Thyroid Disruption and Neurodevelopment in an Adverse Outcome Framework: Translating NAMs - Filling in Gaps

Mary E Gilbert, PhD

Center for Public Health and Environmental Assessment

Office of Research and Development

US Environmental Protection Agency

Research Triangle Park, NC

Food Safety Commission of Japan

Tokyo, Japan

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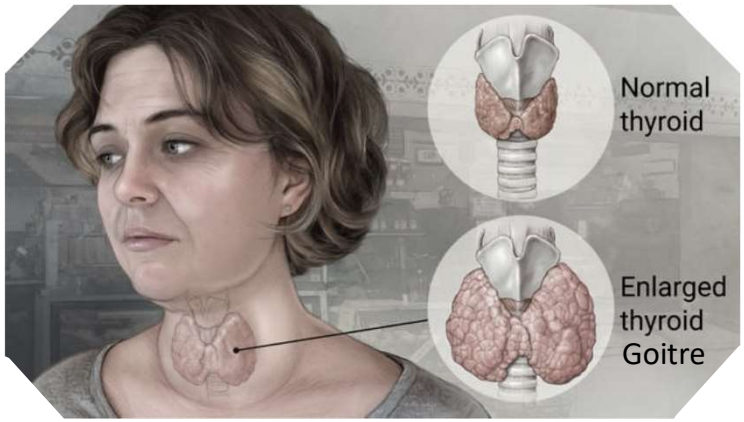
Thyroid Hormones and Physiology

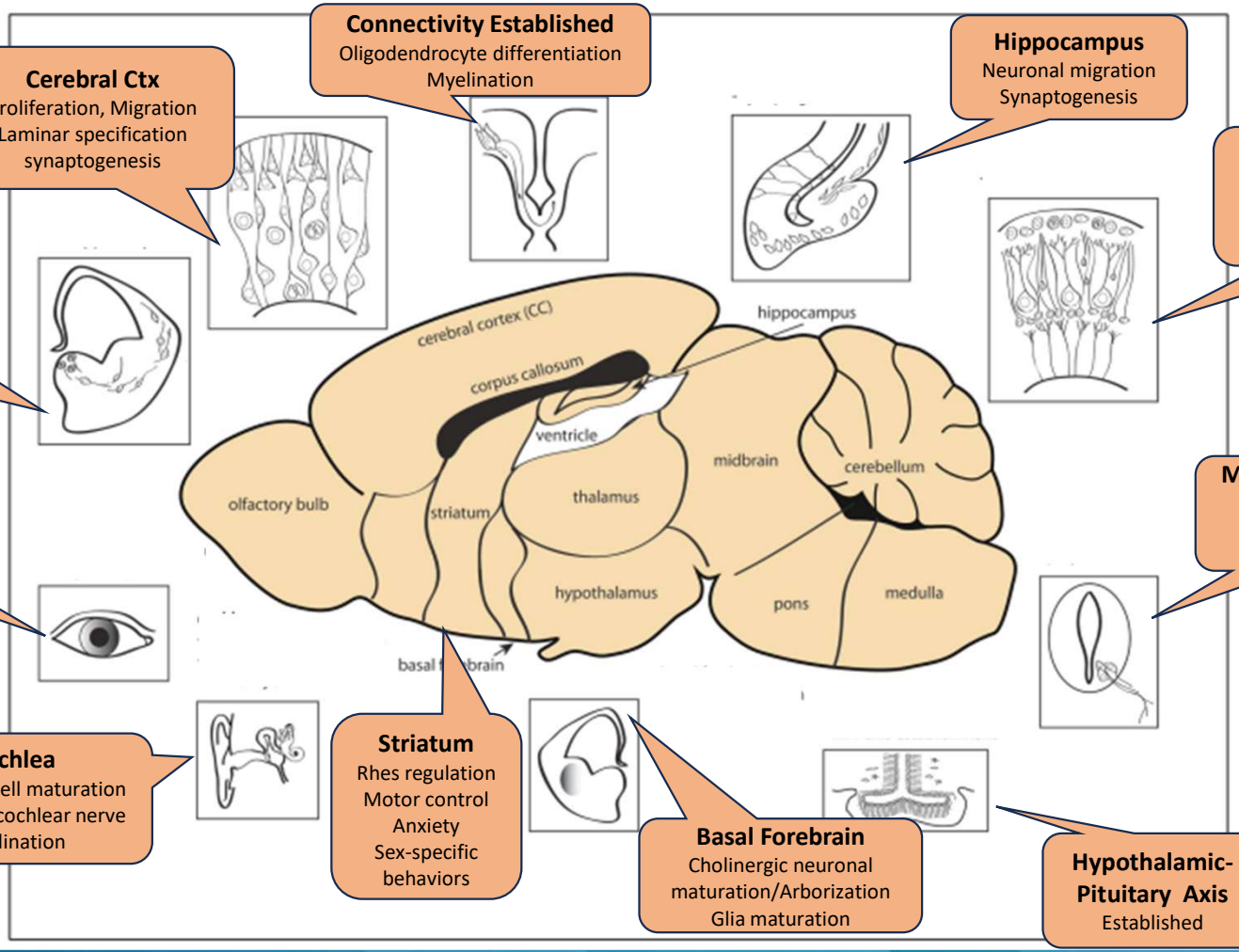
TH regulate processes in the body - Metabolic rate, thermogenesis.

- ***In the Adult:***
  - TH can impact weight gain, mood, cognition
  - ***Effects are largely reversible***



- ***In Fetus, Newborn, Child:***
  - TH mediate many aspects of somatic growth and development
  - TH especially critical for nervous system development
  - ***Effects are permanent***

Thyroid Hormones - Roles in CNS Development



Cerebral Ctx
Proliferation, Migration
Laminar specification
synaptogenesis

Connectivity Established
Oligodendrocyte differentiation
Myelination

Hippocampus
Neuronal migration
Synaptogenesis

Cerebellum
Precursor Proliferation
Purkinje Cell Arborization
GC Migration
Interneuron Generation

Inhibitory Interneurons
Cell Fate Specificity
Migration

Retina
M-cone
Differentiation
M-opsin Expression

Motor Neurons
CorticoSpinal Projections
Established

Cochlea
Inner hair cell maturation
Veistibulo-cochlear nerve myelination

Striatum
Rhes regulation
Motor control
Anxiety
Sex-specific behaviors

Basal Forebrain
Cholinergic neuronal maturation/Arborization
Glia maturation

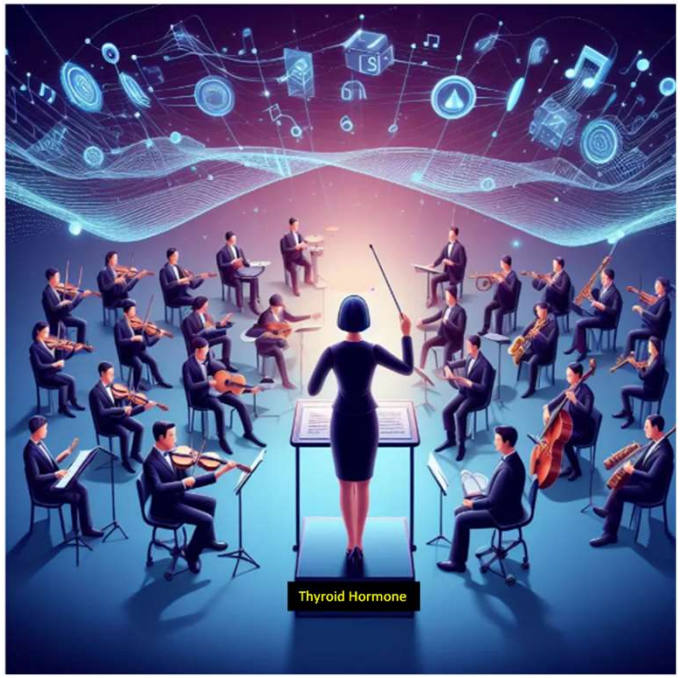
Hypothalamic-Pituitary Axis
Established

Modified from Stepien and Huttner FENDO, 2019

Multiple Processes -Multiple Regions -Independent Timelines

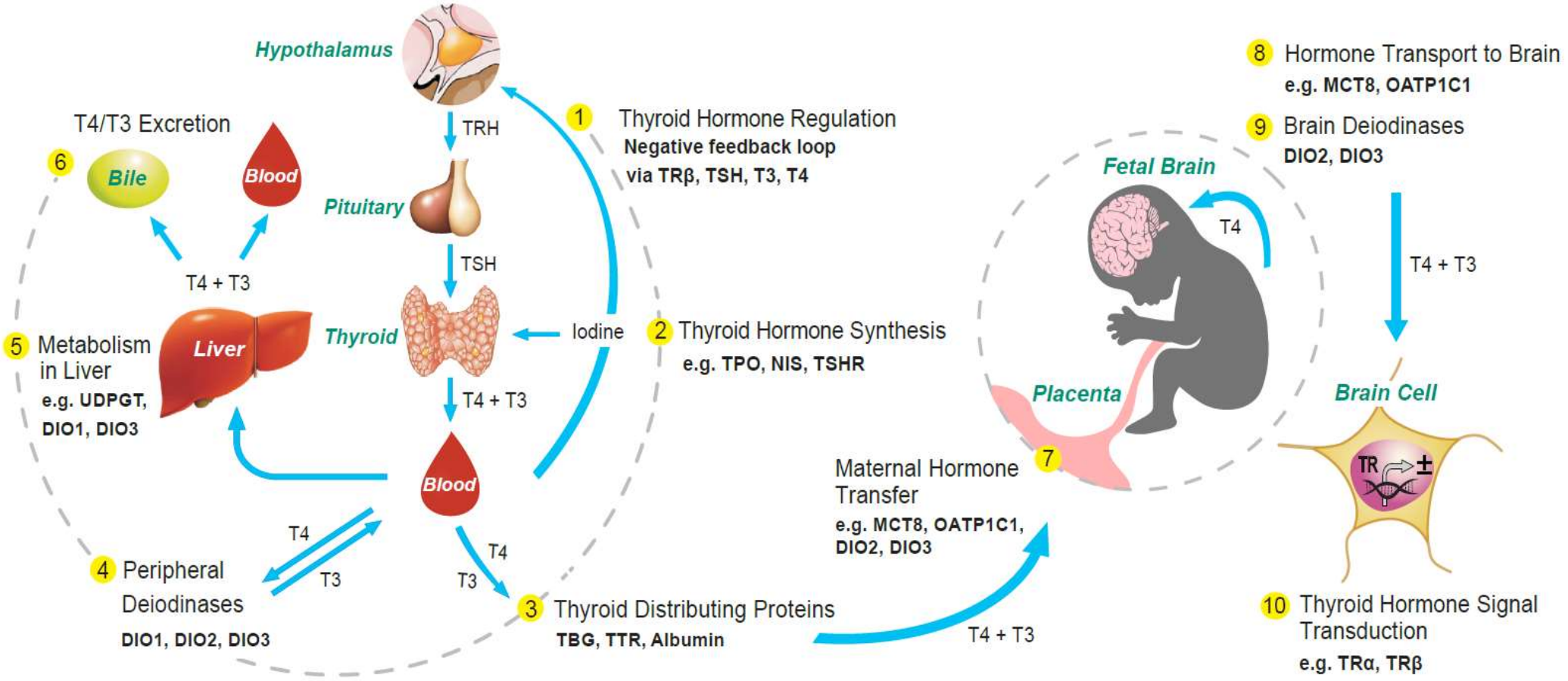
TH and CNS Development – Role as Master Timer

Multiple Processes -Multiple Regions-Independent Timelines

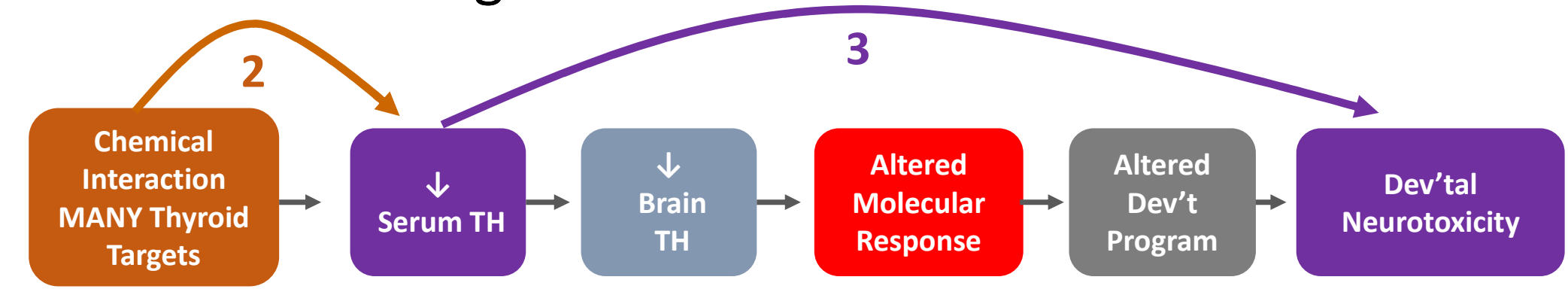


TH is not *ESSENTIAL* to these processes - they may proceed in its relative absence ….BUT…. they just won’t occur at the right time

Multiple Targets Within the Thyroid System



Regulator Concerned for DNT…..



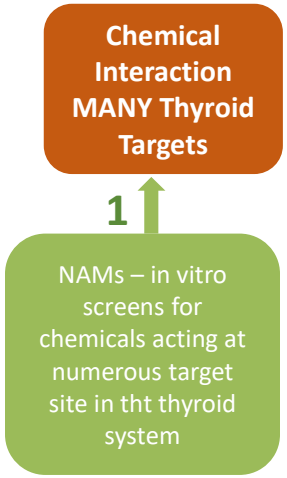
NAMs to the Rescue? THREE Big Gaps to Leap!

