OVERVIEW OF ONGOING THYROID ACTIVITIES IN THE EU AND US

Manon Beekhuijzen

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Introduction

US EPA – ENDOCRINE DISRUPTION SCREENING PROGRAM

EDSP

In 1996 US congress passed the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act amendments (SDWA).

- Requiring US EPA (environmental protection agency) to screen pesticide and commercial chemicals for their potential to produce **estrogenic** effects in humans.

- Advisory Committee expanded EDSP to also include:
  - **androgens** and the **thyroid** system;
  - effects on fish and wildlife

EDSP TIER 1

Assay	Receptor binding				Steriodo-genesis		HPG axis	HPT axis
	A	Anti-E	A	Anti-A	E	A		
In vitro								
ER binding	x	x						
ER transcriptional activation	x							
AR binding			x	x				
Steriodogenesis					x	x		
Aromatase recombinant					x			
In vivo								
Uterotrophic	x							
Hershberger			x	x				
Pubertal male			x	x		x	x	x
Pubertal female	x	x			x		x	x
Fish short-term reproduction	x	x	x	x	x	x	x	
Amphibian metamorphosis								x

EDSP TIER 1

T4 and TSH

Assay	Receptor binding				Steriodo-genesis		HPG axis	HPT axis
	A	Anti-E	A	Anti-A	E	A		
In vitro								
ER binding	x	x						
ER transcriptional activation	x							
AR binding			x	x				
Steriodogenesis					x	x		
Aromatase recombinant					x			
In vivo								
Uterotrophic	x							
Hershberger			x	x				
Pubertal male			x	x		x	x	x
Pubertal female	x	x			x		x	x
Fish short-term reproduction	x	x	x	x	x	x	x	
Amphibian metamorphosis								x

COMPARATIVE THYROID ASSAY

October 24, 2005

Guidance for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals, and Adult Animals

Office of Pesticide Programs
Health Effects Division
Washington DC

Serum hormonal measurements (T4, T3, and TSH) and pathological evaluations of thyroid tissue should be performed on GD 20 dams and fetuses, on neonates on the day of standardization of the litters (PND 4), and on dams and pups on PND 21, for a total of 10 litters per treatment on GD 20 and 20 litters per treatment on PND 4 and PND 21.

COMPARATIVE THYROID ASSAY

A number of pesticide chemicals have been shown to perturb thyroid hormone homeostasis via reduction of circulating thyroid hormones (Hurley *et al.*, 1998). Chemicals that perturb thyroid homeostasis and result in hypothyroidism are known to be associated with neurological disorders and alterations in neurological development, both in animals and humans (Fisher, 2000; Chan and Kilby, 2000; Morreale de Escobar *et al.*, 2000; Zoeller and Rovet, 2004; Anderson *et al.*, 2003). Thus, in the assessment of the toxic characteristics of a thyroid disrupting pesticide, determination of the potential to adversely impact thyroid hormones, thyroid structure, and/or thyroid hormone homeostasis during development is important. Normally, if a neurodevelopmental concern is raised by existing data on a pesticide, a rat developmental neurotoxicity (DNT) study is requested. However, disruption of thyroid homeostasis by thyroid disrupting pesticides is the initial, critical effect that may lead to adverse effects on the developing nervous system. Thus, *in lieu* of the rat DNT study, the special study described herein entails a mechanistic approach to generate specific data on the thyroid (*i.e.*, the primary target of the chemical of interest) to protect the developing nervous system from thyroid hormone disrupting chemicals. The specific purpose of this special study is to generate data to establish NOAELs and LOAELs (or benchmark doses) that may be used to derive RfDs that would be protective of the ability of a chemical to disrupt thyroid function in pregnant females and in the fetus and newborn. This special study will generally be requested based on the results of a study(ies) in adult animals that provide evidence that a pesticide produces effects on thyroid function or structure.

GUIDELINES

OECD Guidance Document No. 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. ENV/JM/MONO(2012)22.

2011

* OECD TG 443 (EOGRTS)

2015

* OECD TG 421 (repro screening study)
* OECD TG 422 (combined 28-d/repro)

2018

* OECD TG 408 (90-day toxicity)
* OECD TG 414 (prenatal developmental study)

ECHA (European Chemicals Agency) and **EFSA** (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC), 2018.

