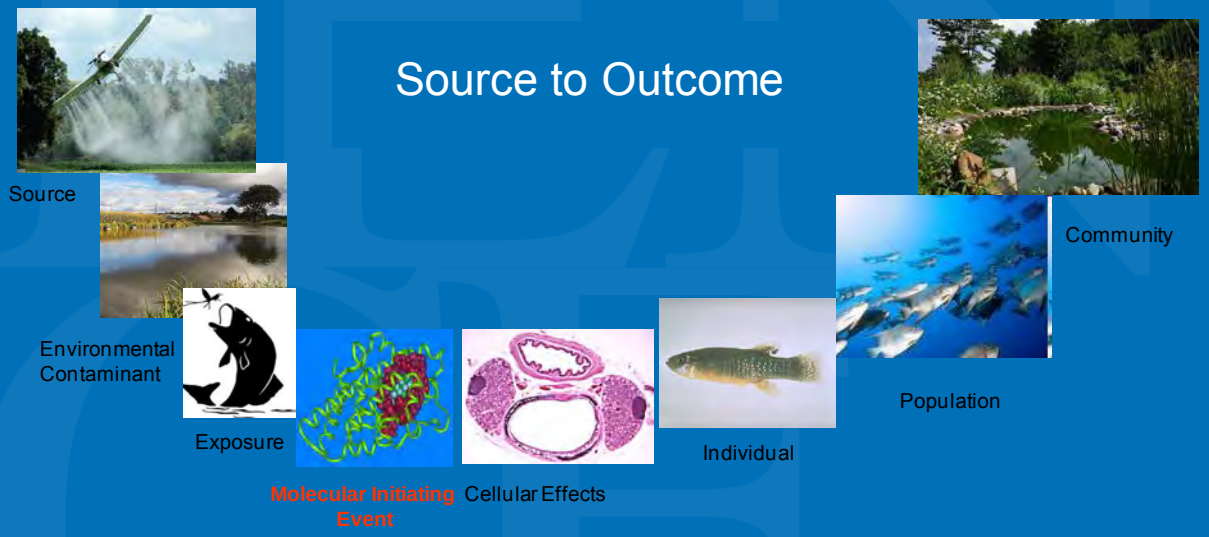
Adverse Outcome Pathways and Their Unifying Role in Developmental Toxicology

Kevin M. Crofton
National Center for Computational Toxicology



Office of Research and Development
National Center for Computational Toxicology

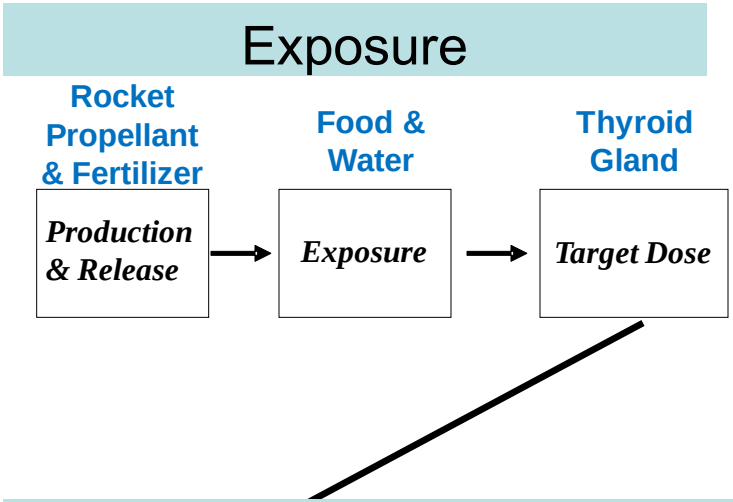
PPDC 21st Century Workshop
2013

Outline

- Introduction to “pathways”
  - Source-to-Outcome Pathway, 21st Century Toxicity Pathway, Mode of action, Adverse Outcome Pathways
  - Example – Perchlorate
- Thyroid Biology 101
- Using AOP to Focus Research on Critical Data Gaps in Thyroid Disruption Induced Adverse Outcomes
  - Life-Stage Specificity and Species extrapolation
  - Provides structure for qualitative & quantitative predictive models
  - Helps indentify data gaps in hazard assessments that, if filled, with reduce uncertainties in decisions
- Take home message
  - AOPs can reduced uncertainty = better predictions of human toxicity

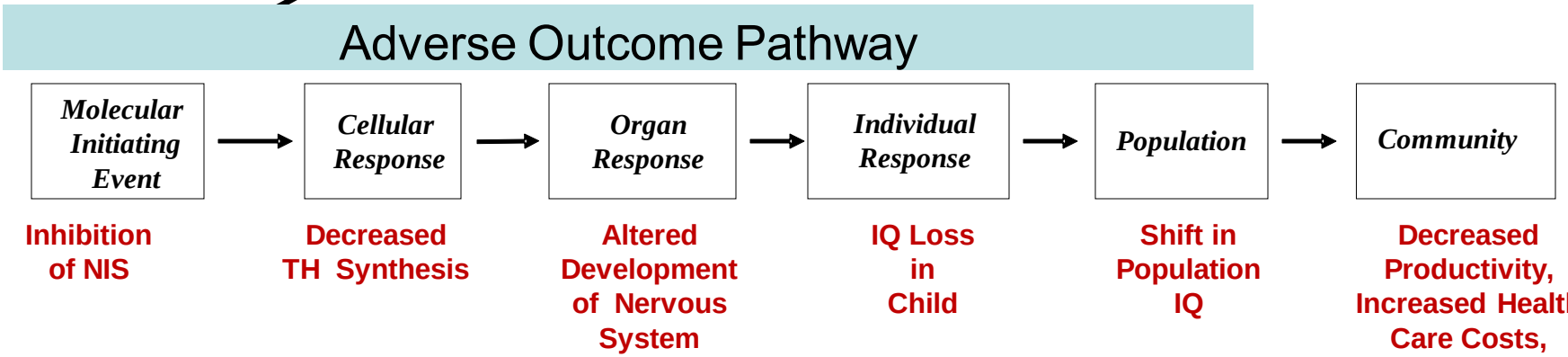
SOP, AOP, MOA, ToxPathway

- **Definition:** The continuum or cascade of measurable events starting from release into the environment and ending at an adverse outcome (USEPA 2003).
- **Example**: Perchlorate



Uses:

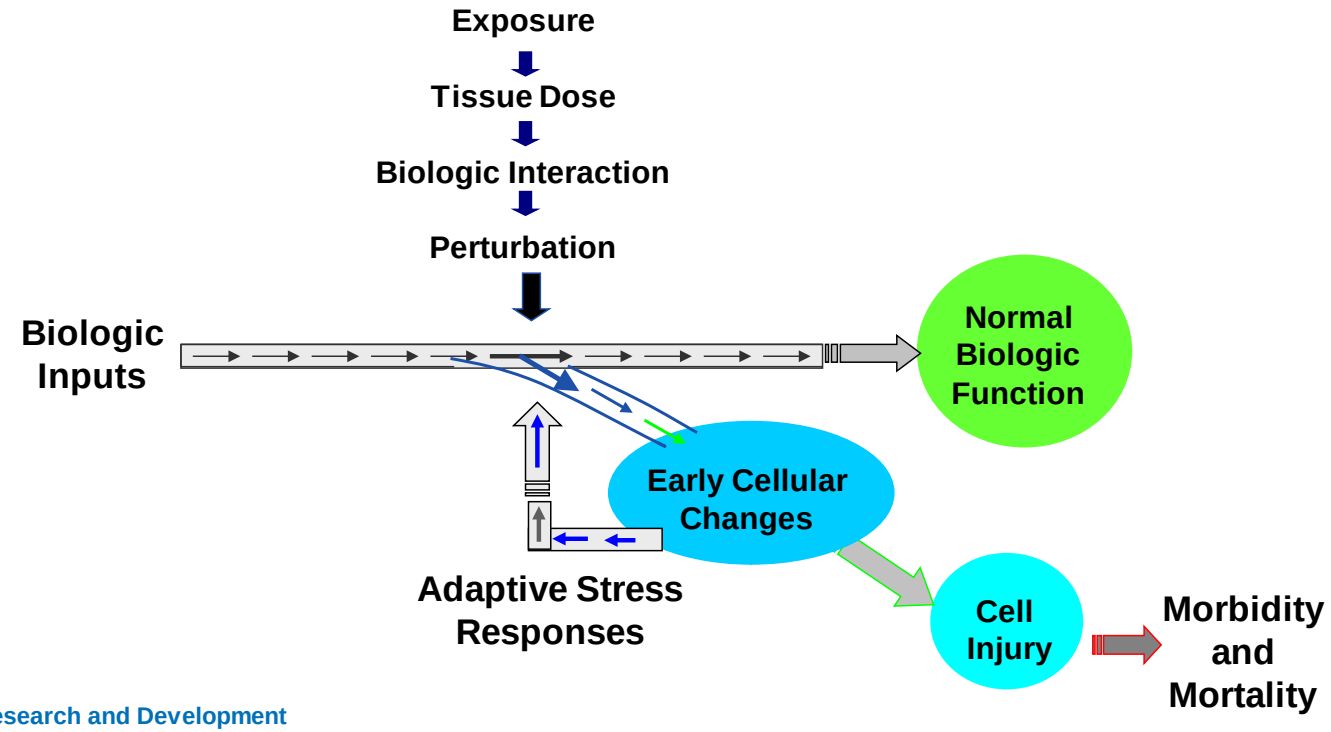
- Qualitatively links actual use of chemicals to adverse outcomes
- Can be used to link exposure, toxicokinetic and toxicodynamic models to qualitatively predict outcomes
- Basis for causative & quantitative models



