Towards a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny

27th November 2023

ECETOC Webinar

Dr. Stephanie Melching-Kollmuss, BASF SE, on behalf of the ECETOC Thyroxine (T4) Task Force

Endocrine Disruption Criteria

"An endocrine disruptor is an exogenous substance or mixture that alters the function (s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." (WHO/IPCS, 2002)

COMMISSION REGULATION (EU) 2018/605

of 19 April 2018

amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties

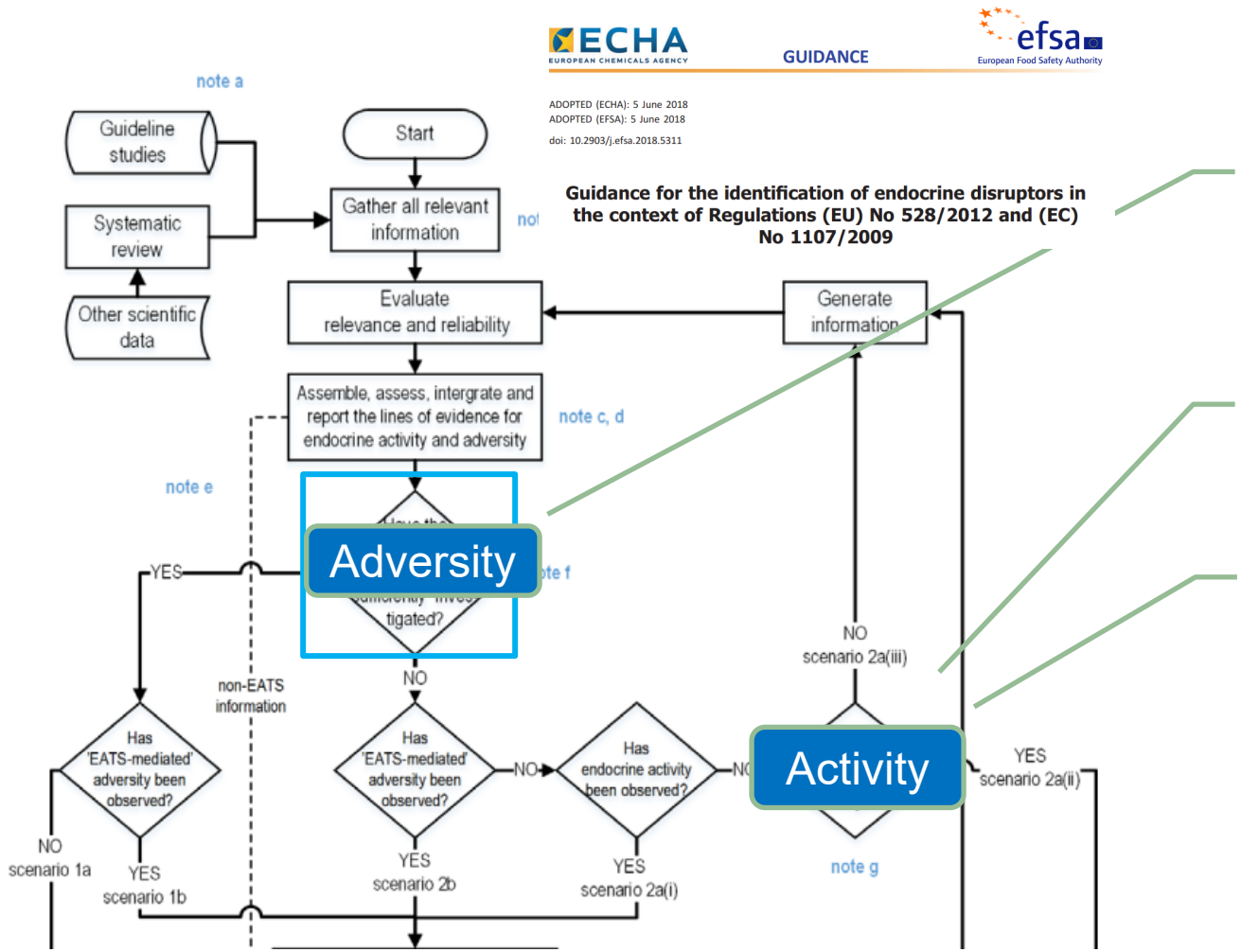
Adverse Effects

Endocrine Activity (Estrogen Androgen Thyroid Steroidogenesis)*

The adverse effect is the consequence of the endocrine activity = (biologically plausible link) - *Correlation (?)*

*according to ECHA/EFSA Guidance, 2018

ECHA/EFSA Guidance, 2018 – does it work for the thyroid?



Thyroid weight
Thyroid histopathology
Thyroid tumors

Thyroid hormone data

Thyroid MIE assays
- TPO
- NIS
- *Enhanced TH clearance (e.g. liver enzyme induction)*
- TR
- Dio
- …

E – estrogen
A – androgen
T – thyroid
S - steroidogenesis

Uncertainties in thyroid disruption assessments

- EATS-parameter: Thyroid weight, thyroid histopathology only (brain parameter are STBNDO parameters)
- Adverse outcome of concern: neurodevelopmental toxicity
  → no standard parameter in tox data sets
- Which parameter are indicative for neurodevelopmental toxicity?
- Thresholds for thyroid hormone changes indicating hypothyroidism
- Correlation between thyroid hormone changes and neurodevelopmental toxicity
- How relevant is the underlying mechanism / MIE?
- Species differences

Guiding questions for the ECETOC Thyroxine (T4) Task Force started work in Fall 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?

ECETOC T4 TF work at a glance

Towards a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny:

Which parameters from **human** studies are most relevant for toxicological assessment? (Sauer et al., **2020**)

How can key events of relevant **adverse outcome pathways (AOPs)** be addressed in toxicological assessments? (Marty et al., **2021**)

How is substance-mediated thyroid hormone imbalance in pregnant / lactating rats or their progeny related to neurodevelopmental effects? (Marty et al., **2022**)

Testing and assessment scheme (Thyroid-NDT-TAS) (Melching-Kollmuss et al., **2023**)

Which parameters from **human studies** are most relevant for tox assessments?

