Identifying thyroid hormone disruptors by establishing qAOPs

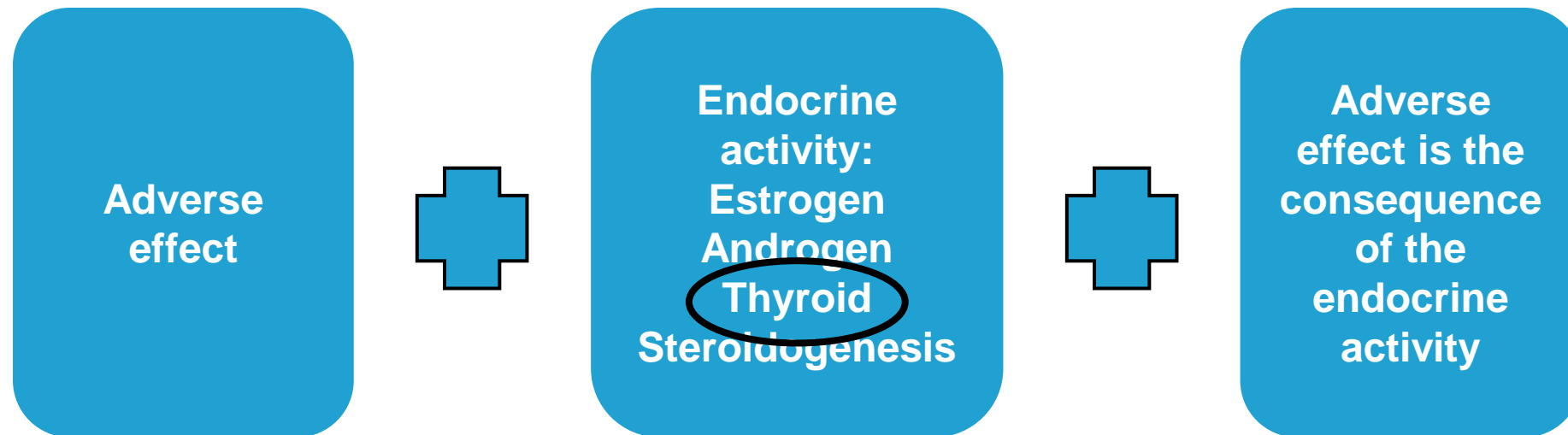**integrating cross-species extrapolations and thresholds**

