Complexity of fetal thyroid hormone economy during gestation: Lessons learned from the assessment of in utero exposure to PFBS

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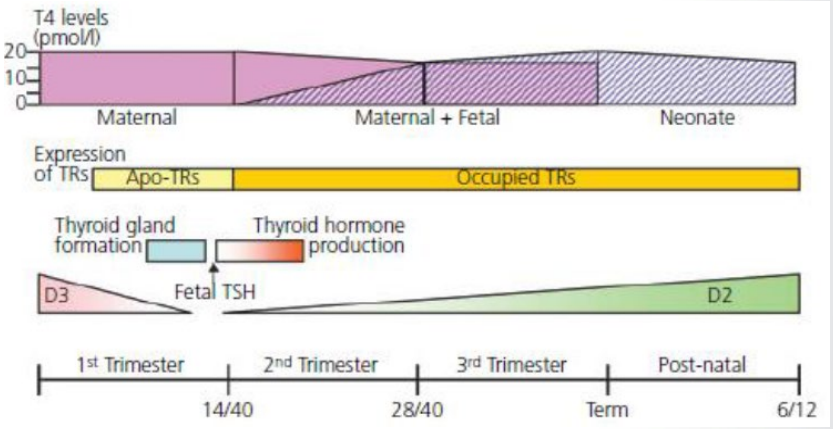
Thyroid hormone during pregnancy

During developmental life stages thyroid hormones are critical in many physiological processes:

- Neurogenesis, synaptogenesis, and myelination
- Thermogenesis
- Pulmonary gas exchange
- Cardiac development

Maternal thyroxine (T_4) is the primary source of thyroid hormone for a developing human fetus in early gestation

- Critical somatic growth and maturation occurs in 1st and early 2nd trimester (e.g., CNS)



Williams, G.R. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *J. Neuroendocrinol.* 20: 784-794

Thyroid hormone during pregnancy

Subclinical hypothyroidism- *elevated TSH levels with normal serum T_4 and T_3 concentrations*

Hypothyroxinemia- *$\downarrow T_4$ with normal serum concentrations of TSH and T_3*

Milder forms of thyroid perturbation are up to 10 times more prevalent in human populations than overt gestational hypothyroidism

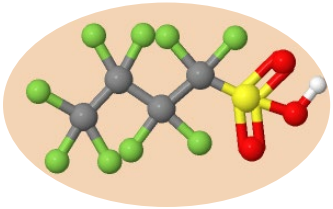
Effects observed in human neonates following in utero thyroid hormone deficiency include:

- Cognitive deficits
- Psychomotor impairment
- Decreased overall size/growth
- Impaired thyroid function

Human Health Assessment of PFBS: Brief Hazard Landscape

- Among the potential effects associated with oral Perfluorobutanesulfonic acid (PFBS) exposure in laboratory rodents, thyroid, developmental, and kidney were identified as hazards
- Thyroid effects were observed in repeat dose study designs in adult rats and mice, and developing mice; primarily decreases in thyroid hormones (T4 and T3)

Principal study:
Feng et al. (2017)
Tox Sci 155(2):409-419



Daily gavage

GD 1-20

Examined GD20

Followed up to PND60

- GD20 Dam effects (no overt tox; ↓TT4*, FT4*, and T3*, ↑ TSH*)

  (*statistically significant compared to control)

Human Health Assessment of PFBS: Brief Hazard Landscape

- Neonatal effects observed in litters of mice exposed to PFBS in utero:
  - ↓BW*
  - delayed eye opening* and vaginal patency*

(*statistically sig. compared to control litters)

- Pubertal/adult offspring effects observed in mice exposed to PFBS in utero:
  - Smaller ovaries
  - ↓ rel. ovarian and uterine weights*
  - ↓ follicles and corpora lutea*
  - Delayed first estrus*
  - Prolonged diestrus*
  - ↓ estrogen (E2) and progesterone (P4)*
  - Chronic hypothyroxinemia [↓T4 and T3*, ↑TSH and hypothalamic TRH mRNA at PND 30 only])

