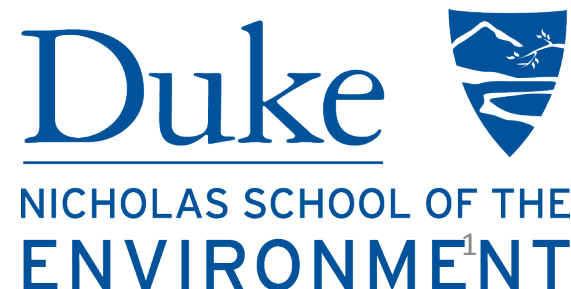


Associations Between Brominated Flame Retardants and Thyroid Hormones in Human Placental Tissues: Sex-Specific Differences?

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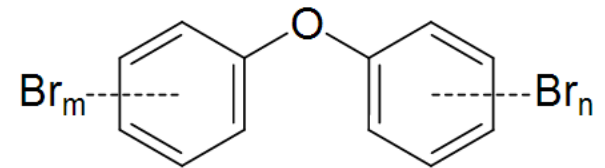
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Brominated Flame Retardants (BFRs)

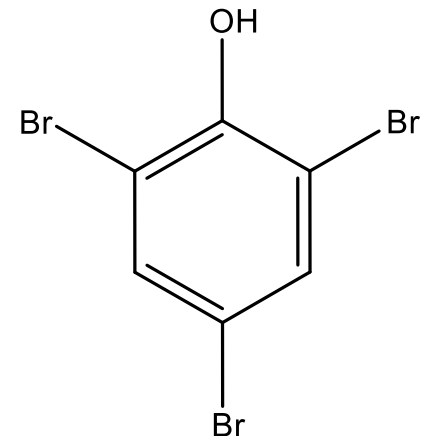
PBDEs:

- Three commercial mixtures (additive):
 - PentaBDE: PUF cushioning
 - OctaBDE: Acrylonitrile and butadiene styrene (electronics)
 - DecaBDE: Polypropylene and HIPS (electronics, automobiles, housing materials)
- Banned in EU in 2002 and added to Stockholm Convention in 2009
 - Penta and Octa voluntarily phased out in US in 2004
 - Deca phased out in US in 2014
- Predominant congeners in environment and human tissue:
 - BDE-47, -99, -100, -153, -154, and -209