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

Adverse effect

➕

**Endocrine activity:
Estrogen
Androgen
Thyroid
Steroidogenesis**

➕

Adverse effect is the consequence of the endocrine activity

Thyroid toxicity assessment according to Criteria and Guidance

COMMISSION REGULATION (EU) 2018/605
of 19 April 2018

ECHA EUROPEAN CHEMICALS AGENCY

efsa European Food Safety Authority

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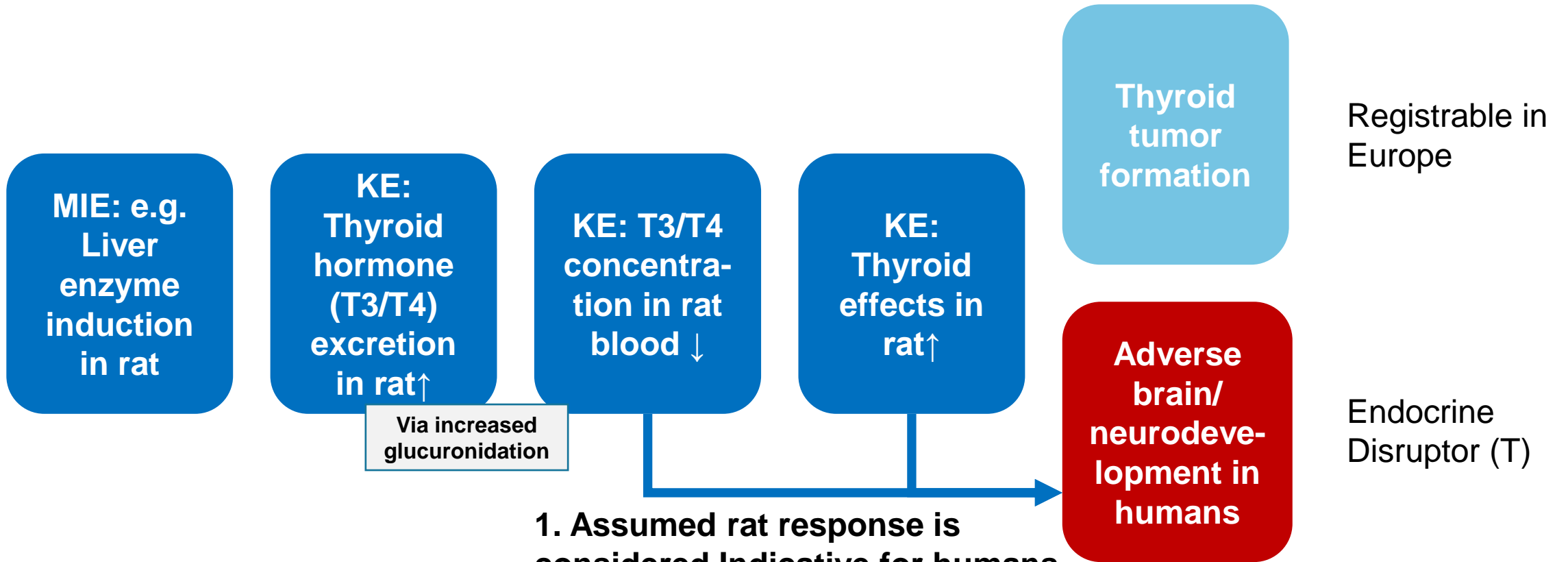Chronic/cancer study
…

Parameters assessed in the tox studies

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

Considered indicative for adverse neurodevelop-ment

If not occurring at systemically toxic doses

On these grounds EFSA has identified thyroid (hormone) disruptors

Current default for thyroid endocrine disruption assessment:

MIE: e.g. Liver enzyme induction in rat

KE: Thyroid hormone (T3/T4) excretion in rat↑

Via increased glucuronidation

KE: T3/T4 concentra-tion in rat blood ↓

KE: Thyroid effects in rat↑

Thyroid tumor formation

Registrable in Europe

Adverse brain/ neurodeve-lopment in humans

Endocrine Disruptor (T)