Toxicity Pathway

- **Definition:** Cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects are termed *toxicity pathways* (NRC 2007).

- **Example – Normal:** Kainate receptors activated by glutamate open ion channels in neurons and regulate ion flux important for neuronal firing.

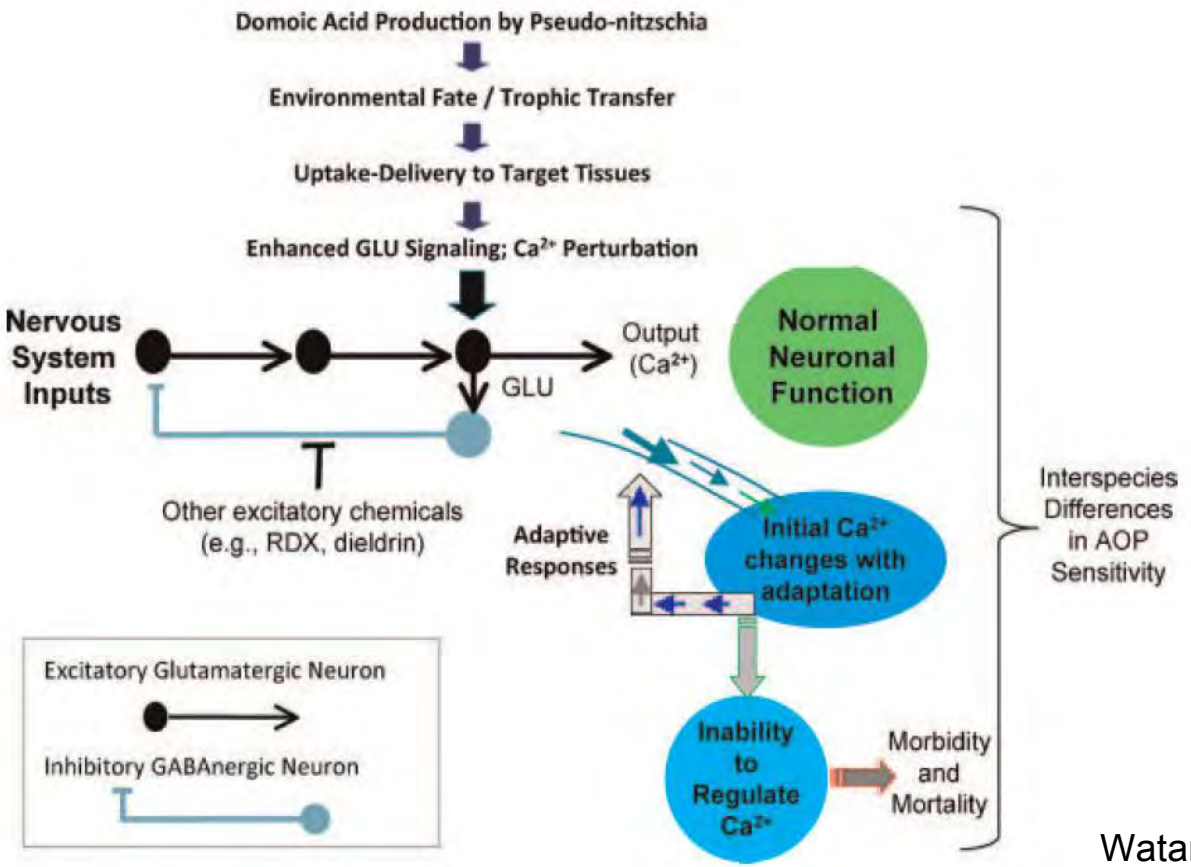


NRC, 2007

Toxicity Pathway

- **Example - Abnormal:** Domoic acid causes glutamate-induced hyperstimulation of neurons, neurons accumulate excess Ca+, and at high enough levels this leads to cell death.

  Use: Computational modeling will allow quantification of the significance of the perturbation. *i.e., how hard does the system need to be hit to overcome compensation and result in cell injury.*



Watanabe et al 2011

Adverse Outcome Pathway

- **Definition:** An adverse outcome pathway (AOP) represents existing knowledge concerning the linkage between a molecular initiating event and an adverse outcome at the individual or population levels (Ankley et al. 2009).

- **Examples**: Chemical antagonism of the interaction of estrogen with the estrogen receptor, depress hepatic production of VTG, thereby decreasing plasma concentrations of the protein, its deposition to developing oocytes in the ovary, and ultimately decreased egg production and repercussions relative to population-level effects

- **Use:**
  - Species concordance (OECD MOA Framework)
  - Identified key events can be qualitatively and quantitatively linked to an adverse outcome and these events can be used in developing chemical categories.

Note: AOPs are not singular – complex interactions can occur

Mode of Action

- A mode-of action describes the key, rate limiting, and quantifiable events that lead to adverse outcomes (IPCS MOA Framework, see Sonich-Mullen et al., 2001)
  - Determine whether animal MOA is plausible and relevant to humans
  - Key events must be measureable and causal
  - Provide guidance on establishing causal relationships between key events (modified Bradford Hill criteria)
  - Must predict adverse outcome from initiating key event (at least qualitatively)
  - Can be species specific