1. Do we have sufficient biological coverage of targets?
- Establishing confidence in NAMs

2. From *in vitro* 'hit' to serum TH
- Establishing translation of NAMs IVIVE

3. From serum to brain
- Establishing bioindicators of TH-dependent AO

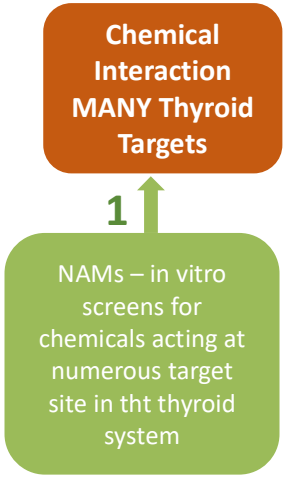
Leap 1: What is a NAM?



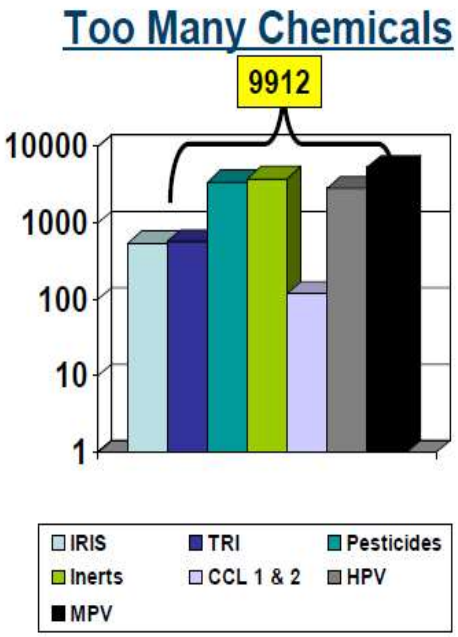
NAMs - New Approach Methodology - Any technology, methodology, approach, or combination of the three that can be used to ***replace, reduce, or refine*** animal toxicity testing.

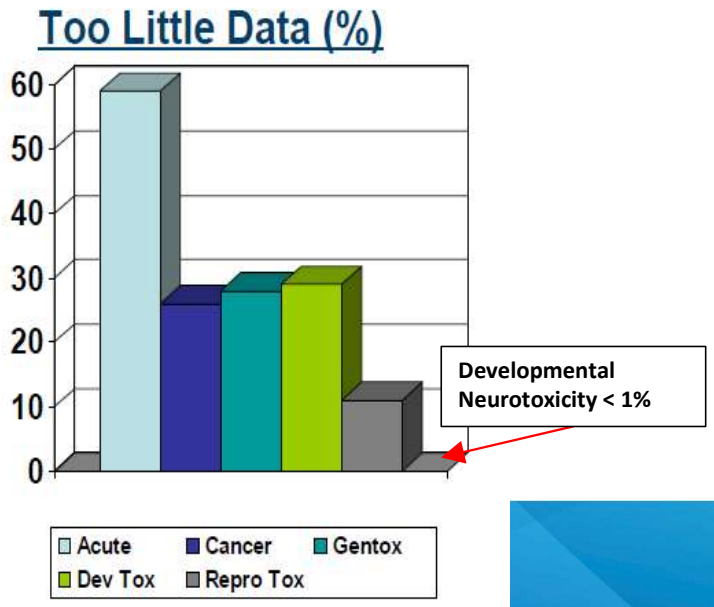
These methods include computational modeling, assays with biological molecules, cells, tissues, or organs, whole-organism assays.

Leap 1: Why do we need NAMs?



- Too many chemicals! Traditional testing is too slow and expensive.
- 3R's: Reduce, Refine, Replace use of animals in testing
- Let's catch up! Existing regulatory assays have not kept pace with advancements in scientific knowledge and technology

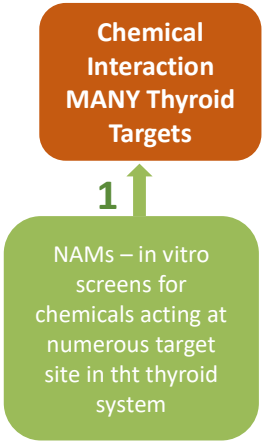




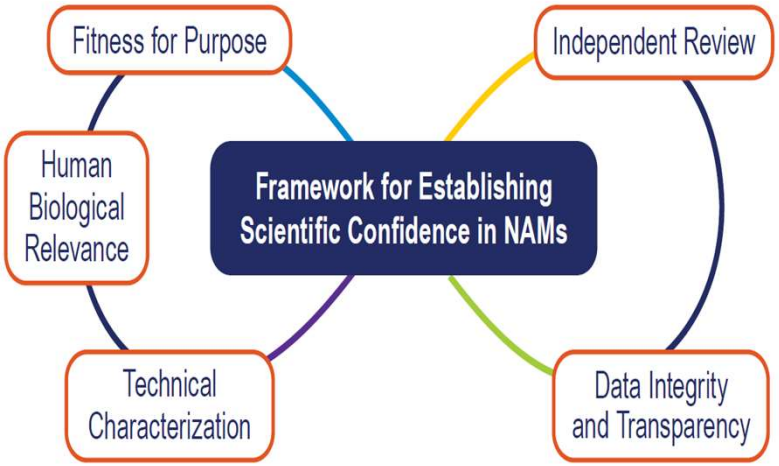
- Needs/congressional mandates/ regulatory drivers at EPA demand a efficient means to identify and prioritize chemicals for testing - e.g., OPP, FIFRA, TSCA, FQPA, EDSP
- **Complexity of thyroid system - many targets! Need screening assays for chemical interference thyroid axis due to concern of DNT**

Leap 1: Roadmap to Building Confidence in NAMs

Criterion for confidence in NAMs



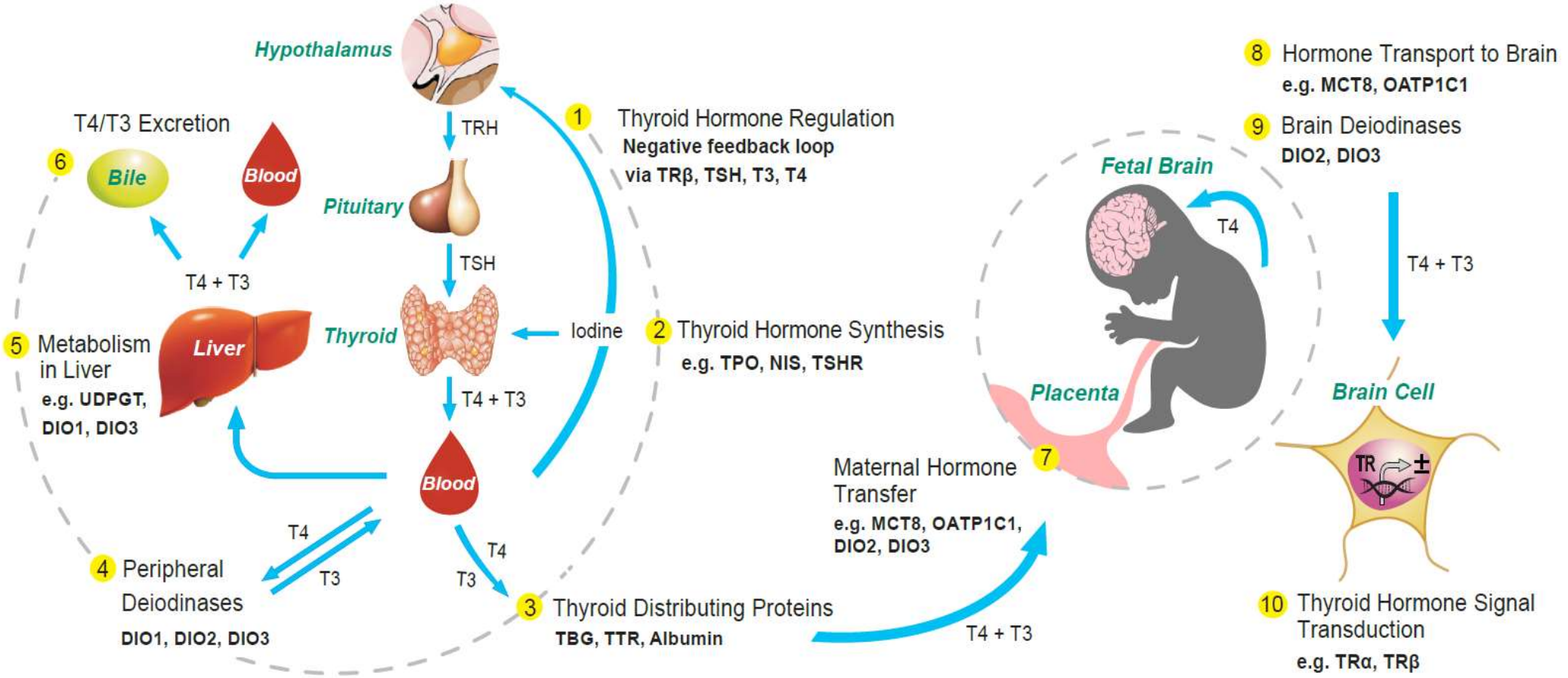
a) Understand the biology- what are the targets?
b) Data integrity and transparency-availability of data, stated uncertainties and limitations
c) Technical characterization-acceptance criteria, assay interference, reproducibility, transferability
d) Fit for purpose – Spectrum of Assessment Applications
e) Human relevance- does the NAM reflect human biology – ie are the KEs relevant to AO?



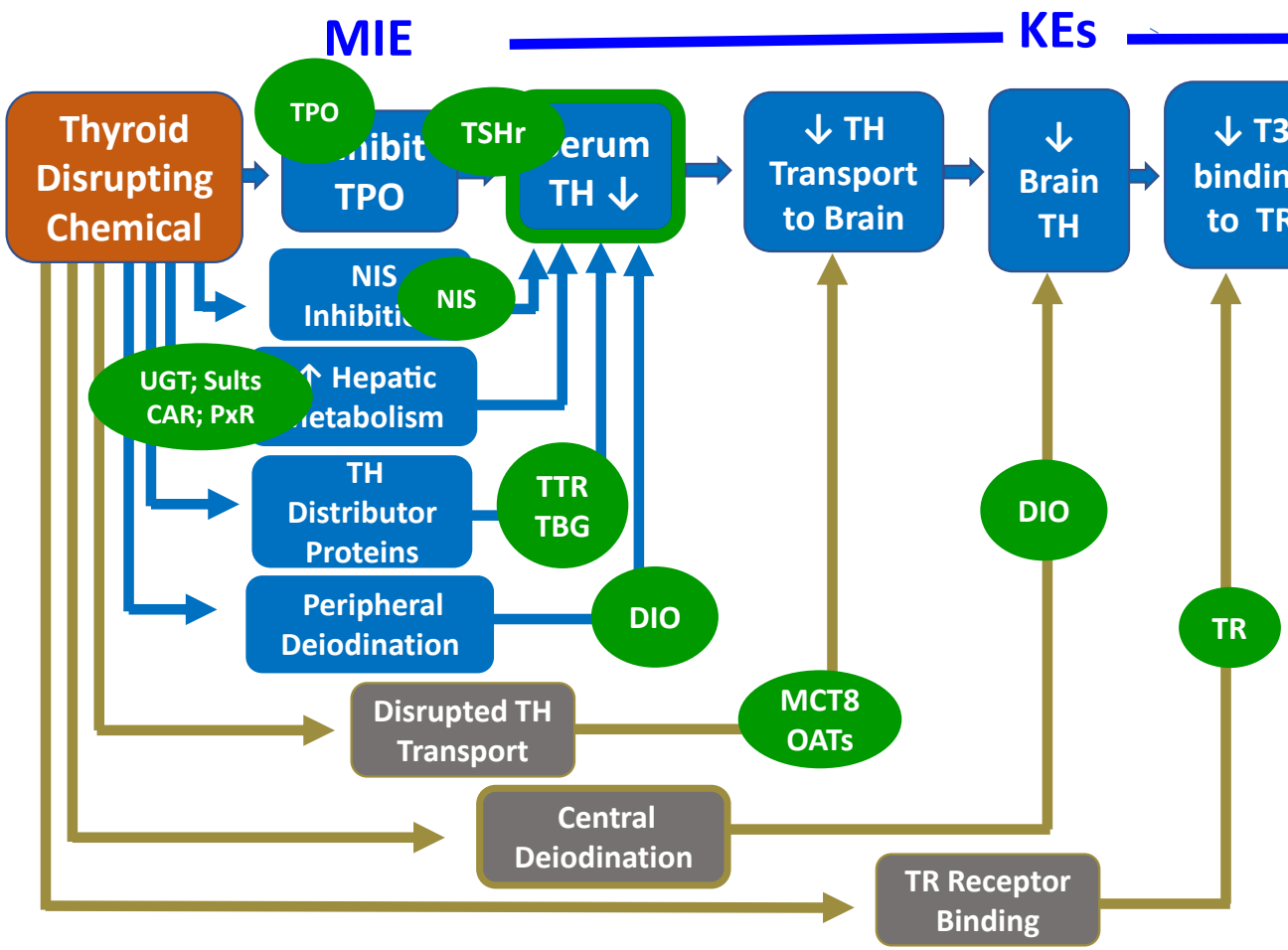
A roadmap has been established

Van der Zalm, et al., Arch Toxicol. 2022 Nov;96(11):2865-2879. doi: 10.1007/s00204-022-03365-4.
Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies Interagency Coordinating Committee on the Validation of Animal Methods (ICCVAM). March 2024

How are we doing in NAMs for thyroid targets?