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
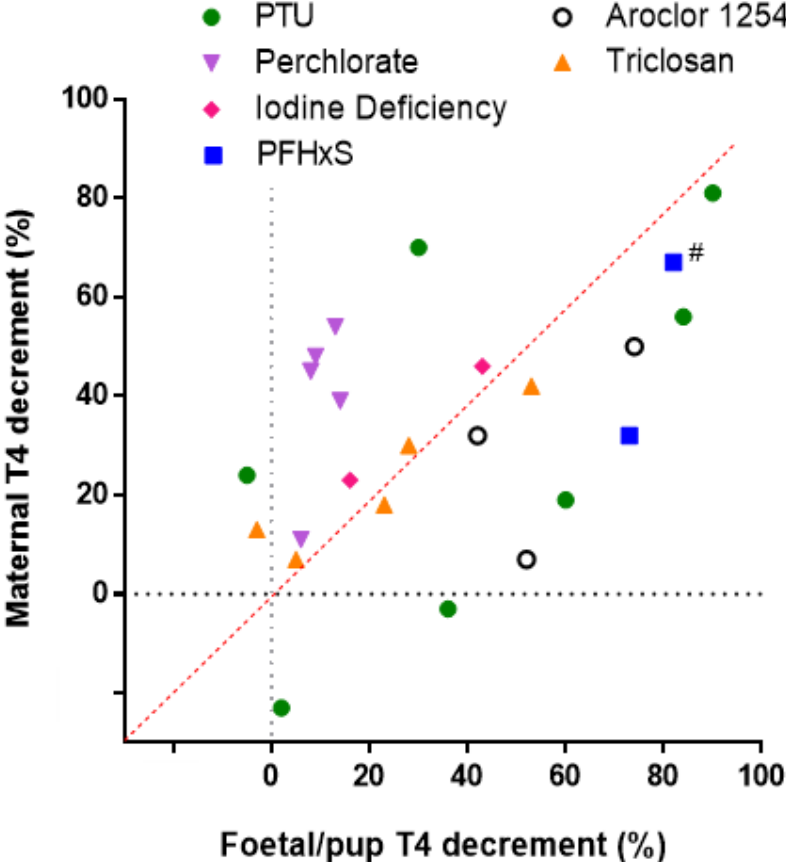
Offspring serum thyroid hormone levels are more decisive compared to maternal THs
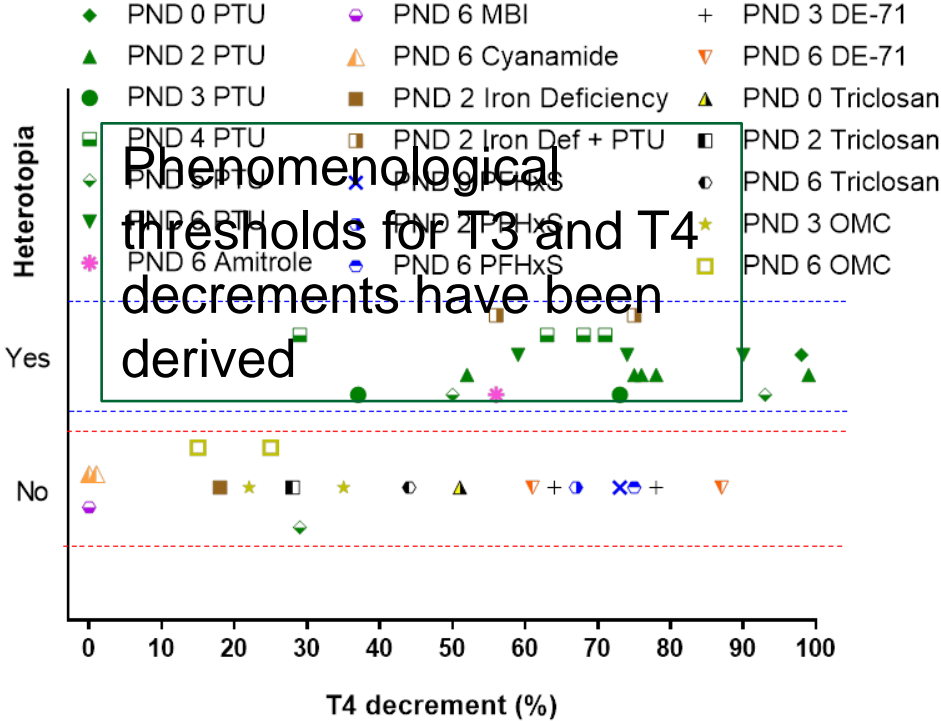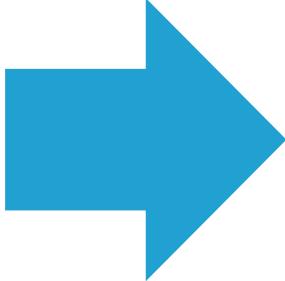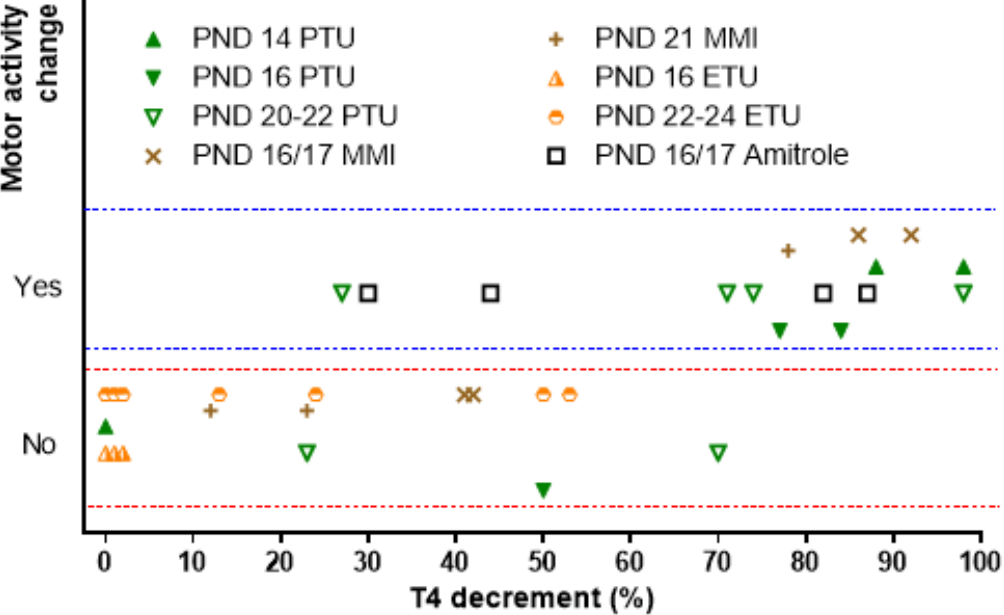
Relevant results from literature evaluations (variety of MIEs assessed)