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

ECHA/EFSA GUIDANCE, 2018

ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority)

- A decrease in T4 in the absence of adverse histological changes should act as a trigger for further studies of F_1 generation depending on the other information available.

- The OPPTS thyroid comparative assay is expected to be conducted based on the results of a study or studies in adult animals that provide evidence that a substance produces effects on thyroid function.

Ongoing thyroid activities

ETS TASK FORCE

- Founded in 2016
- Publication (2018):

The urgency for optimization and harmonization of thyroid hormone analyses and their interpretation in developmental and reproductive toxicology studies

Manon Beekhuijzen[a,*], Steffen Schneider[b], Narinder Barraclough[c], Nina Hallmark[d], Alan Hoberman[e], Sheri Lordi[e], Mary Moxon[f], Deborah Perks[c], Aldert H. Piersma[g], Susan L. Makris[h]

A B S T R A C T

In recent years several OECD test guidelines have been updated and some will be updated shortly with the requirement to measure thyroid hormone levels in the blood of mammalian laboratory species. There is, however, an imperative need for clarification and guidance regarding the collection, assessment, and interpretation of thyroid hormone data for regulatory toxicology and risk assessment. Clarification and guidance is needed for 1) timing and methods of blood collection, 2) standardization and validation of the analytical methods, 3) triggers for additional measurements, 4) the need for T4 measurements in postnatal day (PND) 4 pups, and 5) the interpretation of changes in thyroid hormone levels regarding adversity. Discussions on these topics have already been initiated, and involve expert scientists from a number of international multisector organizations. This paper provides an overview of existing issues, current activities and recommendations for moving forward.

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SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

SOT ROUNDTABLE

- In March 2017
- Roundtable session “Implementing developmental thyroid toxicity guidance into practice: what’s working, what’s not, and how can we do better?”
- Paper recently accepted:

Regul Toxicol Pharmacol. 2019 Apr 21. pii: S0273-2300(19)30109-6. doi: 10.1016/j.yrtph.2019.04.010. [Epub ahead of print]

Practical considerations for developmental thyroid toxicity assessments: What's working, what's not, and how can we do better?

Li AA[1], Makris SL[2], Marty MS[3], Strauss V[4], Gilbert M[5], Blacker A[6], Zorrilla LM[7], Coder PS[8], Hannas B[9], Lordi S[10], Schneider S[11].

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Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

SOT ROUNDTABLE

Abstract

Thyroid hormones (THs; T3 and T4) play a role in development of cardiovascular, reproductive, immune and nervous systems. Thus, interpretation of TH changes from rodent studies (during pregnancy, in fetuses, neonates, and adults) is critical in hazard characterization and risk assessment. A roundtable session at the 2017 Society of Toxicology (SOT) meeting brought together academic, industry and government scientists to share knowledge and different perspectives on technical and data interpretation issues. Data from a limited group of laboratories were compiled for technical discussions on TH measurements, including good practices for reliable serum TH data. Inter-laboratory historical control data, derived from immunoassays or mass spectrometry methods, revealed: 1) assay sensitivities vary within and across methodologies; 2) TH variability is similar across animal ages; 3) laboratories generally achieve sufficiently sensitive TH quantitation levels, although issues remain for lower levels of serum TH and TSH in fetuses and postnatal day 4 pups; thus, assay sensitivity is critical at these life stages. Best practices require detailed validation of rat serum TH measurements across ages to establish assay sensitivity and precision, and identify potential matrix effects. Finally, issues related to data interpretation for biological understanding and risk assessment were discussed, but their resolution remains elusive.

EC/ANSES WORKSHOP, PARIS

- European workshop on thyroid disruption in Paris in March 2017
- The workshop objectives were:
  - To address and discuss interpretations of experimental laboratory studies, wildlife field data as well as human epidemiological data in relation to the identification of thyroid disrupting substances; and
  - To identify ways forward in addressing potential gaps in the test methods in relation to identification of thyroid disrupting substances
- Workshop paper “A. Kortenkamp, O. Martin, A. Baynes, E. Silva, M. Axelstad, U. Hass, Supporting the Organisation of a Workshop on Thyroid Disruption – Final Report, DG Environment, European Commission, 2017”.

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EC/ANSES WORKSHOP, PARIS

In conclusion, altered TH and TSH levels cannot be seen as the only definitive marker of thyroid hormone action, or of thyroid disruption.