Mode of Action

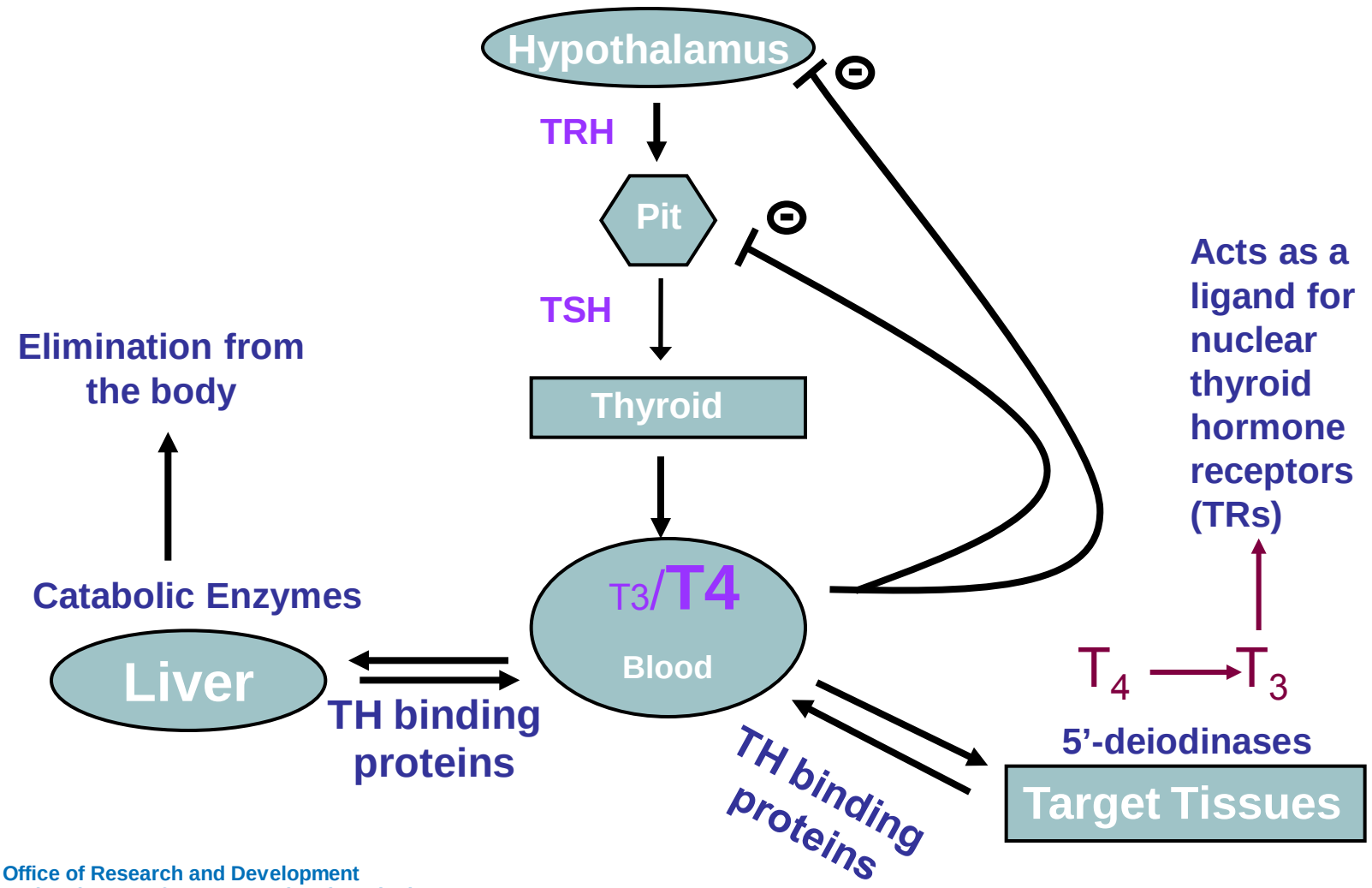
- A mode-of action describes the key, rate limiting, and quantifiable events that lead to adverse outcomes

MOA is a regulatory assessment framework based on the concept of pathways

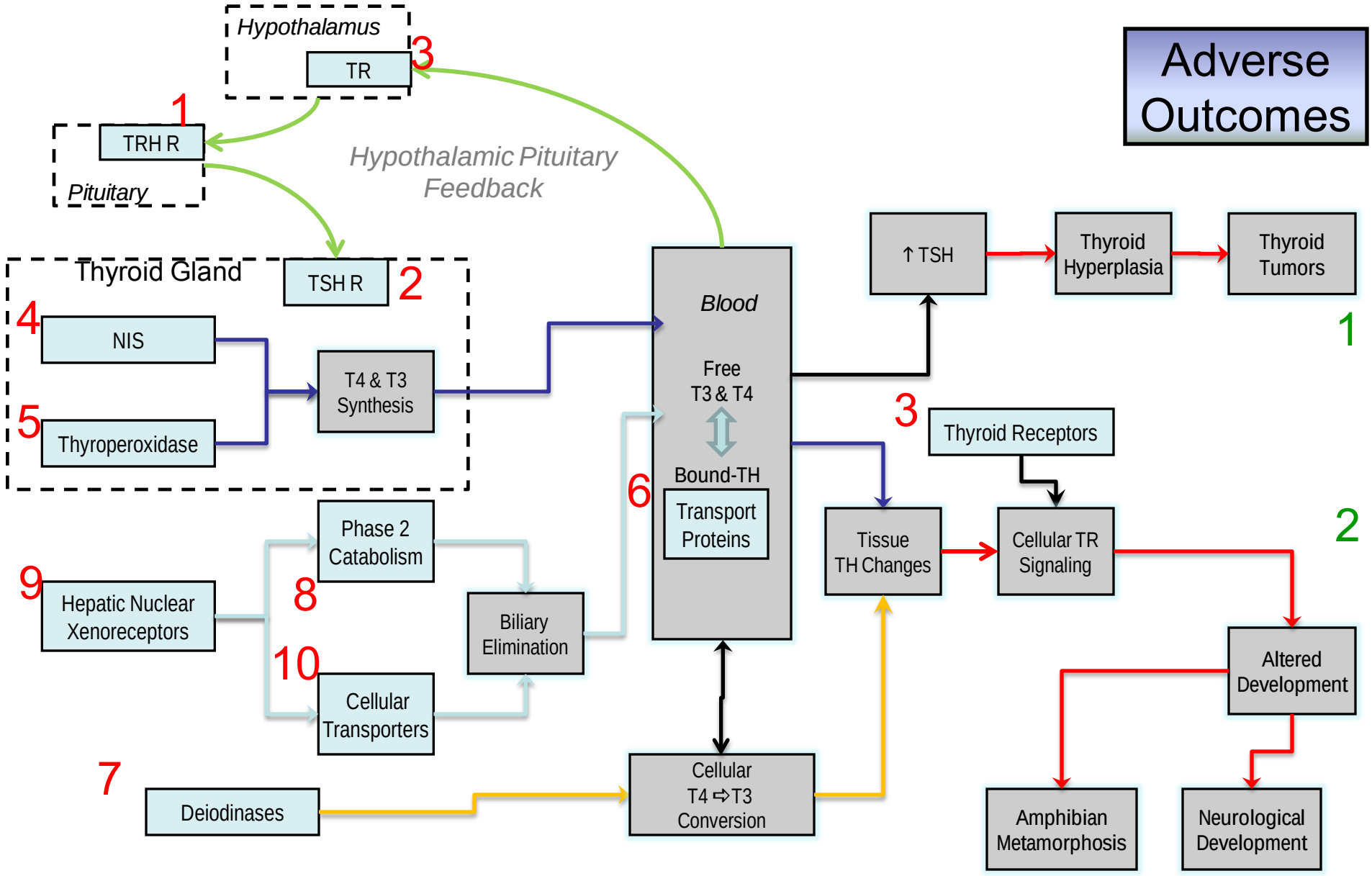
AOP is a conceptual and practical tool to capture descriptions of toxicological processes.