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
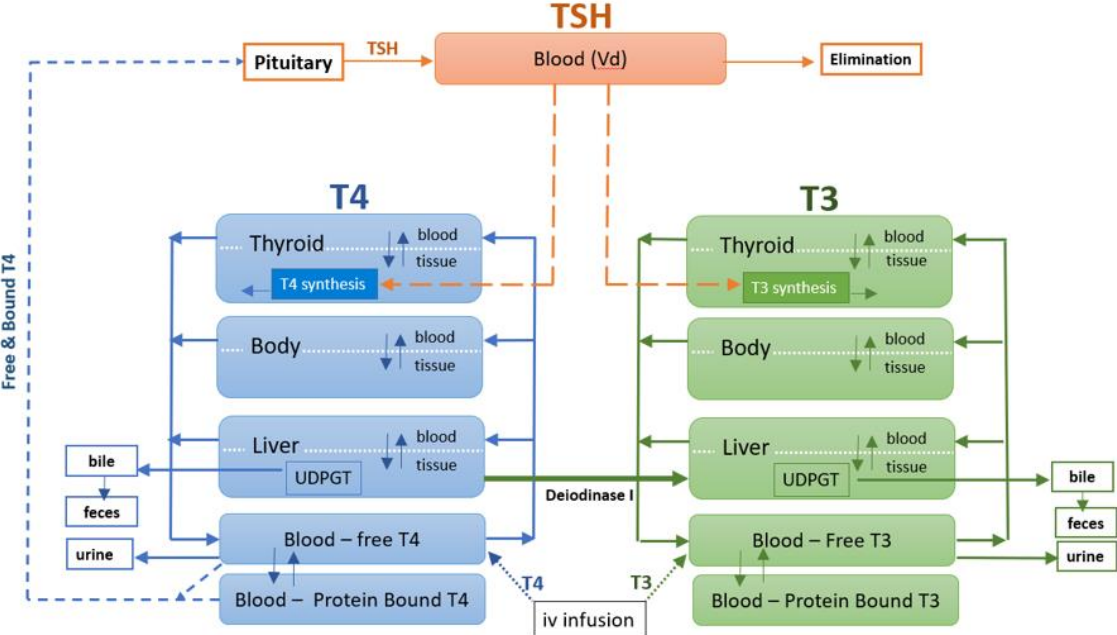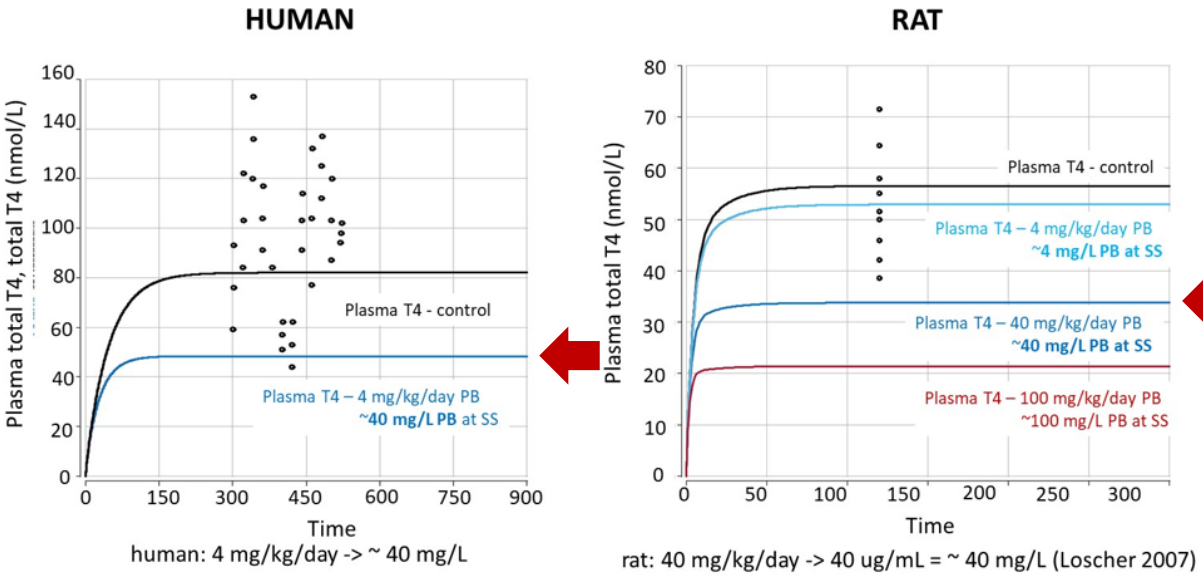

