Science For A Better Life

Using adverse outcome pathway analysis to identify gaps in high-throughput screening for thyroid disruption

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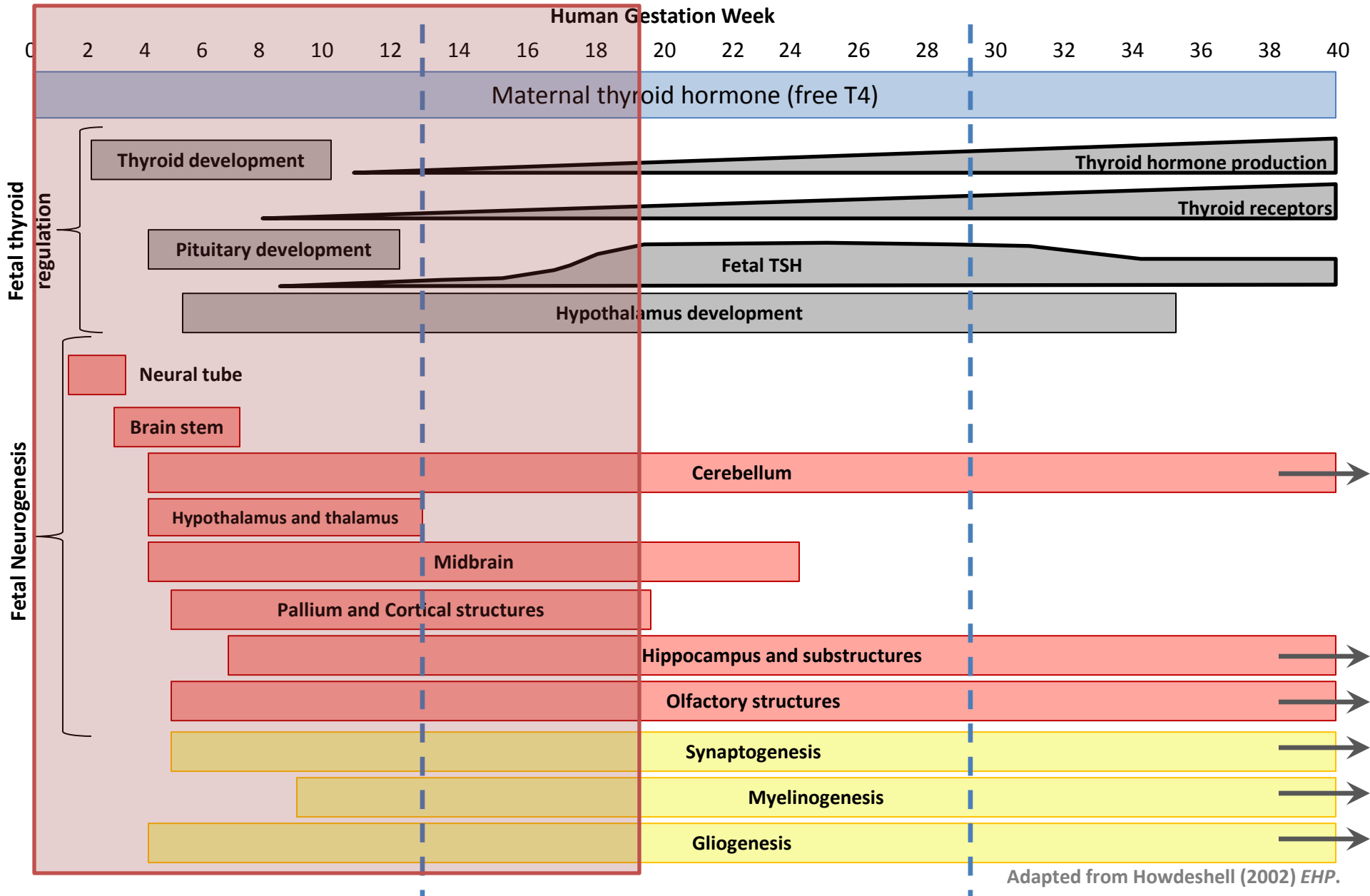
Disclaimer: The views herein are those of the speaker and do not represent Bayer CropScience or the US Environmental Protection Agency.

Objectives

- A view of the importance of thyroid hormone to neurodevelopment in rodents and humans.
- An adverse outcome pathway for thyroid hormone perturbation.
- A comparison of available rodent thyroid data in ToxRefDB versus the available high-throughput screening assay data in ToxCast.
- Opportunities for assay development.
- Current barriers to the use of AOPs in chemical screening.

Maternal thyroid hormones modulate fetal brain development; thus screening for developmental Δ[TH] may identify neurotoxicants.



Adapted from Howdeshell (2002) *EHP*.

Moderate maternal thyroid hormone disruption → Cognitive and brain abnormalities.

(Barone et al., 2000; Berbel et al. 2009; Cuevas et al., 2005; Howdeshell, 2002; Morreale de Escobar et al., 2000; Rice et al., 2000; Zoeller et al., 2000)

