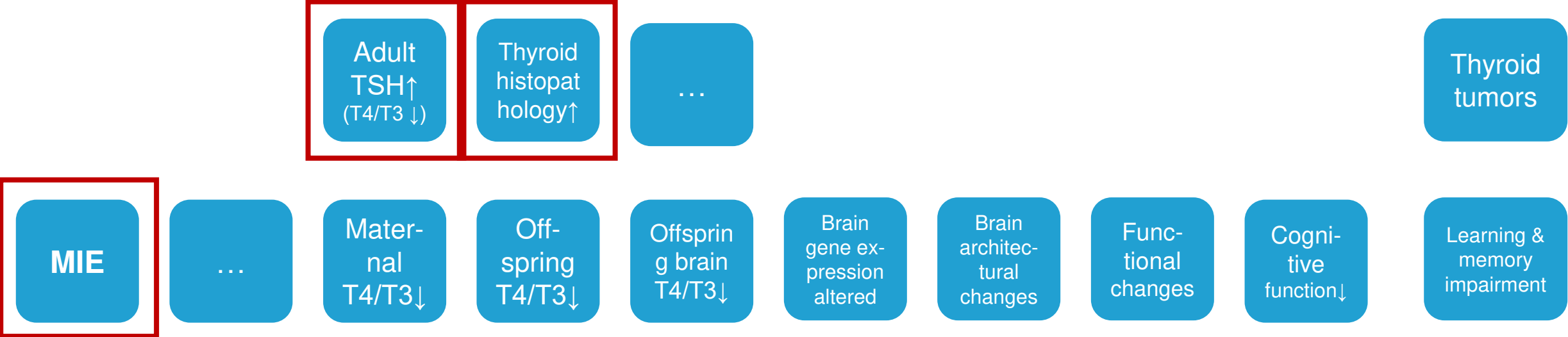
Future plan – modelling T3, T4 in offspring blood / offspring brain in humans vs rats



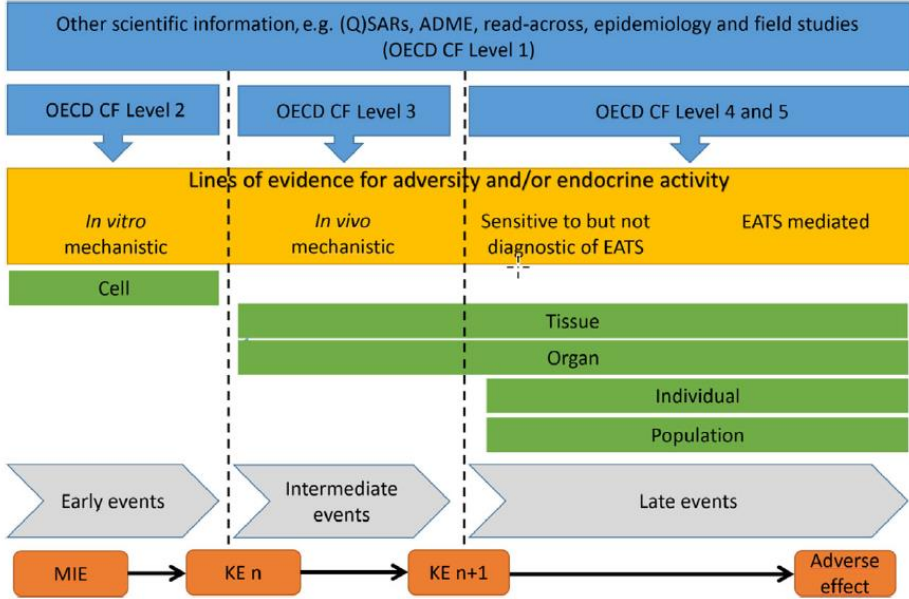
Set up of a Thyroid Hormones (TH) QST Platform

- Include thyroid-stimulating, T4 and T3 synthesis, distribution, clearance, and regulatory feedback in rats and humans
- Include mechanisms for dynamic changes and species specificity in thyroid hormone protein binding
- Simulate and predict TH concentrations in the **fetal (and pup) blood and brain** after in utero / lactational exposure to the chemical in rats and humans
- Take into account realistic physiological compartments for the thyroid hormone network
- Be structurally comparable for healthy humans and rat for cross-species evaluations

