FLASH REPORT (1-Day Symposium)

Background

Perturbation of thyroid hormone levels during gestation and the early post-natal period may cause neurodevelopmental impairment. The evaluation of effects in offspring is therefore relevant for the toxicological assessment of chemicals affecting thyroid function, as reflected in current ECHA/EFSA guidance on the identification of endocrine disruptors.

Following the logic of this guidance, substances meet the ED criteria if they elicit adverse effects, have endocrine activity and if the adverse effects and endocrine activity are linked by an endocrine mode of action (MoA) unless the MoA is not relevant for humans. However, clear regulatory guidance on the assessment of whether substances meet the ED criteria for the thyroid modality considering developmental neurotoxicity (DNT) as an adverse outcome is currently unavailable. This is particularly relevant as a large proportion of substances evaluated under different legislation are shown to affect the thyroid, many through mechanisms not of human relevance.

Accordingly, this symposium had the objective of reviewing the state of the science of thyroid hormone disruption mediated DNT, and its current assessment in regulatory decision-making.

DISCLAIMER:

Representatives from the US EPA, ECHA, EFSA or Member State Competent Authorities expressed no official views or positions on the scientific matters presented during the event or discussed thereafter, hence their participation cannot be taken as agreement/disagreement with the views and positions expressed and documented in this report. This report is intended to accurately reflect the event discussions and conclusions.

Event participation

The Symposium, organised jointly by ECETOC and RSA, was held in person in Copenhagen on September 8th prior to EUROTOX 2024. A well-balanced profile of approximately 60 participants and 10 speakers (regulatory, academia, industry) contributed to the symposium.

Introduction to the Symposium

The speakers and attendees were welcomed to the Symposium. The co-Chairs Bennard van Ravenzwaay representing ECETOC and Christine Walter representing RSA introduced the main intent of the Symposium, to build on the work undertaken by ECETOC and published in four papers[1], reflecting the evolution of the assessment of thyroid hormone disruptors from animal studies to NAMs.

[1] *ECETOC publications are listed at the end of this report*

Key Points

The following key points were highlighted during speaker presentations and in response to attendees' questions.

Main challenges

- The continuing development of scientific understanding and techniques was highlighted, but the current disconnect between the state of the science and hazard-based regulation was also recognised.
- Substances affecting the thyroid are of concern under different regulations. A significant proportion of EOGRTS requested by ECHA have been triggered by concerns relating to the T-modality. A large proportion of substances considered under BPR have ED decisions pending requests for additional data for the T-modality. Additionally, approximately 30% of all pesticide active substances assessed by EFSA affect thyroid hormone levels.
- A large proportion of substances affect the thyroid via an indirect and rodent-specific mode of action (MoA), which is difficult to conclusively demonstrate (to the satisfaction of regulators) without formally validated methods.
- Knowledge of age-specific background levels and normal variability in thyroid hormone levels is essential to proper interpretation. Thyroid hormone levels from neonatal brain (whole brain, cortex and cerebellum) have been investigated in order to facilitate the interpretation of treatment-related effects from normal background variation.

Current uncertainties

- There is no agreement on a threshold for thyroid hormone reduction as a trigger for DNT assessment. ECETOC have proposed a 50% threshold, whereas EFSA consider a decrease of >20% to be of concern; the practicality of this threshold was questioned considering the extent of biological variability and analytical issues associated with measuring thyroid hormone levels.
- Maternal thyroid hormone levels are generally used as a marker of thyroid disruption but are strongly influenced by temporal factors and homeostatic mechanisms, whereas investigations are usually made at a single point in time. The most appropriate analyte (total or free T4, T3 or TSH) was also questioned. Furthermore, due to the lack of correlation between maternal thyroid hormone levels and DNT, investigations should focus on reductions in fetal/pup thyroid hormone levels. Good quality analytical methods are crucial as high variability hampers proper interpretation
- Substances will be classified for ED in CLP Cat. 1 or 2 depending on the strength of the evidence available at that point; it was highlighted that any effects on the thyroid will be considered human-relevant and sufficient for classification, regardless of mechanistic data. This contrasts with the ECHA/EFSA ED guidance which requires a causal relationship. All substances concluded by EFSA to be ED will be assigned to Cat. 1 without additional assessment. However, reassessment by ECHA of substances concluded non-ED by EFSA may still result in ED classification.
- The difficulty in correlating neuropathology with clinical findings was recognised. It was noted that different factors might bias the results/interpretations of neurotoxicity studies e.g. sampling of the correct target organ, sample preparation/processing, brain morphometry, prediction of 3D structures

from 2D measurements, differentiation of treatment-related changes from background findings and/or artefacts. Not all changes seen in animal studies have a direct human correlate; Neuropathological findings may have a variable background incidence, be difficult to quantify and can also be subject to observer bias.

