The "*in vitro* comparative metabolism project" in the pesticide regulatory context

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General background on pesticide regulatory assessment in EU

General background

- The toxicological data-package included:
  - Toxicity studies with rat, mice, dogs, rabbits
  - ADME*, in vivo* metabolism at least in the rats, but not human *in vivo* data

Why are we here?

Two main purposes:

1. To identify human metabolite not properly assessed with laboratory animals.

2. To assess human relevance of toxicological animal data.

The first purpose:

Human metabolites

The EU Legal framework:

- The relevance of generating toxicity data in animal models with **dissimilar metabolic profile** to those found in **humans** shall be addressed, if such metabolic information is available, and taken into consideration for study design and risk assessment
- *"**Comparative in vitro metabolism studies** shall be performed on animal species to be used in pivotal studies and on **human** material (microsomes or intact cell systems) in order to determine the **relevance of the toxicological animal data** and to **guide in the interpretation of findings and in further definition of the testing strategy**"* (Regulation EU 283/2013, OJ: L93/22)
- An **explanation** shall be given or **further tests** shall be carried out where a **metabolite is detected in vitro in human material** and not in the tested animal species.

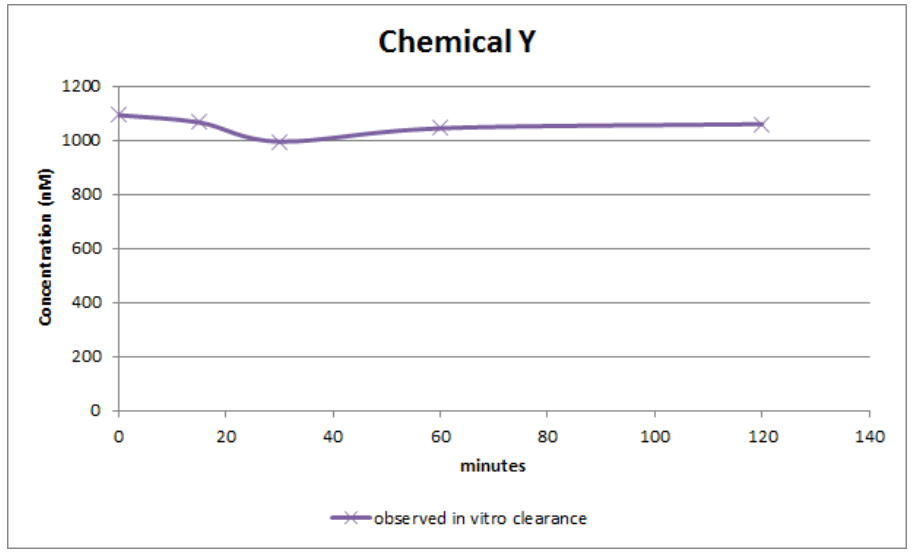
http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32013R0283

Introduction

- These kind of studies are routinely performed for human drugs; no OECD guideline
- If submitted, majority of cases:

1. Microsomes
2. Human and rat only
3. Metabolite profiling

Theoretical example to illustrate the need for guidance



No metabolism observed.

There are 2 possibilities:

The chemical is not metabolised at all

Metabolism is not observed because technical limitations of the method (e.g chemical not soluble in cell medium, cells not viable, limited incubation time, enzymes not expressed, etc.)

Key issues:

- What should be the criteria for selecting the most appropriate test system according what we know about the specific active substance?
- What are the advantages and disadvantages of using the different test system?
- What are the species to be included?
- What are the minimum criteria for the conduction, acceptance and interpretation of the studies?
- What is the most appropriate analytical method?
- How to deal with quantitative differences?
- Finally to define the term unique human metabolite.

