An AOP Network for Thyroid Hormone Disruption and Adverse Outcomes

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Outline

1. **AOP and AOP Networks**
   - What are they?

2. **Thyroid AOP Network**
   - What's is it good for?

3. **Issues and Challenges for the Future**

Adverse Outcome Pathways (AOPs)

- The OECD's Adverse Outcome Pathway (AOP) concept provides a framework for assembling information on how biological pathways can be perturbed by chemical stressors (see Ankley et al. Environ Toxicol Chem 29:730-41, 2010)
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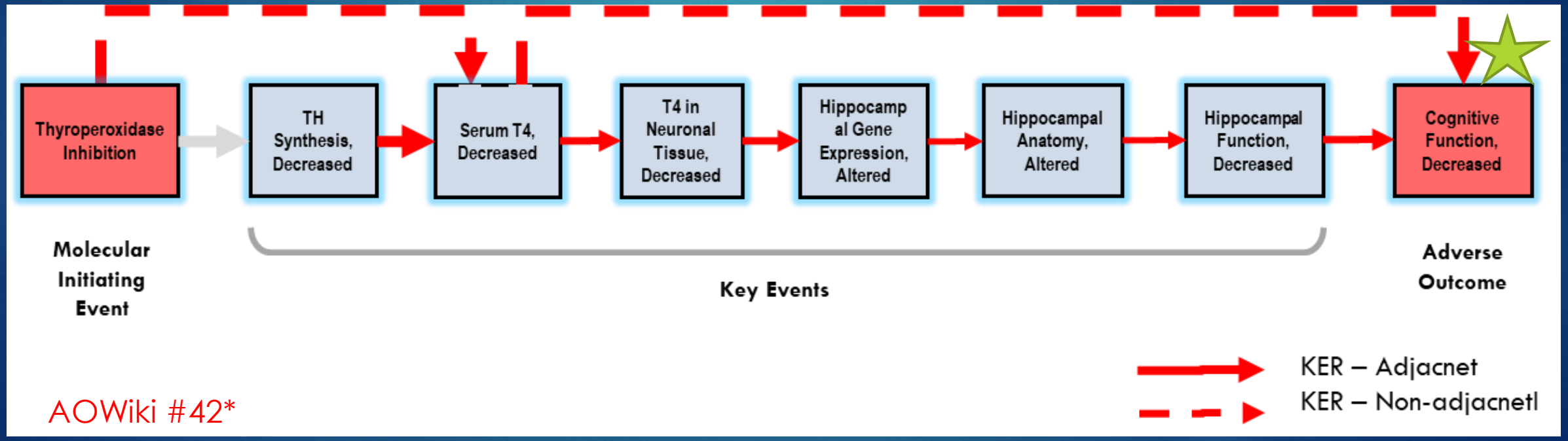
Example – Thyroperoxidase and Neurodevelopment



AOWiki #42*

*https://aopwiki.org/aops/42

Thyroid AOPs
Currently Under Development on the AOPWiki

- **Currently has 19 AOPs that involve thyroid hormones**
- **MIEs include**
  - Sodium iodine transporter (NIS)
  - Thyroperoxidase (TPO)
    - Example of MIE that leads to multiple AOs
      - Frog metamorphosis
      - Fish reproduction
      - Mammalian neurodevelopment
      - Rat thyroid follicular tumors
  - Transthyretin
  - Hepatic nuclear receptors
  - Type I, II and III iodothyronine deiodinases
  - Iodotyrosine deiodinase (IYD)

AOP Networks

- **Note that biology is not 'linear' – there are interactions and feedback between cells and tissues**
  - AOP Networks are one way to move from linear thinking to better model biology

- **AOP networks**
  - defined as an assembly of two or more AOPs that share one or more key events *(see Villeneuve et al. Environ Toxicol Chem, 37:1734-48, 2018)*
  - should more realistically represent interactions of normal biological systems and thus the potential effects of chemical stressors.