These insights should have significant consequences for the interpretation of data in the context of identifying a substance as thyroid disruptor (and by extension, as an endocrine disruptor). In order to properly identify all thyroid disrupting chemicals, information in chemical dossiers should be supplemented by data on down-stream effects of an adverse nature, such as effects on the developing brain, or on other TH target organs.

However, the current test guidelines in the OECD Conceptual Framework for endocrine disruptors (e.g. TG 421/422, 426, 443) lack such parameters indicative of down-stream adverse effects diagnostic of thyroid disruption. This hampers the reliable identification of substances as thyroid disruptors.

Important perspectives for improving test guidelines are the inclusion of endpoints for the identification of adverse effects on brain function and brain morphology in TG 421/422, 426 and 443, since traditionally used behavioural assays may not be sensitive enough to identify all thyroid disrupting chemicals. Also necessary is the inclusion of endpoints representative of TH action at the cellular level, *e.g.* nerve cell migration and differentiation, or altered expression of TH-dependent genes. Some of these gaps can be filled by including standard endpoints in existing test guidelines (e.g. by making determinations of T4 levels mandatory in TG 407, 408), others require the implementation of new endpoints as well as research and development efforts.

EC WORKSHOP, BRUSSELS

- Workshop in Brussels in May 2017
- Workshop paper “Setting priorities for further development and validation of test methods and testing approaches for evaluating endocrine disruptors, 2018.”

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EC WORKSHOP, BRUSSELS

At the workshop, a number of concerns for diseases/conditions potentially related to exposure to endocrine disrupting substances with respect to both human and environmental health were discussed. Although all of the adverse outcomes were recognised as being important, the human and environmental health groups were able to come to a general consensus about the diseases/conditions currently of highest priority.

For human health, this was those related to thyroid-related developmental neurotoxicity, metabolic dysfunction, female reproduction and male reproduction. For environmental health, this was those related to reproductive health, growth and development.

At the workshop, a range of ideas for addressing gaps in available test guidelines to better identify substances that may contribute to the etiology of diseases or conditions related to endocrine disruption were also discussed. These ideas could be broadly categorised into five main themes:

- Theme 1: The development of more *in vitro* assays,
- Theme 2: Enhancing the current test guidelines by refining them and performing additional measurements/analyses,
- Theme 3: The development of new and appropriate *in vivo* methods,
- Theme 4: Modifications of regulatory data requirements,
- Theme 5: More research.

Bundesinstitut für Risikobewertung

BfR meeting

- International expert hearing in 2017 in Berlin
- Publication (accepted March 14 2019)

Report from the BfR expert hearing on practicability of hormonal measurements: recommendations for experimental design of toxicological studies with integrated hormonal end points

Olena Kucheryavenko[1] · Glenn Lurman[1] · Anja Lehmann[1] · Juliana Braz[1,3] · Lars Niemann[1] · Ibrahim Chahoud[4] · Alberto Mantovani[5] · Helen Håkansson[6] · Steffen Schneider[7] · Volker Strauss[7] · Pragati S. Coder[8] · Alexius Freyberger[9] · John C. O'Connor[10] · Martina Rauch[2,14] · Kostja Renko[4] · Marize L. M. Solano[1,11] · Niklas Andersson[12] · Olivier Blanck[13] · Vera Ritz[1] · Roland Solecki[1]

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Bundesinstitut für Risikobewertung