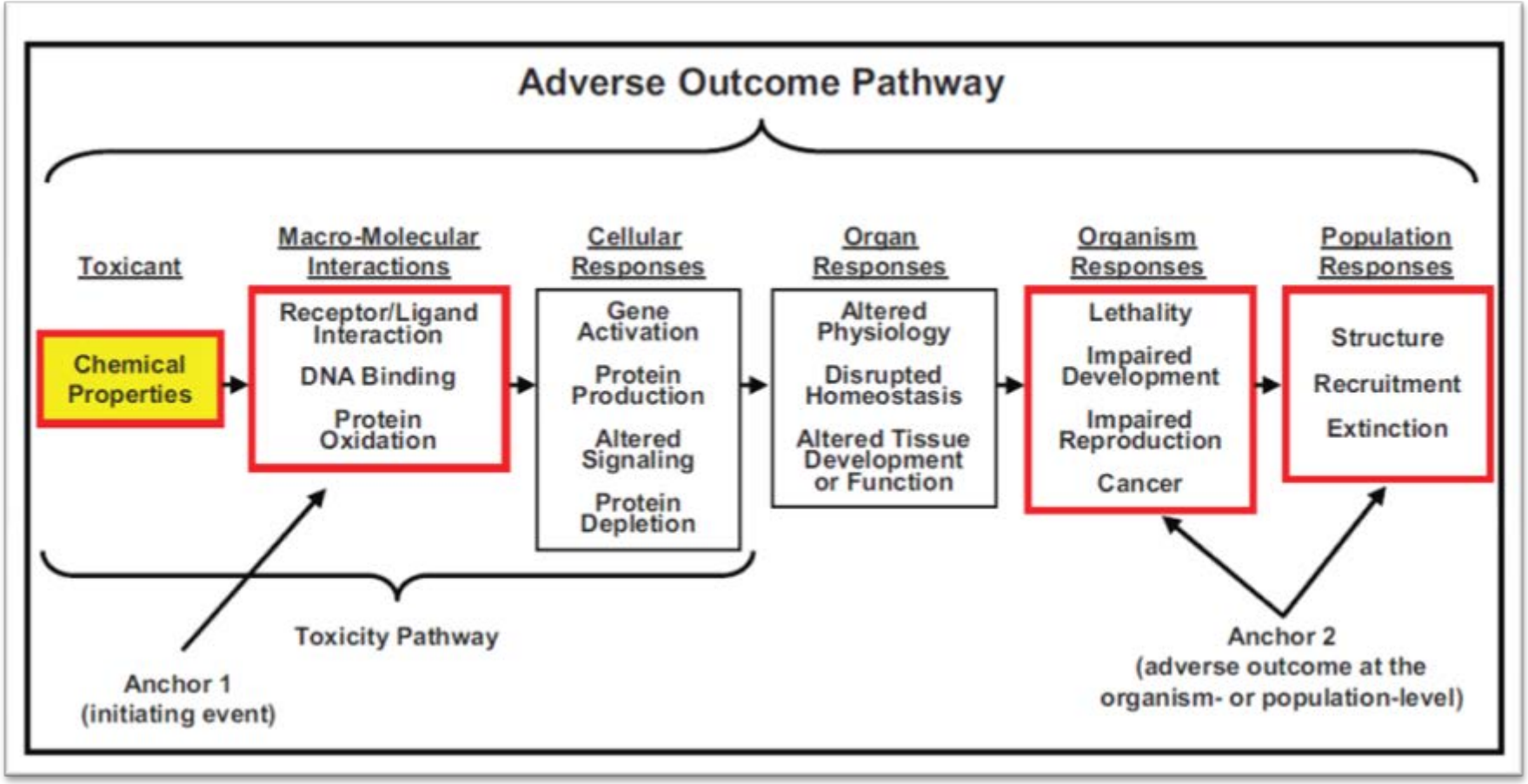
Human maternal hypothyroxinemia in the first trimester

- ↓ behavioral response @ **3 wks** (Kooistra et al. 2006);
- ↓ psychomotor development @ **10, 12, 24 mos** (Pop et al., 1999; Pop et al., 2003);
- ↓ motor coordination/socialization at **18 mos** (Berbel et al. 2009);
- Language delays at **18 and 30 mos** (Henrichs et al. 2010);
- Small IQ point ↓ at **7-9 yrs** (Haddow et al. 1999).

Rat models of maternal T4 insufficiency

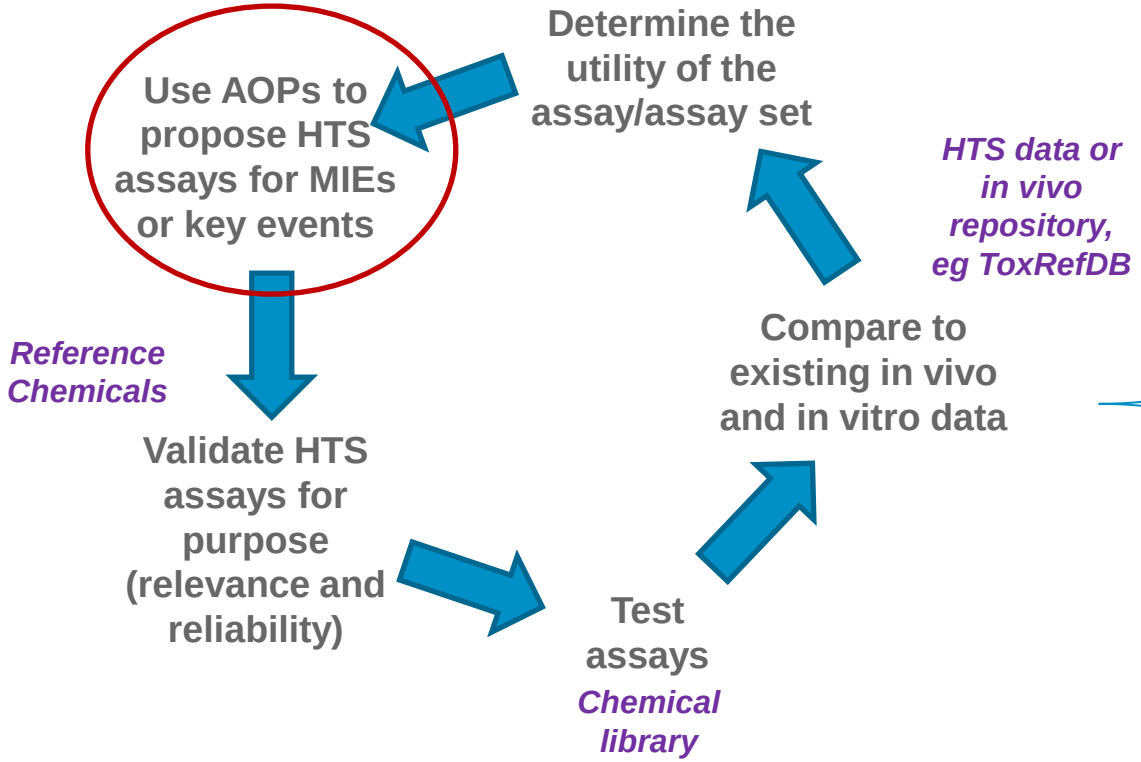
- Δ **cytoarchitecture** (Auso et al., 2004; Cuevas et al., 2005; Lavado-Autric et al., 2003; Sharlin et al. 2008);
- Δ **synaptic calcium regulation and myelination** (Ibarrola et al., 1997; Iniguez et al., 1996);
- **Gene expression Δ :synaptic calcium/transmission, myelination, and developmental cell adhesion** (Morreale de Escobar et al., 2008; Morreale de Escobar et al., 2000; Morreale de Escobar et al., 2004, Royland et al.,2008).