Conceptual Framework for AOP Networks

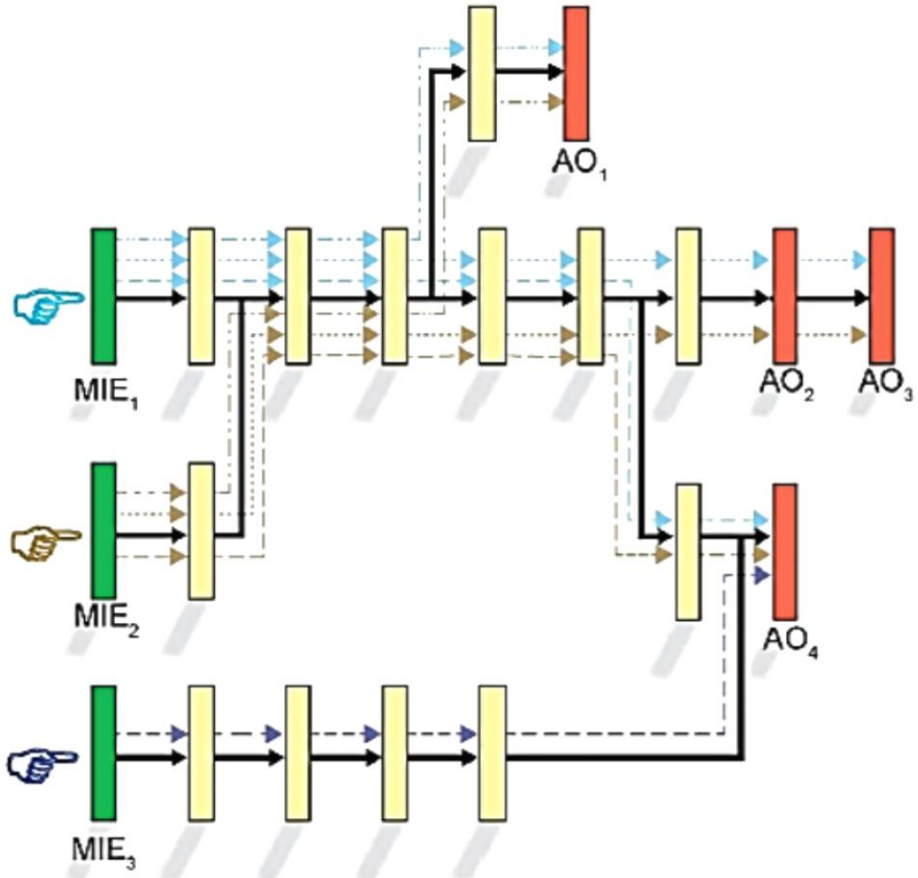
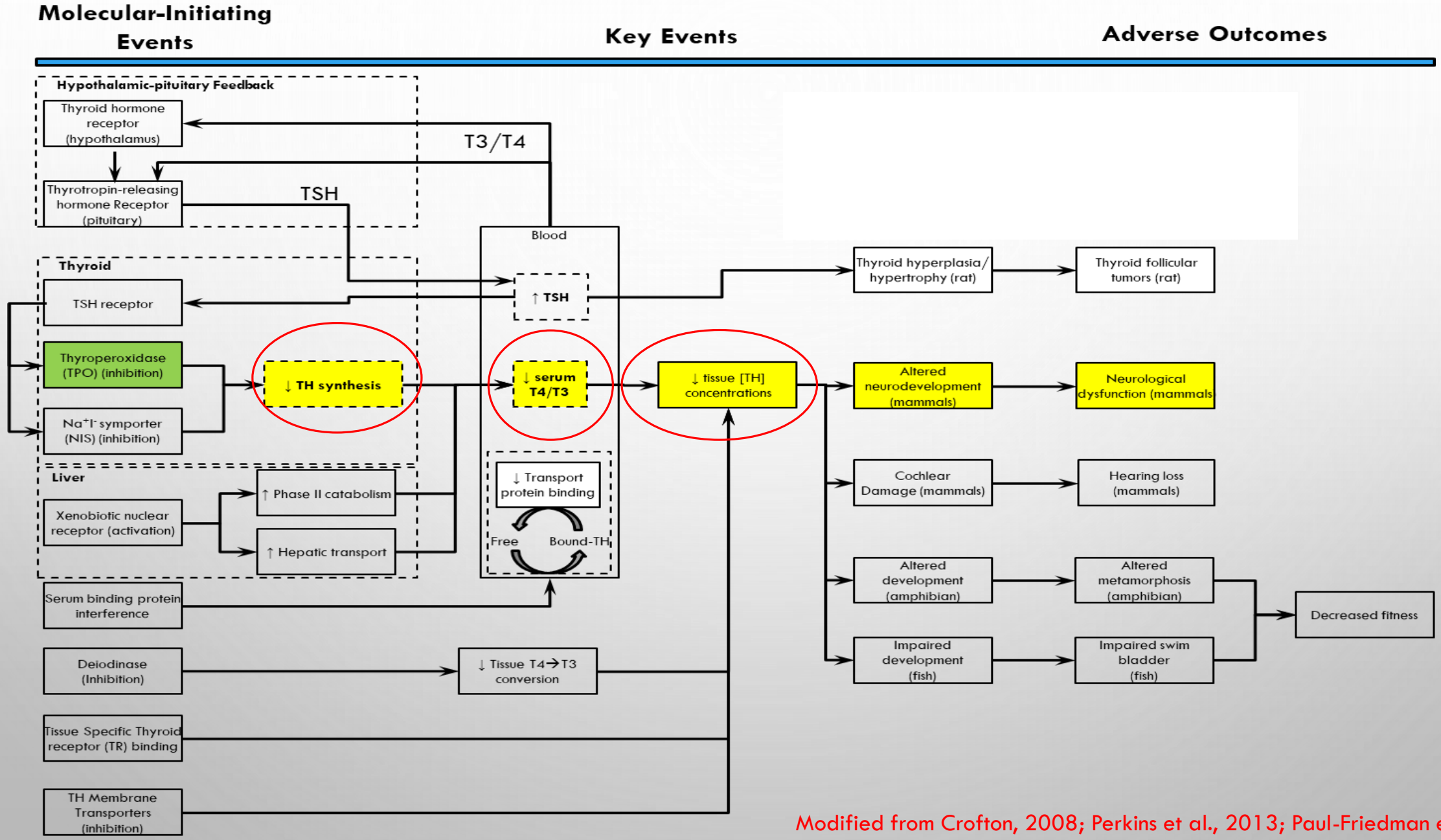