Leap 1: AOPs Guide NAM Development and Utility



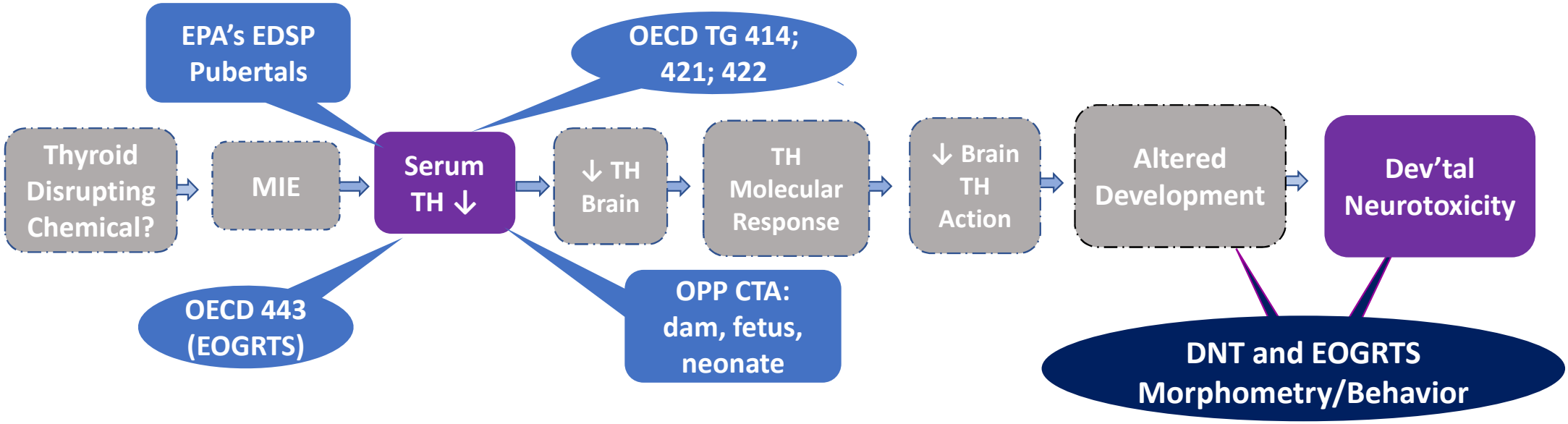
Progress - NAMs 'Exist' for Multiple Sites of Chemical Interaction

…. but …..

- missing targets remain
- acceptance criteria lacking for many
- no orthogonal/confirmatory assays

Leap 3: Translating Serum TH to DNT

Current Mammalian Guidelines: Reliance Thyroid Histopathology & Serum TH



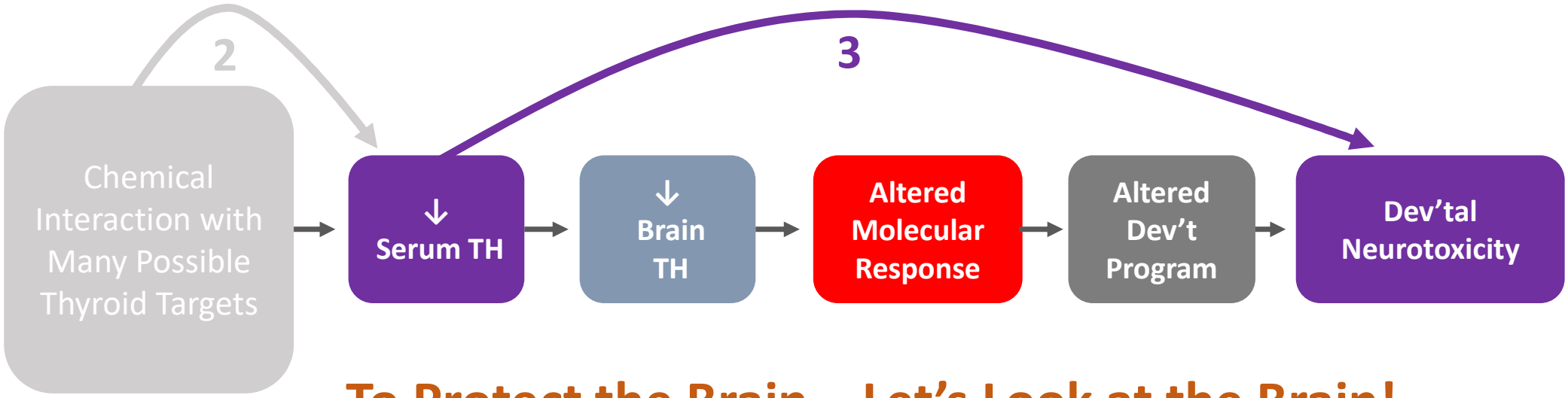
Serum TH – Common KE

- Included in Many Guideline Studies
- Easy to Measure, Clinically Relevant

TH-Relevant Outcomes?

- Brain Morphometry
- Motor Activity, Acoustic Startle, Learning & Memory

Leap 3: AOPs- Improve Translation from Serum to DNT



To Protect the Brain – Let's Look at the Brain!

AOP - Assemble Available Knowledge From Target to Adversity

Start with well characterized goitrogen, PTU

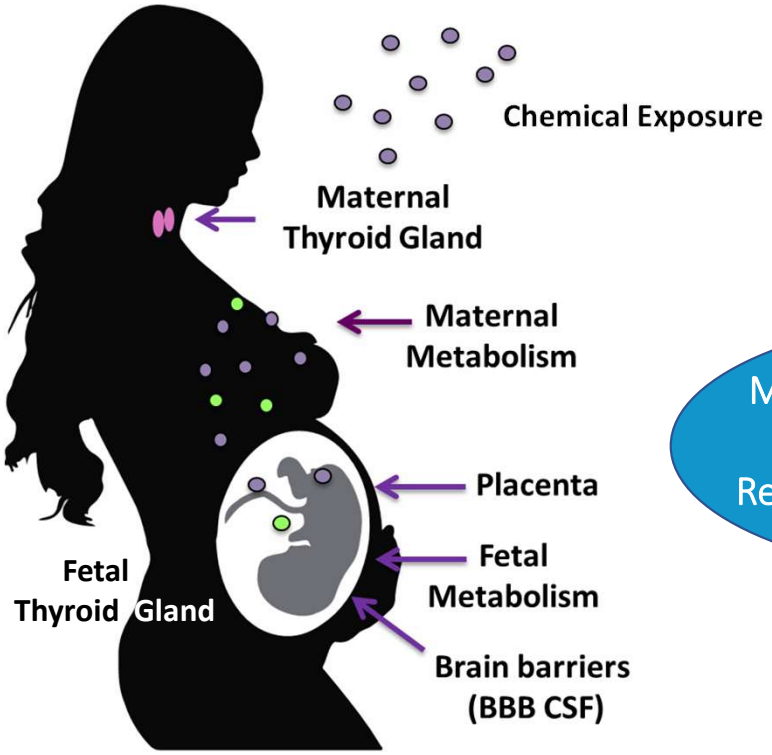
Exploring lower end of the dose response curve

Filling in the gaps from serum to brain function

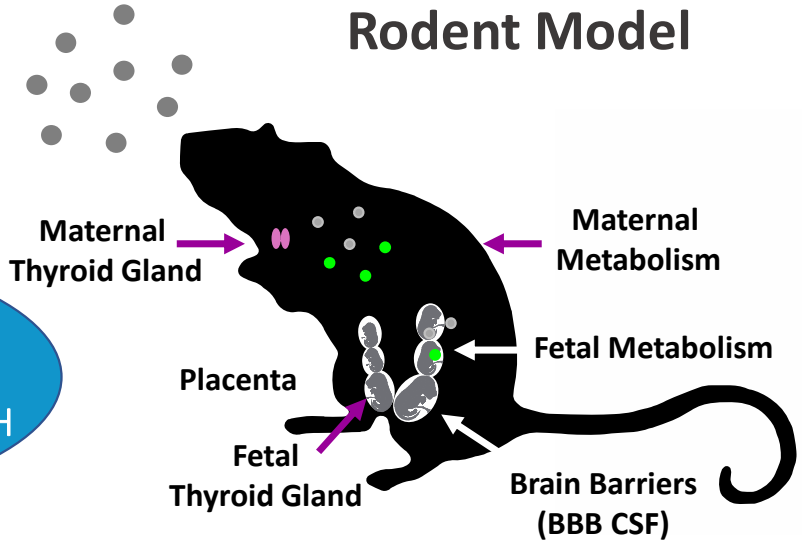
Identify Intermediate Markers of TH-dependent neurodevelopment

Environmental Thyroid Disruptors and Neurotoxicity

Rodent Models Mimic The Complexities of Thyroid Biology During Development



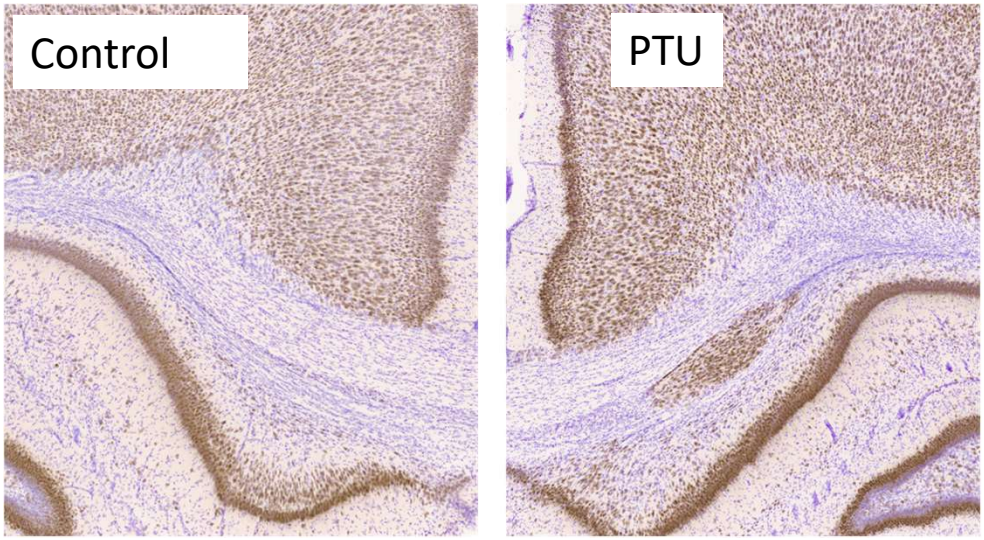
Maternal and Fetal Metabolism
Placental and Brain Barriers
Reliance on Maternal Sources TH



We have learned HPT/Brain from rodent models using high dose PTU – BUT - neurological effects in rodents with *moderate* changes in serum TH remain elusive

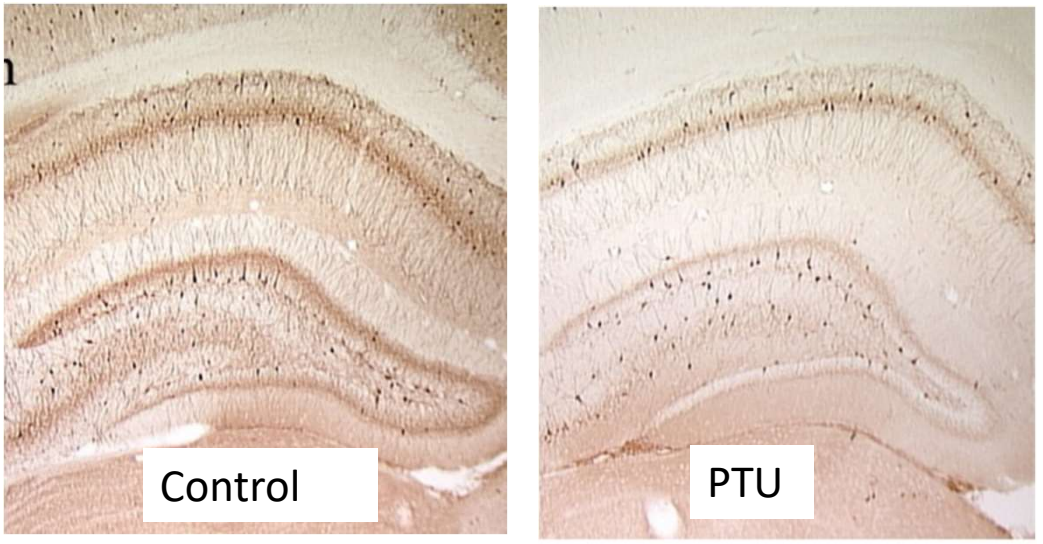
‘Intermediary’ TH-Dependent Brain Metrics

TH and Neuronal Migration



TH Insufficiency - Periventricular Heterotopia
An aberrant cluster of ectopic neurons