EU Regulatory actions:

- Further assessment needed, strategy to be developed, **out of the scope** for this workshop

The second purpose:

Human relevance

2° Purpose: Background

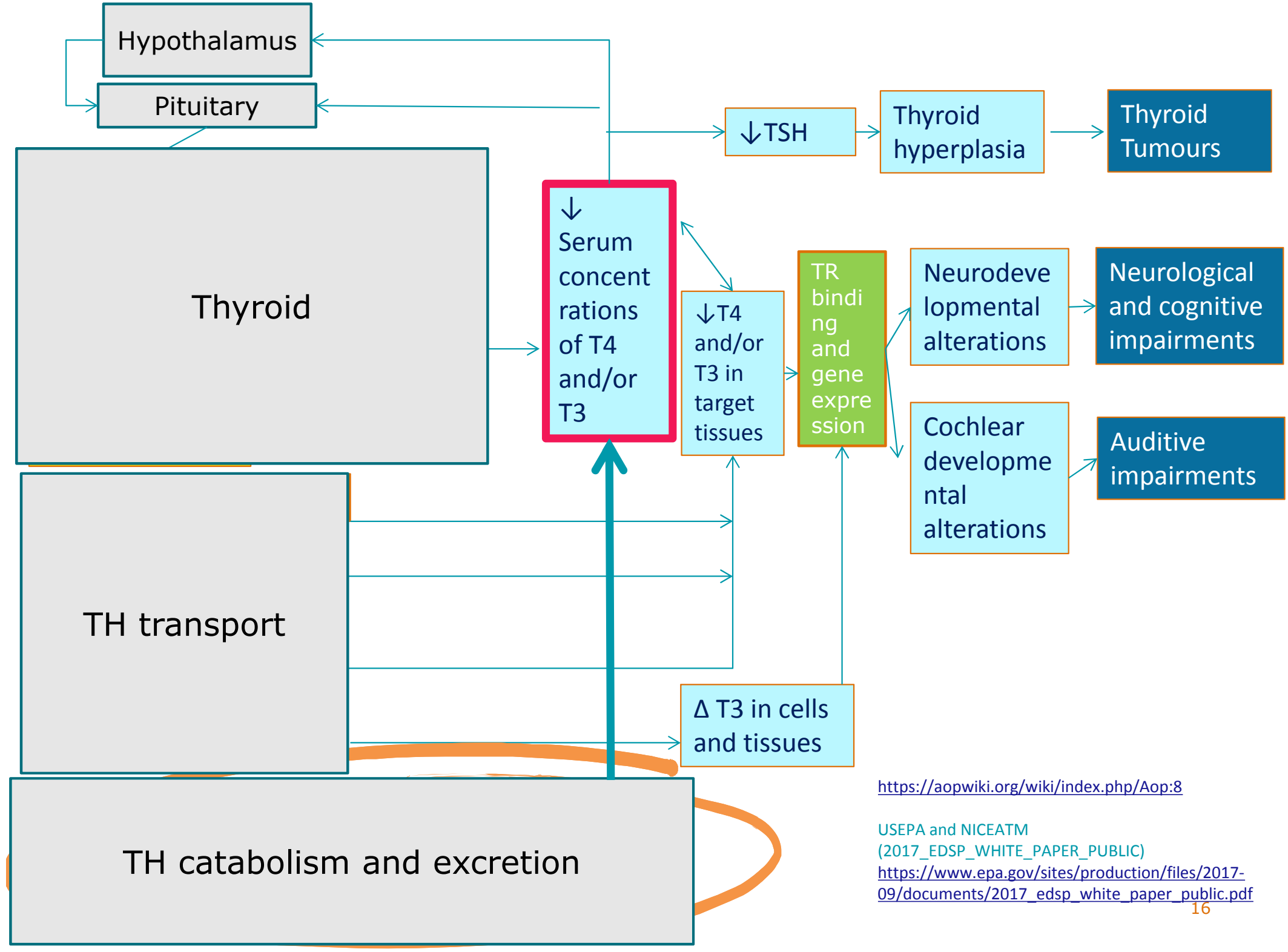
- This kind of studies have been submitted in the past as part of weight of evidence for excluding human relevance for rat and mice tumours
- Human relevance of thyroid effects secondary to liver enzyme induction
- Cut-off criteria and ECHA/EFSA GD on endocrine disruptors (appendix A)
- https://www.efsa.europa.eu/it/efsajournal/pub/5311