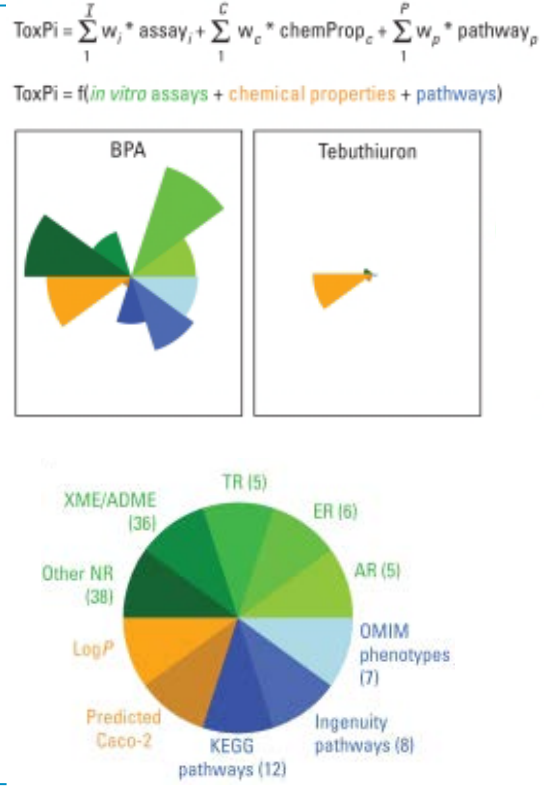
AOPs provide a framework to illustrate existing understanding of how an initial chemical action is potentially associated with apical outcomes.



Reproduced from Ankley et al. (2010) *ETC*

AOP analysis, high-throughput screening, and informing predictive models iteratively





Cycle of assay and assay set development to generate the best available information for predictive models and prioritization
(Adapted from EPA Factsheet, Monica Linnenbrink)

Reproduced from Reif et al. (2010) EHP

The TH system is regulated at several nodes, each corresponding to a potential MIE.