Key issues to be addressed/areas for future work

- Substances inducing increased turnover of thyroid hormones via CAR/PXR activation show strong species differences; a threshold can be demonstrated and humans are clearly less sensitive. Thyroid tumours resulting from this indirect MoA are considered not to be of human relevance, but this is not currently the case for other effects resulting from the same MoA. Further collaboration would be helpful to validate in vitro thyroid or CAR/PXR MoA assays, and therefore gain regulatory acceptance.
- Humanised mice models are currently considered critical in demonstrating a lack of human relevance for substances acting via CAR/PXR activation. More recently, 2D-sandwich cultures of primary hepatocytes have been developed to demonstrate species-specific responses to hepatic receptor-mediated alterations in T4 glucuronidation. Basal T4 glucuronidation activity is much higher in rat compared to human hepatocytes; while some increase in activity may be seen for human hepatocytes, overall activity remains comparatively very low. Consequently, delta activity is arguably the more relevant parameter and can be used to demonstrate species differences.
- There are two options for testing strategies for substances that impact thyroid hormone levels: investigation of the human relevance for the underlying MoA for the thyroid hormone reductions, or generation of more hormone and DNT data to gather more information on a possible adverse outcome (reduced thyroid hormone levels in pup brains). The CTA is currently the gold standard for the follow-up of thyroid hormone reductions in adult animals, but it is hoped that the future use of NAMs can be used to reduce the reliance on animal testing.
- There is a need to find suitable markers and common endpoints for thyroid-related DNT in humans and rodents. The important criteria that need to be fulfilled for a robust biomarker for thyroid-related DNT were summarised as follows: correlation with brain TH levels; be quantitative, reproducible and applicable to humans; be measurable in developing animals (ideally) using translatable methods
- Effects observed on neurodevelopment in studies with PTU (as a model thyroid disrupting substance) must be confirmed as related to reduced thyroid hormones and therefore relevant to other thyroid-disrupting substances.
- Thyroid histopathology (follicular hypertrophy and hyperplasia) commonly seen in animal studies shows a graded response and may be difficult to interpret consistently. More consistent responses are seen with semi-automated 3-dimensional morphometric analysis that adds some information about the histopathological severity changes observed, with limited observer variation.

Closing remarks

There clearly remains a significant amount of effort required to build a robust and regulatory acceptable approach in this domain. ECETOC will remain mobilized to provide a platform for fruitful dialogue between interested parties with the aim of advancing the scientific aspects of this topic.

Event programme

Item	Start	End	Agenda item	Who
1	11:30 AM	12:00 PM	Arrival and registration at the Copenhagen Island Hotel	Organising committee
2	12:00 PM	12:30 PM	**Welcome Lunch** (Registration to continue through lunch)	
3	12:30 PM	12:40 PM	**Welcome, introduction and symposium objectives**	Bennard van Ravenzwaay (ECETOC) Christine Walter (RSA)
4	12:40 PM	1:00 PM	**Testing and assessment of thyroid-hormone related neurodevelopmental toxicity – the ECETOC-CLE Thyroid NDT-TAS**	Stephanie Melching-Kollmuss (BASF)
5	1:00 PM	1:20 PM	**Experiences with Thyroid ED assessment – EFSA ED Database**	Martina Panzarea (EFSA)
6	1:20 PM	1:40 PM	**Experiences with Thyroid ED assessment & new CLP ED Criteria**	Niklas Andersson (ECHA)
7	1:40 PM	2:00 PM	**In vitro and in vivo investigation of pesticide effects on the T-axis**	Philip Marx-Stoelting (BfR)
8	2:00 PM	2:20 PM	**Coffee break**	
9	2:20 PM	2:40 PM	**Use of the ECETOC-CLE Thyroid NDT-TAS to Support Identification and Classification of Thyroid Hormone Disruptors**	Helen Tinwell (Bayer)
10	2:40 PM	3:00 PM	**How to identify adverse neurodevelopmental toxicity in laboratory animals**	Heike-Antje Marxfeld (BASF)
11	3:00 PM	3:20 PM	**Decisive new endpoints indicative of abnormal neurodevelopment**	Katie O'Shaugnessy (US EPA)
12	3:20 PM	3:40 PM	**Assessment of thyroid hormone alterations in rat brain and plasma**	Christiane Hindrichs (BASF)
13	3:40 PM	4:00 PM	**Species-specific thyroxine (T4) metabolism and response to nuclear-receptor activators in long-term cultured hepatocytes**	Lysiane Richert & Laure Asselin (KaLy-Cell)
14	4:00 PM	4:15 PM	**Summarise and closing remarks**	Bennard van Ravenzwaay (ECETOC) Christine Walter (RSA)

ECETOC publications

Sauer UG *et al*. (2020). Toward a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny - Part I: Which parameters from human studies are most relevant for toxicological assessments? *Crit Rev Toxicol* 50(9):740-763. doi:10.1080/10408444.2020.1839380

Marty MS *et al*. (2021). Towards a science-based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effects in the progeny - Part II: How can key events of relevant adverse outcome pathways be addressed in toxicological assessments? *Crit Rev Toxicol* 51(4):328-358. doi:10.1080/10408444.2021.1910625

Marty MS *et al*. (2022). 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? *Crit Rev Toxicol* 52(7): 546-617. doi:10.1080/10408444.2022.2130166

Melching-Kollmuss S *et al*. (2023). 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). *Crit Rev Toxicol* 53(6):339-371. doi:10.1080/10408444.2023.2231033

Rapporteurs

Kathrin	Bothe	Bayer, DE
David	Andrew	RSA, UK

Organising Committee

Phil	Botham	Syngenta, UK
Stephanie	Melching-Kollmuss	BASF, DE
Bennard	van Ravenzwaay	Wageningen University, DE
Blanca	Serrano Ramón	ECETOC, BE
Andrea	Salvadori	ECETOC, BE
Francesca	Uguccioni	ECETOC, BE
Sergio	León Pérez	ECETOC, BE
Alison	McCondichie	RSA, UK
Jen	Buchanan	RSA, UK
Liz	Macquarrie	RSA, UK
David	Andrew	RSA, UK

Contact

Sergio León Pérez, ECETOC
sergio.perez@ecetoc.org