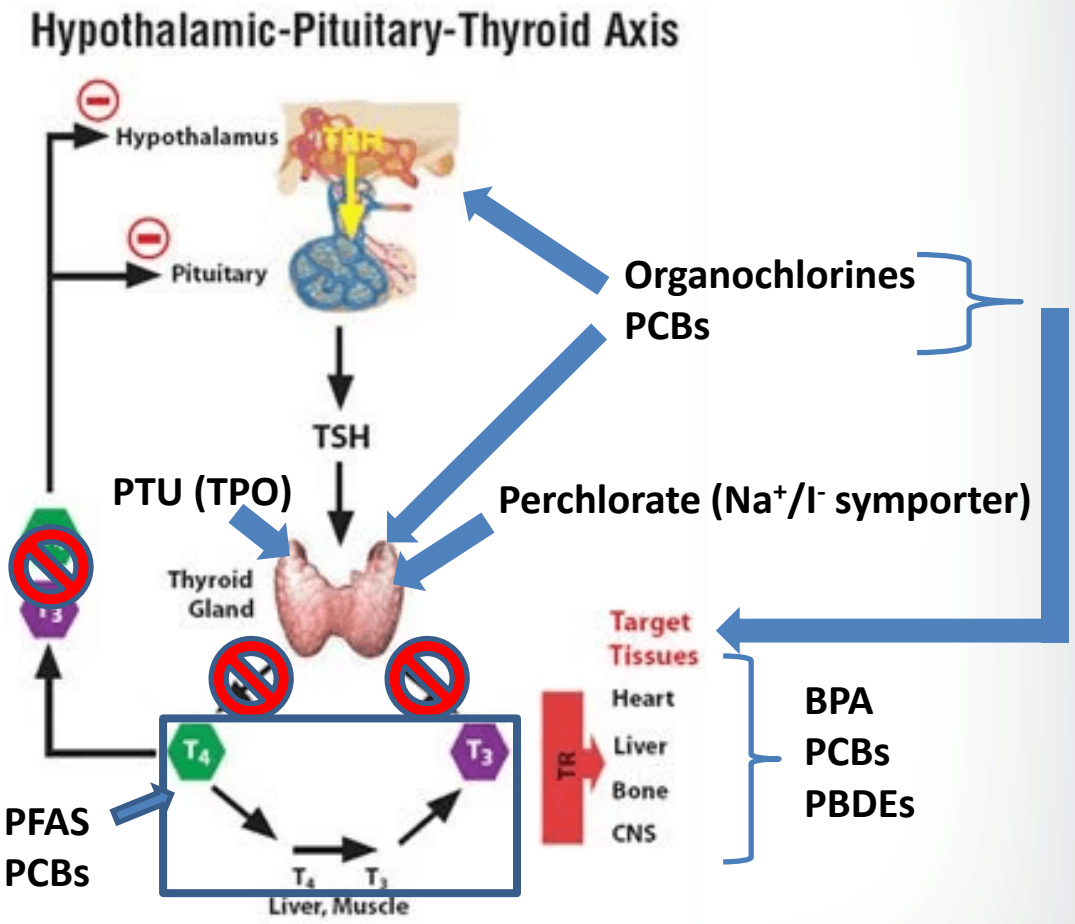
(*statistically sig. compared to control)

❖ *Across PFBS studies examining TH, ↓ T4 and T3 was not consistently associated with reflex increases in TSH; nor were there alterations in thyroid tissue weight, or histopathology. This is consistent with the human clinical condition "hypothyroxinemia"*

Do all thyroid-disrupting chemicals act alike?