ToxPi predictive tool for endocrine disruptors

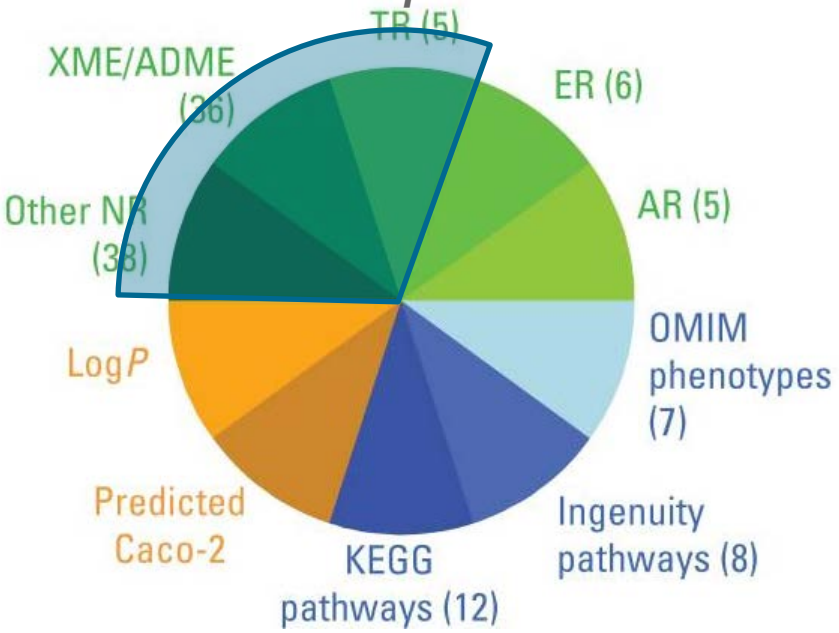
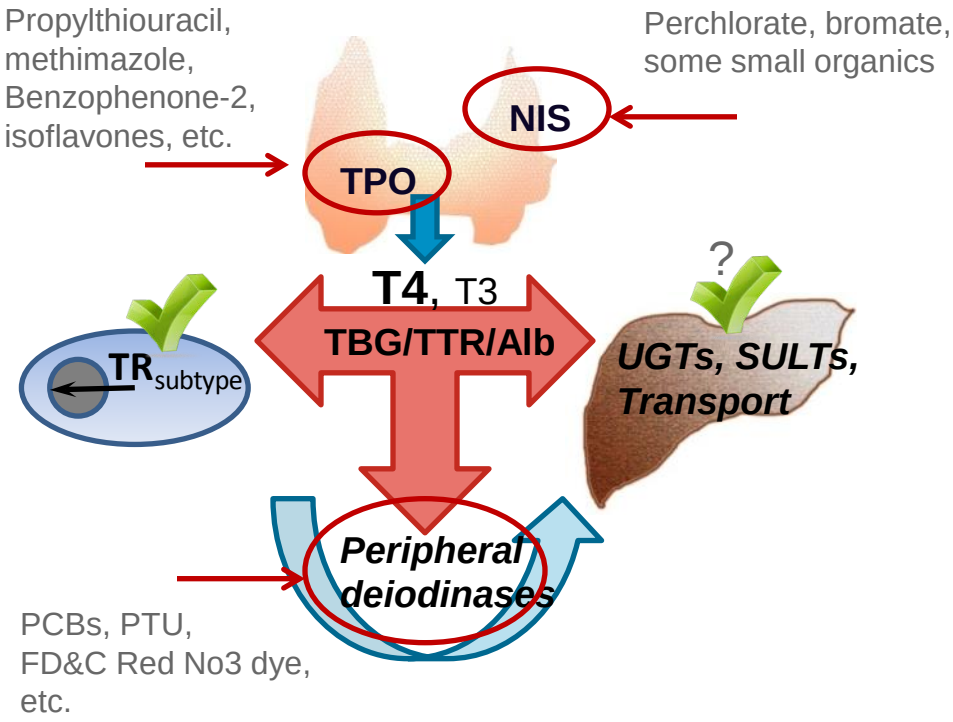
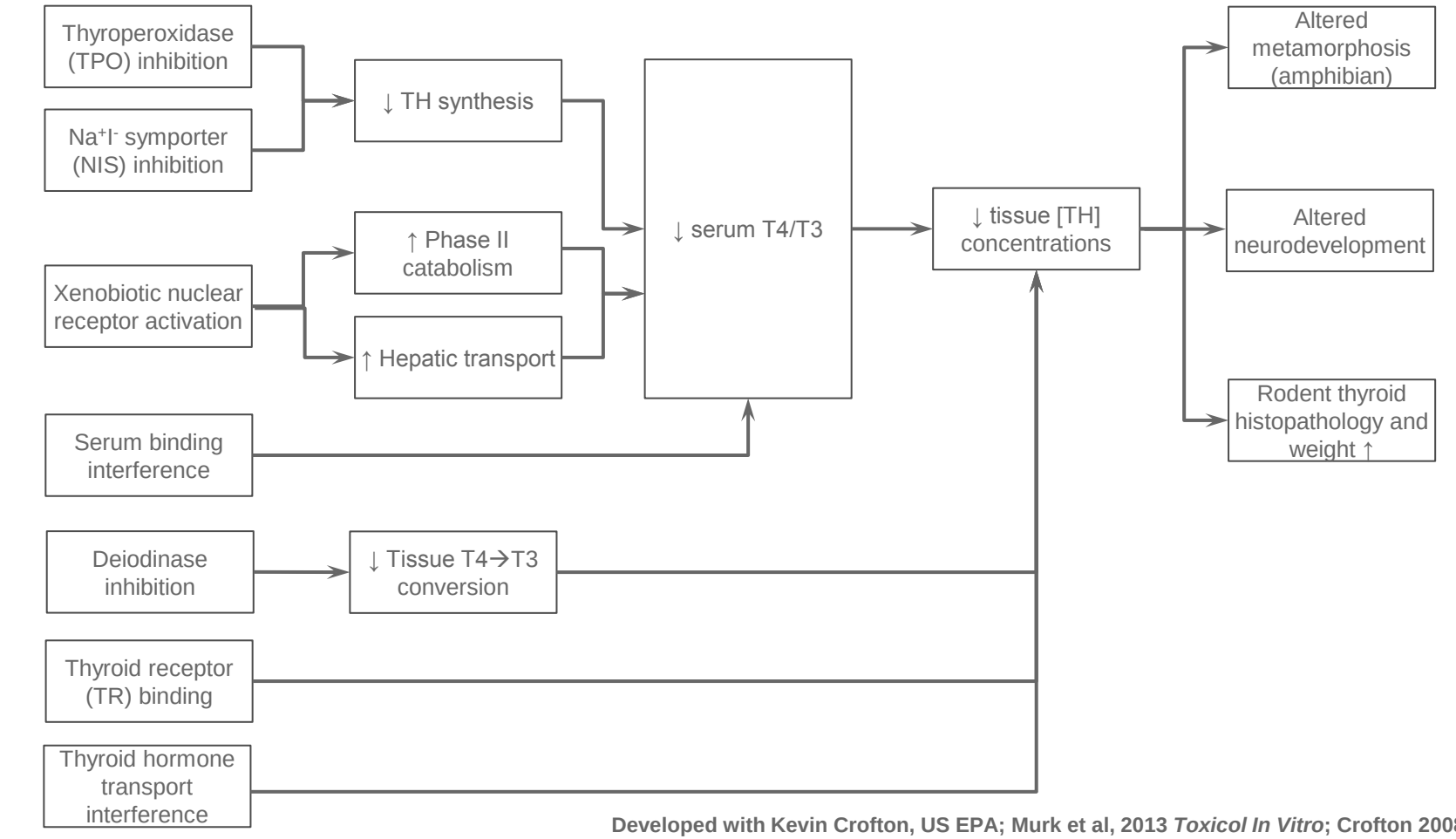


Figure reproduced from Reif et al. 2010 EHP



Several MIEs do not correspond to a medium- or high-throughput screening assay in ToxCast/Tox21.

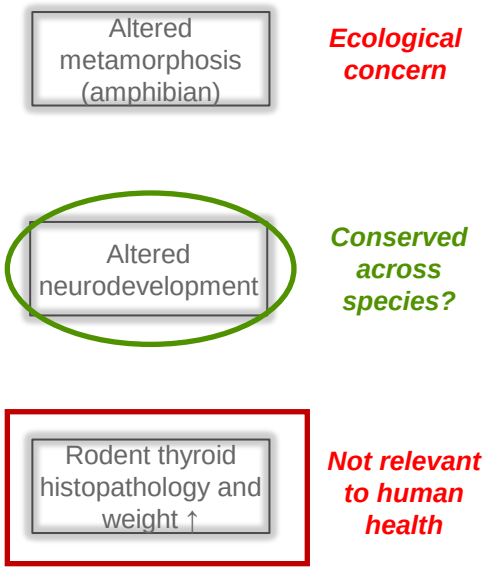
Multiple molecular-initiating events converge on common key events to proceed to adverse outcomes.



Developed with Kevin Crofton, US EPA; Murk et al, 2013 *Toxicol In Vitro*; Crofton 2008 *Int J Androl.*

Rodent thyroid tumors resultant to TSH stimulation are not relevant to human carcinogenesis.