Relevant elements of a thyroid-hormone related AOP



e.g.
 TPO / NIS inhibition
 Liver nuclear receptor activation
 Interaction with TH binding hormone in serum



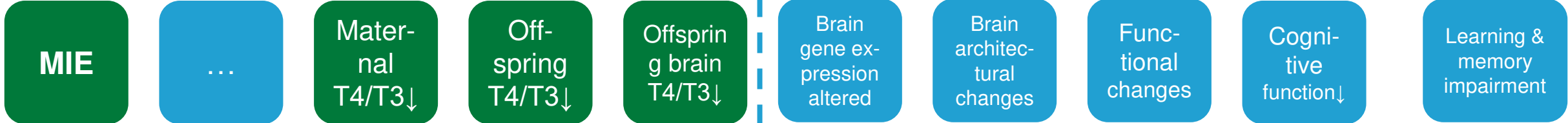
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AOPs for rats vs humans

T4: 20 % decrement associated with heterotopia for **PTU** (Hassan et al., 2017)

Rat



T4: 50% / 60% decrement associated with sign. neurodevelopmental findings (**variety of MIEs** assessed)

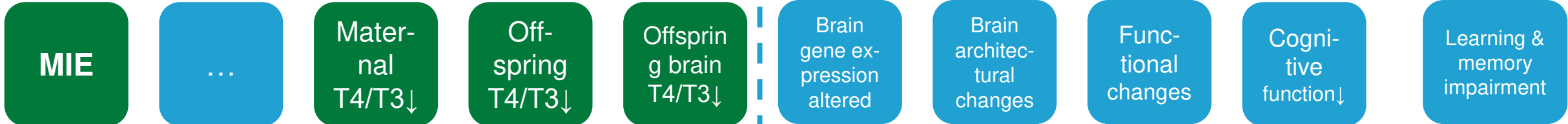
Phase II Liver enzyme induction: Rat vs human hepatocyte responses

PBK models: Rat vs human responses after exposure to substances

Likely relevant quantitative association

- TH levels
- TR occupancies
- Dio 3 activity

Human



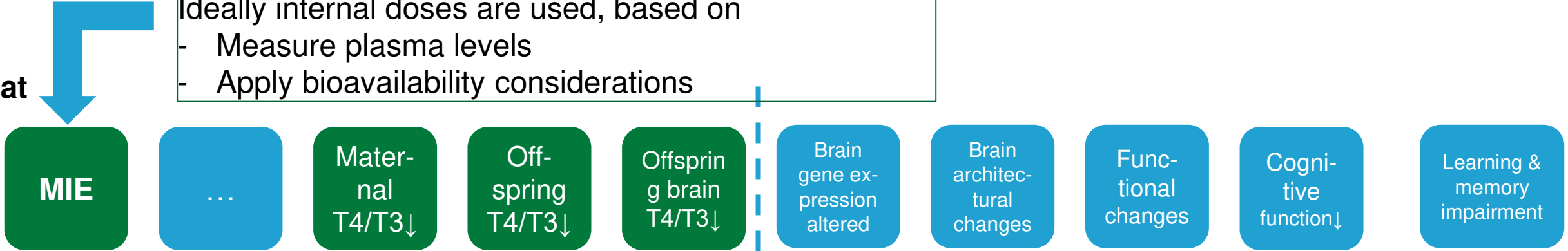
TH – Thyroid hormone
 TR – Thyroid hormone receptor
 Dio 3 – Deiodinase Type 3

AOPs for rats vs humans

Consider toxicokinetics of the test substances applied:
 Ideally internal doses are used, based on