BfR meeting

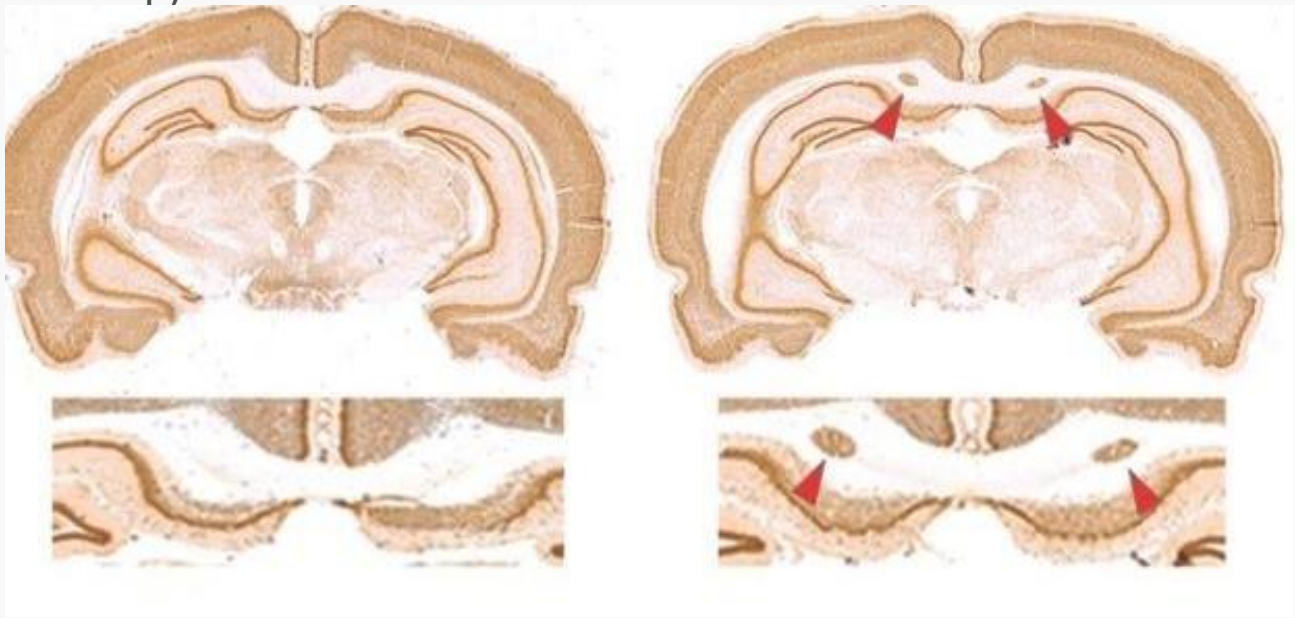
Abstract

This publication summarizes discussions that were held during an international expert hearing organized by the German Federal Institute for Risk Assessment (BfR) in Berlin, Germany, in October 2017. The expert hearing was dedicated to providing practical guidance for the measurement of circulating hormones in regulatory toxicology studies. Adequate measurements of circulating hormones have become more important given the regulatory requirement to assess the potential for endocrine disrupting properties for all substances covered by the plant protection products and biocidal products regulations in the European Union (EU). The main focus was the hypothalamus–pituitary–thyroid axis (HPT) and the hypothalamus–pituitary–gonadal axis (HPG). Insulin, insulin-like growth factor 1 (IGF-1), parathyroid hormone (PTH) and vitamins A and D were also discussed. During the hearing, the experts agreed on specific recommendations for design, conduct and evaluation of acceptability of studies measuring thyroid hormones, thyroid stimulating hormone and reproductive hormones as well as provided some recommendations for insulin and IGF-1. Experts concluded that hormonal measurements as part of the test guidelines (TGs) of the Organisation for Economic Co-operation and Development (OECD) were necessary on the condition that quality criteria to guarantee reliability and reproducibility of measurements are adhered to. Inclusion of the female reproductive hormones in OECD TGs was not recommended unless the design of the study was modified to appropriately measure hormone concentrations. The current report aims at promoting standardization of the experimental designs of hormonal assays to allow their integration in OECD TGs and highlights research needs for better identification of endocrine disruptors using hormone measurements.

EC PROJECT

Thyroid feasibility study

- The objective of this project is to develop study protocols for thyroid disruptor testing in the mammalian system to improve the identification of substances disturbing the thyroid system, either by enhancing already existing test guidelines and/or by developing a new test guideline.
- Project Leader is Brunel University (Andreas Kortenkamp)
- First meeting in April 2018
  Deadline final report in June 2019
- Current focus on cortical heterotopia*



* Correct neuronal migration is dependent on TH supply;
TH insufficiency leads to defective migration, with clusters of neurons occurring in untypical places (heterotopia)

ESTP

6th ESTP International Expert Workshop:
"Adversity of Thyroid Follicular Epithelial Hypertrophy/Hyperplasia, Differentiation between direct and indirect mechanisms and their implication for risk assessment"

- Primary goals:
  - Summarize common mechanisms of TFEH/H and present example cases for direct and indirect modes of action;
  - Discuss adversity considerations for TFEH/H, species differences/human relevance, and implications for classification as endocrine disrupting compound;
  - Propose information requirements to support review and interpretation of TFEH/H with examples of information/testing strategies.
- F2F May 16-17th 2018 in Berlin
- Monthly preparatory teleconferences before and after workshop
- 27 international experts and members of the ESTP
- Publication planned for Tox. Path., with endorsement by major national and international societies
- Planned Posters - at STP and JSTP meetings

ECETOC – SPECIAL T4 TASK FORCE

European Centre For Ecotoxicology and toxicology of Chemicals

- In Summer 2018, ECETOC convened a 'Special T4 Task Force'.
- It is the main objective of this Task Force to serve to overcome the scientific uncertainty triggered by the EFSA and ECHA Guidance with regard to the assessment of thyroid disruption.