Figure 2. Conceptual representation of an AOP network of seven AOPs. AOP_1 linking MIE_1 to AO_1. AOP_2 linking MIE_1 to AO_3. AOP_3 linking MIE_2 to AO_1. AOP_4 linking MIE_2 to AO_3. AOP_5 linking MIE_1 to AO_4. AOP_6 linking MIE_2 to AO_4. AOP_7 linking MIE_3 to AO_4.

Courtesy of Dan Villeneuve

A Thyroid AOP Network



Modified from Crofton, 2008; Perkins et al., 2013; Paul-Friedman et al. 2016

So What is a Thyroid AOP Network Good For?

1) Identification of MIEs important for systems with common KEs and AOs (e.g., Thyroid, Perkins et al., 2013)

- Used to identify MIEs and prioritize for assay development

  (upcoming talk by Mike Hornung)

2) Should more realistically represent interactions of normal biological systems and thus the potential effects of chemical stressors

- Can incorporate feedback systems (e.g., Inflammation, Villeneuve et al. 2018)
- Need to incorporation compensatory processes (e.g., DI 2 upregulation in brain)

3) Allows identification of relevant MIEs, KEs and AOs across Taxa (e.g., cross species)

4) Theoretical future use

- Theoretically could be used to cumulate hazard for co-exposure to chemicals that hit multiple MIEs with common AO

MIE Identification for Assay Development

Table from a 2011 workshop that developed a "mechanism based testing strategy" for *in vitro* assays (Murk et al., 2013)

- Identifies MIEs (e.g., TR, TPO, NIS) and relevance to thyroid pathways
- Provides status of current technologies (low, med, high throughput)

This was followed by a 2014 OECD Scoping Document for thyroid modulators (OECD, 2014)