- Free T4 (fT4) and TSH are most frequently measured in humans, total T4 and T3 (tT4, tT3) and TSH in rat studies
- Association between altered maternal serum fT4 and/or TSH and increased risk for child neurodevelopmental impairment **confirmed**
- No evidence for a substance-mediated UGT induction leading to increased TH clearance, let alone to child neurodevelopmental impairment in humans
- Broad variety of neurodevelopmental parameters in human studies; no most sensitive parameter identified

ECETOC T4 TF MS #3 Authors

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REVIEW ARTICLE

Towards a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny—part III: how is substance-mediated thyroid hormone imbalance in pregnant/lactating rats or their progeny related to neurodevelopmental effects?

M. Sue Marty[a], Ursula G. Sauer[b], Alex Charlton[c], Rashin Ghaffari[d], Davy Guignard[e]*, Nina Hallmark[f], Bethany R. Hannas[d]*, Sylvia Jacobi[g], Heike-Antje Marxfeld[h], Stephanie Melching-Kollmuss[i], Larry P. Sheets[j], Daniel Urbisch[i]*, Philip A. Botham[c] and Bennard van Ravenzwaay[k]

[a]Dow, Inc., Midland, MI, USA; [b]Scientific Consultancy–Animal Welfare, Neubiberg, Germany; [c]Syngenta, Jealott's Hill, Bracknell, UK; [d]Corteva Agriscience, Newark, DE, USA; [e]Bayer CropScience, Sophia Antipolis, France; [f]Bayer AG, Monheim, Germany; [g]Albemarle, Louvain-la-Neuve, Belgium; [h]BASF SE, Ludwigshafen, Germany; [i]BASF SE, Limburgerhof, Germany; [j]Bayer CropScience, Chesterfield, MO, USA; [k]Environmental Sciences Consulting, Altrip, Germany

Review #3: Thyroid- and Brain-related Effects in Gestationally/Lactationally Exposed Rats

Goal: Investigate patterns of thyroid- and brain-related effects in rats with gestational/lactational exposure to 14 substances causing thyroid hormone imbalance by 4 different MOAs

Methods

- Four MoA-driven case studies for substances that:

  (1) inhibit thyroid peroxidase (TPO)

  (2) inhibit sodium-iodide symporter (NIS) (+ dietary iodine deficiency)

  (3) enhance thyroid hormone (TH) clearance

  (4) affect deiodinase 1 (DIO1) activity

- Both TG-compliant and investigational rat toxicity studies
- Brain-related parameters: motor activity, cognitive function, acoustic startle response, hearing function, periventricular heterotopia, electrophysiology and brain gene expression

Review #3: Thyroid- and Brain-related Effects in Gestationally/Lactationally Exposed Rats

- Relationship between thyroid hormone imbalance in pregnant/lactating rats and/or their pups and neurodevelopmental effects
  - Sensitive thyroid and nervous system endpoints
  - Whether MoA impacts neurodevelopmental outcome
  - Magnitude of thyroid hormone change that affects neurodevelopment
  - How to consider systemic toxicity

Review #3: Thyroid- and Brain-related Effects in Gestationally/Lactationally Exposed Rats

Thyroid-related effects (valuable to have multiple endpoints):

- Serum T4, T3, TSH*
- Thyroid histopathology*
- Thyroid weight is not sensitive*

Neurodevelopmental effects on:

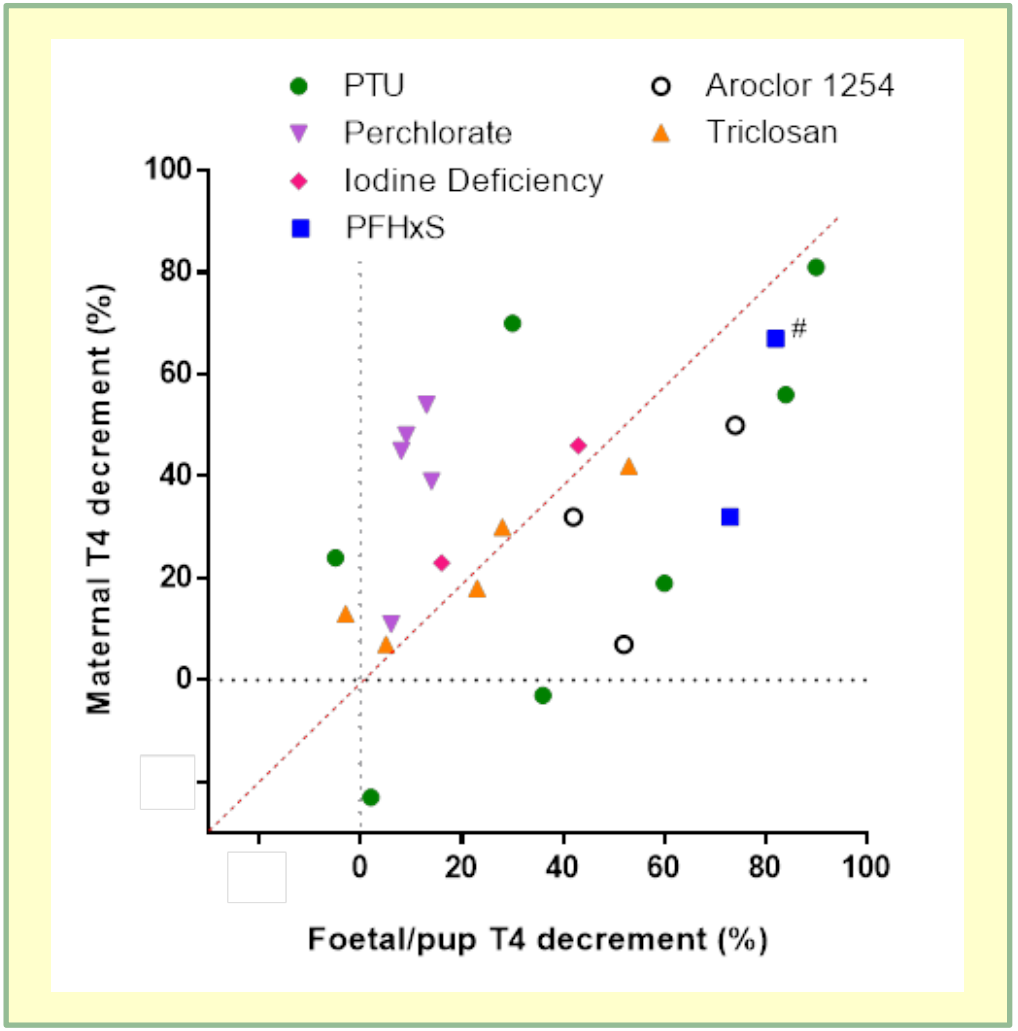
- Motor activity**
- Cognitive function (not the most sensitive)*
- Acoustic startle response and hearing function**
- Heterotopia**