In pursuing this main objective, the ECETOC Special Task Force T4 is engaged in the following activities:

1. Collate epidemiological evidence for how serum thyroid hormone changes lead to neurodevelopmental impairment in humans;
2. Establish how (a) rat thyroid histopathological findings and thyroid hormone changes; and (b) rat thyroid hormone changes and neurodevelopmental changes are correlated;
3. Identify and compare the specific steps of the pathways that lead to thyroid disruption in humans and rodents.

For each of these topics, the ECETOC Special T4 Task Force is considering the current understanding of endocrine biology and ongoing related research work. The outcome of the Task Force work, that will be published as ECETOC Report, shall contribute to the establishment of specific guidance on to reliably predict if a chemical has the potential to disrupt the thyroid hormone system in humans.

Workshop expected in Q4 2019.

CEFIC LRI

(Long-range Research Initiative)

- Species comparison in liver-mediated thyroid and thyroid-related toxicities.
- Project Leader is RIVM (Aldert Piersma)
- In vivo phase of Part 1 will run in Q3 2019.

Project Structure

Part 1 of this project will concentrate on characterizing dose-response profiles for liver enzyme induction, thyroid hormones, and thyroid-related effects, in the rat model for model compounds known to cause thyroid toxicity through a secondary, hepatic, mode of action.

Part 2 will develop a model (or models), using data generated in Part 1 of the project, to facilitate translation of outcomes in rodent toxicity studies to likely effects in humans accounting for species differences or similarities in this liver mediated thyroid adverse outcome pathways (AOPs).

HORIZON 2020

The EU Framework Programme for Research and Innovation

Cluster for endocrine disruption

ATHENA	**Assays for the identification of Thyroid Hormone axis-disrupting chemicals: Elaborating Novel Assessment strategies**
GOLIATH	Beating Goliath: Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds
FREIA	Female Reproductive toxicity of EDCs: a human evidence-based screening and Identification Approach
SCREENED	A multistage model of thyroid gland function for screening endocrine-disrupting chemicals in a biologically sex-specific manner
ENDpoiNTs	Novel Testing Strategies for Endocrine Disruptors in the Context of Developmental NeuroToxicity
OBERON	An integrative strategy of testing systems for identification of EDs related to metabolic disorders
ERGO	Breaking down the wall between human health and environmental testing of endocrine disrupters: EndocRine Guideline Optimisation
EDCMET	Metabolic effects of Endocrine Disrupting Chemicals: novel testing METhods and adverse outcome pathways

HORIZON 2020 ATHENA

The EU Framework Programme for Research and Innovation

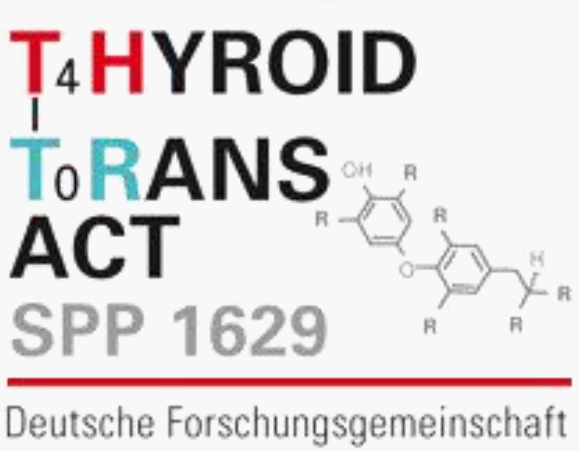
- **A**ssays for the identification of **T**hyroid **H**ormone axis-disrupting chemicals: **E**laborating **N**ovel **A**ssessment strategies
- The ATHENA consortium aims to mobilize the scientific progress made in recent years to close critical gaps left open in test methods for thyroid hormone axis disrupting-chemicals.
- They will develop new methods for incorporation into existing OECD test guidelines that can capture the consequences of maternal thyroid hormone deficiency on the developing brain, due to disruption of delivery of thyroid hormones to the fetus.
- Project leader is Brunel University (Andreas Kortenkamp)
- 2019 - 2023

THYROID TRANS ACT

Translation of Thyroid Hormone Actions beyond Classical Concepts.