- Measure plasma levels
- Apply bioavailability considerations

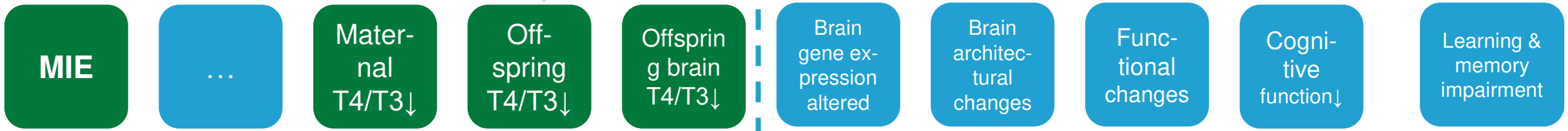
Rat



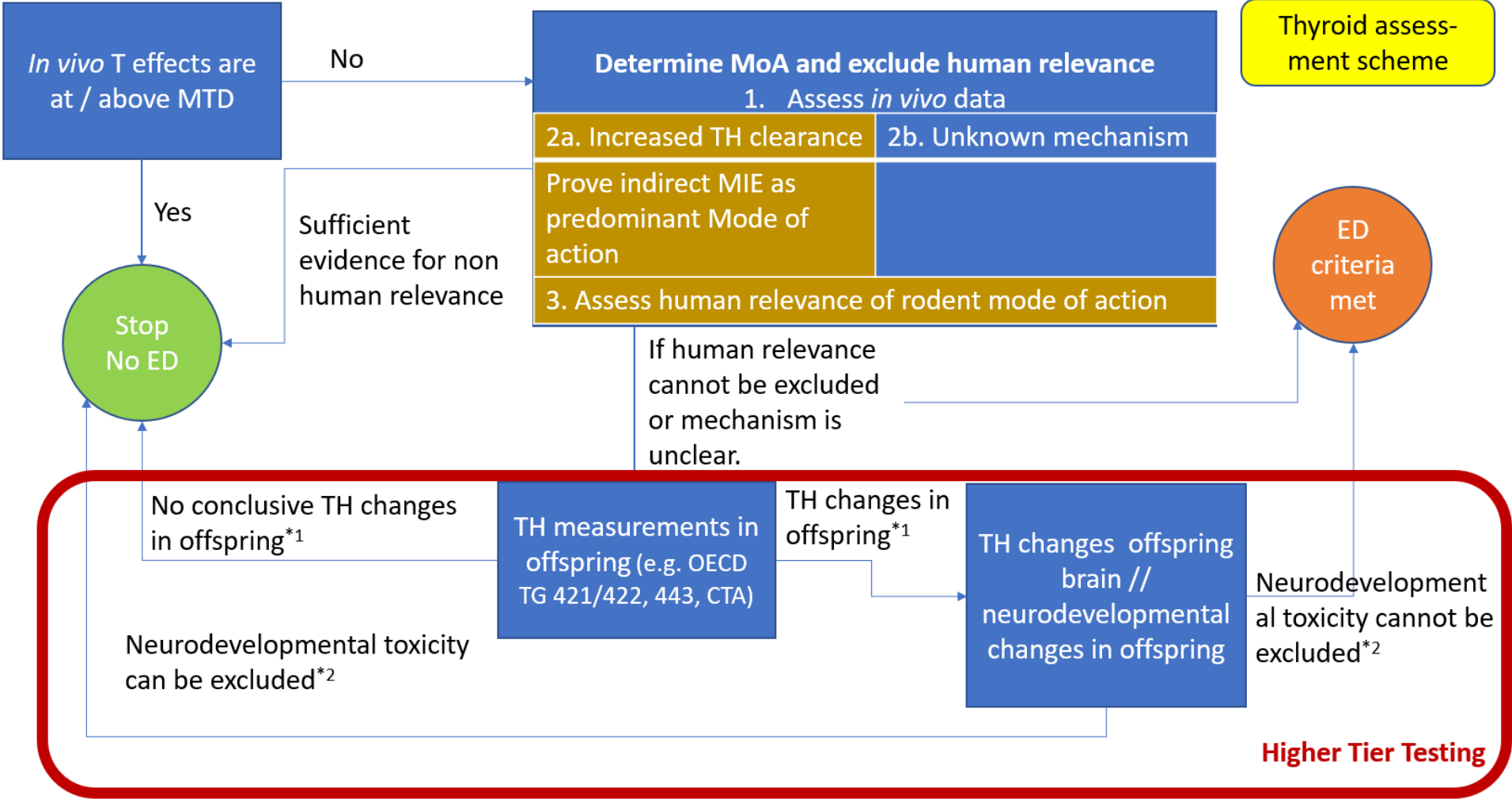
Phase II Liver enzyme induction:
 Rat vs human hepatocyte responses

PBK models:
 Rat vs human responses after exposure to substances

Human



Application of qAOP for Thyroid assessment / testing



*¹Consider thresholds

*²Consider relevance / sensitivity of parameters indicating neurodevelopmental toxicity

Acknowledgements

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ECETOC Team

- To only name a few:
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- Ben van Ravenzwaay
- ...





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