  - Can be species specific

Thyroid Biology 101
Synthesis, Regulation, Action and Catabolism



AOPs for Thyroid Disruption - From MIE to Adverse Outcome



TDCs - Two Major Adverse Outcomes of Regulatory Concern

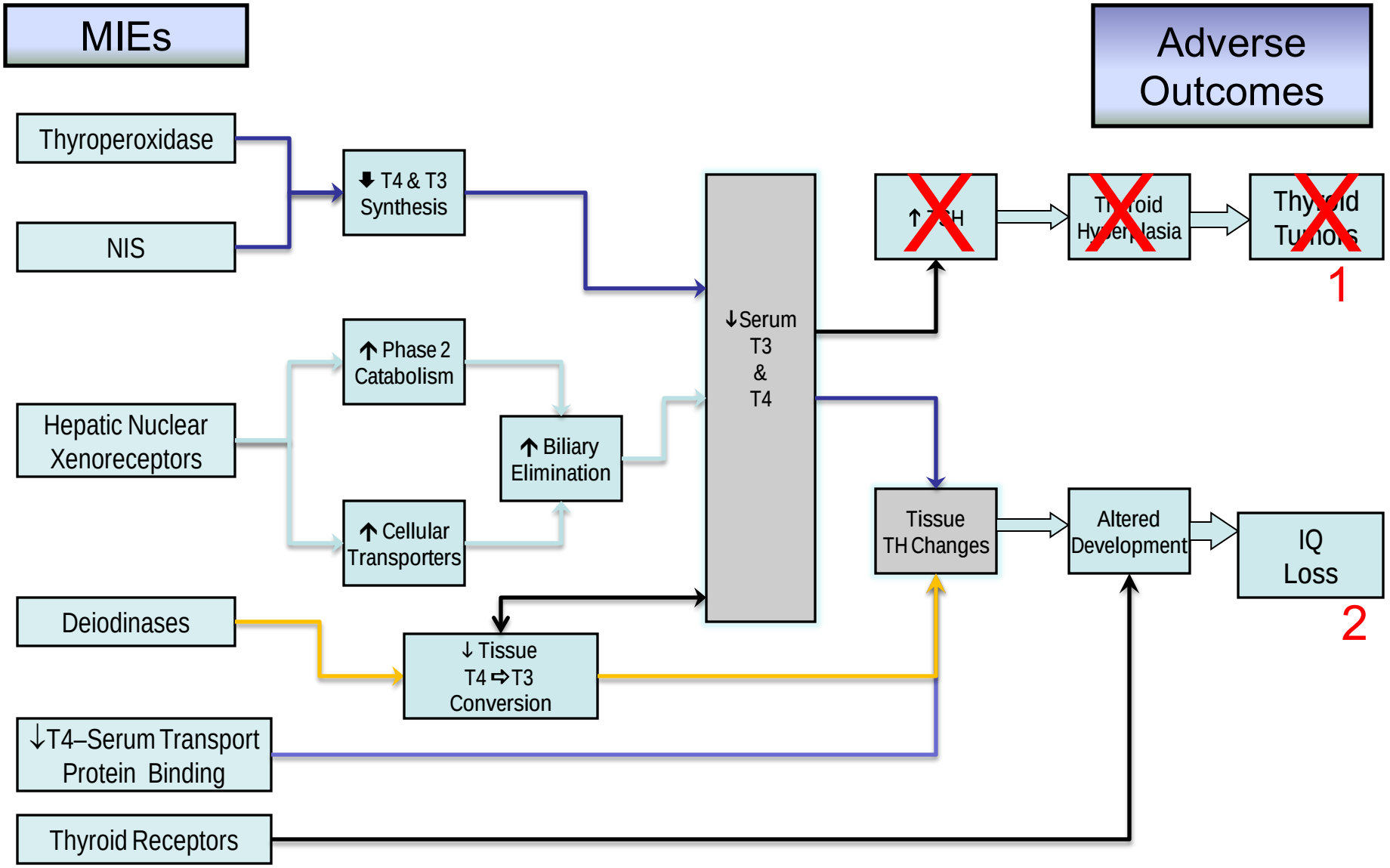
- Pathway #1
  - Upregulation of the HPT feedback system
  - Yields thyroid hypertrophy, hyperplasia and thyroid follicular tumors in rats
  - This AOP is not relevant to humans due to substantial species differences in

We're worried about IQ in kids, not thyroid tumors
It's serum TH not TSH that matters!

- Pathway #2
  - Decreased serum TH, decreased tissue TH, decreased tissue mRNA and protein synthesis, during development leads to:
    - Amphibians – altered metamorphosis
    - Humans - decreased IQ (MOA relevant to humans)
  - This AOP has be shown to be relevant to rodents and humans (Crofton & Zoeller, Crit Rev. Toxicol. 2005)

AOPs for Thyroid Disruption

Multiple MIEs and Species Specific Outcomes



AOPs – What are they good for?

1. **Improved predictions of toxicity via decreased uncertainty**
   - Increases level of confidence in the relationship between measured data and adverse outcomes that is critical for risk assessments
2. **Can be Life-Stage specific**
3. **Enhance species to species extrapolation**
4. **Identification of Data Gaps**
   - Construction of an AOP should identify data gaps i.e., critical needs to build a useful model
5. **Provide molecular targets for development of in vitro screening assays**

6. **Holy Grail is development of predictive computational models**
   - If the MIE predicts the Adverse Outcome – then you don't need to measure the outcome

Example:
Linking Liver to thyroid hormones to developmental neurotoxicity

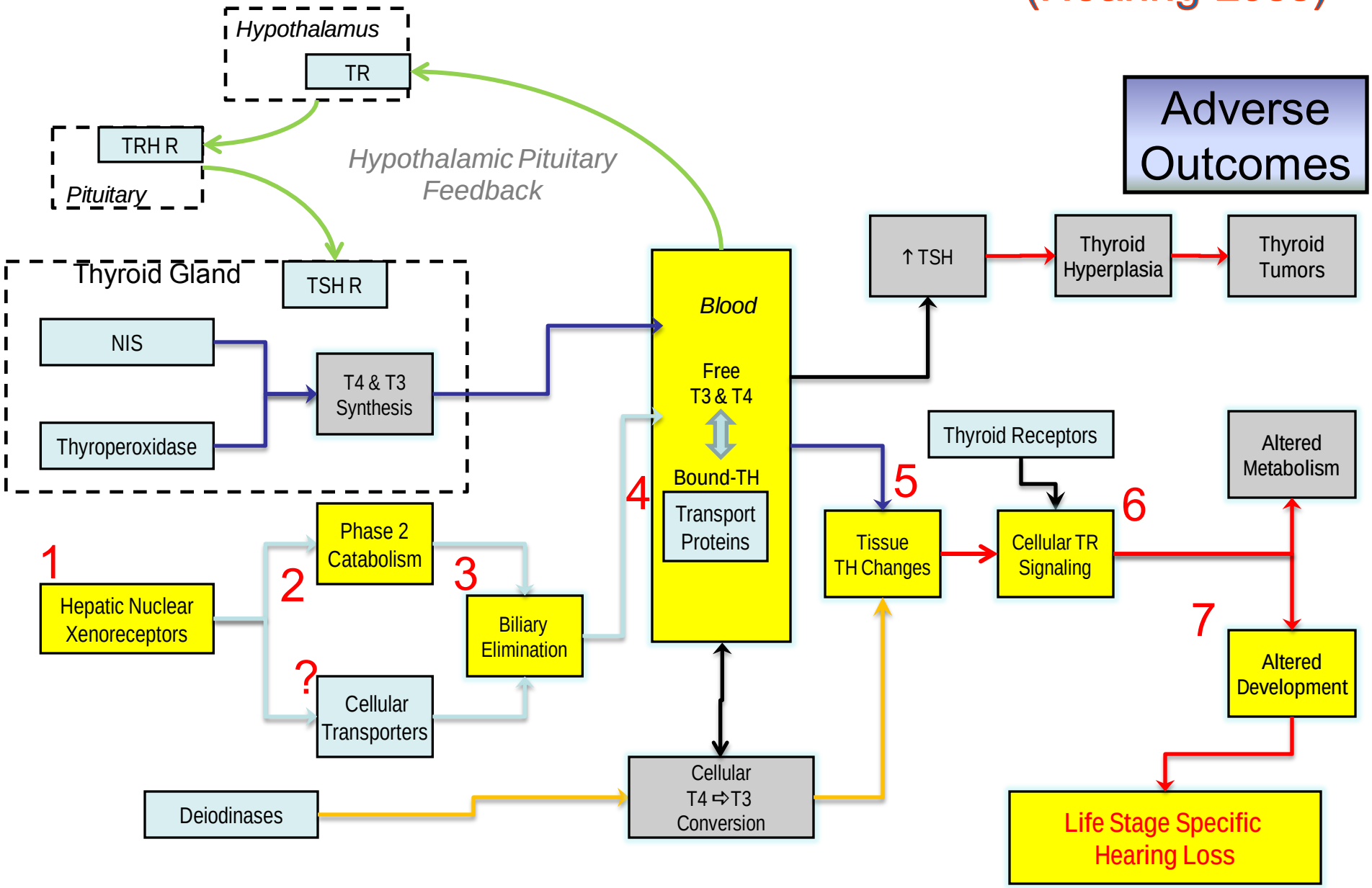
Life Stage Specificity

Description of Postulated AOP

- **MIE**
  - Activation of hepatic nuclear receptors (i.e., CAR/PXR)
- **Key Events**
  - Upregulation Phase II and III metabolism
  - Increased hepatic clearance of TH
  - Decreased Serum T3, T4
  - Decreased Tissue TH concentrations during critical developmental periods
  - Altered expression of TH-responsive genes in developing brain
- **Adverse Outcome**
  - Altered neurological development – case study = hearing loss