Similar for systemic toxicity: WoE is needed

*OECD TG 426 DNT study
*OECD TG 443 EOGRTS

Review #3: Thyroid- and Brain-related Effects in Gestationally/Lactationally Exposed Rats

- MoAs not related to specific patterns of brain-related effects
  - Magnitude and timing of TH decrement
- Offspring T4, T3, TSH levels (not maternal) predict likelihood of neurodevelopmental toxicity
  - Maternal serum T4 and pup serum T4 on GD 20-PND 0 are shown



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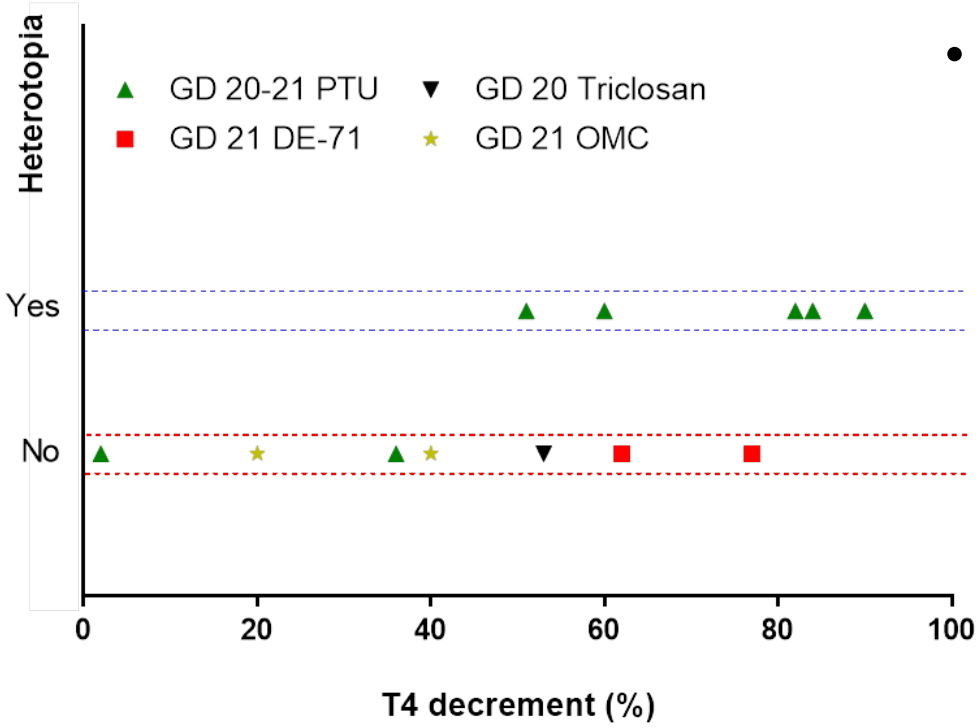


- Brain TH levels may be useful
  - Serum TH decrements and neurodevelopmental outcome are not always correlated even when TH is measured during critical windows

Review #3: Thyroid- and Brain-related Effects in Gestationally/Lactationally Exposed Rats

- Thresholds: ≥ 60%/50% offspring serum T4 ↓, and ≥ 20% & statistically significant offspring serum T3 ↓ appear to indicate ↑ likelihood for NDT in rats
  - Hypothesized thresholds (Timing is important)

Relevant Conclusions and Implications for Toxicological Assessments

Main Observations	Implications for Assessment Strategy
Changes in maternal TH do not necessarily correspond to TH changes in rat offspring	Thyroid hormone disruption in offspring should **not** be decided based on TH data alone (or TH in repeated dose studies)
No single TH parameter alone is decisive	Pattern of thyroid effects is more decisive
Possibly ≥ 50/60% **offspring** T4 decrements are associated with neurodevelopmental findings in rats	Rat DNT / EOGRT studies can identify TH related neurodevelopmental effects
Extent of TH imbalance appears more important than MIE to predict neurodevelopmental impairment in rat	The extent of TH effect in offspring, pattern of T effects and/or pattern of neurodevelopmental parameter changes should be used to assess ED disruption.
There is not one most sensitive neuro-developmental parameter (humans or rats)	Investigations on species differences need to be conducted earlier in the AOP (e.g. based on identified MIEs).