The AOPs for THs disruption

- Molecular and cellular processes associated with thyroid homeostasis are known to be altered by xenobiotics and include: hypothalamic and pituitary feedback control; iodine transport and syntheses of thyroid hormones in the thyroid; serum transport of THs; cellular uptake and metabolism of THs; activation of thyroid hormone receptors; and catabolism and excretion of THs.
- The molecular targets for these processes, as well as downstream consequences, can be represented as adverse outcome pathways (AOPs).

- When the experimental data from in vivo toxicity studies indicated that one of the potential key events in one of these thyroid-relevant AOPs involves increased TH catabolism via liver enzyme induction, it is acknowledged that given the higher sensitivity of the rat, human relevance ***might*** be excluded and this should be experimentally demonstrated.

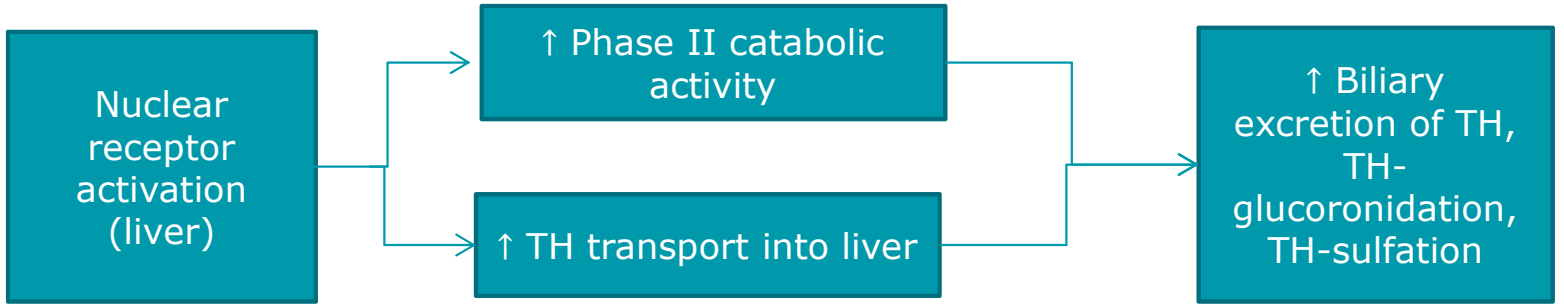
USEPA and NICEATM (2017_EDSP_WHITE_PAPER_PUBLIC)
https://www.epa.gov/sites/production/files/2017-09/documents/2017_edsp_white_paper_public.pdf



https://aopwiki.org/wiki/index.php/Aop:8

USEPA and NICEATM (2017_EDSP_WHITE_PAPER_PUBLIC) https://www.epa.gov/sites/production/files/2017-09/documents/2017_edsp_white_paper_public.pdf

Key issues:



What key events should be measured and how they should be measured?

- Nuclear receptor activation: binding/transactivation?
- Phase I induction: CYP induction
- Phase II induction: UDPGT activity
- Overall thyroid clearance: i.e. T3, T4-glucoronidation, T4-sulfation

What test system should be used?

- Fresh or Cryopreserved hepatocytes/Line cells (HepaRG)
- How to evaluate induction?
- Qualitative
- Quantitative (categorisation)

Overall aim of the workshop

Outcome of the workshop

- Foundation stone for developing further **EFSA guidance** for conduction and interpretation of *in vitro* comparative metabolism studies (in close collaboration with JRC, OECD and ECHA)
- Pave the way for a **common understanding** on how to evaluate liver mediated effects in the area of endocrine disruption, in this case thyroid effects when using *in vitro* comparative metabolism studies and within a weight of evidence approach

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