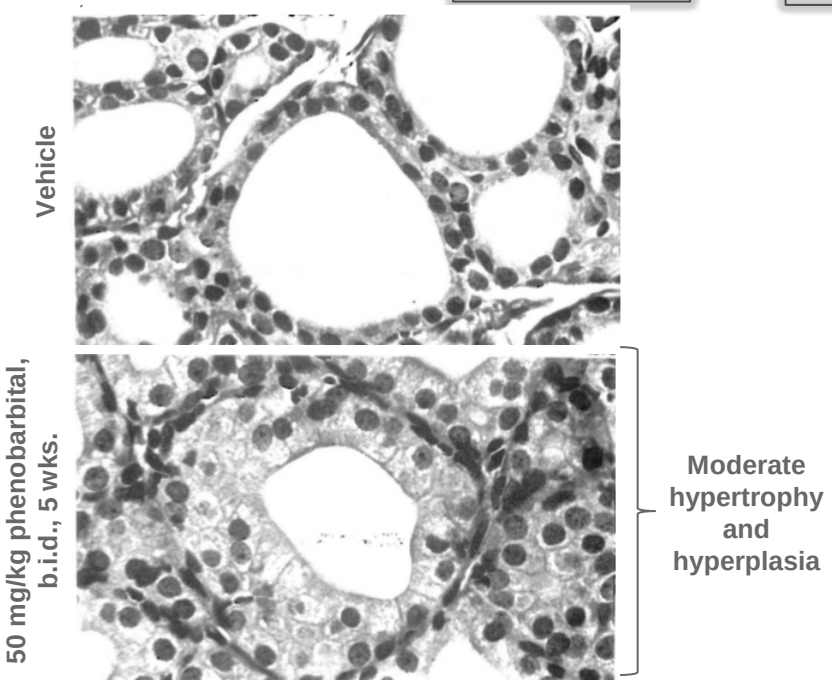
Parameter	Rodent	Human	Sources
Differentiated tumor types	Follicular	Follicular (20%) or papillary (60%)	[1]Williams (1995) Mechanisms and pathogenesis of thyroid cancer in animals and man. Mutation Research; 333: 123-129. [2]McClain (1989) The Significance of Hepatic Microsomal Enzyme Induction and Altered Thyroid Function inRats: Implications for Thyroid Gland Neoplasia. Toxicologic Pathology; 17(2): 294-206. [3]Hill et al. (1998) Risk assessment of Thyroid Follicular Cell Tumors. Environmental Health Perspectives; 106(8): 447-457. [4]Hard (1998) Recent Developments in the Investigation of Thyroid Regulation and Thyroid Carcinogenesis. Research Reviews; 106(8): 427-436.
Etiology	TSH stimulation (nonmutagenic) or mutagenic agents	Mutagenic agents (coupled with growth stimulation)	
Serum TSH	6-60X	1X	



- **Tumors as a biomarker:** tumors themselves are not relevant to humans, but they may indicate the potential for thyroid hormone perturbation across species.
- **Limitations of the biomarker:** Changes in T4 and/or T3, particularly transient changes or changes only in T4, may not result in a rodent thyroid tumor.

Rodent thyroid tumors may be a biomarker for potential Δ[TH].

Phenobarbital example: rodent

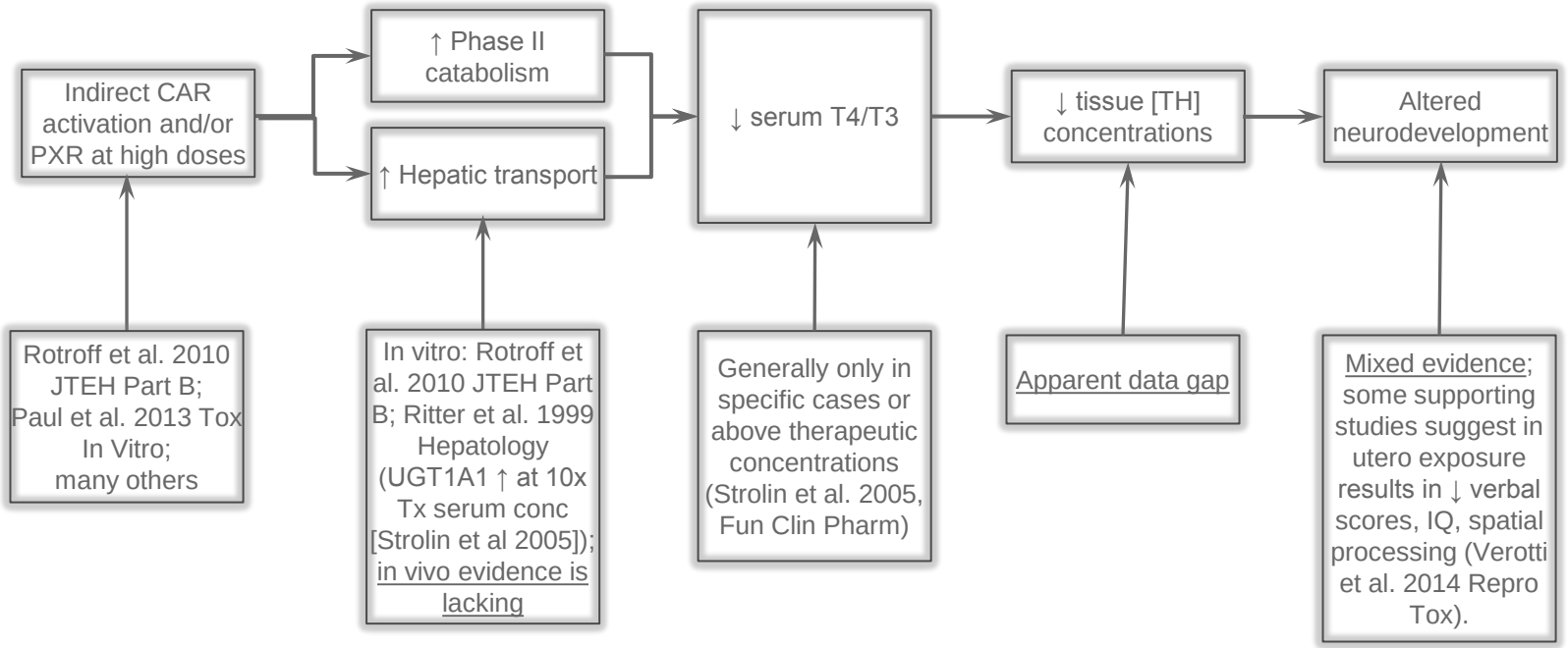


Reproduced from Smith PJ et al. (1991) Tox Path.