AOP- Adverse Outcome Pathway
DNT – Developmental neurotoxicity (OECD TG 426)
EOGRT – Extended-One-Generation-Toxicity (OECD TG 443)

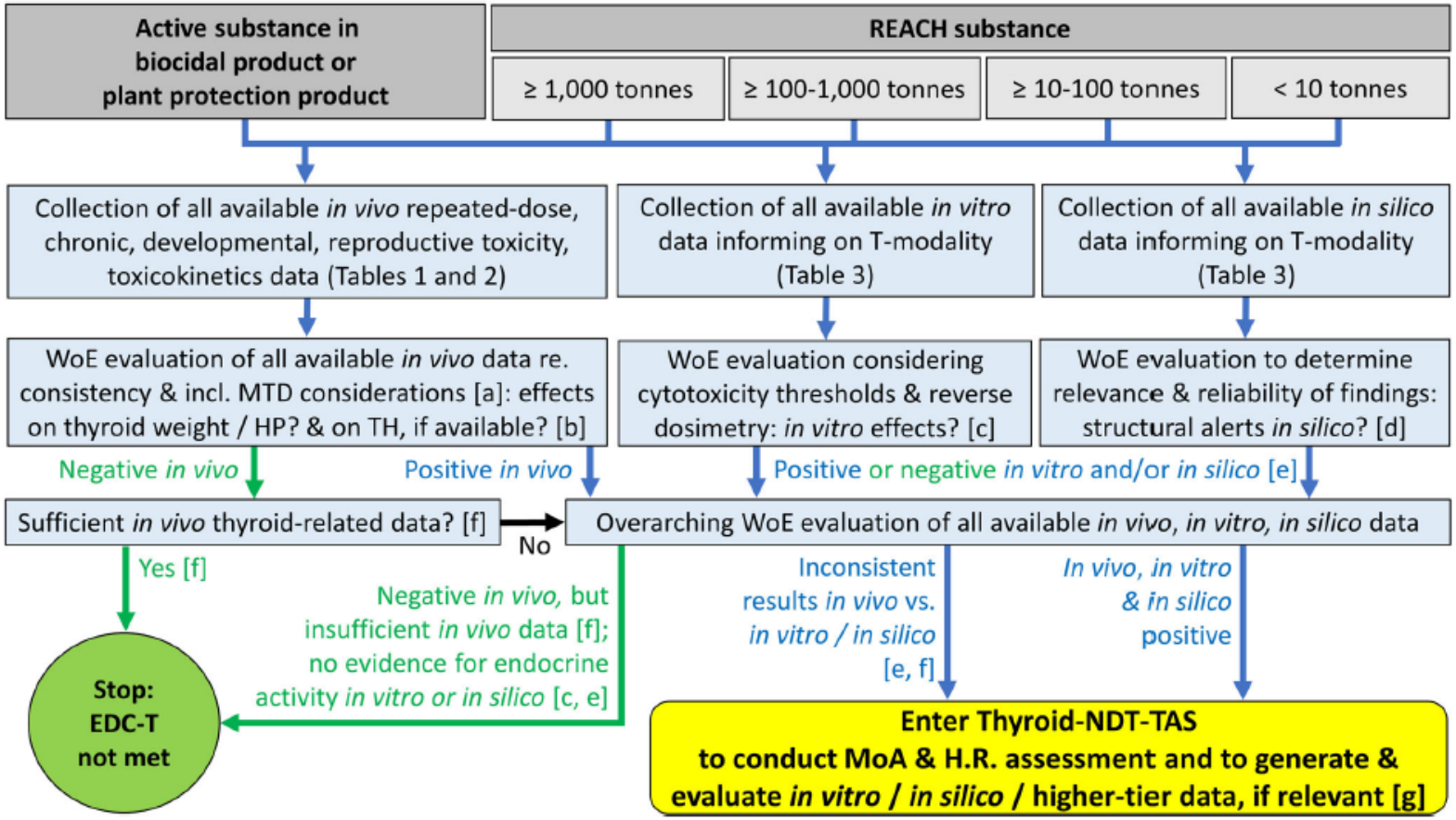
START WITH ASSESSING THYROID FUNCTION

Thyroid function assessment

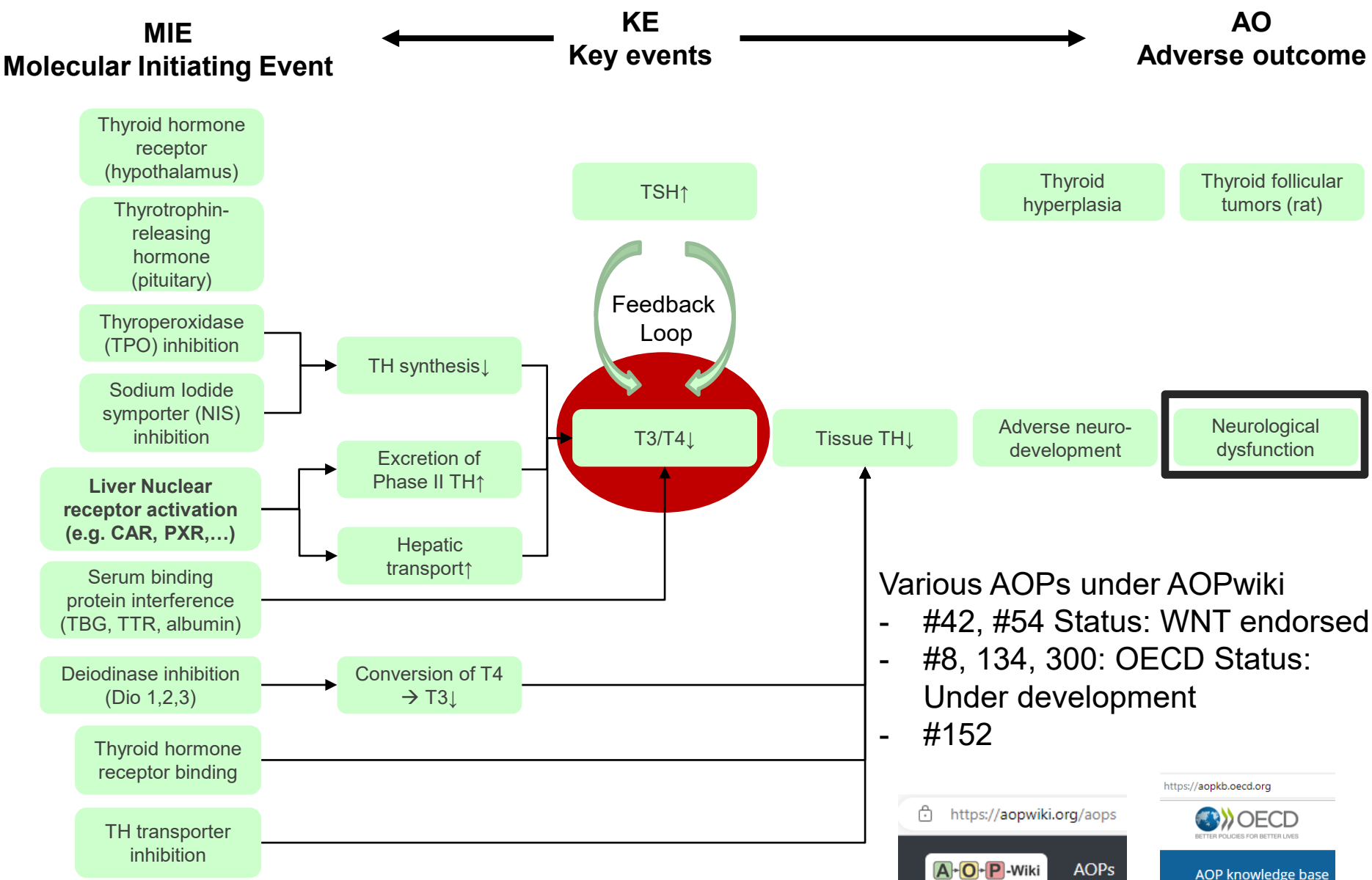
- Thyroid hormones
- Thyroid weight
- Thyroid histopathology
- In vitro assays (TPO, NIS, …)
- *QSARs*