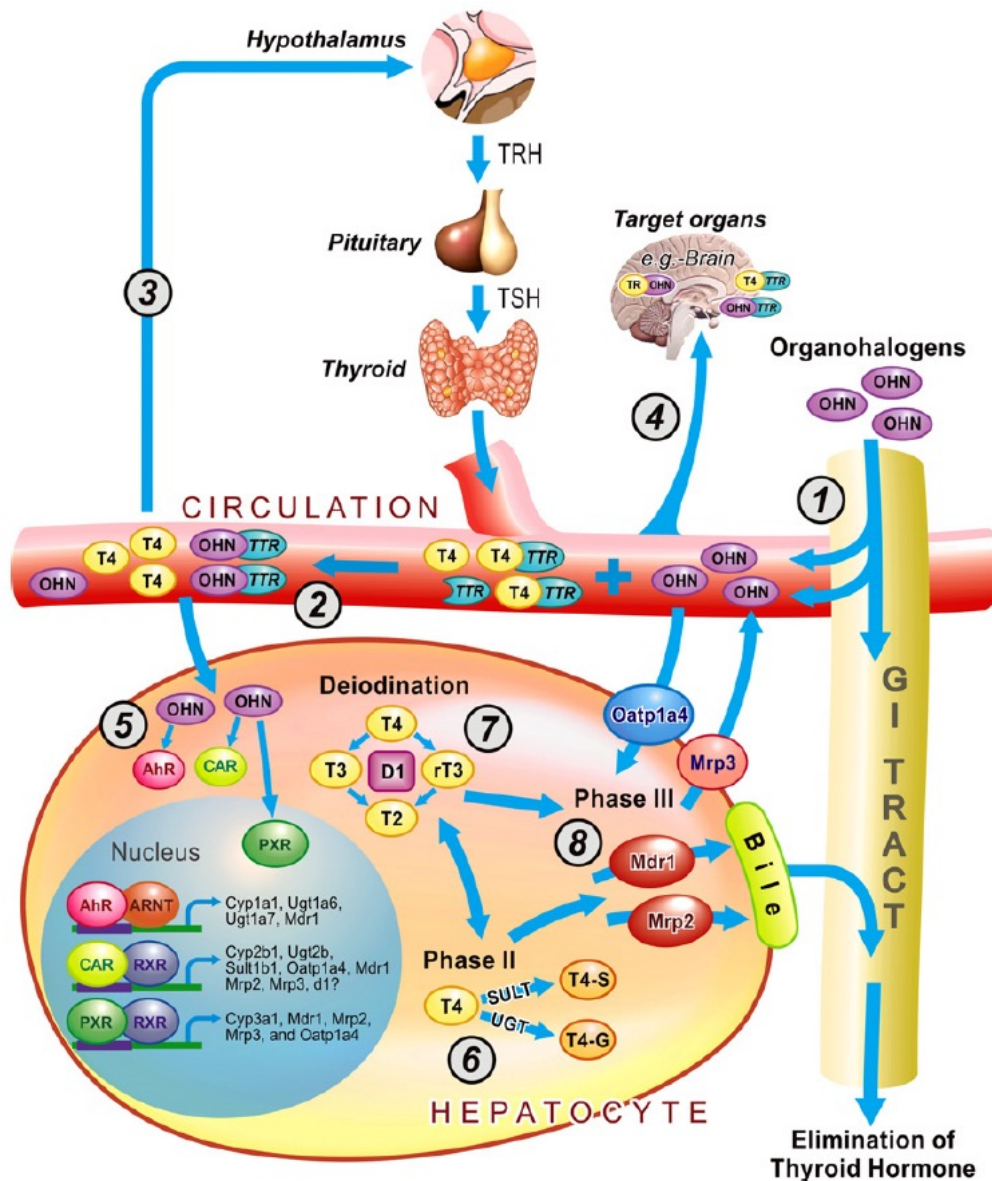


2,4,6-Tribromophenol:

- Used as a
 - Fungicide
 - Reactive FR
 - Intermediate in the production of other BFRs
 - Metabolites of PBDEs
- Natural sources from marine algae
- 2,4,6-TBP measured in marine wildlife, however very few human biomonitoring and toxicology studies