Figure 2: Structure of the Thyroid Hormone Model (THM)

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

Quality Scientific Solutions, LLC.

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 (mg/kg/day)	THM-Predicted[3] (% of Control)	Observed[4] (% 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)

[1]BMDs 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).

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

[3]THM-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.

[4]THM-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

| Adult TSH↑ (T4/T3 ↓) | Thyroid histopathology↑ | … | | | | | | | Thyroid tumors |

| MIE | … | Maternal T4/T3↓ | Off-spring T4/T3↓ | Offspring brain T4/T3↓ | Brain gene expression altered | Brain architectural changes | Functional changes | Cognitive function↓ | Learning & memory impairment |
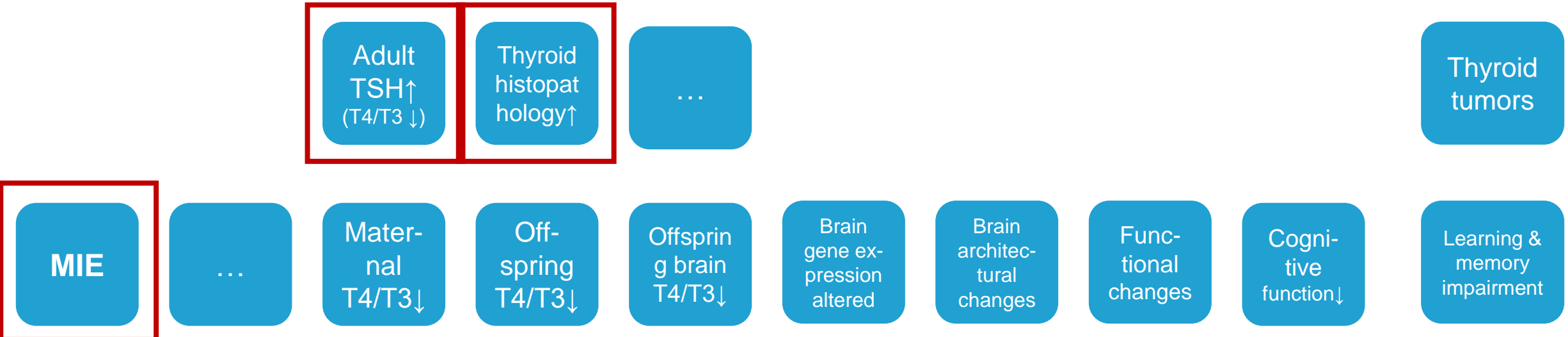
e.g.
TPO / NIS inhibition
Liver nuclear receptor activation
Interaction with TH binding hormone in serum