Thyroid Function-related **N**eurodevelopmental **T**oxicity **T**esting and **A**ssessment **S**cheme (Thyroid-NDT-TAS)

This works with data-rich and data-poor substances!

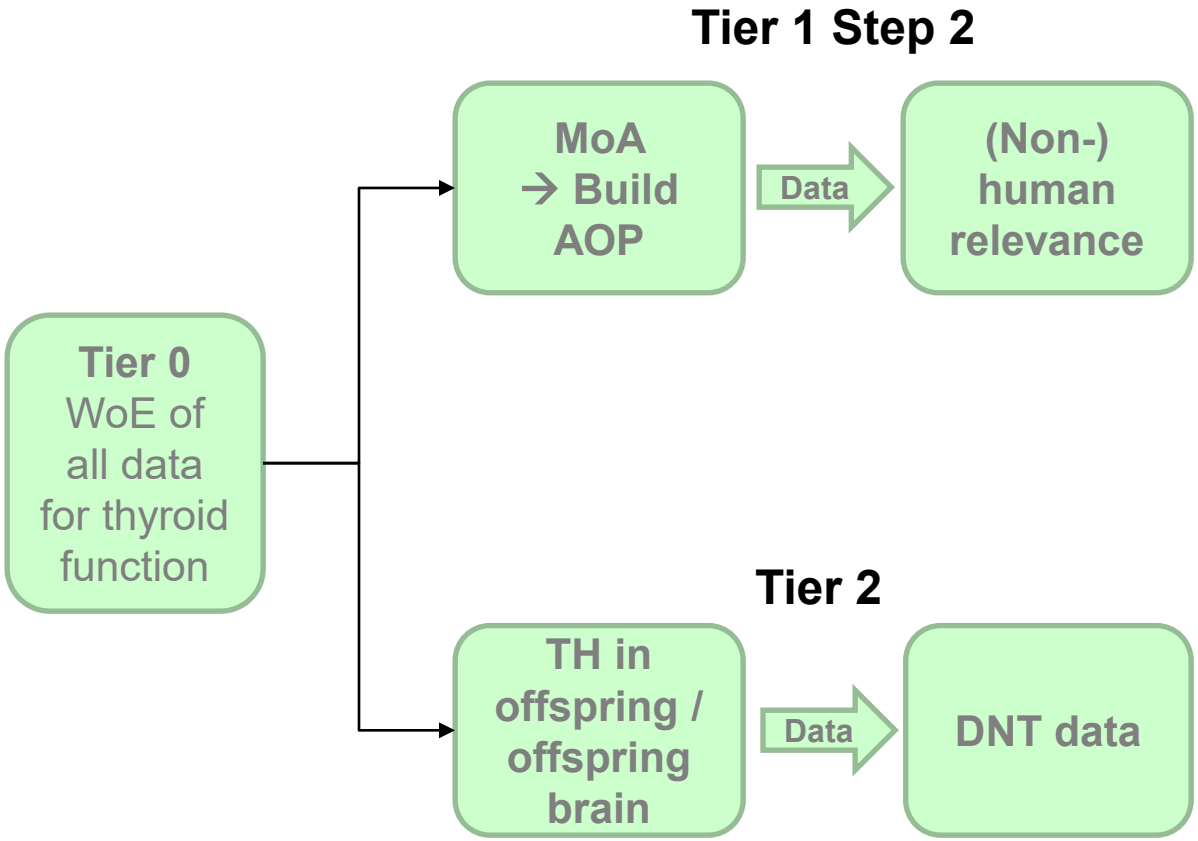


ASSESSMENT SCHEME FOR NEURODEVELOPMENTAL TOXICITY - TESTING NEEDS



WNT: OECD Working Group of National Coordinators of the Test Guideline program

Thyroid Function-related **N**eurodevelopmental **T**oxicity **T**esting and **A**ssessment **S**cheme (Thyroid-NDT-TAS)