THYROID TRANS ACT is a scientific program which targets TH action beyond the classical concepts by asking the central question: What defines healthy and diseased thyroid function?

Priority project of German group (Deutsche Forschungsgemeinschaft).



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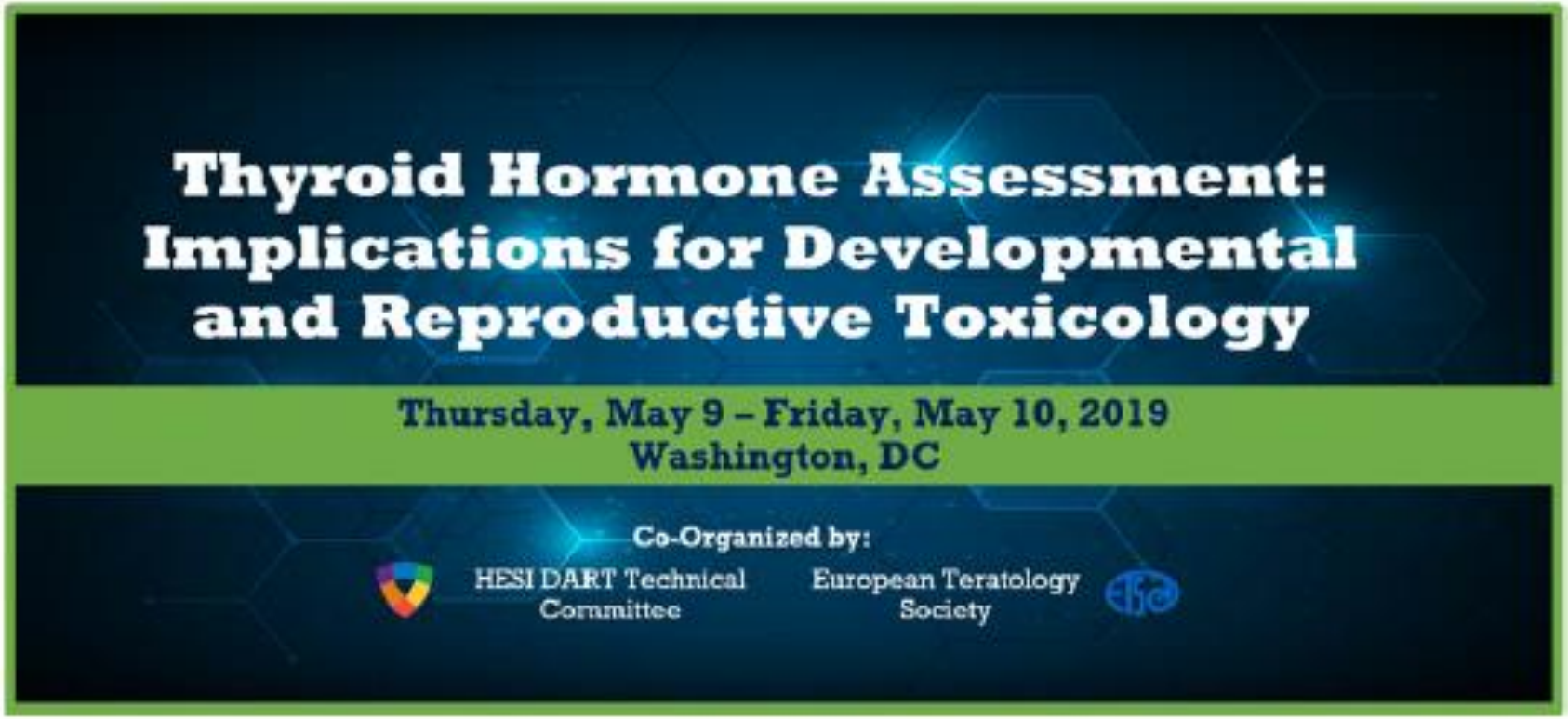
HESI DART – ETS TASK FORCE

HESI DART – ETS Task Force

- Survey (2018)
- Current workshop (2019)



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