Other scientific information, e.g. (Q)SARs, ADME, read-across, epidemiology and field studies (OECD CF Level 1)

| OECD CF Level 2 | OECD CF Level 3 | OECD CF Level 4 and 5 |

Lines of evidence for adversity and/or endocrine activity

| In vitro mechanistic | In vivo mechanistic | Sensitive to but not diagnostic of EATS | EATS mediated |

Cell

Tissue
Organ
Individual
Population

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of 19 April 2018

Early events → Intermediate events → Late events

MIE → KE n → KE n+1 → Adverse effect
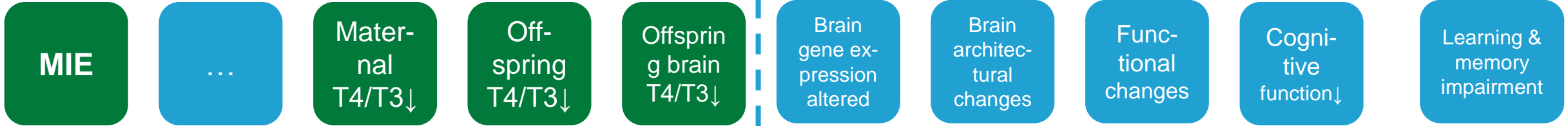
AOPs for rats vs humans

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

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

Rat

| MIE | … | Mater-nal T4/T3↓ | Off-spring T4/T3↓ | Offspring brain T4/T3↓ | Brain gene expression altered | Brain architec-tural changes | Func-tional changes | Cogni-tive function↓ | Learning & memory impairment |

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

| MIE | … | Mater-nal T4/T3↓ | Off-spring T4/T3↓ | Offspring brain T4/T3↓ | Brain gene expression altered | Brain architec-tural changes | Func-tional changes | Cogni-tive function↓ | Learning & memory impairment |

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

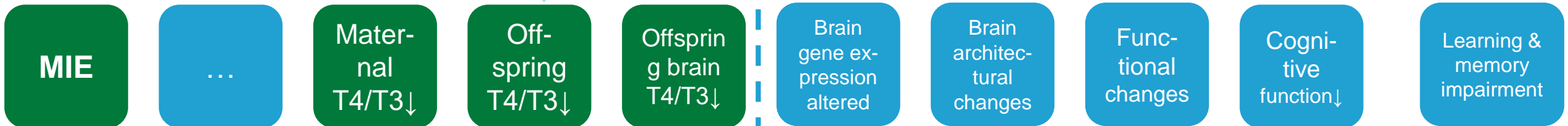| MIE | … | Mater-nal T4/T3↓ | Off-spring T4/T3↓ | Offspring brain T4/T3↓ | Brain gene expression altered | Brain architectural changes | Functional changes | Cognitive function↓ | Learning & memory impairment |

Phase II Liver enzyme induction:
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Human