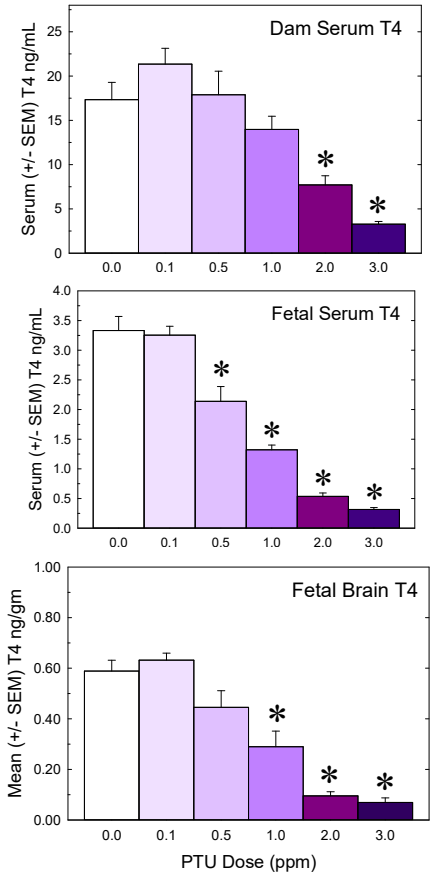
TH and Inhibitory Interneurons



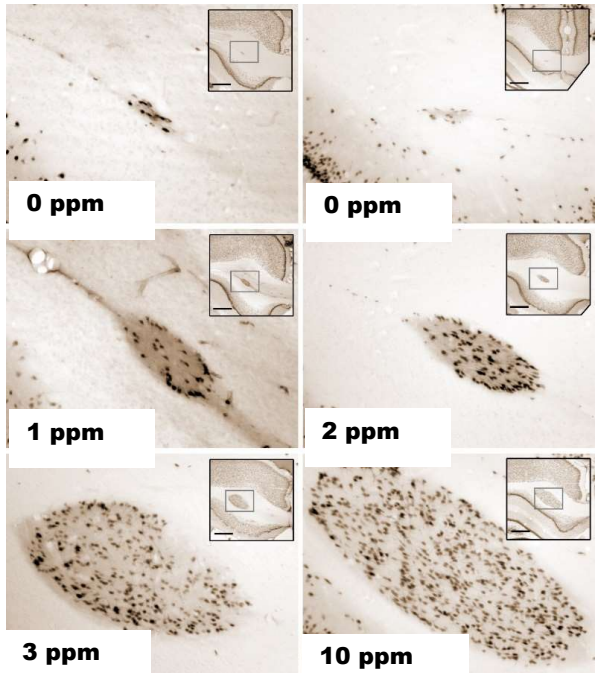
TH Insufficiency and Parvalbumin Interneurons
Parvalbumin+ cells reduced in brain

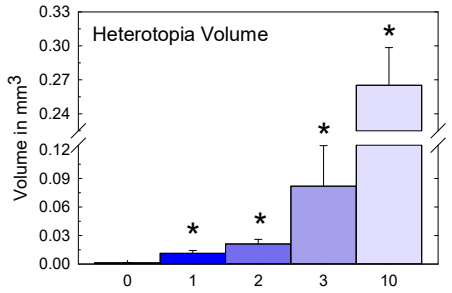
Heterotopia: Migration Errors From TH Insufficiency

Key Features of PTU Model of Developmental Hypothyroidism



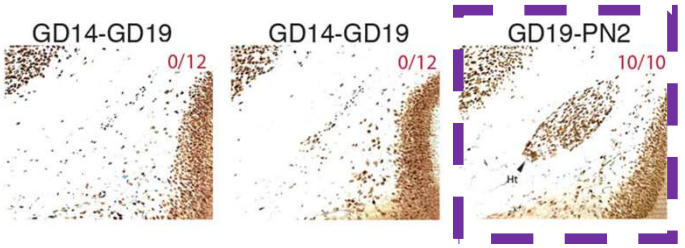
- Dam and fetal serum T4 ↓
- Fetus more sensitive than dam
- Fetal Brain T4 ↓



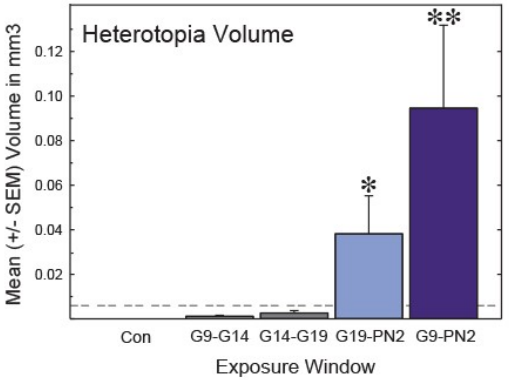


- Evident at doses that do not reduce serum T4 in pregnant dam
- Size dose-dependently increased

Critical Window in Beginning Late Gestation Heterotopia Persists to Adulthood

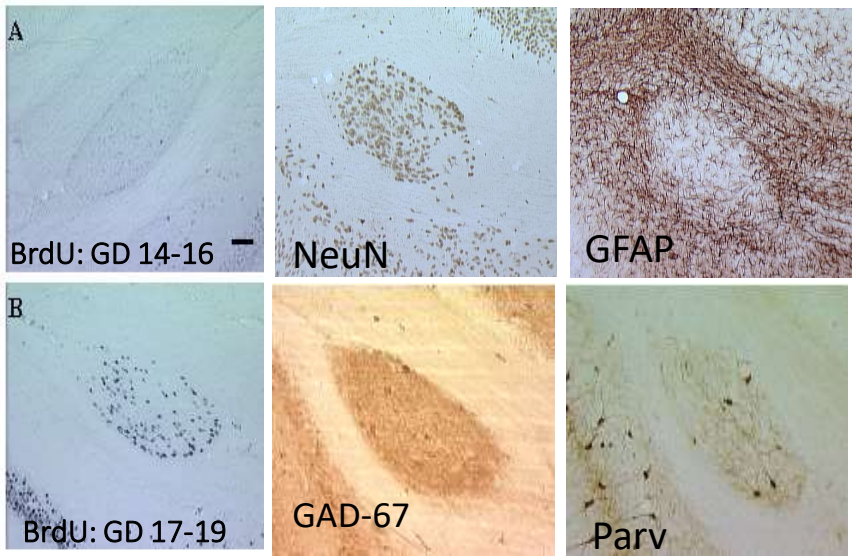


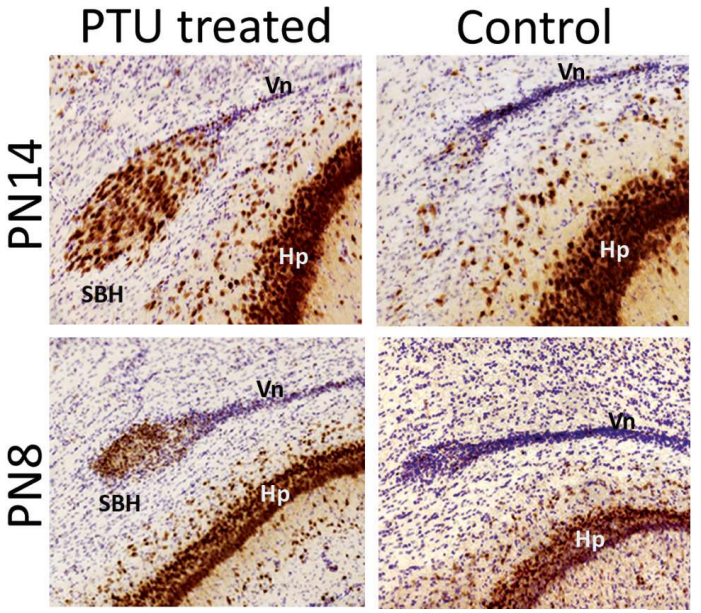
5-day Perinatal Exposure is Sufficient

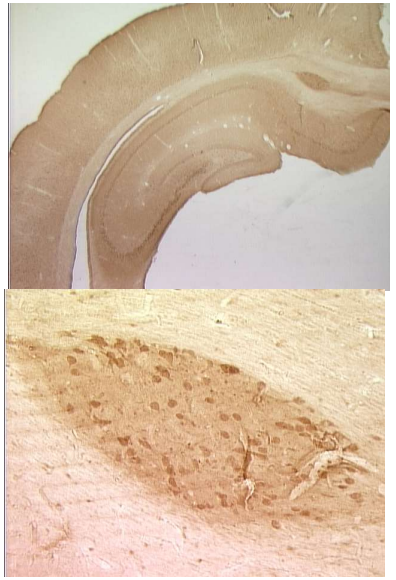


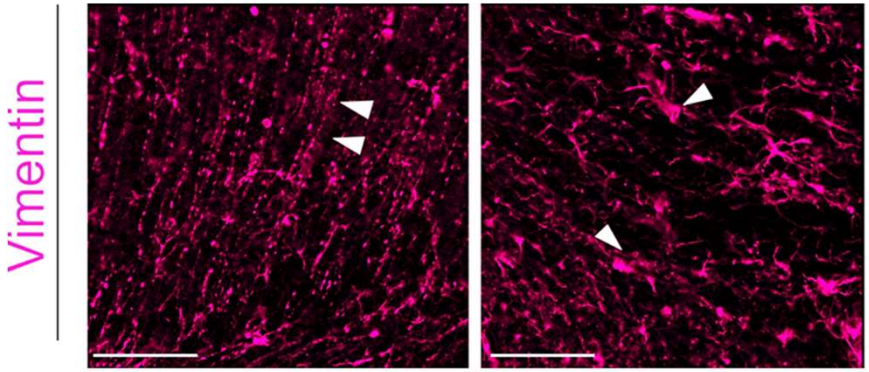
Hassan et al., 2017
Gilbert et al., 2014;
O'Shaughnessy et al., 2019

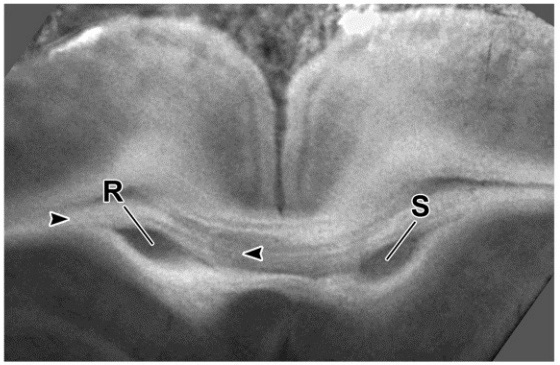
Heterotopia Fun Facts ….. with Implications

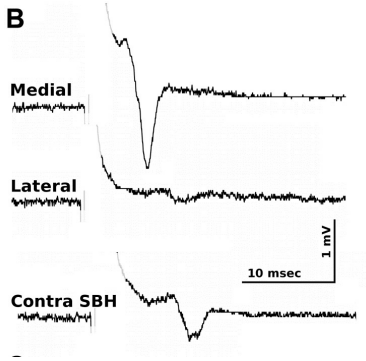






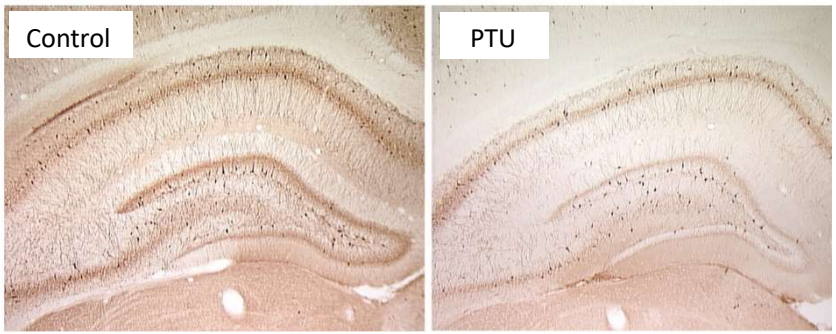




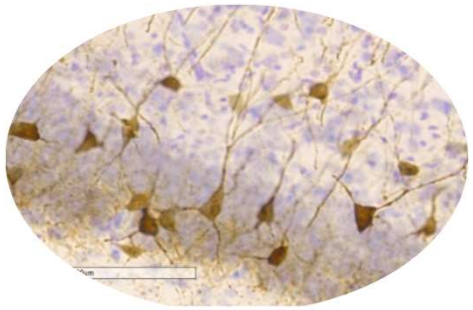


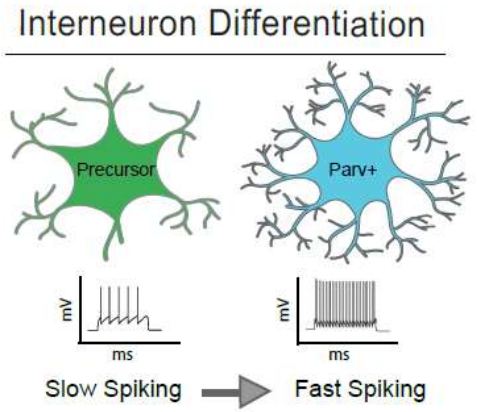
Goodman and Gilbert, Endocrinology, 2008; Gilbert et al., JNeuroEndo., 2014; O'Shaughnessy et al., SciReports, 2019; O'Shaughnessy et al., FrontEndo, 2023

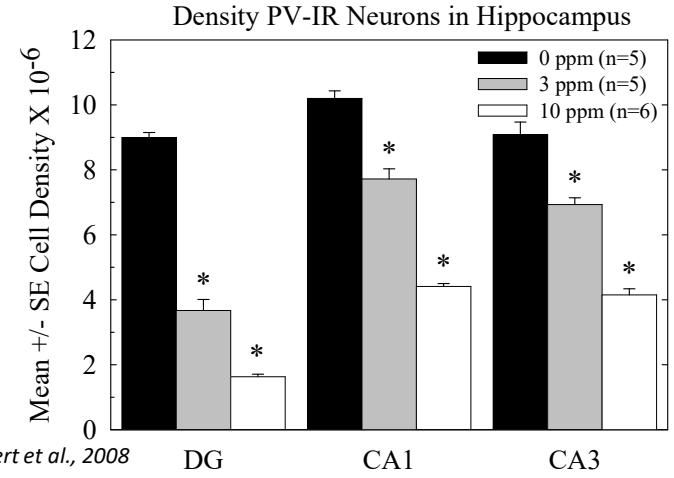
Pv⁺ Expression Altered in PTU model of Developmental Hypothyroidism