Many rodent studies available to support neurological changes, including:
↓brain weight, cerebellum weight, Purkinje and granular cells in the cerebellum, granule cells in the hippocampus, performance in motor activity/learning tests
(reviewed by Slikker and Chang, Handbook of Developmental Toxicology and Costa et al. 2005 Ann Rev Pharm Tox)

Rodent thyroid tumors may be a biomarker for potential Δ[TH].

Phenobarbital example: moderate human plausibility



Perhaps difficult to separate apical outcomes from potential GABA-mimetic or direct neurological effects.

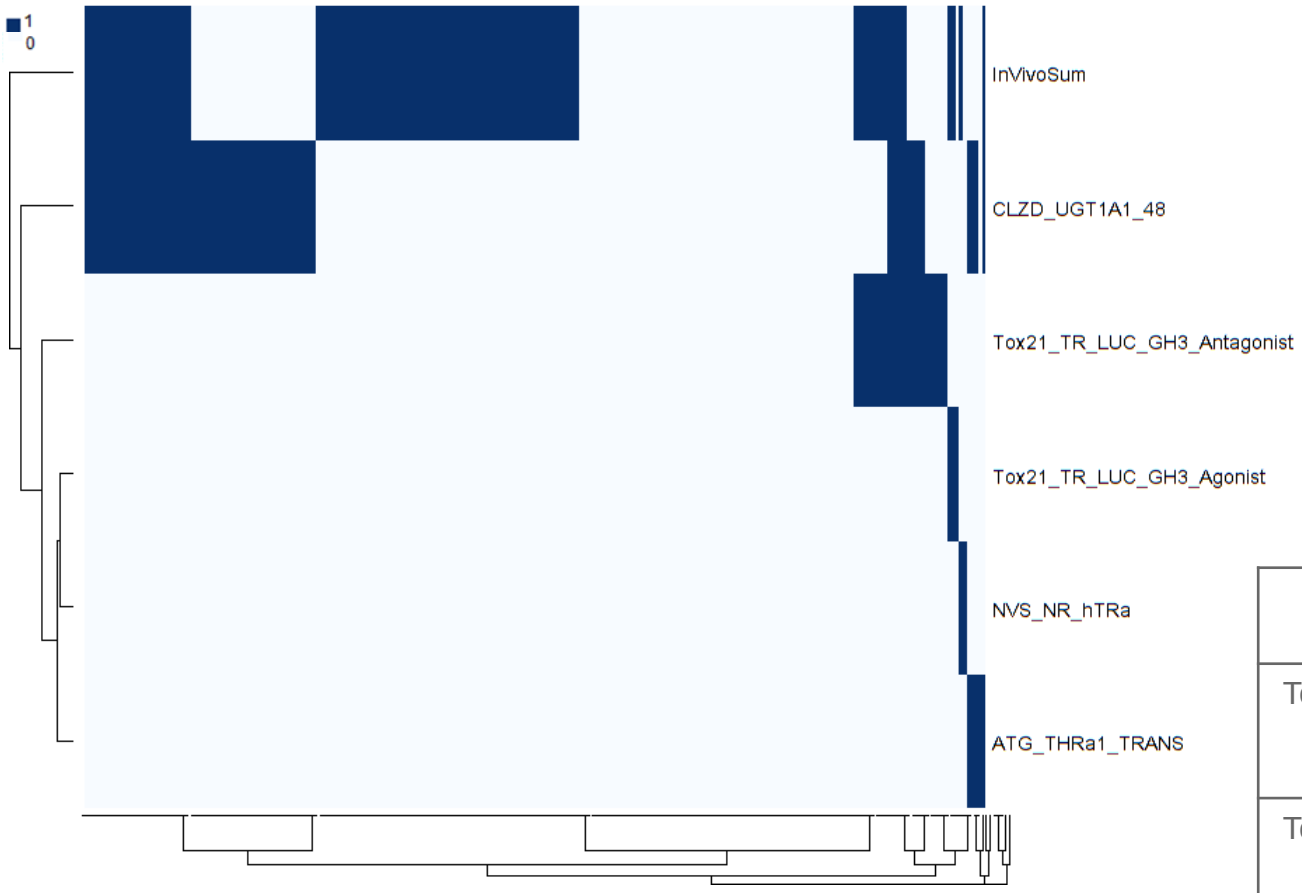
Similar AOP for PCBs or other chemicals also have data gaps despite some human plausibility, the link to effects in humans is weak (Crofton & Zoeller 2005 Crit Rev in Toxicol; Zoeller & Crofton 2005 Crit Rev in Toxicol).

Anchoring HTS assay data from ToxCast to ToxRefDB thyroid endpoints could illustrate how well current screening efforts capture potential thyroid disruption.

Study type	Species	Endpoints
Subchronic	Rat, Dog	Thyroid gland weight, nonproliferative pathology, proliferative pathology, thyroid gland effect (e.g., gross change)
Chronic	Rat, Mouse	Thyroid gland weight, nonproliferative pathology, proliferative pathology, thyroid gland effect (gross change)
Multi-generation	Rat	Nonproliferative pathology, thyroid gland weight, thyroid tumor