Significant literature base reveals interaction of environmental chemicals along entirety of HPT-axis:

- Inhibition of TRH synthesis in hypothalamus (OCPs, PCBs)
- ↓ Iodine uptake (perchlorate, thiocyanate)
- ↓ TPO activity (PTU, methimazole)
- Interference with TSHR binding (OCPs, PCBs)
- Competitive binding with TBPs (PFAS, PCBs)
- Alteration of THR-dependent gene expression (BPA, PCBs, PBDEs)
- Enhanced hepatic metabolism of TH (Acetochlor, PCBs)

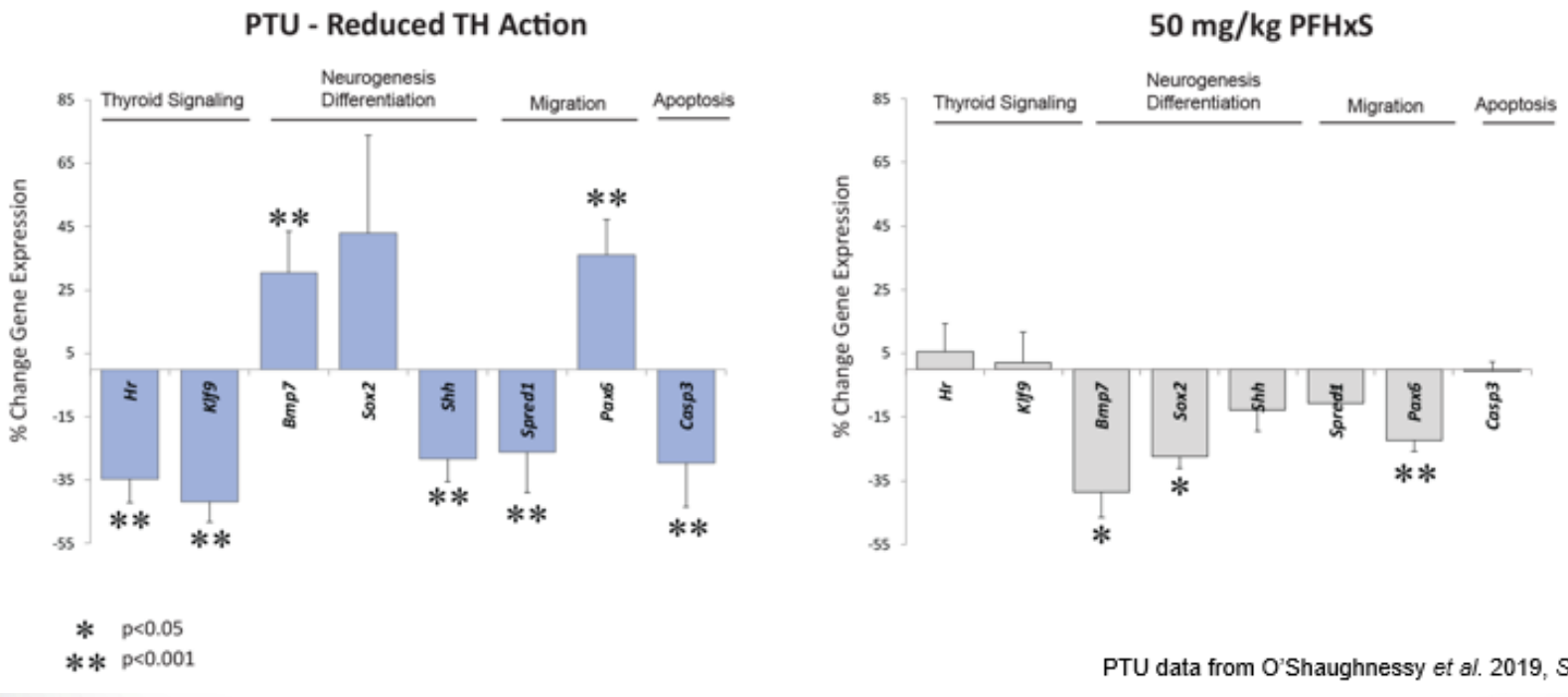


For review see: Lyn Patrick. (2009). *Alt Med Rev* 14(4):326-346; Boas et al. (2009). *Curr Opin Endocrinol Diabetes* 16(5):385-391

Direct thyroid acting versus downstream perturbation

Does it make a difference where/how the HPT-axis is perturbed?