Both informed EPA research program to prioritize and develop HTS assays

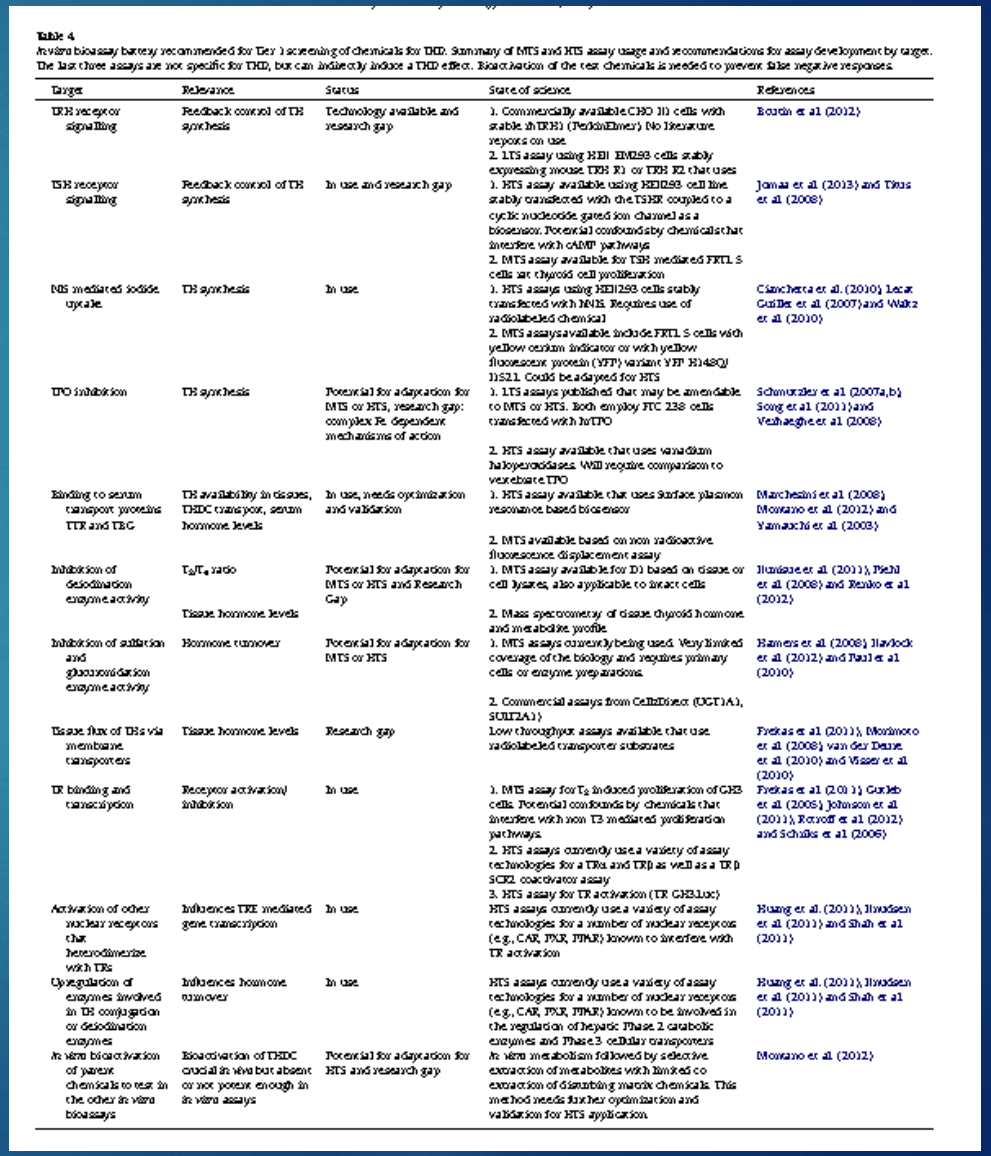
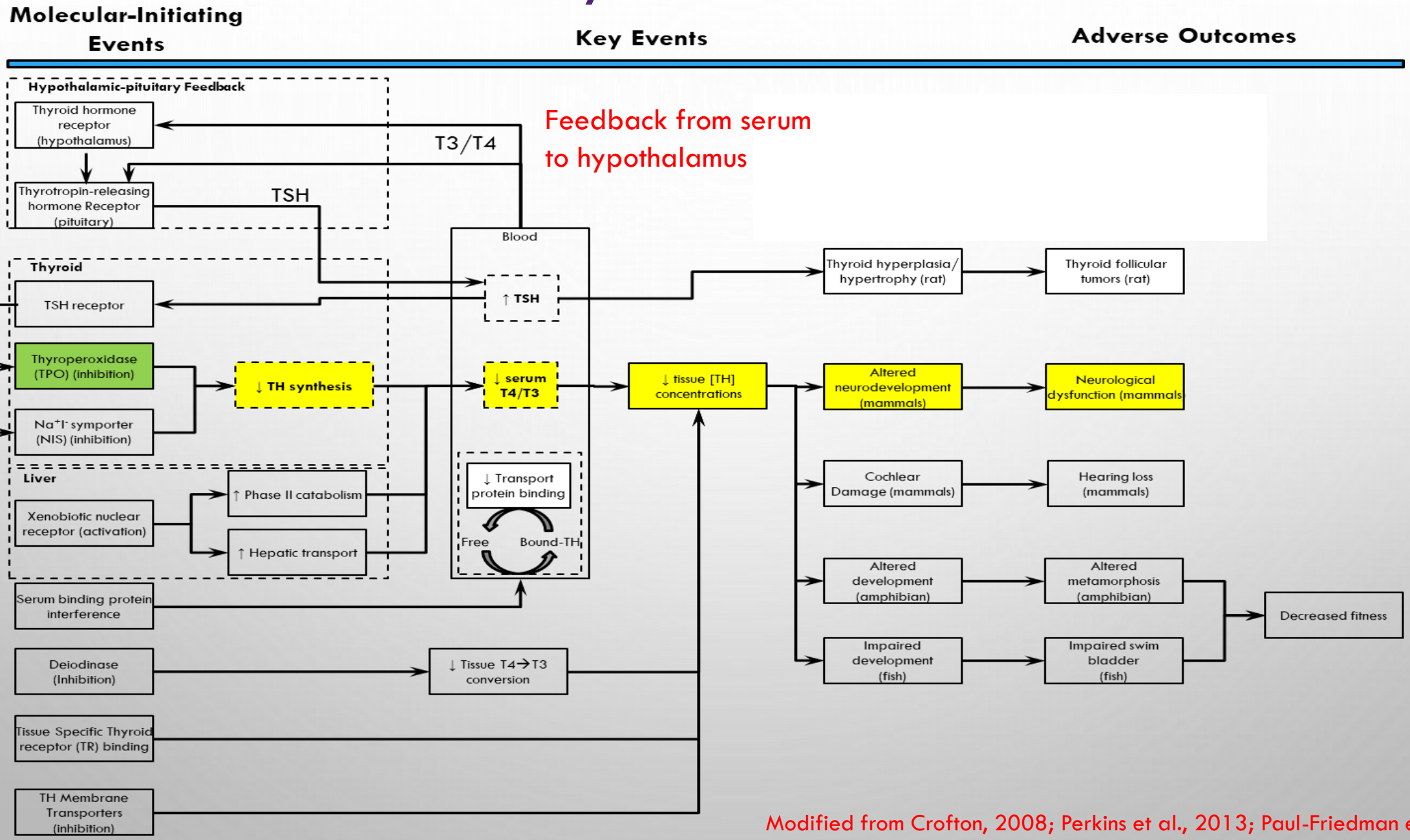