Modes of Action Affecting Thyroid Regulation

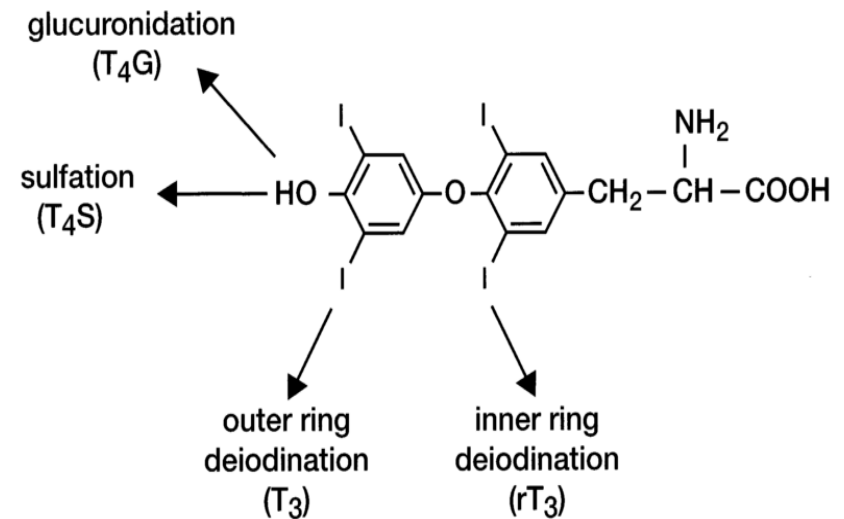


2. PBDE metabolites displace T4 from serum transporters (Meerts et al., 2000);
4. Transporters deliver PBDEs or metabolites to brain where agonism/antagonism with nuclear receptors may occur;
5. Upregulation of xenobiotic metabolizing enzymes (XMEs) (Szabo et al 2009)