Note: TSH not involved

Case Study – Hepatic NR and Developmental Neurotoxicity (Hearing Loss)



Experimental Support for Linking MIE, Key Events, and Adverse Outcome

Table 1: Key Events in the Animal AOP – PHAH Example

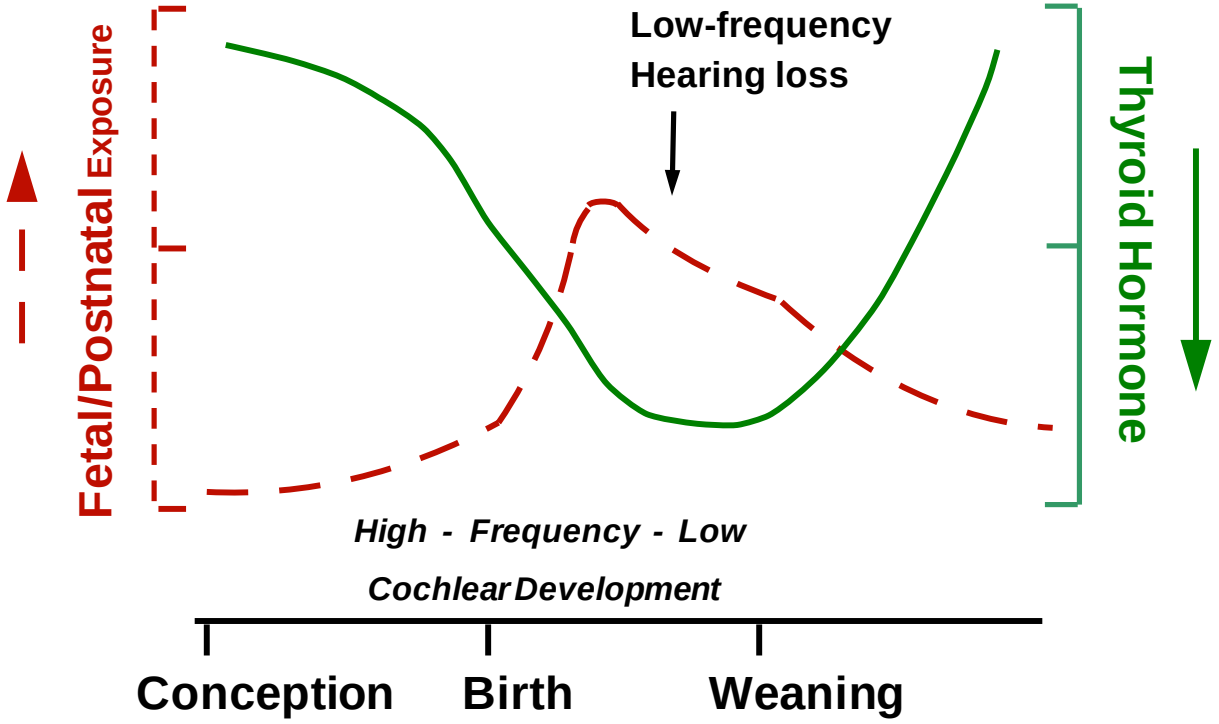
Key Event	Experimental Evidence	References
1. Activation of Hepatic Ah, CAR and PXR pathways	**YES: A lot of data demonstrating (***In vivo* and *in vitro)* evidence for activation by PHAHs Knockout models prevent subsequent key event	(Safe, 1994; Schuetz et al., 1998; Honkakoski et al., 2003)
2. Induction of Phase II Catabolism	**YES**: Hepatic NR agonists induce hepatic UGTs	(Ahotupa et al., 1978; Ganem etal., 1999; Oppenheimer et al., 1968; McClain et al.,1989; Hood and Klaassen, 2000)
3. Biliary Elimination	**YES:** Many PHAHs increase biliary elimination of conjugated T4.	(Batomsky, 1974; Beestra et al., 1991; Vansell and Klaassen, 2001; 2002)
4. Developmental Hypothyroxinemia	**YES: In vivo** decreases in thyroxine during early critical postnatal period; thyroxine replacement ameliorates functional loss – *many chemicals and many MIEs do this* *T4 Therapy reverses this effect*	(Morse et al., 1993; Morse et al., 1996; Goldey et al., 1995a; Goldey & Crofton, 1998; Crofton et al., 2000a.)
5. Decreased Target Tissue TH Concentrations	**No Data:** No data available for effects of PHAHss on cochlear T3. Limited data suggest induction of Type II deiodinases prevents decreases in fetal cortical T3 levels PCB exposure. *(lots of evidence from other THD Chemicals)*	(Morse et al., 1996)
6. Decreases in TR Regulated Cochlear Proteins	**No Data:** No evidence in cochlea.	*Data gap for PHAHs*
7. Structural Damage in the Cochlea	**YES:** *In vivo* evidence of missing hair cells in apical turns of cochlea *(also evidence from other TH Chemicals)*	(Crofton et al., 2000b)
8. Loss of cochlear function	**YES:** Loss of low-frequency hearing using behavioral audiometry, brain stem auditory evoked potentials and otoacoustic emissions. *(lots of evidence from other THD Chemicals)*	(Goldey et al., 1995; Crofton et al., 2000b; Herr et al., 1996; Laskey et al., 2002)

Example of Temporal relationship (lifestage)

Critical-Period Model for Highly Lipophilic Chemicals

Exposure and Key Event Align Temporally with Critical Window

- Exposure occurs mainly postnatally due to lactational transfer
- Impact on thyroid hormones in mostly postnatal
- Therefore, impact on developing cochlea occurs during low-frequency development

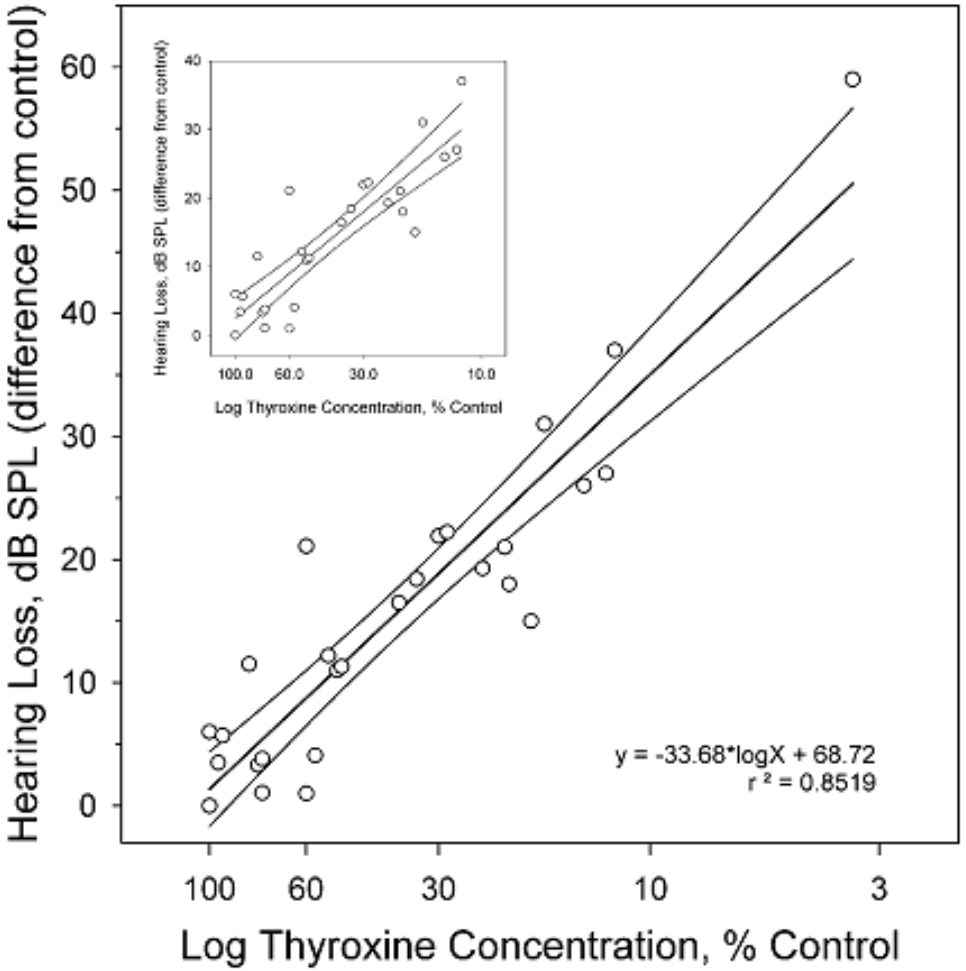


Example of Correlative relationship between Key Events

Serum Thyroxine on PND14 Predicts Low-Frequency Hearing Loss in Adults

Data from multiple developmental studies over a ten year with various Chemicals

- Propylthiouracil
- PCBs
- Dioxins
- Brominated flame retardants



Identification of Data Gaps
&
Cross Species Extrapolation

- Construction of an AOP should identify data gaps i.e., critical needs to build a useful model
  - Identification of missing empirical data for some key events
  - Lack of identification of MIEs – some AOPs start with key events
  - Filling gaps should reduce uncertainties in decisions