PFHxS Gene Expression is Distinct



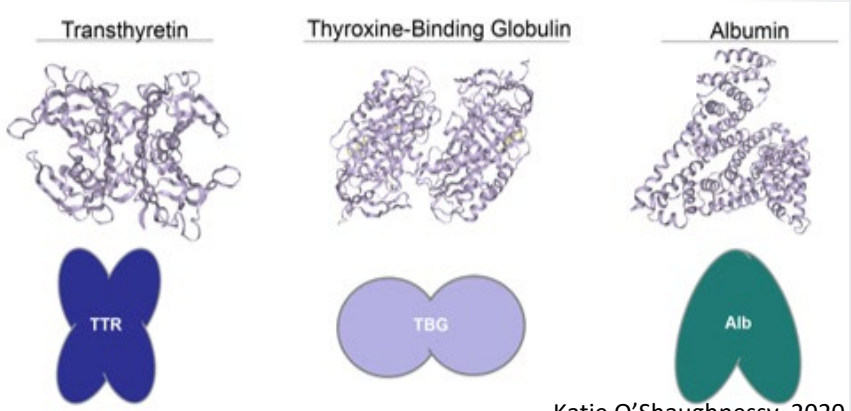
PTU data from O'Shaughnessy *et al.* 2019, *Scientific Reports*.

PFAS induced hypothyroxinemia and fetal microenvironment

- Within the context of early developmental life stages, there are several commonalities in HPT dynamics between humans and rodents:
  - Thyroid hormone binding proteins (TBPs)
  - Placental deiodinase (built in TH gatekeeping function)

Two carrier proteins—thyroid binding globulin (TBG) and transthyretin (TTR) are primarily responsible for storage and transit of T_4 in mammals:

- TBG is the primary carrier of T_4 in humans across all life stages
- In fetal and infant rats, TBG is also the primary carrier of T_4
- As rats transition to adulthood, TTR takes over as the primary carrier of T_4



Katie O'Shaughnessy, 2020

PFAS induced hypothyroxinemia and fetal microenvironment

Basal thyroid hormone dynamics

Katie O'Shaughnessy, 2020

- >90% of T4 in the blood is bound to TBPs
- Unbound T4 = FT4
- Unbound T4 (or T3) at the placenta is metabolized by deiodinases
- Bound T4 traverses the placental stroma, subsequently released, and made available for fetal uptake

PFAS-mediated thyroid hormone dynamics

- PFAS compete with T4 for carrier binding
- Results in excess FT4 being cleared by mother (systemic and placental)
- Less bound T4 presented to placenta for transfer to fetus

For review see: B. Blake and S. Fenton. (2020). *Toxicology* 443:152565; Weiss et al. (2009). *Tox Sci* 109(2):206-216