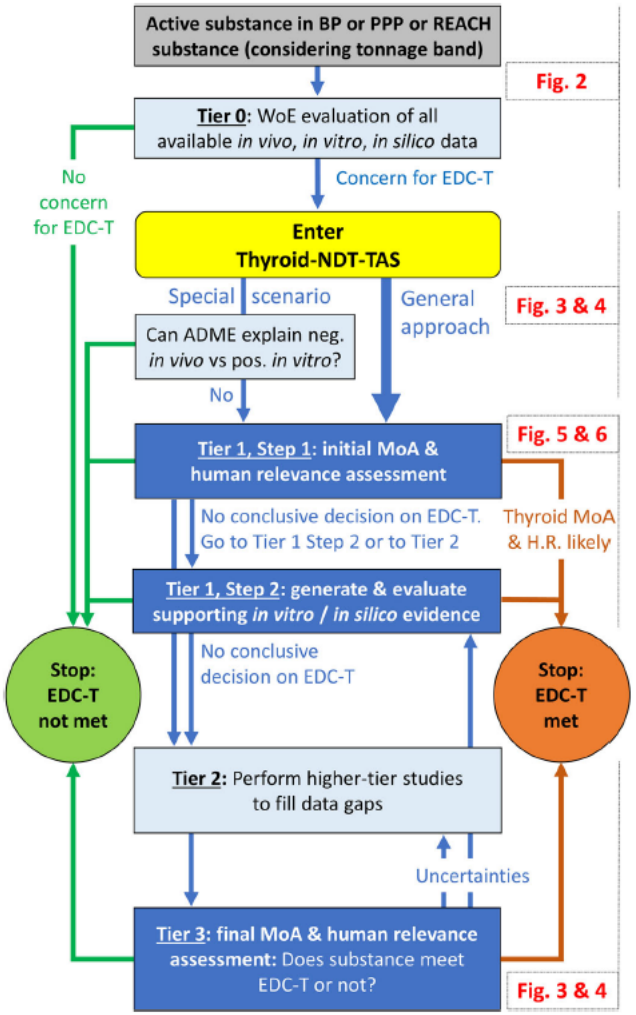


Figure 1. Overview of the ECETOC and CLE Thyroid-NDT-TAS (see Figures 2–6 for details). BP: biocidal product; EDC-T: endocrine disruptor criteria for the thyroid modality; MoA: mode-of-action; PPP: plant protection product; REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals; WoE: weight-of-evidence.

Thyroid Function-related **N**eurodevelopmental **T**oxicity **T**esting and **A**ssessment **S**cheme (Thyroid-NDT-TAS)

Tier 0
WoE of all data for thyroid function

Tier 1 Step 2

Critical Reviews in Toxicology

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Towards a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny – Part IV: the ECETOC and CLE Proposal for a Thyroid Function-Related Neurodevelopmental Toxicity Testing and Assessment Scheme (Thyroid-NDT-TAS)

Stephanie Melching-Kollmuss, Kathrin Bothe, Alex Charlton, Babunilayam Gangadharan, Rashin Ghaffari, Sylvia Jacobi, Sue Marty, Heike-Antje Marxfeld, Elizabeth F. McInnes, Ursula G. Sauer, Larry P. Sheets, Christian Strupp, Helen Tinwell, Christiane Wiemann, Philip A. Botham & Bennard van Ravenzwaay

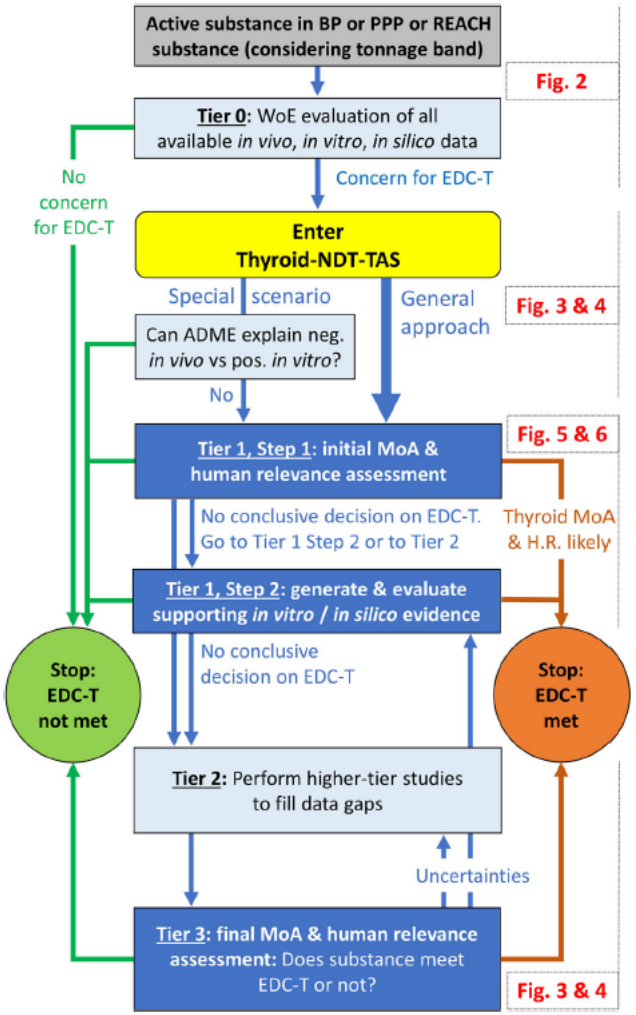
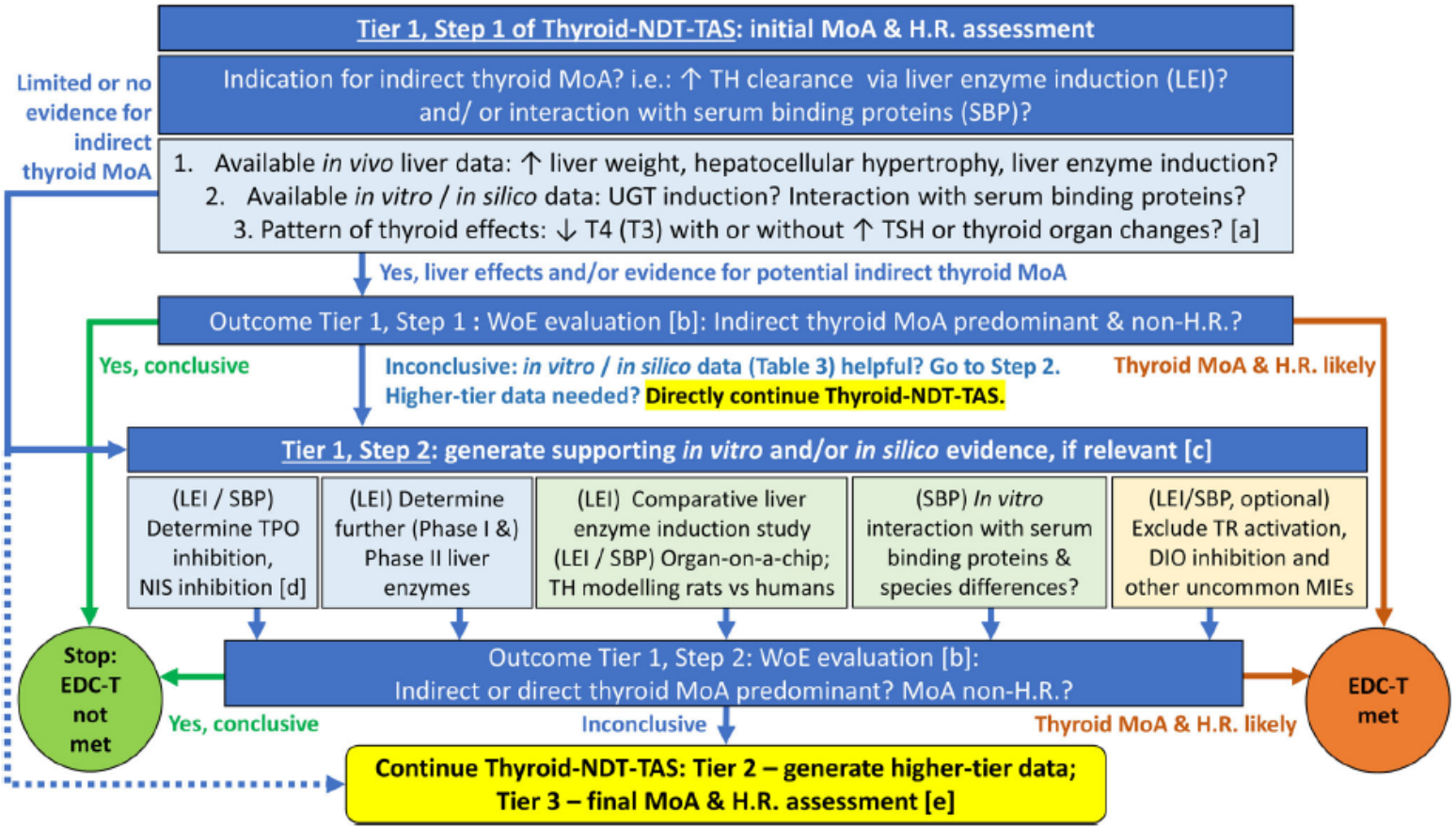