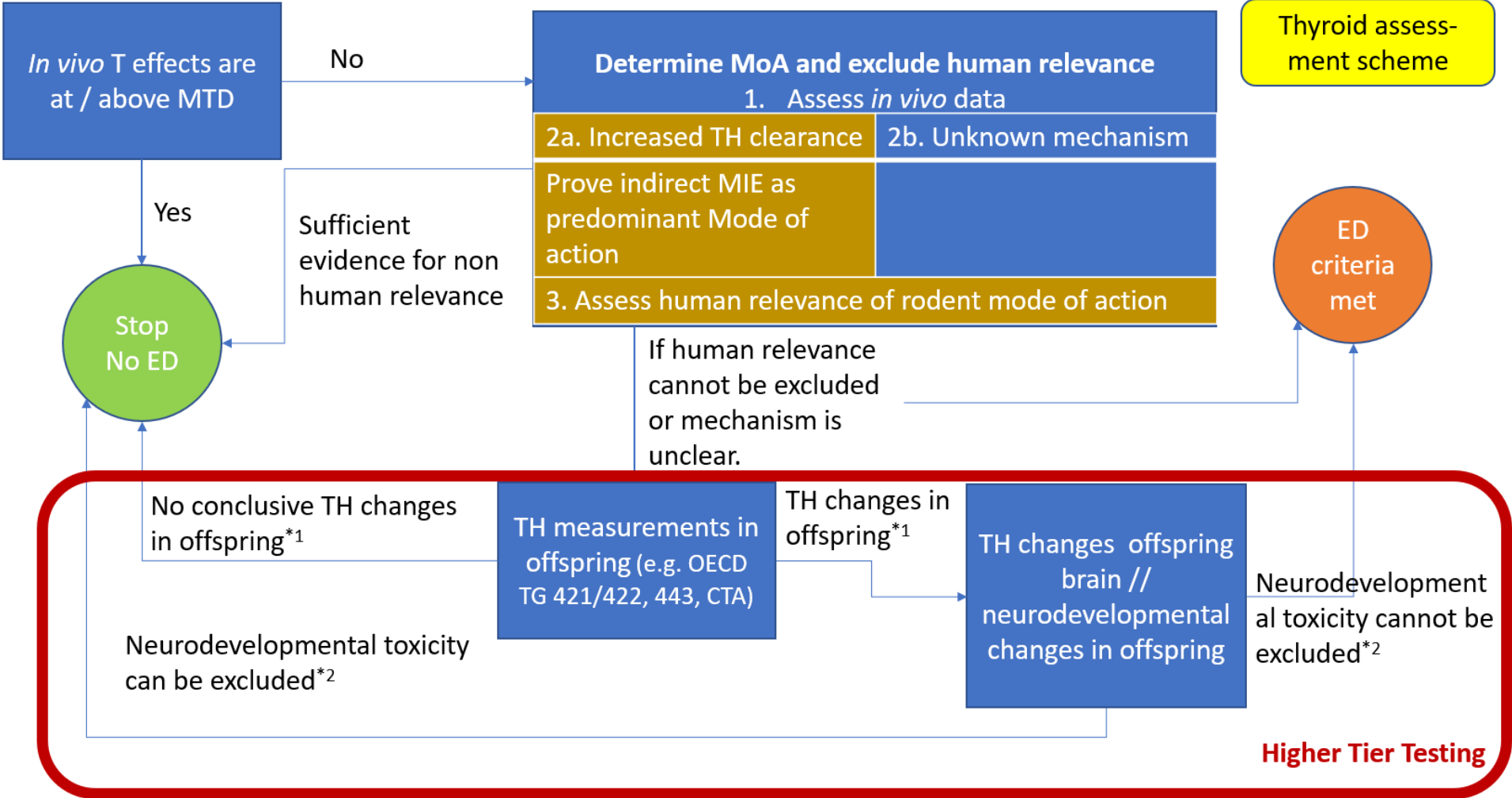| MIE | … | Mater-nal T4/T3↓ | Off-spring T4/T3↓ | Offspring brain T4/T3↓ | Brain gene expression altered | Brain architectural changes | Functional changes | Cognitive function↓ | Learning & memory impairment |

Application of qAOP for Thyroid assessment / testing

In vivo T effects are at / above MTD

No → Determine MoA and exclude human relevance
1. Assess in vivo data

2a. Increased TH clearance | 2b. Unknown mechanism

Prove indirect MIE as predominant Mode of action

3. Assess human relevance of rodent mode of action

Thyroid assessment scheme

Yes

Sufficient evidence for non human relevance

Stop No ED

ED criteria met

If human relevance cannot be excluded or mechanism is unclear.

No conclusive TH changes in offspring[*1]

TH measurements in offspring (e.g. OECD TG 421/422, 443, CTA)

TH changes in offspring[*1]

TH changes offspring brain // neurodevelopmental changes in offspring

Neurodevelopmental toxicity cannot be excluded[*2]

Neurodevelopmental toxicity can be excluded[*2]

Higher Tier Testing

[*1]Consider thresholds
[*2]Consider relevance / sensitivity of parameters indicating neurodevelopmental toxicity

Melching-Kollmuss et al., 2022 (Poster / Symposium at ICT)

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