6. XMEs conjugate T4; increased or decreased clearance of THs (Butt et al., in 2013);
7. Disruption of Deiodinase Activity by PBDE metabolites (Butt et al., 2011)

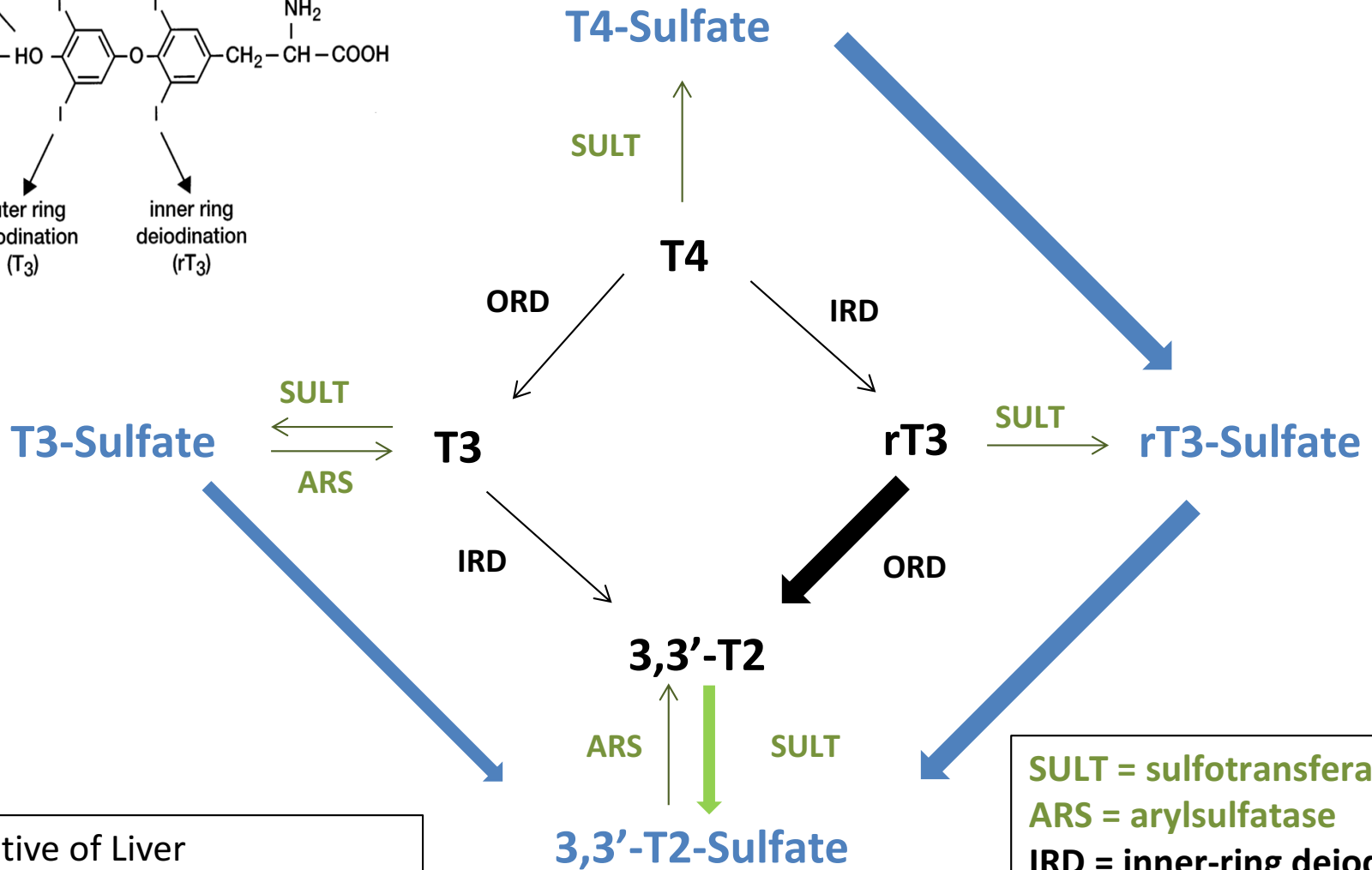
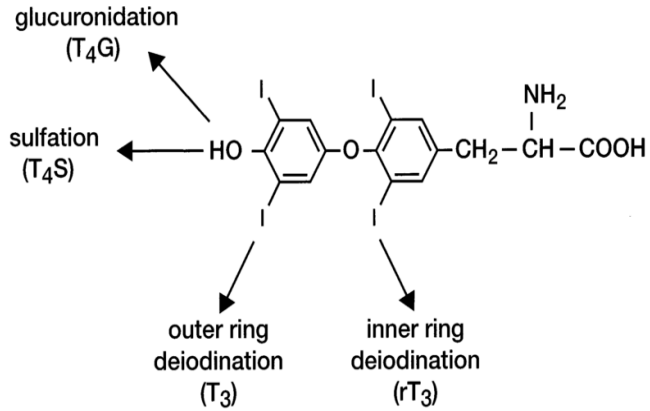
(From Kodavanti and Curras-Collazo, 2010)