*239 chemicals with Phase I, Phase II, and ToxRefDB data

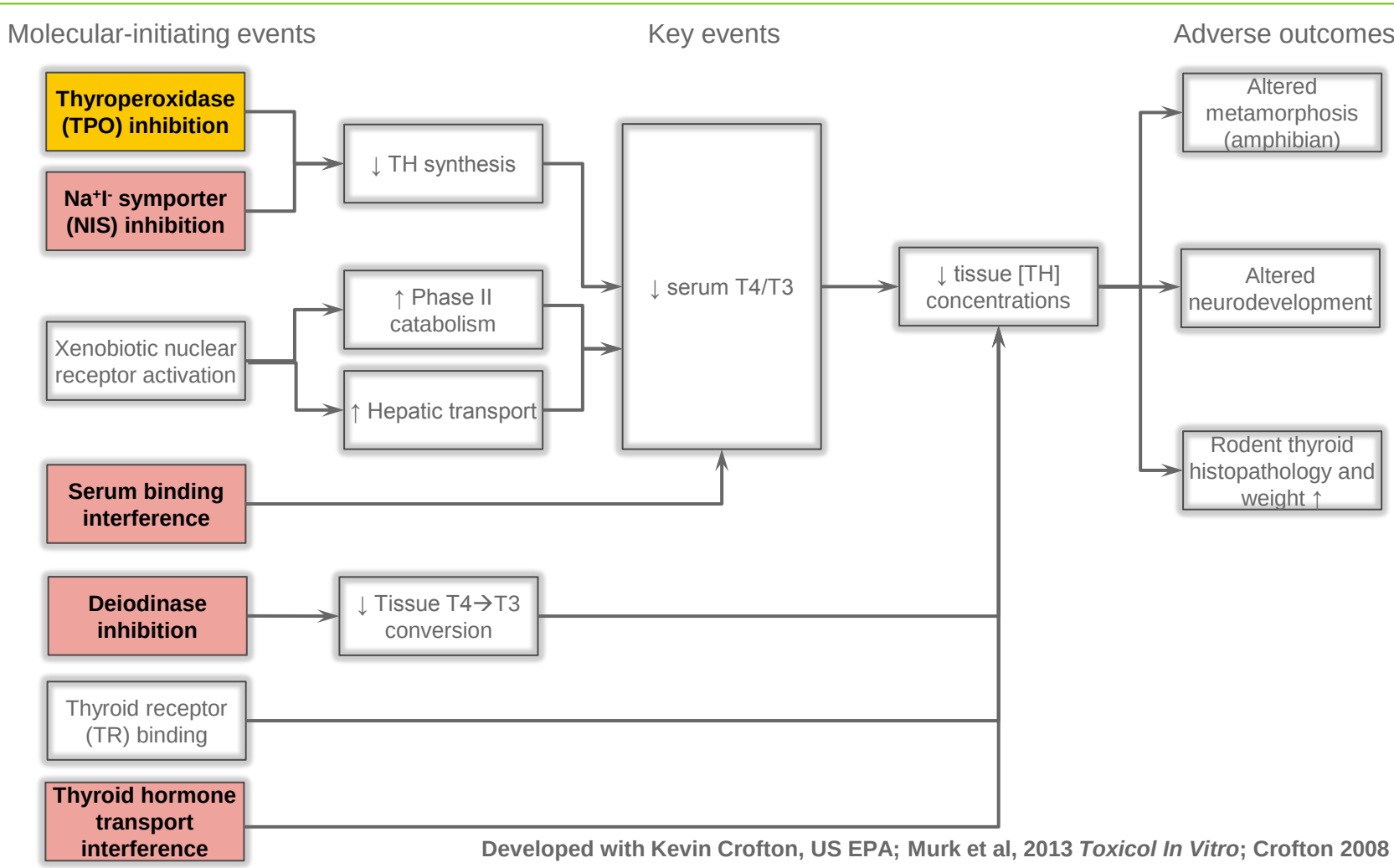
A binary view of thyroid changes in ToxRefDB versus *in vitro* bioactivity prediction.



Lack of concordance between *in vivo* thyroid effects and current assay battery underscores that important MIEs are missing from the assay battery.

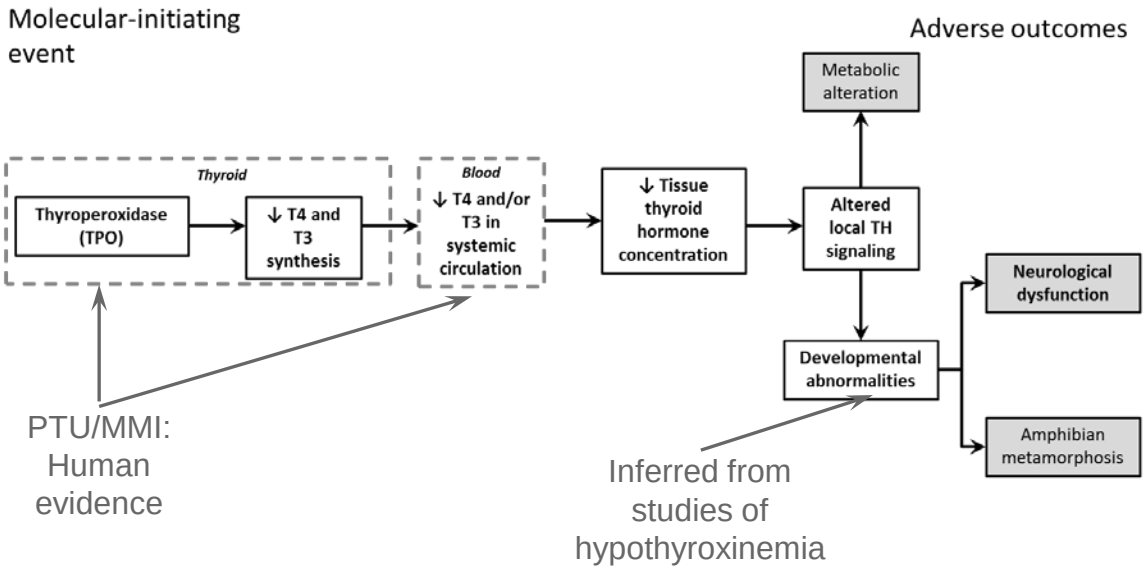
	ToxRefDB (+)	ToxRefDB (-)
ToxCast (+)	True Positive	False Positive (?)
ToxCast (-)	False Negative	True Negative (?)

Missing MIEs from the current screening battery for ToxCast/Tox21.



Developed with Kevin Crofton, US EPA; Murk et al, 2013 *Toxicol In Vitro*; Crofton 2008 *Int J Androl.*

Addition of assays for MIEs including TPO may improve prediction for rodent thyroid pathology and/or Δ[TH].