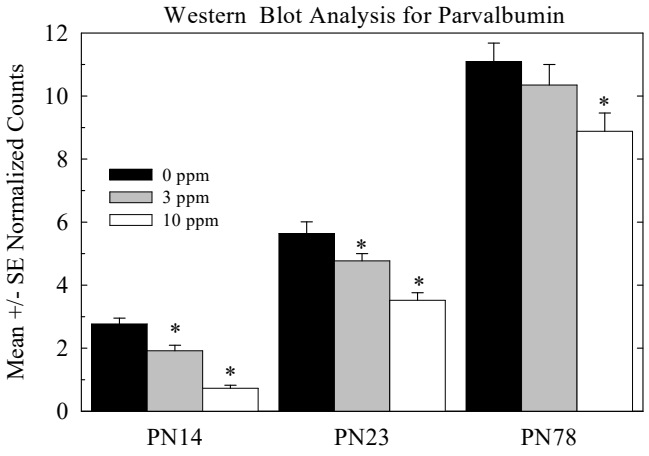
PV+ cells reduced in hippocampus

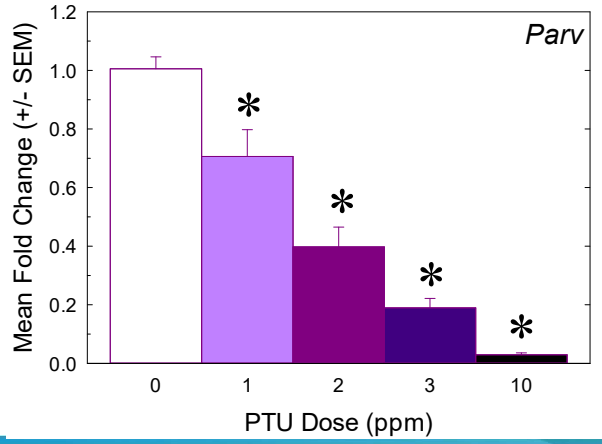






Gilbert et al., 2008



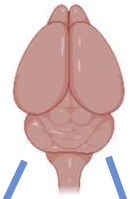


Pv+ cell number, Pv protein concentration, *Pv* gene expression are all reduced in PTU model

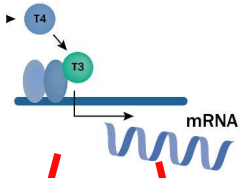
Leap 3: Intermediary TH-Dependent Biomarkers of Altered Brain Development -Filling some gaps from serum to brain

TPO Inhibition *Low Dose PTU Model* → **↓ Serum TH** → **↓ Brain TH** → **Altered Molecular Response** → **Altered Neurodev't** → **Dev'tal Neurotoxicity**

Serum TH
- Low levels
- Low volumes
- Sensitive assays

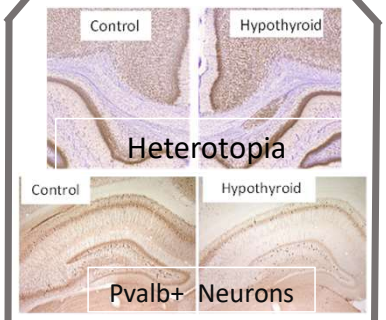


Brain TH
- Low levels
- Small mass
- Sensitive assays



TH Action

Transcriptional Readouts
- Vary by region
- Vary by age

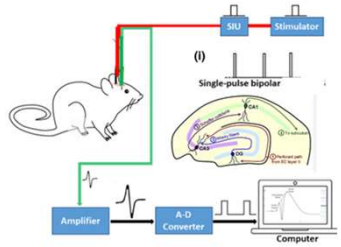


TH-Dependent Structural Defects

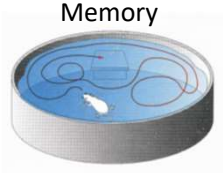
Cell Fate Specificity
Neuronal Migration
- Vary by region
- Vary by age

TH Mediated Functional Deficits

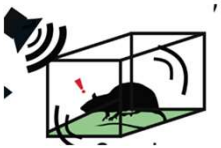
Neurophysiology ReadOuts
Basis of Communication



Learning
Memory

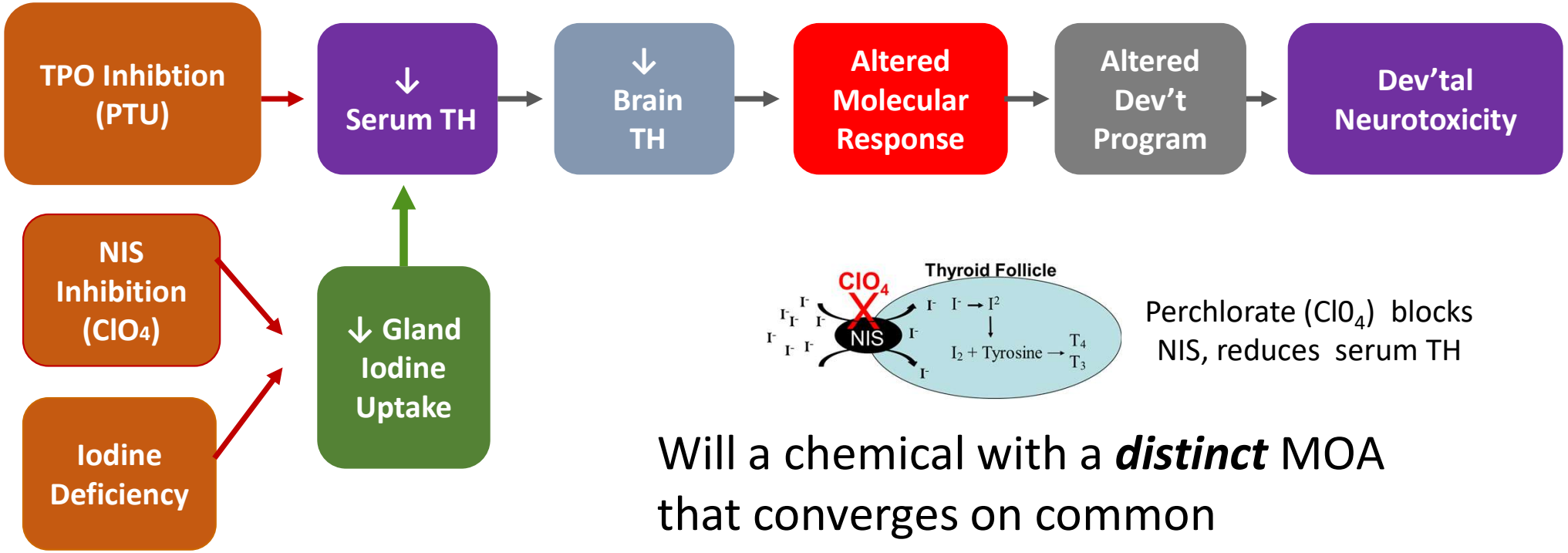


ASR
PPI
Sensory Gating



Applicability to Other MIEs??

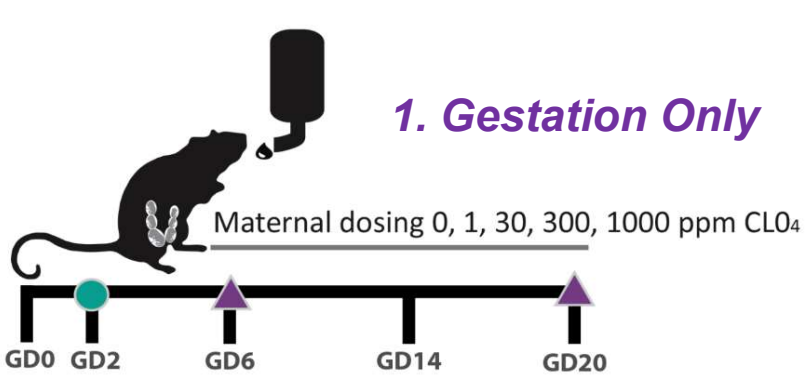
AOPs - Testing the hypothesis – Case study with ClO_4

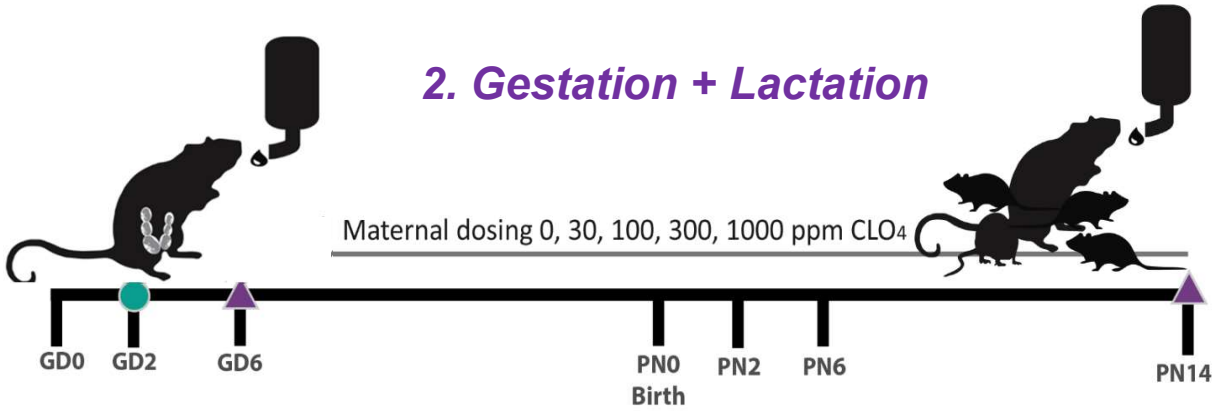


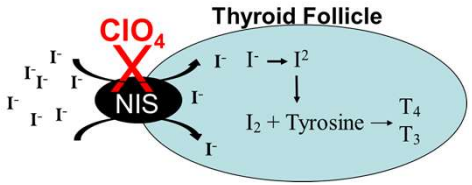
Perchlorate (ClO_4) blocks NIS, reduces serum TH

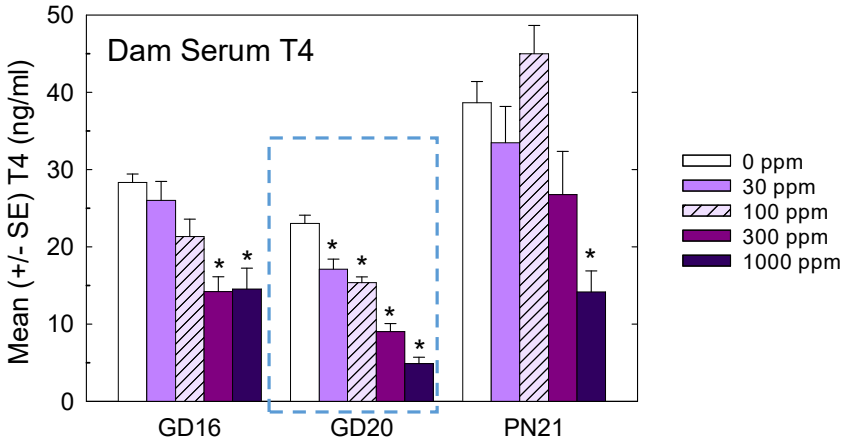
Will a chemical with a ***distinct*** MOA that converges on common downstream KE induce similar AO?