Thyroid Hormone Metabolism

- Bioactivated/inactivated in peripheral tissues
- *Deiodinase (DI)*
 - Microsomal, membrane-bound
 - Three isoforms with different catalytic capacities and tissue localization
 - IRD and/or ORD
 - DIO3 predominantly expressed in placenta
- *Sulfotransferase (SULT)*
 - Cytosolic
 - Class of phase II metabolism enzymes
 - Increase polarity/hydrophilicity for excretion
 - Bioactivate/inactivate endogenous compounds/xenobiotics
 - Multiple isoforms and broad substrate specificity
 - SULTs inactivate THs



Integrated TH Metabolism

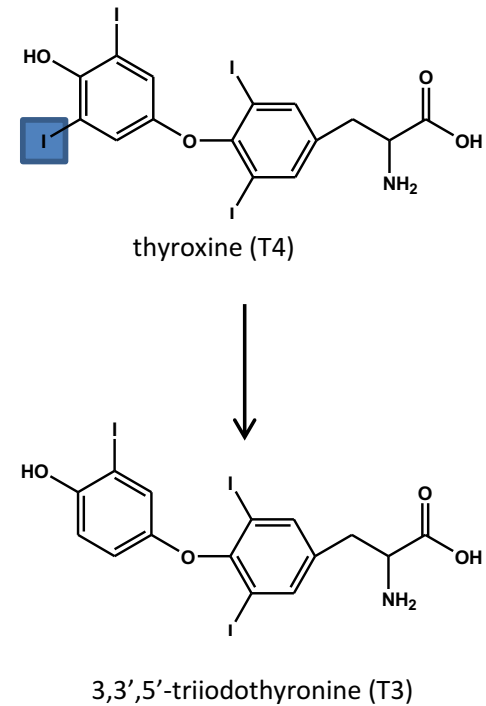
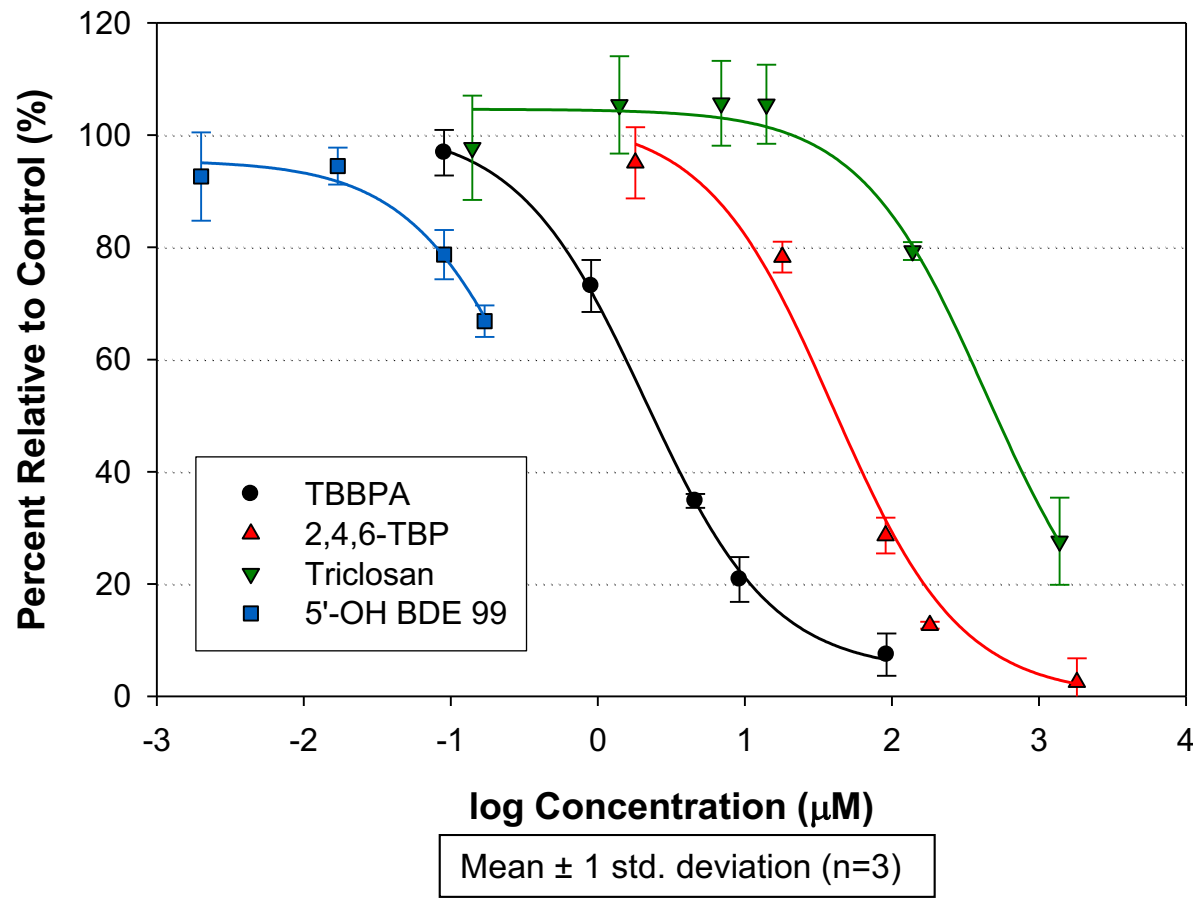


Representative of Liver
Modified from Visser & Kester, 2002

SULT = sulfotransferase
ARS = arylsulfatase
IRD = inner-ring deiodinase
ORD = outer-ring deiodinase