Figure 1. Overview of the ECETOC and CLE Thyroid-NDT-TAS (see Figures 2–6 for details). BP: biocidal product; EDC-T: endocrine disruptor criteria for the thyroid modality; MoA: mode-of-action; PPP: plant protection product; REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals; WoE: weight-of-evidence.

Thyroid Function-related **N**eurodevelopmental **T**oxicity **T**esting and **A**ssessment **S**cheme (Thyroid-NDT-TAS)

Non-human relevance assessment for Case study 3 – increased TH clearance
Liver enzyme induction (LEI) or interaction with serum binding proteins (SBP)



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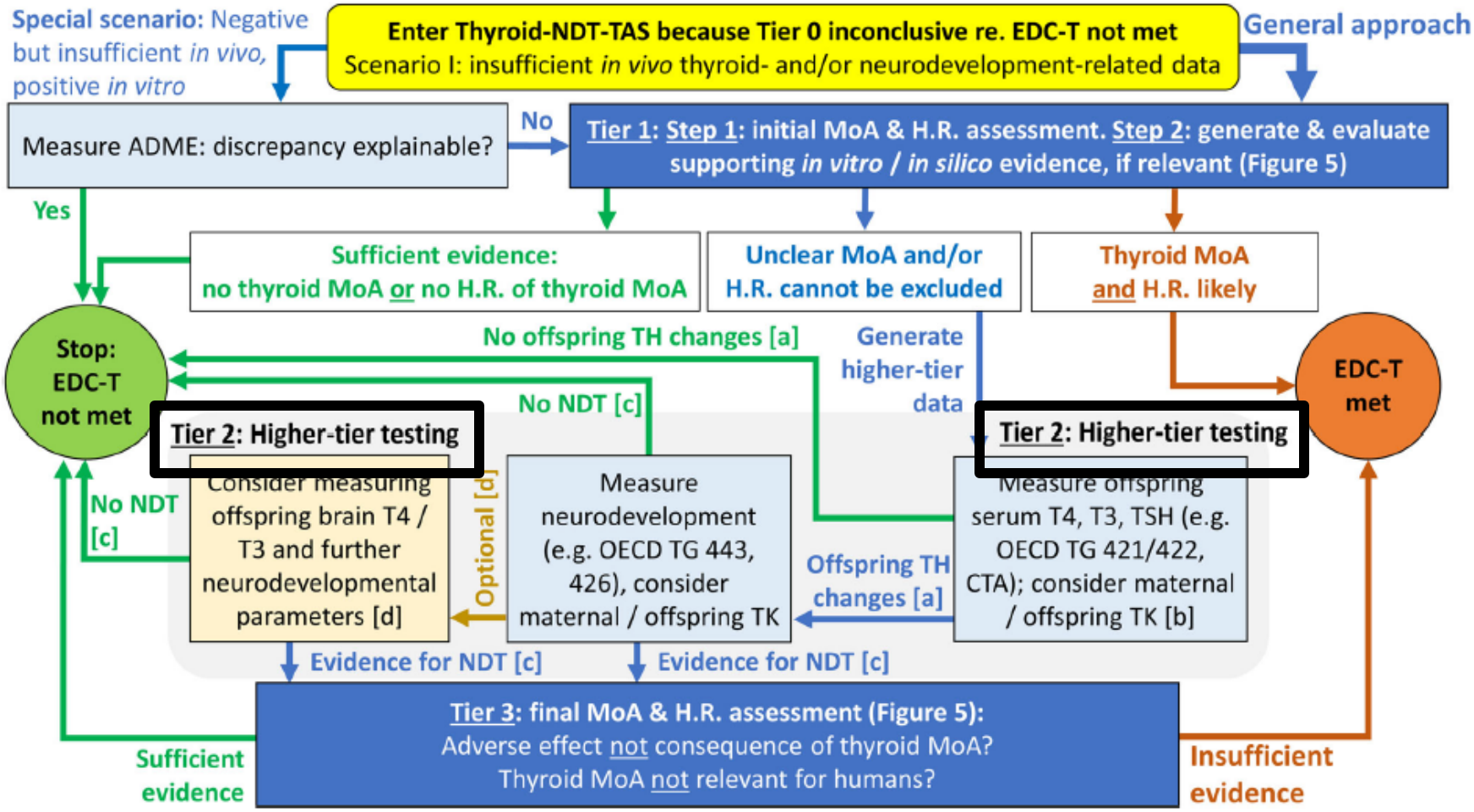