Table 4
In vitro bioassay battery recommended for Tier 1 screening of chemicals for THD. Summary of MTS and HTS assay usage and recommendations for assay development by target. The last three assays are not specific for THD, but can indirectly induce a THD effect. Bioactivation of the test chemicals is needed to prevent false negative responses.

Target	Relevance	Status	State of science	References
TRH receptor signalling	Feedback control of TH synthesis	Technology available and research gap	1. Commercially available CHO 10 cells with stable hTRH1 (PerkinElmer) No literature reports on use. 2. LTS assay using HEK HM293 cells stably expressing mouse TRH R1 or TRH R2 that uses	Bourdin et al (2012)
TSH receptor signalling	Feedback control of TH synthesis	In use and research gap	1. MTS assay available using HEK293 cell line stably transfected with the TSHR coupled to a cyclic nucleotide gated ion channel as a biosensor. Potential confounds by chemicals that interfere with cAMP pathways 2. MTS assay available for TSH mediated FRTL 5 cells for thyroid cell proliferation	Jomaa et al (2013) and Titus et al (2008)
NIS mediated iodide uptake	TH synthesis	In use	1. MTS assays using HEK293 cells stably transfected with NIS. Requires use of radiolabeled chemical 2. MTS assays available include FRTL 5 cells with yellow cerium indicator or with yellow fluorescent protein (YFP) variant YFP H148Q/I152L Could be adapted for HTS	Cánchera et al. (2010) Lecat Guillet et al (2007) and Waltz et al (2010)
TPO inhibition	TH synthesis	Potential for adaptation for MTS or HTS, research gap: complex Fe dependent mechanisms of action	1. LTS assays published that may be amendable to MTS or HTS. Both employ FTC 238 cells transfected with hTPO 2. MTS assay available that uses vanadium haloperoxidases. Will require comparison to vertebrate TPO	Schmutzler et al (2007a,b) Song et al (2011) and Vanhaecke et al (2008)
Binding to serum transport proteins TTR and TBG	TH availability in tissues, THDC transport, serum hormone levels	In use, needs optimization and validation	1. MTS assay available that uses Surface plasmon resonance based biosensor 2. MTS available based on non radioactive fluorescence displacement assay	Marchesini et al (2008) Mowaino et al (2012) and Yamauchi et al (2003)
Inhibition of detoxification enzyme activity	T_3/T_4 ratio Tissue hormone levels	Potential for adaptation for MTS or HTS and Research Gap	1. MTS assay available for D1 based on tissue or cell lysates, also applicable to intact cells 2. Mass spectrometry of tissue thyroid hormone and metabolite profile	Hamase et al. (2011), Piehl et al (2008) and Renko et al (2012)
Inhibition of sulfation and glucuronidation enzyme activity	Hormone turnover	Potential for adaptation for MTS or HTS	1. MTS assays currently being used. Very limited coverage of the biology and requires primary cells or enzyme preparations. 2. Commercial assays from CellzDirect (UGT1A1, SULT2A1)	Hamers et al (2006), Hallock et al (2012) and Paul et al (2010)
Tissue flux of THs via membrane transporters	Tissue hormone levels	Research gap	Low throughput assays available that use radiolabeled transporter substrates	Friesema et al (2001), Monimoto et al (2008), van der Deure et al (2010) and Visser et al (2010)
TR binding and transcription	Receptor activation/ inhibition	In use	1. MTS assay for T_3 induced proliferation of GH3 cells. Potential confounds by chemicals that interfere with non TR mediated proliferation pathways. 2. MTS assays currently use a variety of assay technologies for a TRα and TRβ as well as a TRβ SCRL coactivator assay 3. MTS assay for TR activation (TR GH3.Luc)	Friesema et al (2001), Gutleb et al (2005), Johnson et al (2011), Kovoff et al (2012) and Schultz et al (2006)
Activation of other nuclear receptors that heterodimerize with TRs	Influences TRE mediated gene transcription	In use	MTS assays currently use a variety of assay technologies for a number of nuclear receptors (e.g., CAR, PXR, PPAR) known to interfere with TR activation	Huang et al. (2011), Hamdsen et al (2011) and Shah et al (2011)
Upregulation of enzymes involved in TH conjugation or detoxification enzymes	Influences hormone turnover	In use	MTS assays currently use a variety of assay technologies for a number of nuclear receptors (e.g., CAR, PXR, PPAR) known to be involved in the regulation of hepatic Phase 2 catabolic enzymes and Phase 3 cellular transporters	Huang et al. (2011), Hamdsen et al (2011) and Shah et al (2011)
In vitro bioactivation of parent chemicals to test in the other *in vitro* bioassays	Bioactivation of THDC crucial *in vivo* but absent or not potent enough in *in vitro* assays	Potential for adaptation for HTS and research gap	*In vitro* metabolism followed by selective extraction of metabolites with limited co extraction of disturbing matrix chemicals. This method needs further optimization and validation for HTS application.	Mowaino et al. (2012)