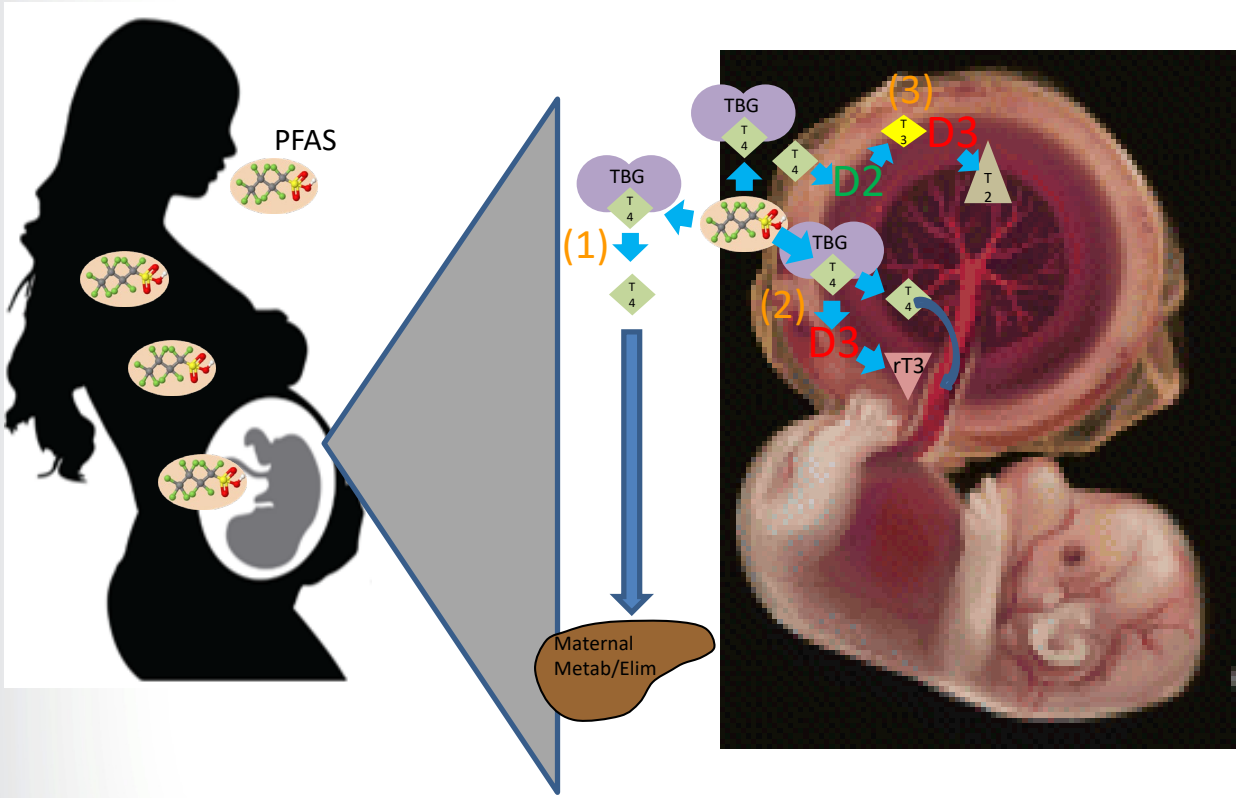
Placental TH gatekeeper function during in utero development

- A tightly regulated transfer of maternal thyroid hormone to a fetus is paramount to proper development of multiple tissues and organ systems (e.g., nervous system), especially during the early trimesters
- The placenta has transporters and deiodinases that collectively act as a gatekeeper to maintain an optimal T_4 microenvironment in the fetal compartment
- Deiodinase 3 (D3) is highly expressed in human uterus, placenta, and amniotic membrane
- D3 is also highly expressed in the rodent uterus and is highly induced during pregnancy

❖ *D3 serves a critical role of regulating thyroid hormone transfer to the fetus through the deiodination of T_4 to transcriptionally inactive reverse triiodothyronine (rT_3) or T_3 to inactive 3,5-diiodo-L-thyronine (T_2)*

For review see: DA Fisher. (1997). *Clin Obstet Gynecol* 40:16-31; Wasco et al. (2003). *Endocrinology* 144:4253-4261.

Postulated pathway for disruption of maternal-fetal thyroid hormone economy



T4 displaced from TBPs may be:

1) Metabolized and excreted by maternal liver
2) Converted to inactive reverse T3 by placental D3
3) Converted to active T3 (by D2) but is then subject to further metabolism to T2 by placental D3

**In hypothyroxinemia, clinically a mother may appear to be euthyroid, however the fetus could be critically deficient in TH*

Maternal: ↓TT4 and FT4; TH-dependent tissues are euthyroid so no signal back to hypothalamus (little-to-no reflex TSH response)

Fetus: ↓ delivery/availability of T4 during critical in utero programming windows; functional maturation of fetal HPT occurs gestational wks 11-35

Challenges

- How much of a decrease in fetal TH = physiological outcome (overt vs. DNT, etc.); differential sensitivities for TH as a function of both dose and time
- Trimester-dependent sensitivity (experimental animal gestational exposure study designs examining a relationship between thyroid hormone perturbations and health outcomes should entail exposure at least starting at GD0, but optimally exposure for days prior to pregnancy)
- THR dynamics in the fetal compartment (i.e., potential for compensation?)
- Chemical-specific effects on placenta? Placental insufficiency dose-response and time-dependency during pregnancy? Impacts across subsequent lifestages in offspring??

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