Inhibition of Thyroxine Deiodination by Flame Retardants

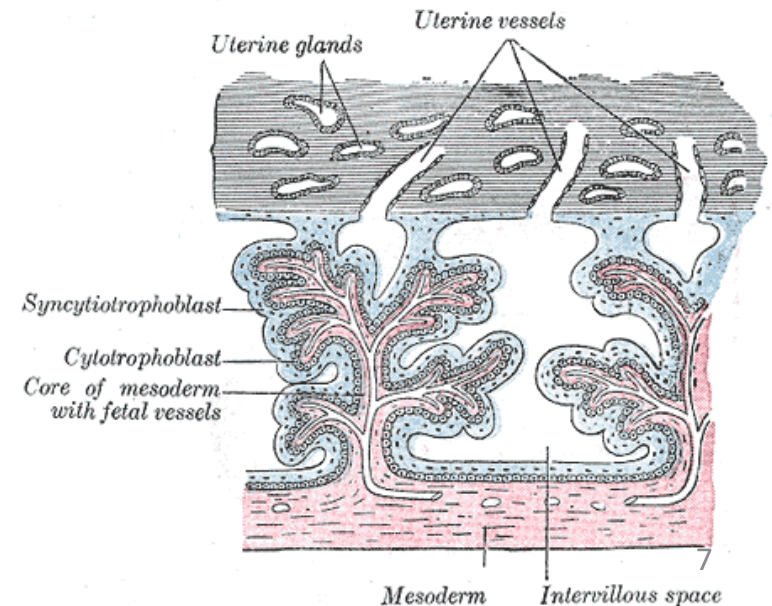
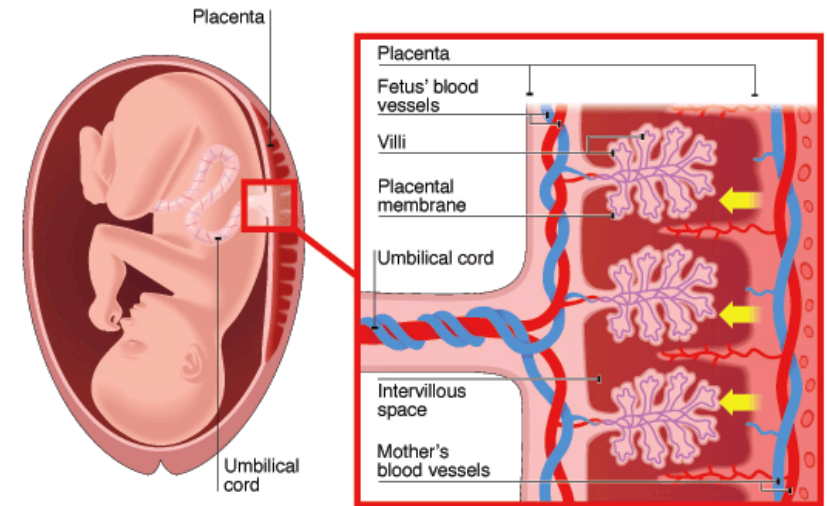
(Butt et al., 2011)



In Vitro Experiments Conducted with Pooled Human Liver Microsomal Samples

Human Placenta

- Fetomaternal organ that connects the developing fetus to the uterine wall
- Disc-shaped, 22 cm long, 2.5 cm thick, 500 grams, thickest at the center
- Facilitates nutrient uptake, thermo-regulation of the fetus, waste elimination, and gas exchange via the mother's blood supply, and produce hormones to support pregnancy
- Fetal TH production begins at 14-16 weeks gestation
 - THs are critical for fetal neurodevelopment
 - DIO Type 3 helps regulate T3 delivery and recirculate iodine supplies