Murk et al Toxicology In Vitro 27:1320-1346 (2013)
OECD, ENV/JM/MONO (2104)23

A "Thyroid" AOP Network

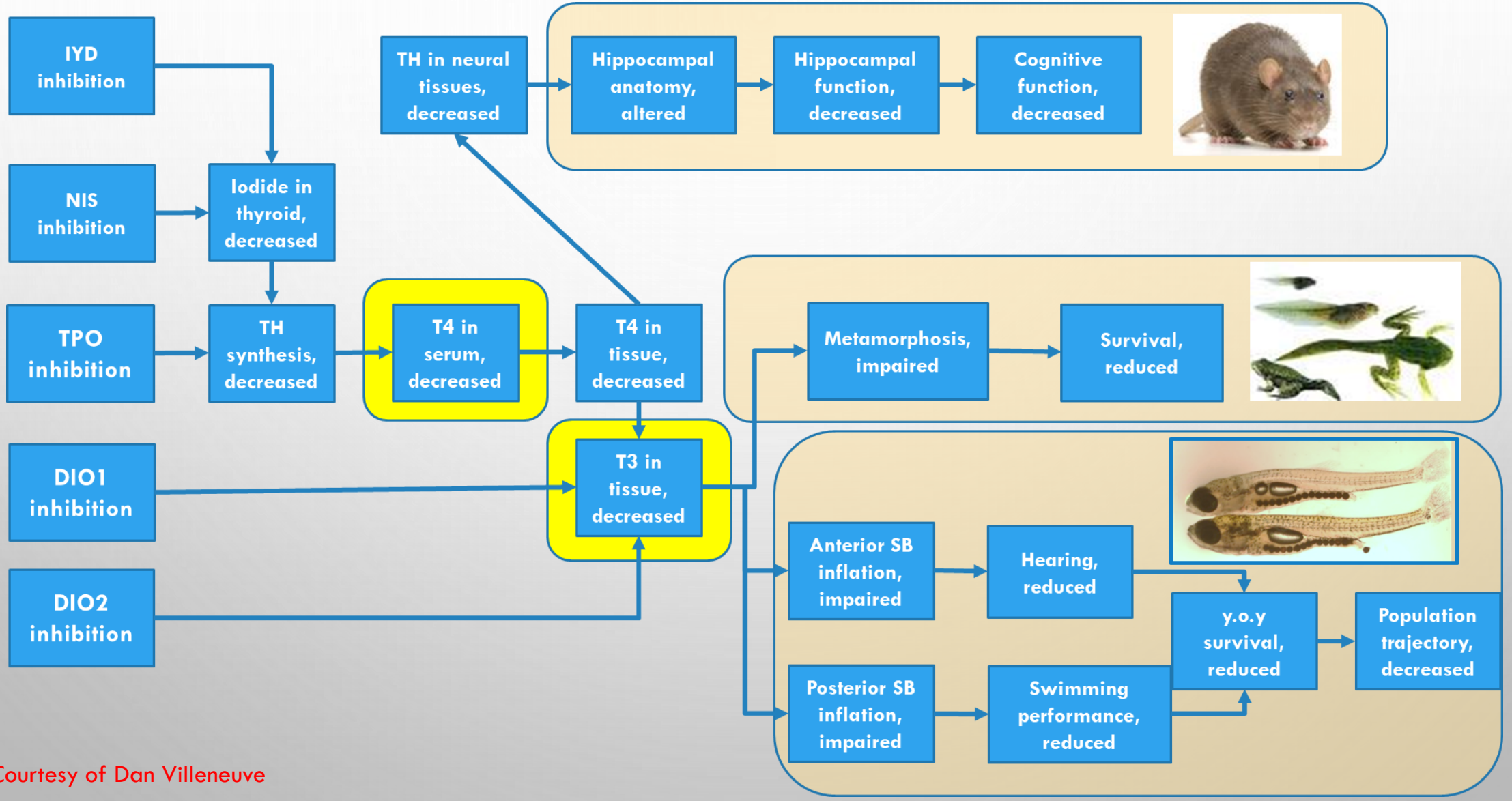


Feedback from serum to hypothalamus

Modified from Crofton, 2008; Perkins et al., 2013; Paul-Friedman et al. 2016

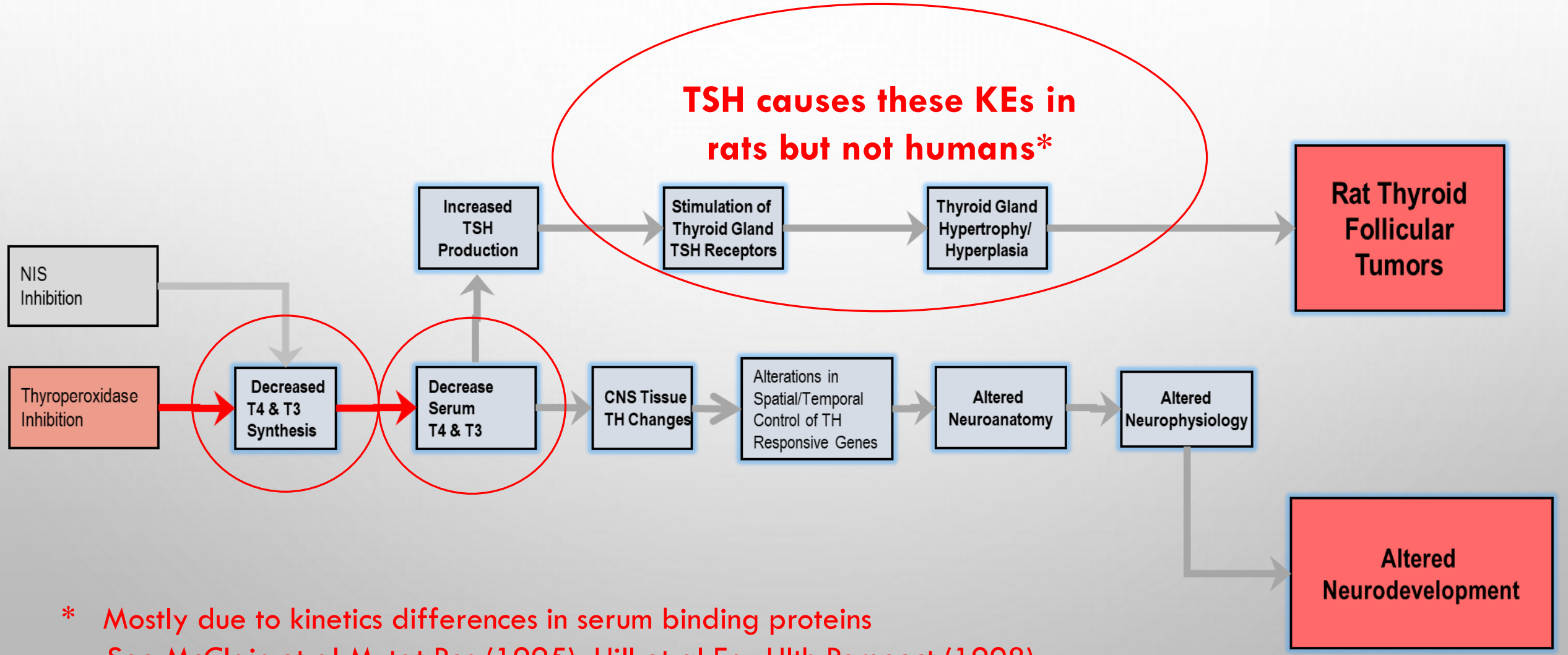
Relevant Hazards Across Taxa

Mammals, Amphibians and Fish



Courtesy of Dan Villeneuve

Lack of Human Relevance for A Rodent AO Due to Different KEs



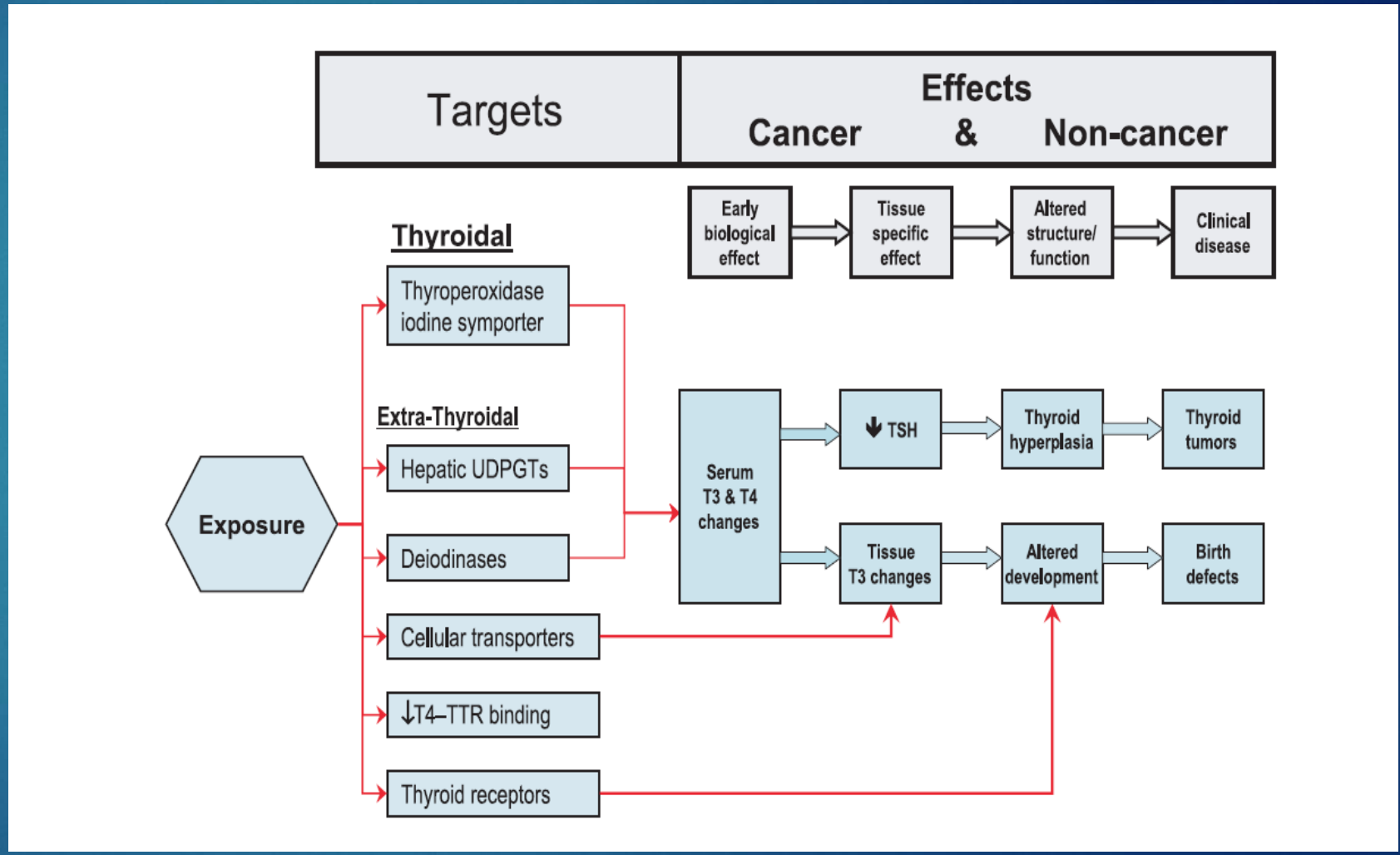
* Mostly due to kinetics differences in serum binding proteins
See McClain et al Mutat Res (1995); Hill et al Env Hlth Perspect (1998)

Cumulate Hazard?

Report from 2007 4th Copenhagen Workshop on Endocrine Disrupters

- Outlined inactions of some thyroid MOA pathways
- Major uncertainty identified as need for models *"to better predict effects of mixtures containing xenobiotics that affect multiple targets with common downstream effects"*

- Difficult problem and no real progress for thyroid disruptors



Crofton, Int J Androl. 2008

Thyroid AOPs and AOP Networks

Some Issues and Challenges

- Crowd-sourcing AOP development
  - Need more input on current AOPWiki entries
  - Only one Thyroid AOP close to endorsement by OECD
  - Need to incorporate compensatory processes
  - Some pathways are not being worked on (e.g, TR, cellular transporters) and many are 'stalled' in early development

- Qualitative vs Quantitative
  - Most all AOPs are qualitative – describe the biology
  - Some work on quantification of MIE and early KEs for NIS AOP (Fisher and colleagues)

- Regulatory use and interpretation of HTS data
  - First application likely to be prioritization
  - Holy Grail is prediction of AO from QSAR or MIE data

- Mixtures
  - Still an unresolved issue10 years later…..

Thanks for Listening