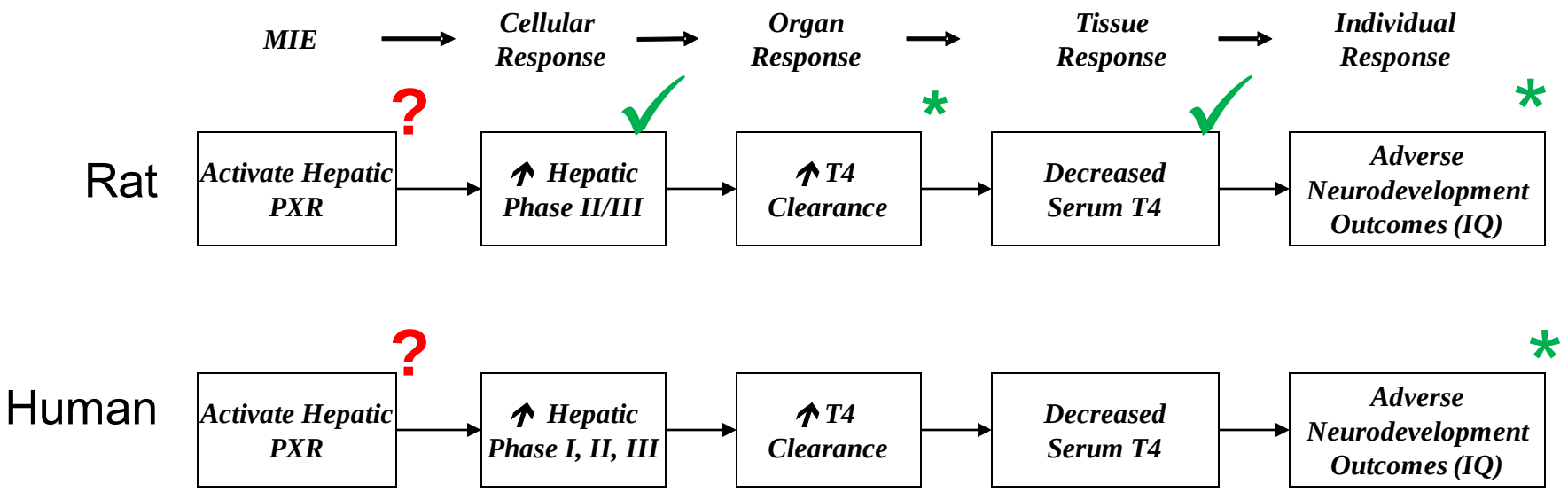
- Enhance species to species extrapolation
  - Compare chemical effects on species specific MIEs or key events

Triclosan as an example

Molecular Initiating Event - Cross Species

Triclosan

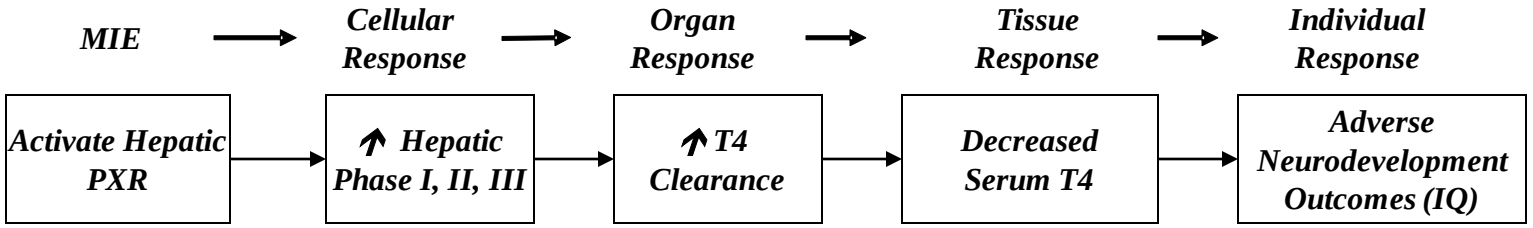
- ***Regulatory Driver: In vivo rodent studies demonstrate that triclosan decreases thyroid hormones and increases hepatic enzyme important for T4 regulation in rats***
  - ***Uncertainty: Does it do so in humans? No data = assumption yes***
- ***AOP is well accepted for many drugs and environmental chemicals***
  - ***Evidence that MIE in humans is activation of PXR (Jacobs et al., 2005)***
- ***Hypothesis: MIE is activation of hepatic PXR***



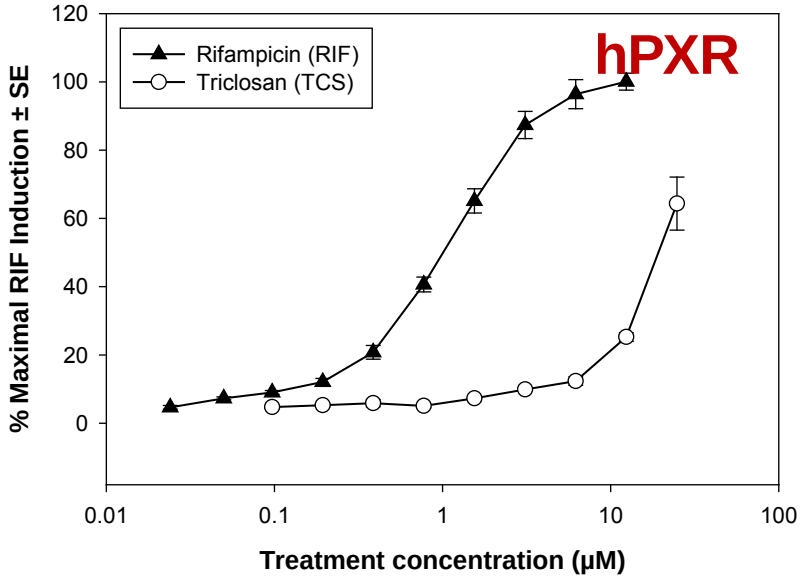
Molecular Initiating Event - Cross Species