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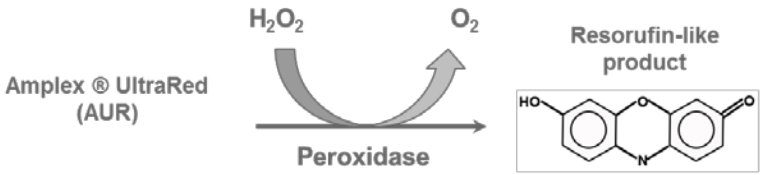

Development of a Thyroperoxidase Inhibition Assay for High-Throughput Screening

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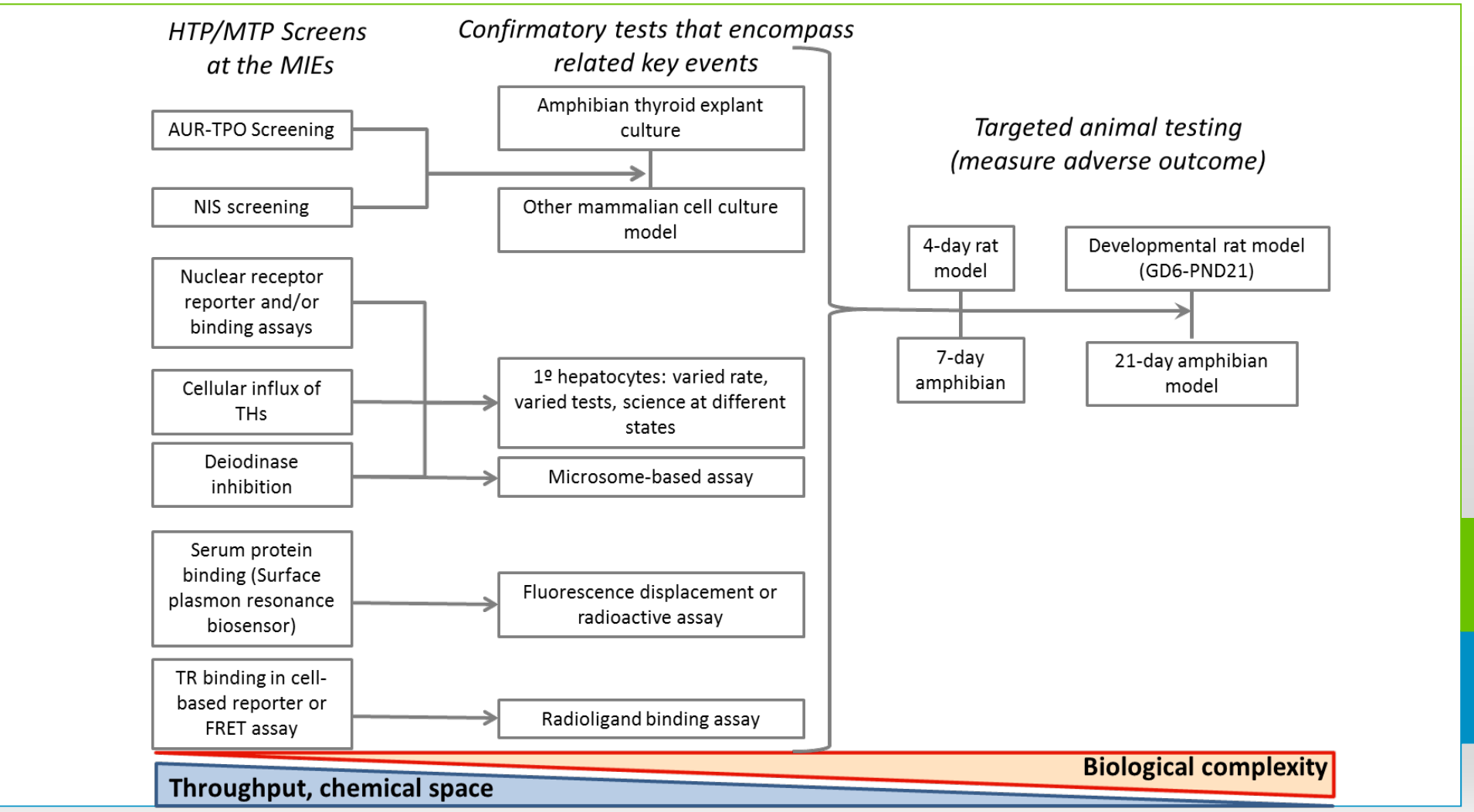
A hypothetical schematic of how an initial screening battery could relate to confirmatory models.



Current barriers to the use of thyroid AOPs in a screening and prioritization context

Barrier	Action merited
Internationally-accepted AOPs for thyroid disruption with clear delineation of data gaps.	Work-in-progress with the OECD and the AOP-Wiki to establish AOPs for thyroperoxidase inhibition and xenobiotic catabolism; more effort should be applied to cover all MIEs (Murk et al., 2013).
Not enough MIEs from thyroid AOP are covered by assay approaches.	Increased screening activity, increased assay development, investment of resources to run lower throughput screening models, increased development of QSAR models for use in screening for particular MIEs rather than for apical thyroid outcomes.
Lack of quantitative linkages between MIE and key events in thyroid AOPs.	Quantitative evaluation of key events in thyroid perturbation in *in vitro* and *in vivo* models.
Lack of defined targeted, confirmatory models, and a decision-tree for how HTS assay relate to these models in a workflow.	Quantitative and qualitative assessment of how models relate; development and characterization of more complex medium-throughput models (*in vitro* and *in vivo*).
Lack of definitive evidence of human concordance for apical outcomes.	Plausibility of interrelated key events should be assessed in models, but mechanistic human data on the concordance of apical outcomes will likely not be available.

Ideal outcome: Combine AOP-based hazard prediction with exposure prediction to prioritize chemicals for any further evaluation in models for greater confidence.

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Thank you!

Especially colleagues at Bayer CropScience, Human Safety Regulatory Toxicology and Kevin Crofton, US EPA

Katie Paul August 2014