Perchlorate Exposure Scenarios – ~CTA Study Design







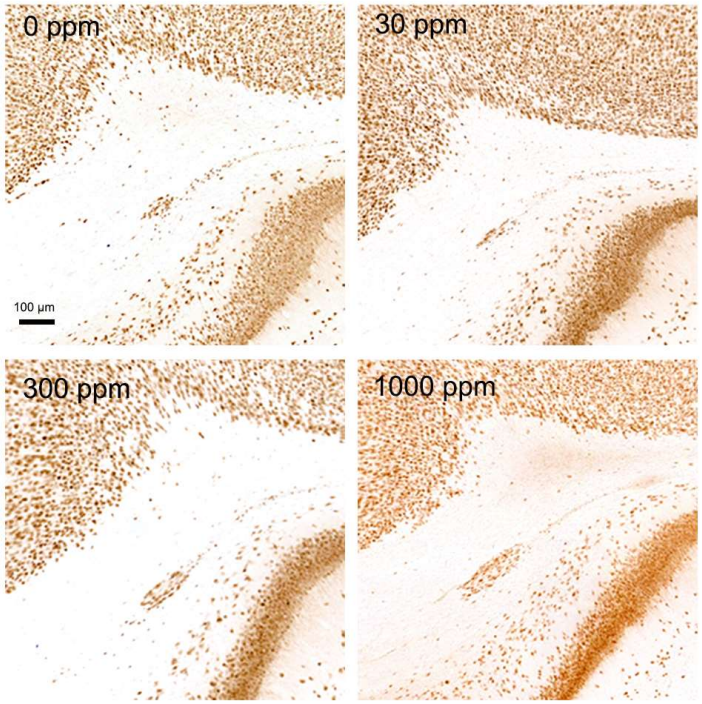


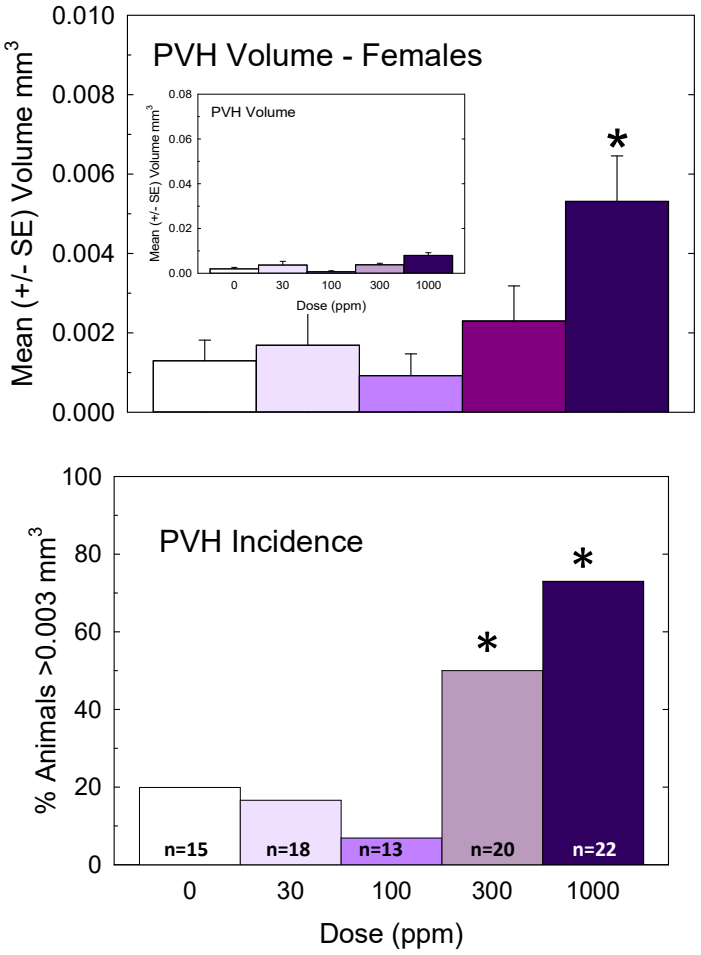
Gilbert et al., Tox Sci 2022

Perchlorate Reduced Maternal Serum TH

Gilbert et al., Tox Sci 2024

Perchlorate Induces a Small Heterotopia



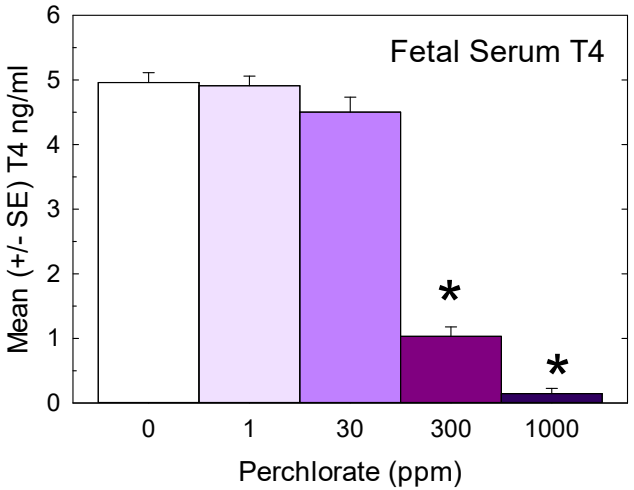


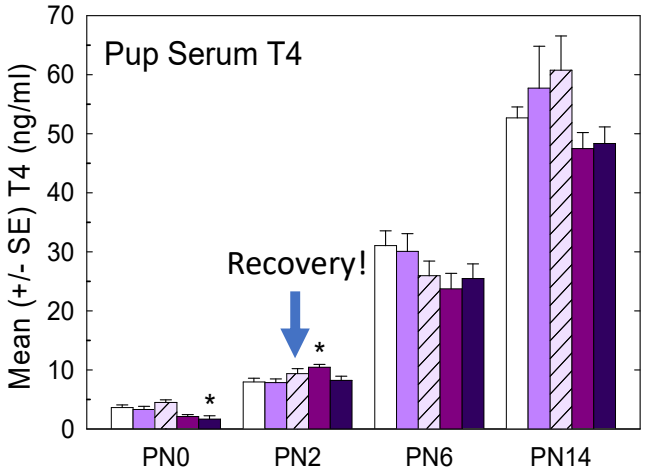
Gilbert et al., Toxics, 2023

Despite Maternal T4 Deficits Heterotopia Small – WHY?

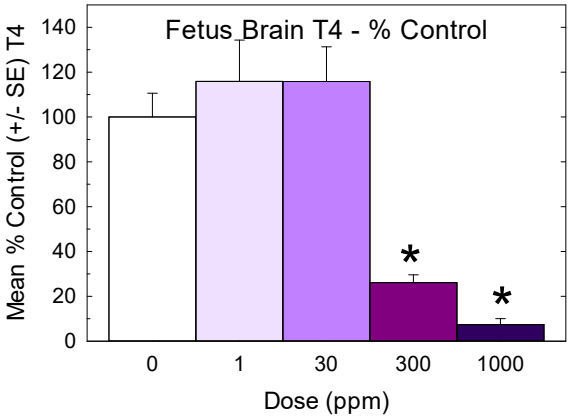
Despite Maternal T4 Deficits Heterotopia Small – WHY?

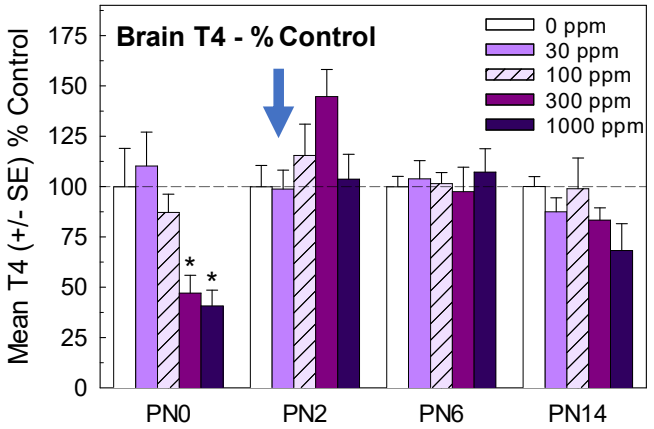
Serum TH reduced in fetus, but not pup





Brain TH reduced in fetus, but not pup





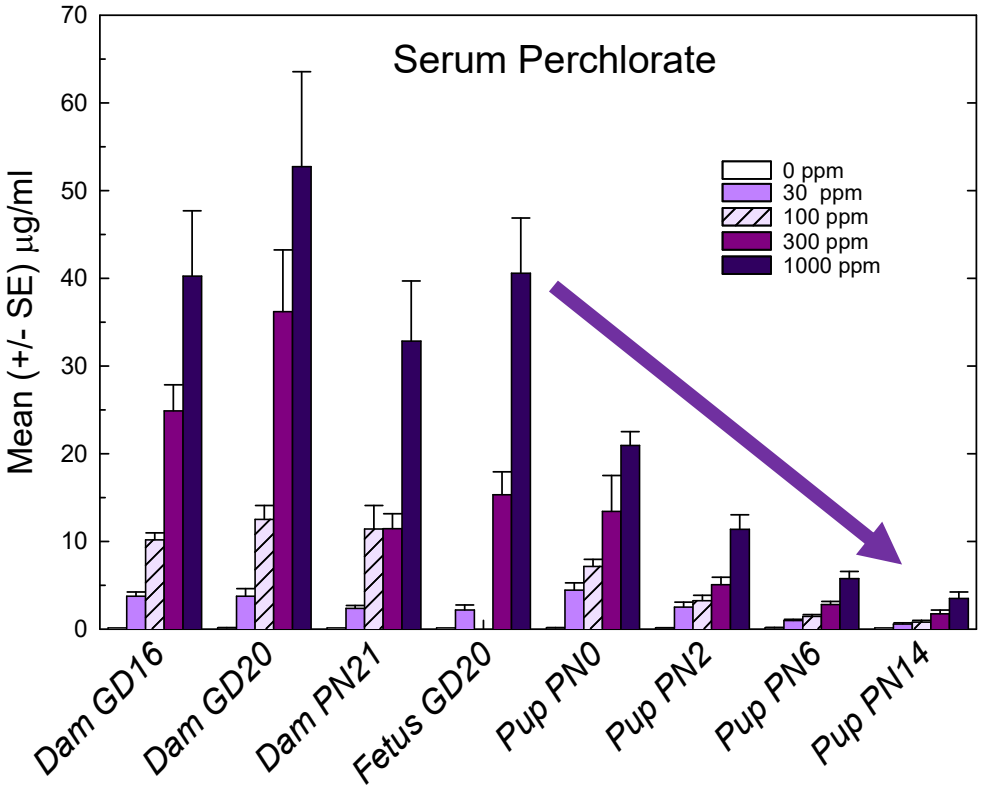
Gilbert et al., Tox Sci 2022

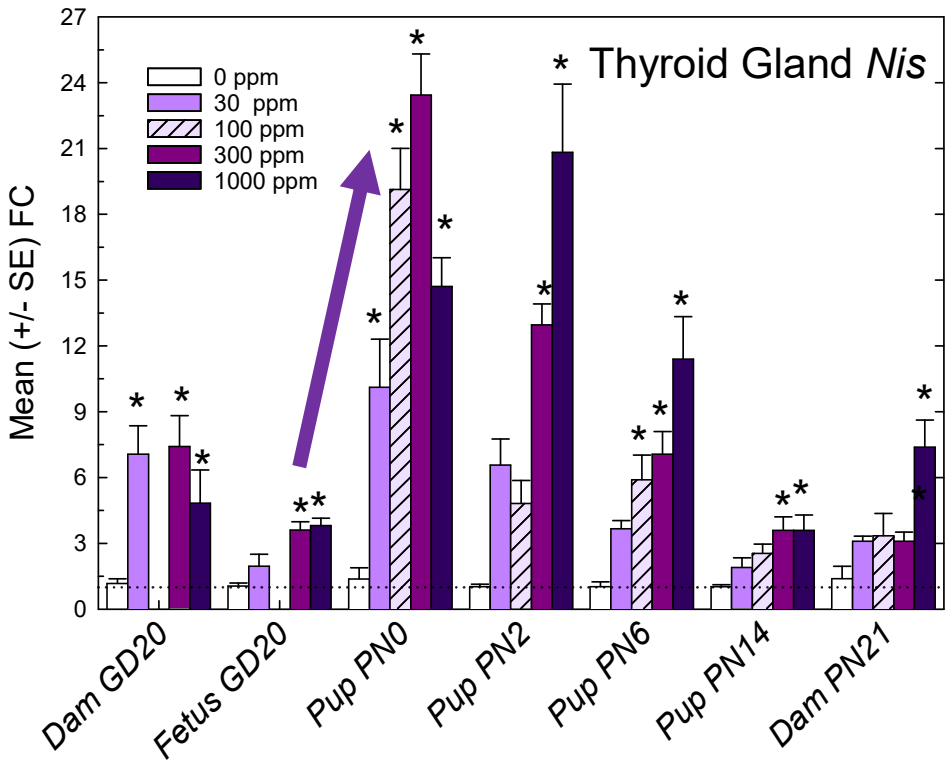
Unlike PTU – Pup serum and brain TH recover – WHY?

Gilbert et al., Tox Sci 2024

Unlike PTU – Pup serum and brain TH recover – WHY?

Reduced Exposure – Augmented Iodine Uptake

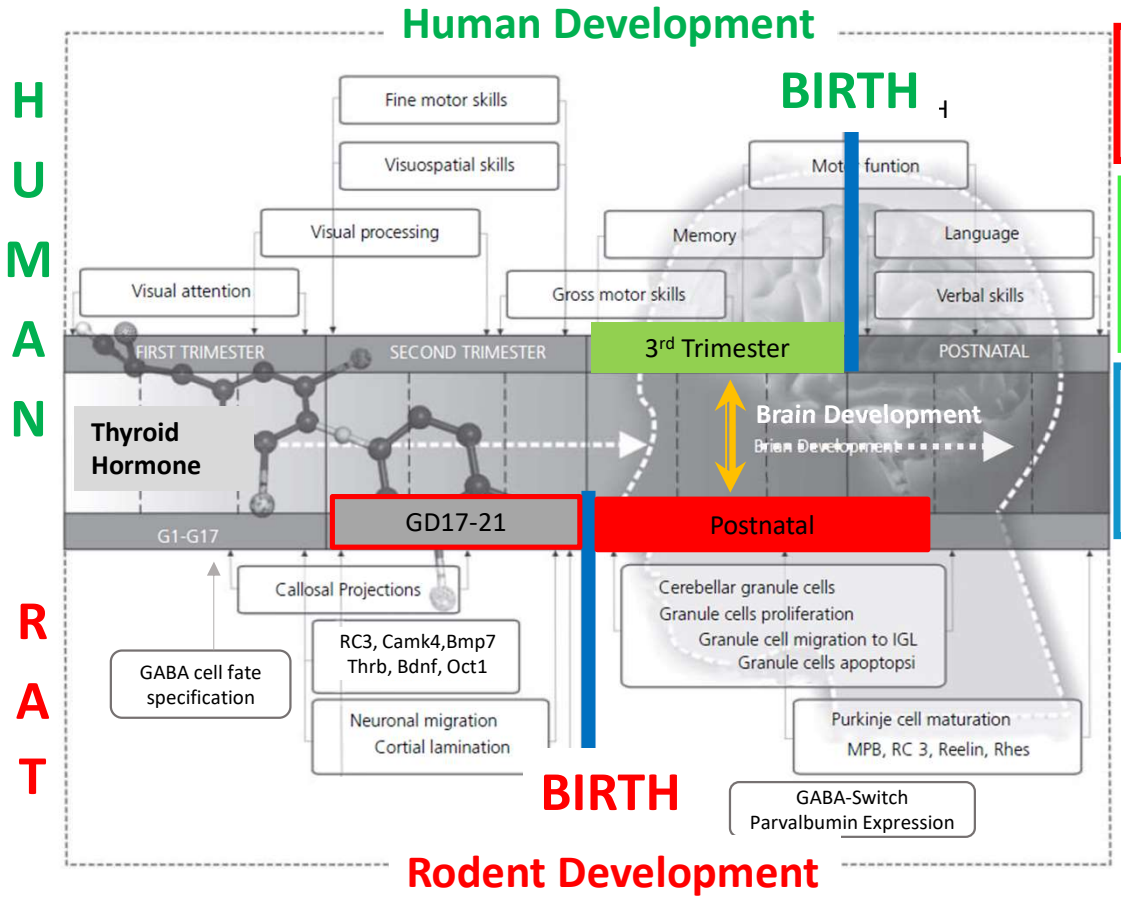




Much lower concentrations of ClO_4 in serum of pups relative to fetus/dams - limited transfer/availability?