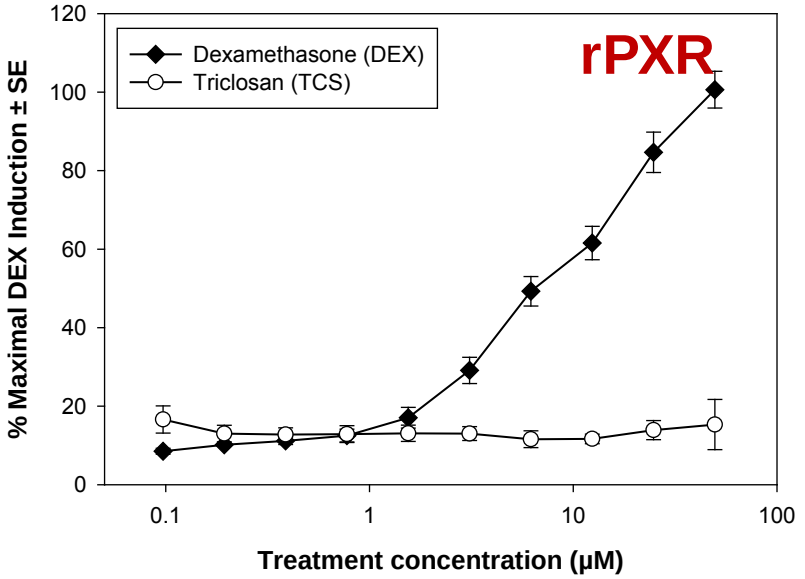
Triclosan

- ***Hypothesis: MIE is activation of hepatic PXR***



In vitro: TCS activates human PXR *but not rat PXR*



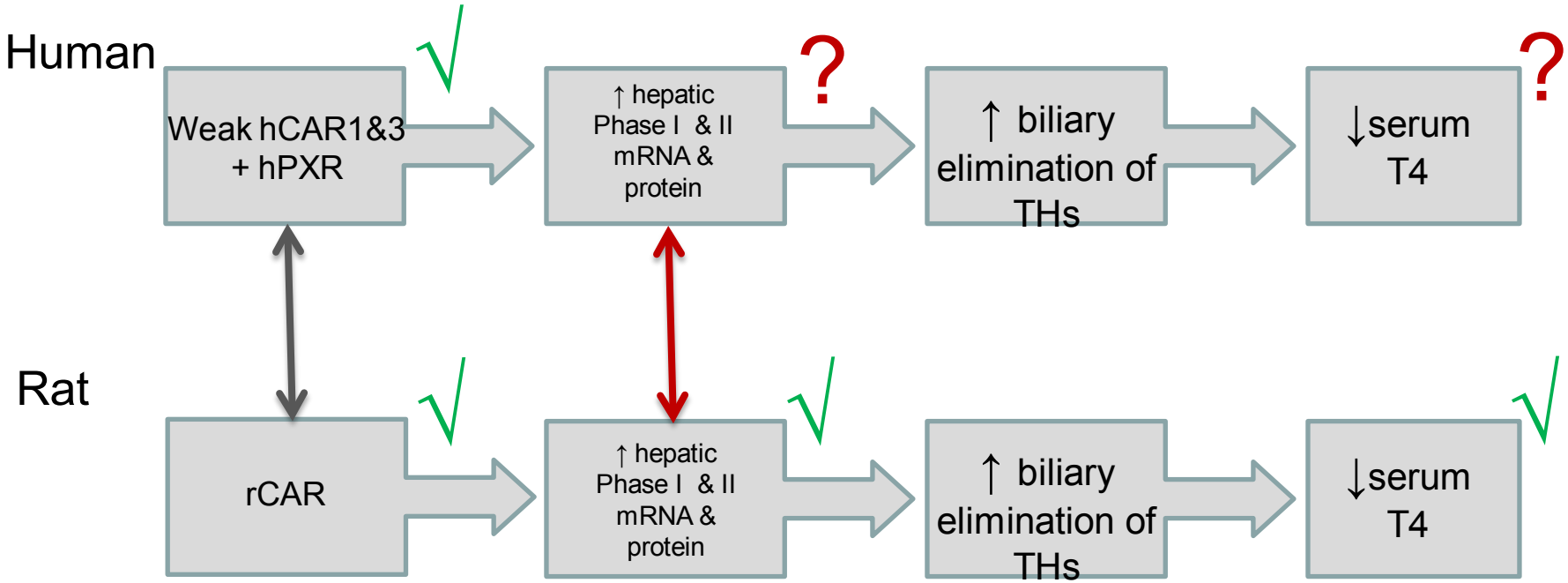


Paul et al., submitted

Triclosan Species Extrapolation - Remaining Uncertainty

- **Differential effects of triclosan on hepatic MIEs**
  - Human – both hCAR and HPXR activated in vitro
  - Rat – only rCAR activated in vitro

- **Are humans more sensitive? Unknown…. Use in vitro cell cultures to check**



Paul et al., 2013

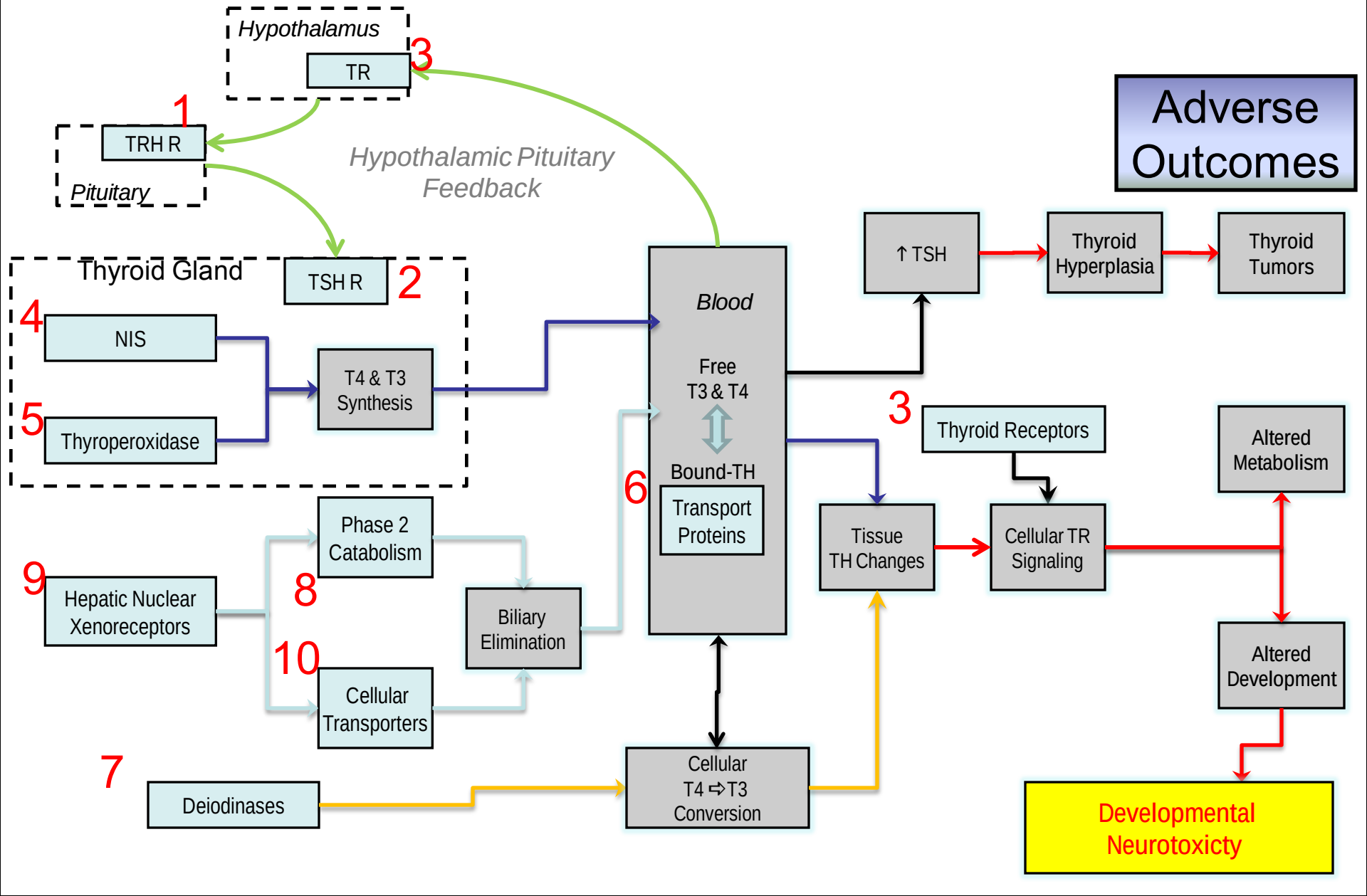
AOP and High-Throughput Screening What's the Promise?