USE OF THE THYROID-NDT-TAS TO HELP CATEGORIZE FOR ED

Endocrine Disruption Criteria – what stays?

Regulation 1107/2009 – …placing plant protection products on the market

An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005.

In force: 20 October 2018

Commission Delegated Regulation, amending EC 1272/2008 – Classification, Labelling, …

Endocrine Disruption for Human Health Cat. 1

May cause endocrine disruption in humans

Endocrine Disruption for Human Health Cat. 2

Suspected of causing endocrine disruption in humans

Where there is evidence conclusively demonstrating that the adverse effects are not relevant to humans, the substance shall not be considered an endocrine disruptor for human health.

In force: 20 April 2023

Future Endocrine Disruption Criteria under EU CLP

- **There needs to be evidence for all three elements of the criteria (adverse effect, endocrine activity, biologically plausible link)**
- **ECHA Guidance expected in Summer 2024**

Cat. 1	Cat. 2	No ED
ED Yes Human relevant Effects are specific adverse ED activity shown Plausible link shown	Insufficient weight of evidence for Cat. 1 (e.g. human relevance, specifi-city of effects or ED activity, plausible link	**ED No**

Use the Thyroid-NDT-TAS to guide ED Categorizations

- Thyroid function effect pattern: Thyroid weight increases, Thyroid follicular cell hypertrophy/hyperplasia, Thyroid hormone changes adults / dams, Thyroid hormone changes offspring
- Possible standard neurodevelopmental toxicity parameter pattern: Brain morphometry, brain histopathology, motor activity, learning and memory
- Possible non-standard neurodevelopmental toxicity parameters: Brain thyroid hormones in offspring, brain gene changes
- MIEs have been studied – non-human relevance studies have been conducted

General Weight of Evidence Considerations

High Weight of Evidence

- Effect(s) seen in >1 species
- Effect(s) seen in more than study / several timepoints
- Effect(s) seen with a clear dose-response
- Effect(s) seen also seen without overt toxicity
- Pattern of effect

Low Weight of Evidence

- Effect(s) seen in one species
- Effect(s) seen in one study
- Effect(s) seen without dose response
- Effect(s) seen at high systemically toxic doses only
- Only isolated effect seen

Case Examples

- Pesticide Active Ingredients
- Extensive Data sets
  - 28-, 90-day, long-term studies rats, mice, dogs
  - Modern 2-Gen, or EOGRT, or DNT study
  - TH data in adults / offspring
  - Mode of action studies → Mode of action identification
  - Non-human relevance studies

Case Examples

Example 6	T	TH increase at low doses without adversity, TH decrease at high doses, evidence for liver-mediated effects, direct T MoA negative, Adversity: Thyroid histopathology; CTA at doses higher than in dietary regulatory studies, In vitro comparative liver enzyme induction assay no human relevance	HH 2	Insufficient severity of the effect, lack of human relevance
Example 7	T	Compound releasing a metabolite which is TPO inhibitor; Thyroid histopathology, thyroid hormone in multiple species. TH in offspring (PND 4 and 21) were unaffected.	HH 2	Most sensitive population unaffected

Case Examples

Example 8	T	Thyroid histopathology and hormones in adults, no TH change in offspring, liver mediated MoA, direct MoA DNT cohorts & learning and memory showed no adversity pattern of DNT-related effects.	No ED	No adverse effect pattern with regard to neurodevelopmental toxicity. No change in offspring thyroid hormones.
Example 9	T	Thyroid histopathology and hormones in adults, thyroid tumors, DNT negative, liver-mediated MoA, negative direct MoAs; , In vitro comparative liver enzyme induction assay no human relevance	No ED	No adverse effect pattern with regard to neurodevelopmental toxicity. Non-human relevance shown in vitro.

Outlook

- RSA / ECETOC Thyroid Workshop in September 2024 (back-to-back to Eurotox)
- Publication Case Examples planned (CLE)
- CLE project on robust PBPK models to explore species differences (e.g. TH levels)
- CLE project to collect reference compound data tested in comparative liver enzyme induction studies → publication planned

Authors of the CLE / ECETOC Testing scheme

- Stephanie Melching-Kollmuss, BASF SE
- Kathrin Bothe, Bayer Crop Science
- Alex Charlton, Syngenta Crop Protection
- Babunilayam Gangadharan, Bayer Crop Science
- Rashin Ghaffari, Corteva Agriscience
- Sylvia Jacobi, SJ-Consult
- Sue Marty, Dow Inc
- Heike-Antje Marxfeld, BASF SE
- Elizabeth McInnes, Syngenta Crop Protection
- Ursula Sauer, Scientific Consultancy – Animal Welfare
- Larry Sheets, Bayer Crop Science
- Christian Strupp, Gowan Crop Protection
- Helen Tinwell, Bayer Crop Science
- Christiane Wiemann, BASF SE
- Phil Botham, Syngenta Crop Protection, ECETOC Steward
- Bennard van Ravenzwaay, Environmental Sciences Consulting, ECETOC Steward

THANK YOU FOR YOUR ATTENTION

QUESTIONS?