Huge upregulation of *Nis* in newborn relative to fetus – increase in iodine uptake

Of Rats and Men- Differing Timelines of Brain Development



RAT: TH-dependent neurodevelopmental events begin just before birth

HUMAN: Analogous TH-modulated brain development in the ***human fetal brain*** *occurs* ***postnatally*** in the rat

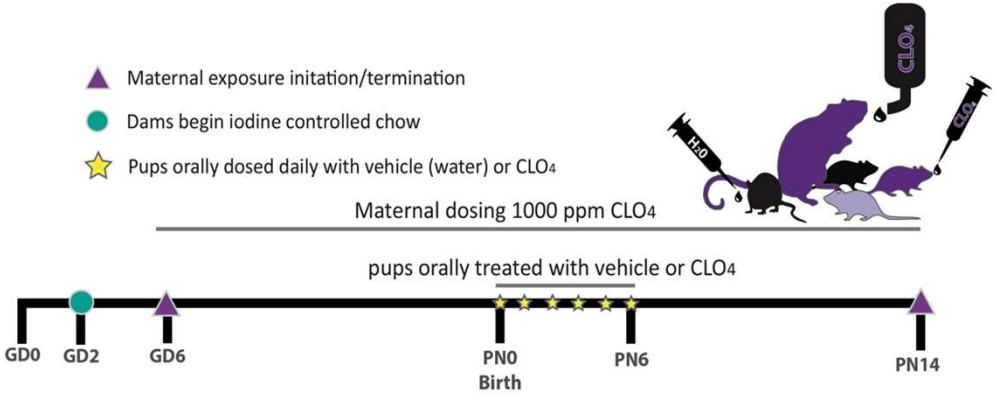
Marked changes in exposure and pharmacokinetics at parturition in **rat do not occur in human** fetus for another 3 months as brain continues to develop

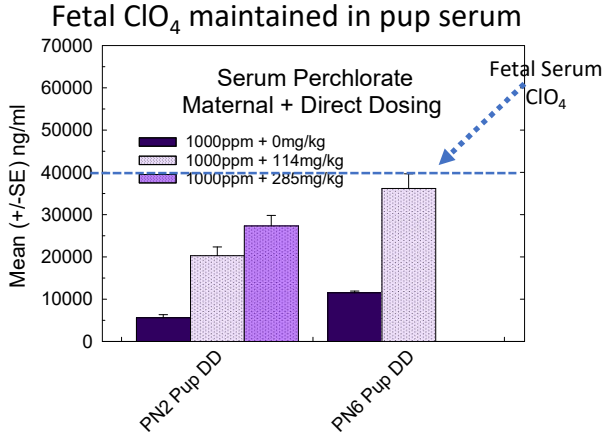
Chemically-induced alterations in the thyroid milieu measured in the fetal rat would be maintained during the extended period of in utero brain development in humans.

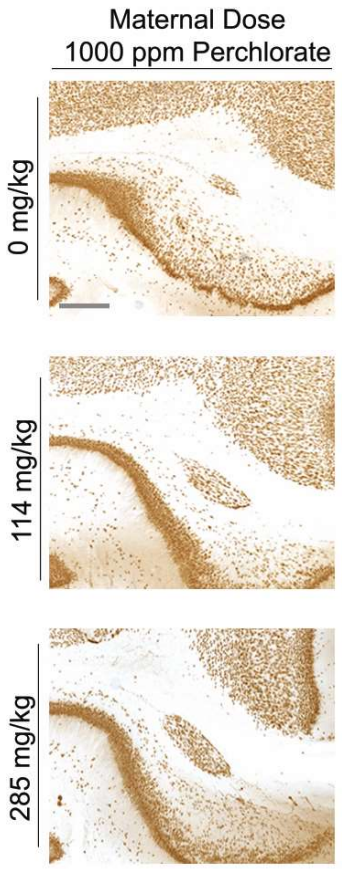
What if we were to 'extend' the 'fetal thyroid environment' in the rat to parallel human?

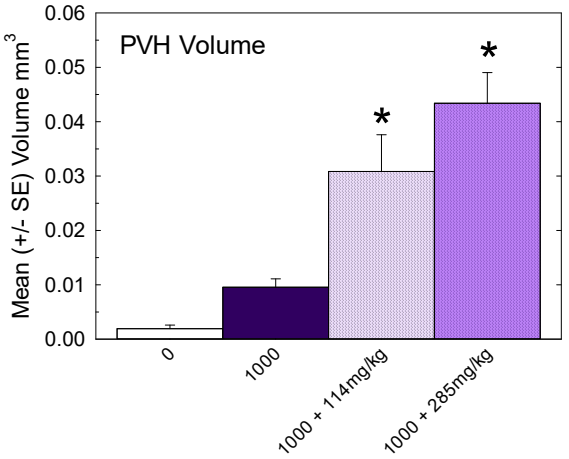
Zoeller and Rovet, 2010
With modifications

Maternal ClO_4 and Direct Dosing to Pup - Emulating 3rd Trimester in Human Brain Development



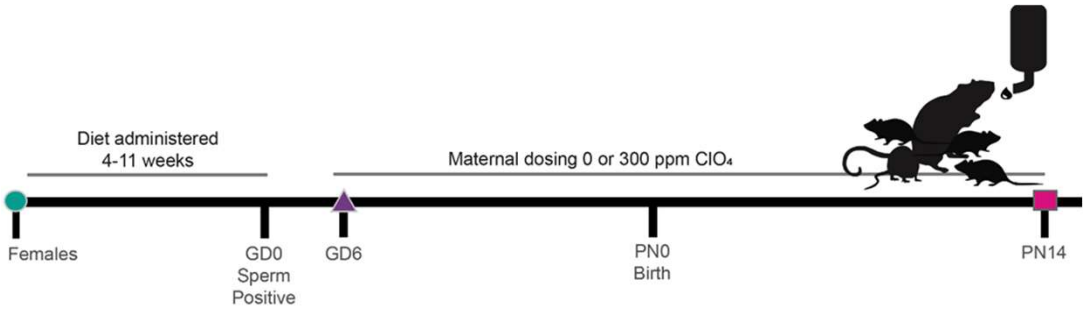




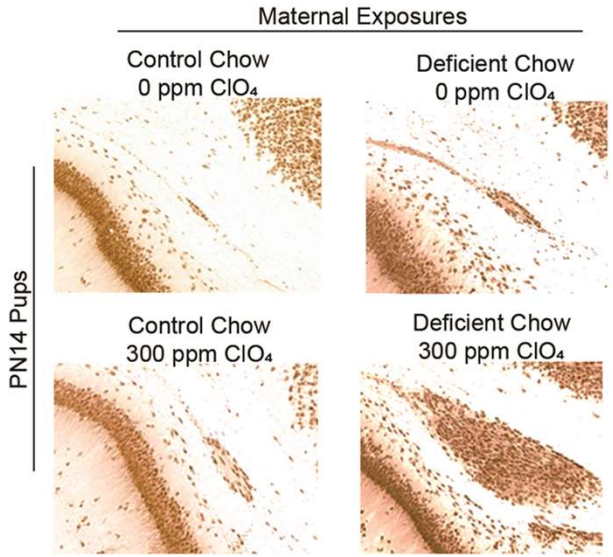


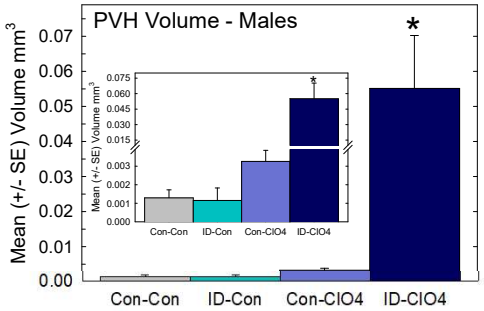
Gilbert et al., Toxics, 2023

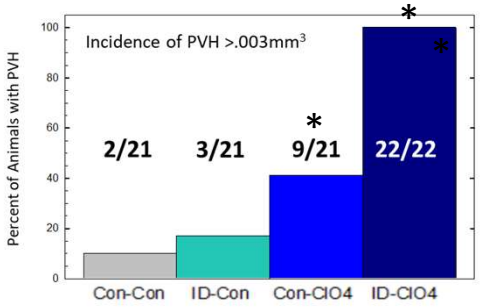
Dietary Iodine Deficiency and ClO_4

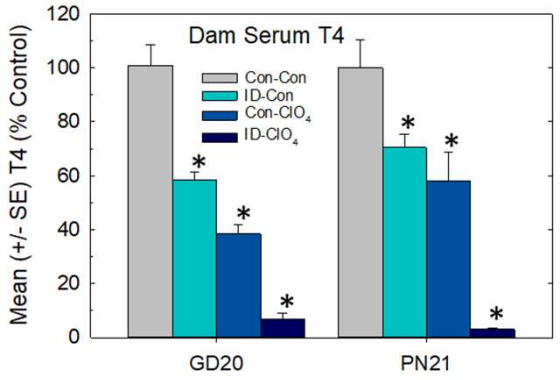


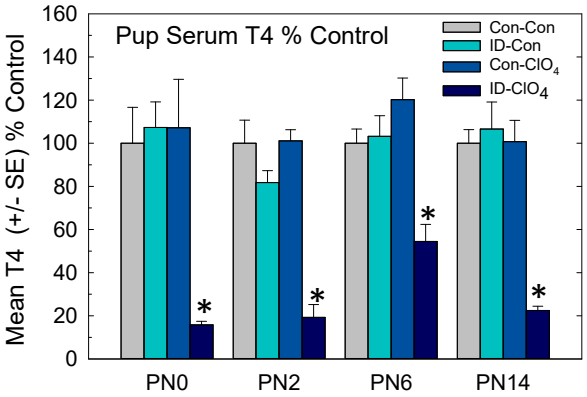
Chow-Water
Con-Con
ID-Con
Con-ClO_4
ID-ClO_4