Premise: The AOP is well developed and based on empirically established links between the MIE and the Adverse Health and Community Outcomes

Promise: Upstream MIEs and Key Events can be used in lieu of costly in vivo testing

- ***Health predictions can be done based on upstream events***
  - e.g., serum T4 during fetal development is a predictor of IQ in children, and TPO inhibition is correlated with serum hormones
  - Already being done in regulatory arena
- ***Can be used to prioritize follow up testing needs***
  - Data from high-throughput methods can represent a true 'first-tier' screen for the thousands of chemicals currently lacking data

MIEs as Molecular Screening Targets

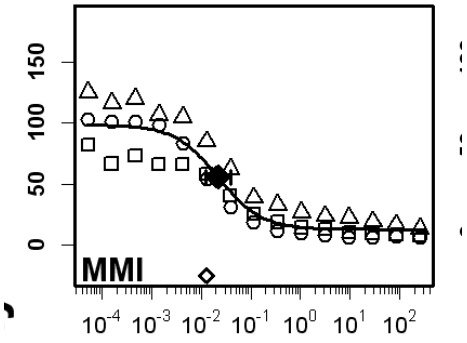


Screening for THDs – State of the Science*

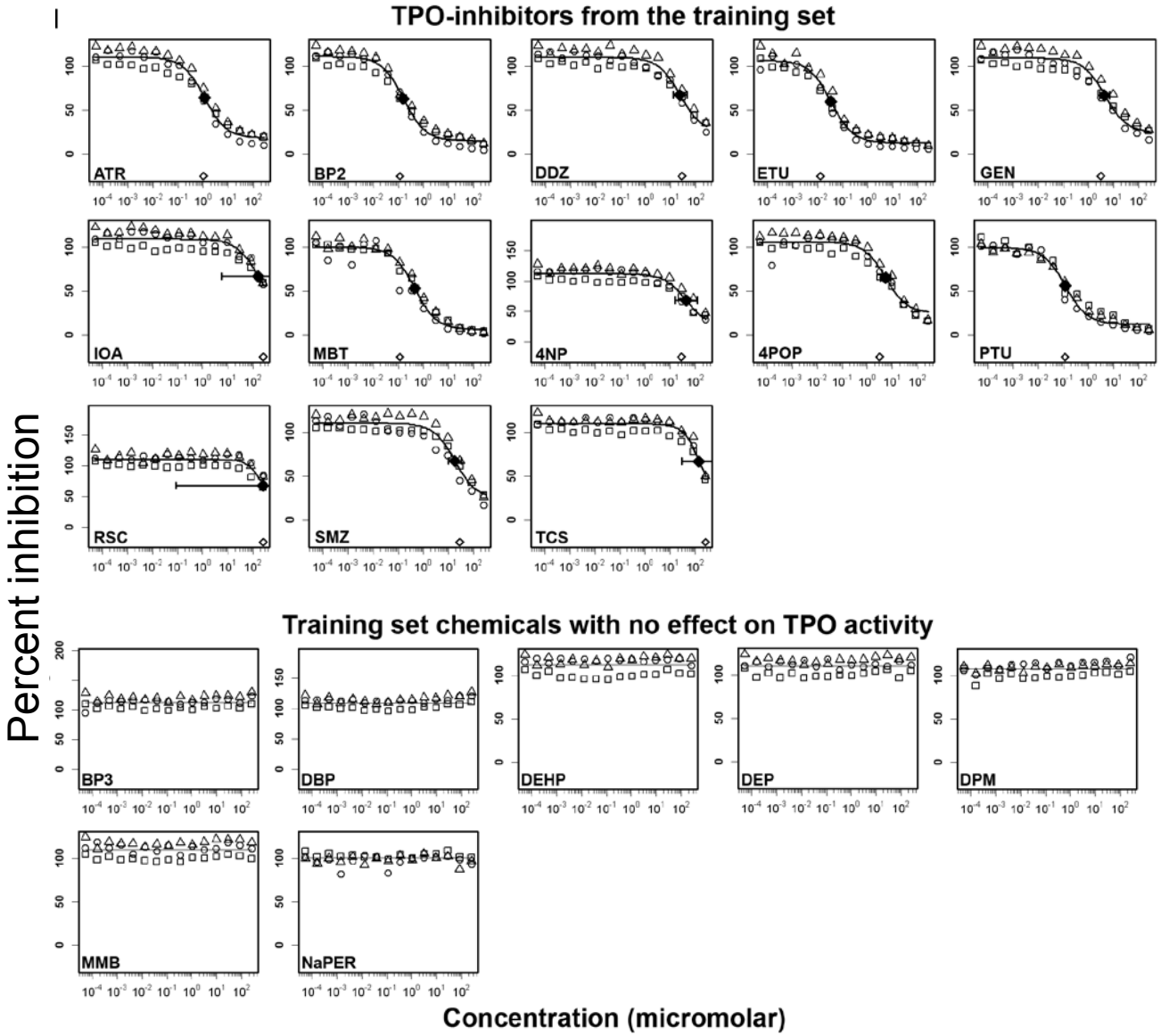
	Screening Technology			
Target	**Existing**	**Adaptable**	**R & D**	**Comments**
TSH Receptor Signaling			X	
TRH Receptor Signally	X			
NIS Mediated Iodine uptake		X		No commercial source
TPO inhibition			X	ORD method under development
Secretion from Thyroid Gland			X	
Transport proteins		X		Two methods, no commercial source
Deiodination			X	
Sulfation and Glucuronidation		X		Low-med throughput
Nuclear Receptors	X			Already done for >1000 chemicals
TH Membrane Transporters			X	
TR binding and transcription	X			Already done for >>8500 chemicals

*adapted from Murk et al., 2013

Positive control



- ***21-chemical training set***
- ***Automated 384-wp testing format***
- ***Automated data work flow for analyses (R.version 2.15.1)***



Paul et al., 2013

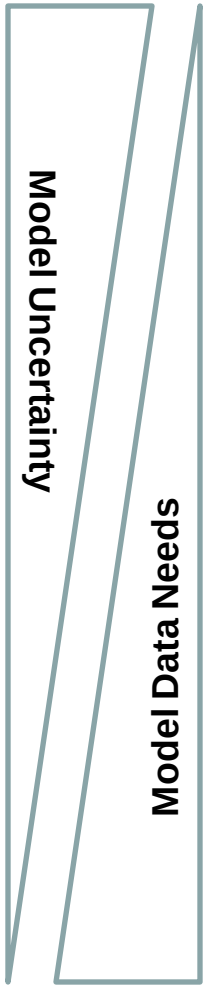
Challenges for Development and Use of AOPs

- Thyroid system disruptions are not the adverse outcome pathways

- For Example: What are all the MIEs and Pathways for environmental contaminants that cause developmental neurotoxicity?
  - Linking MIEs to adverse outcomes can be very difficult for some DNT outcomes
    - e.g., sodium channel disruption induced behavioral effects (pyrethroids)
  - Lack of known pathobiology of outcomes can makes this very difficult
    - where is the nucleus in the brain that is responsible for autismm or IQ loss, or ADHD (what are the MIEs that lead to IQ loss?)
- Development of quantitative predictive models can require a LOT of data
  - How much of a change in the MIE is needed to overcome compensatory processes? (%change in serum T4 and changes in brain tissue T3?

- How can we make use of 'partial' AOPs to reduce uncertainty in risk decisions?

- AOPs are not singular linear pathways, they are complex sets of interacting processes – it only gets more complicated – need to be prudent in deciding how much of a pathway is needed for a purpose



AOP Continuum

- **Simple correlative**
  - Links between inherent chemical properties and outcomes
- **Qualitative**
  - Known links between key events and outcomes
  - No complete set of key events – contains correlative "leaps"
  - Causative
- **Quantitative**
  - *Predictive quantitative models*

Domains of Application

Toolbox Categories

Read Across

Prioritization and/or Targeted Testing

Quantitative Risk Assessments

Examples

LogP and fish mortality

Neuronal-glia differentiation and developmental neurotoxicity

Qualitative Thyroid/DNT AOP

Quantitative models from MIE to health and community impacts

Announcement – AOPWiki

An AOPWiki is being developed and a beta version is being tested – public release later this year

- Developed as a joint project between:
  – OECD, EU Joint Research Center, Italy, US EPA, US Army Engineering Research and Development Center, Vicksburg MS

- Provides a ‘user-friendly’ interface for ‘crowd sourcing the development of AOPs

Link - www.aopwiki.org

Contacts: Edwards.Stephen@epa.gov
Clemens.Wittwehr@ec.europa.eu

Thanks for Listening