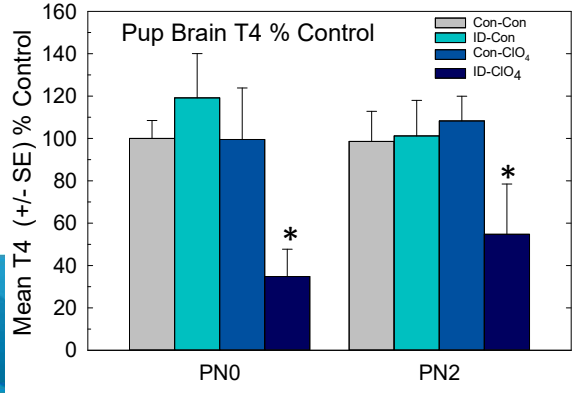






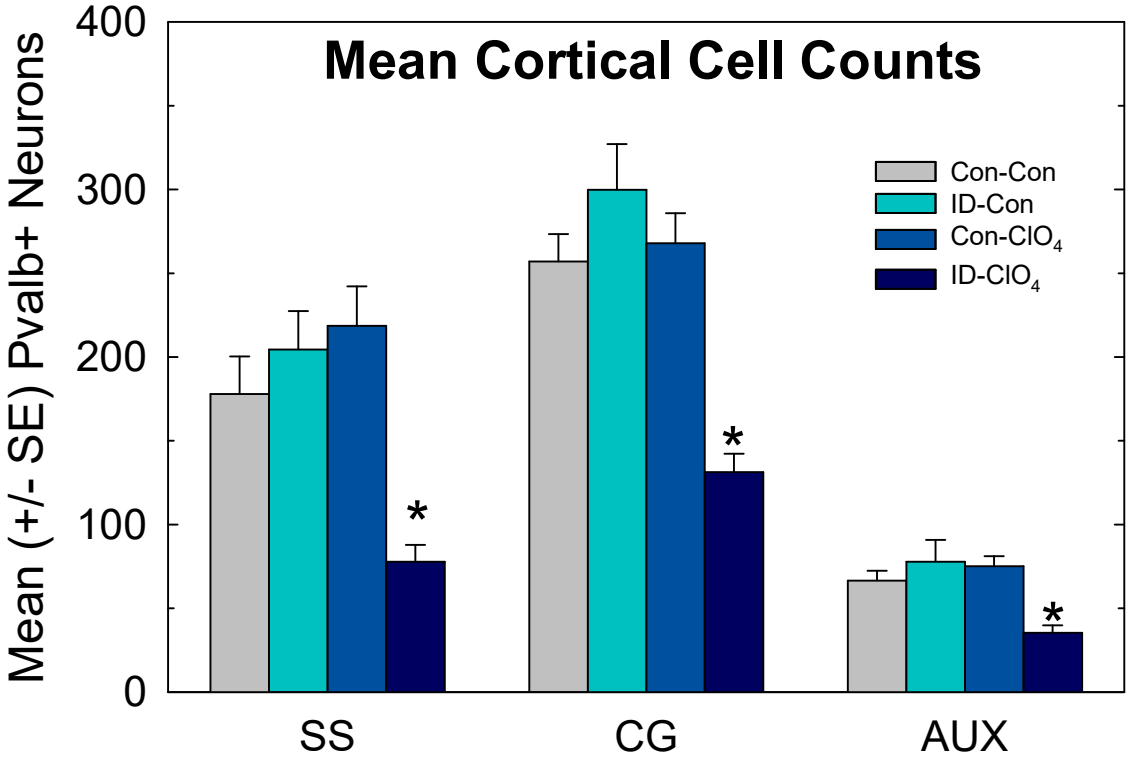




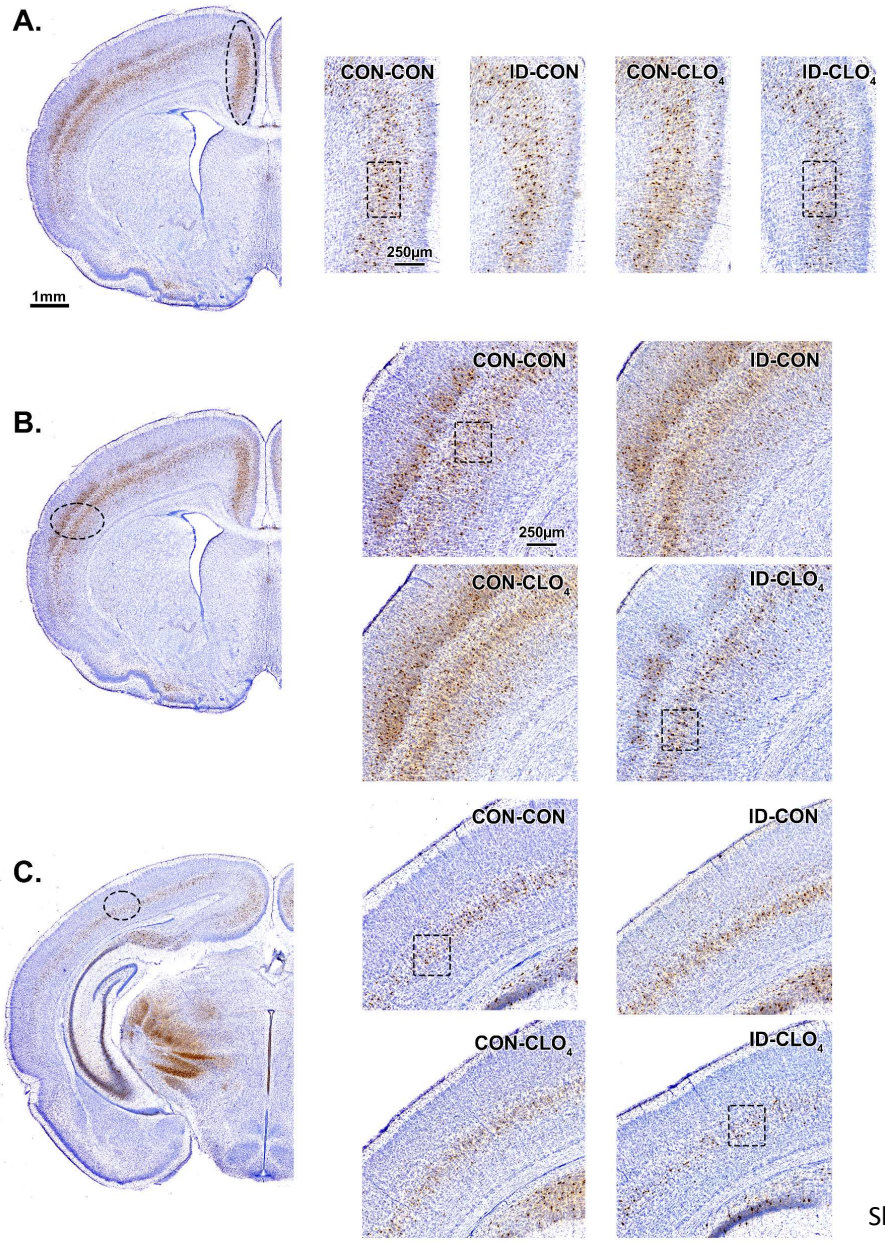


Gilbert et al, Toxics, 2023

Pvalb+ Neurons- Reduced in Cortex



Gilbert et al, Toxics, 2024

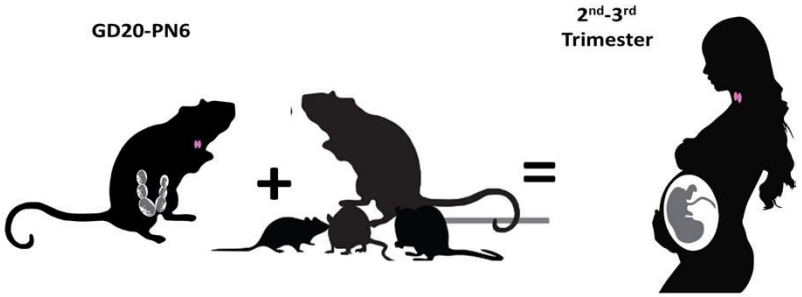


Summary And Conclusions

- Structural changes in brain from TH disruption are not limited to prototype pharmaceuticals or to one thyroid MOA

- Structural, functional, and behavioral impairments greatly exacerbated when ClO_4 is delivered under conditions of marginal ID

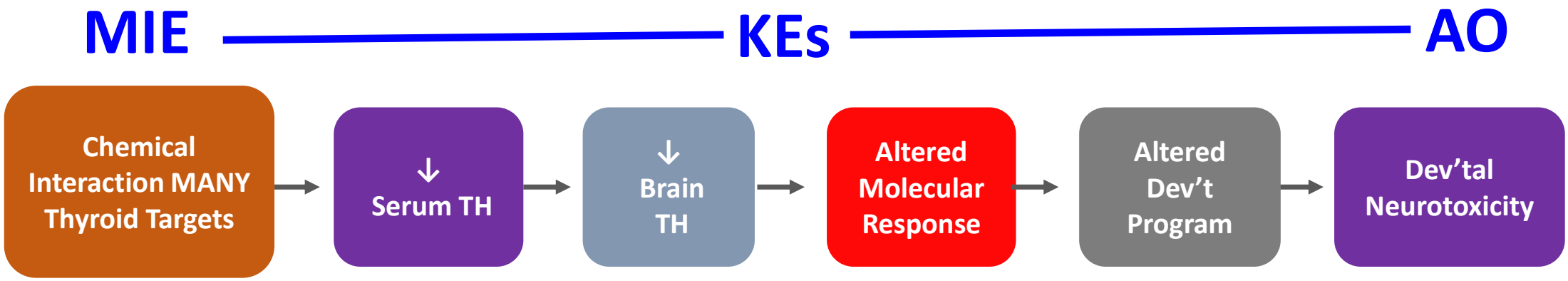
- Humans may be more sensitive to these impairments than rodents due to extended period of brain development that occurs in utero in humans



- Fetal measures of TH dysfunction in rodent models especially significant!!
- **Underscore the utility of the CTA and importance of fetal measures of TH in serum and brain**

Can we expand the utility of the CTA by incorporating some upstream brain markers?

Testing for Thyroid DNT within AOP Framework?



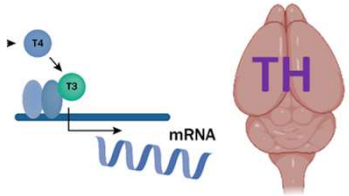
Where do we stand?

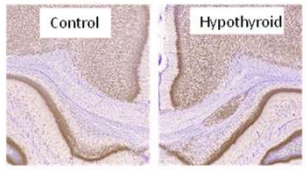
Progress …. Yet Significant Challenges Remain

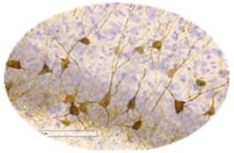
in vitro NAMs for Thyroid MOA:

- Assays developed for most relevant MIEs
- Successful applications in identification for prioritization
- Some success in MOA, WOE, thyroid hazard characterization
- Acceptance criteria, transferability yet to be achieved for universal adoption

in vivo NAMs for Thyroid-Mediated DNT:

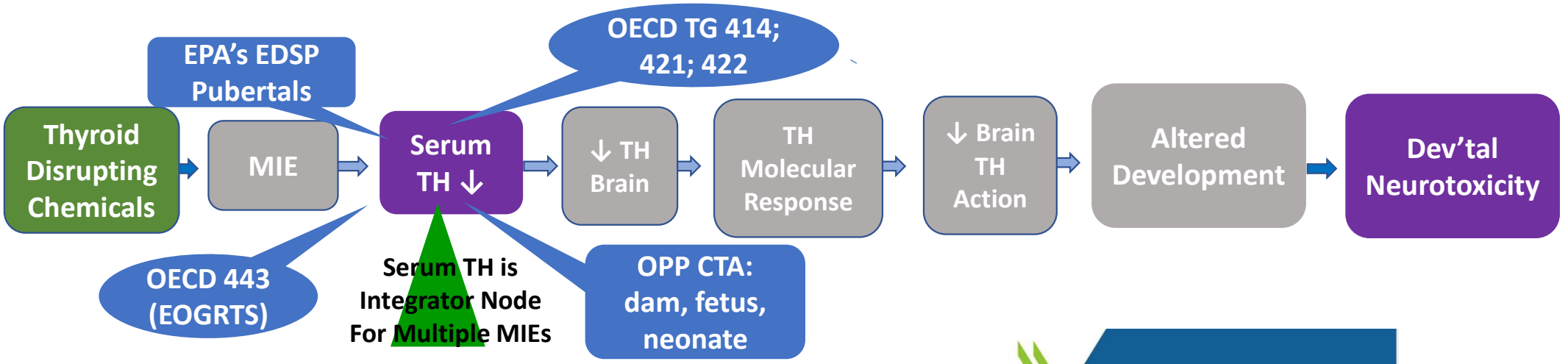


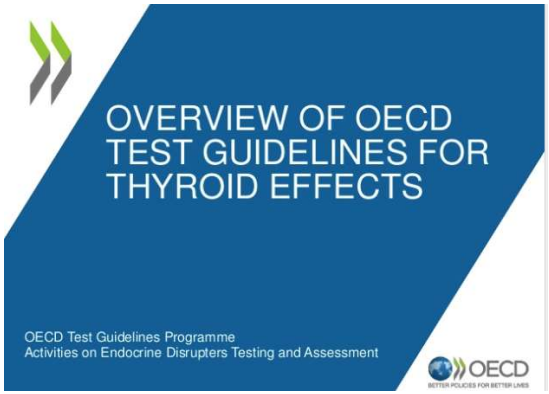




- Two very complex systems
- Defining *in vivo* phenotypes to anchor mechanistic study
- Building confidence in upstream biomarkers of DNT
- Rat:Human Differences in timing of brain development
- Utility of the CTA for hazard ID
- Expanding CTA to encompass intermediary brain endpoints?

Revisiting Current Mammalian Guidelines



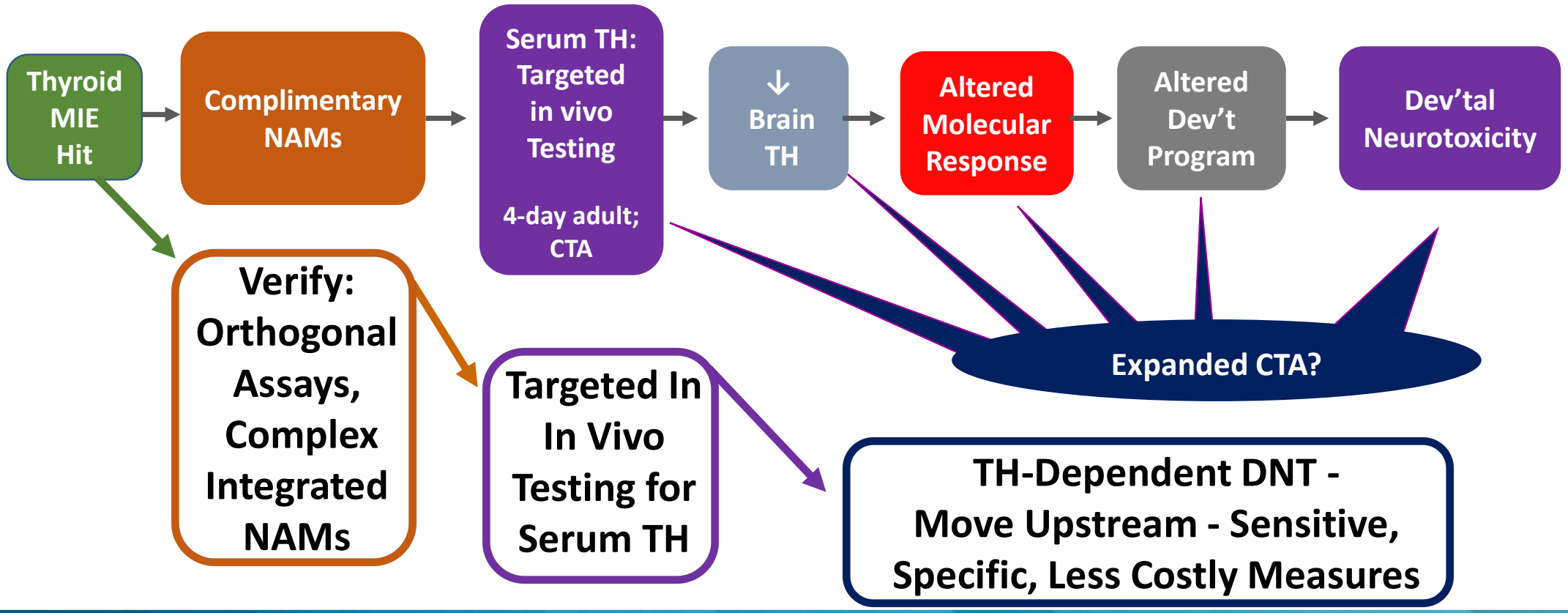


Serum TH Measures Taken in Several TG

- **Clinically Relevant, Easily Measured**

….. But not necessarily taken at the right time, largely optional at critical development timepoints, often not using sufficiently sensitive and robust assays

Envisioning Incorporation of NAMs into Hazard Assessment: A Step-Down Tiered Approach?



Thank You for Your Attention!

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