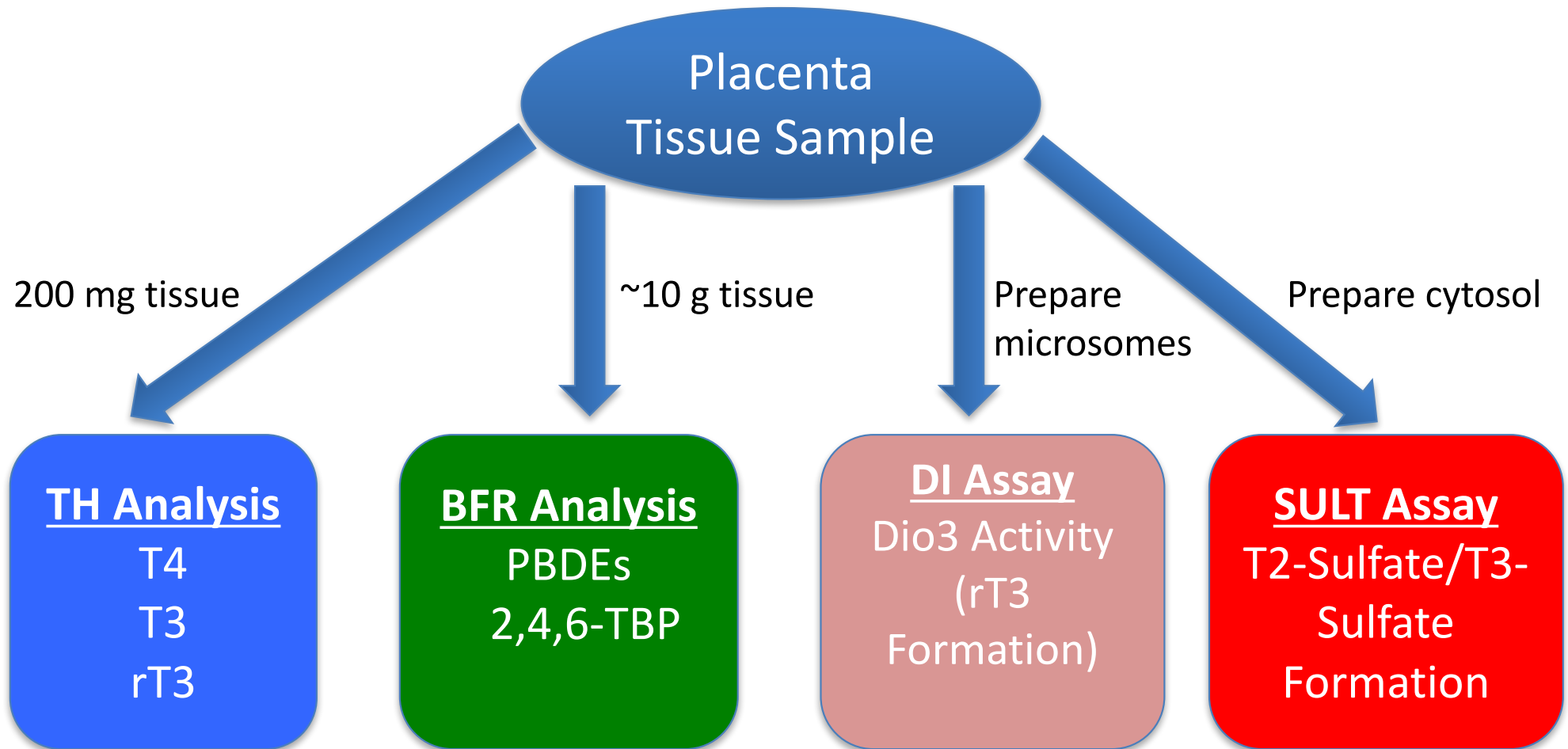
Could Exposure to BFRs Affect Thyroid Function in Human Placental Tissues?

Study Cohort

- 102 placenta tissue subsamples collected at delivery
- Collected from Duke University Medical Center (2010-2011)
- Cohort is part of **SCEDDBO/Healthy Pregnancy Healthy Baby Study** – “determine how the interaction of environmental, social, and host factors contributes to disparities in birth outcomes between African-American and white women in North Carolina”
- Demographics:
 - 68% non-Hispanic black
 - Age range: 18-40 years old
 - 9.3% with private health insurance



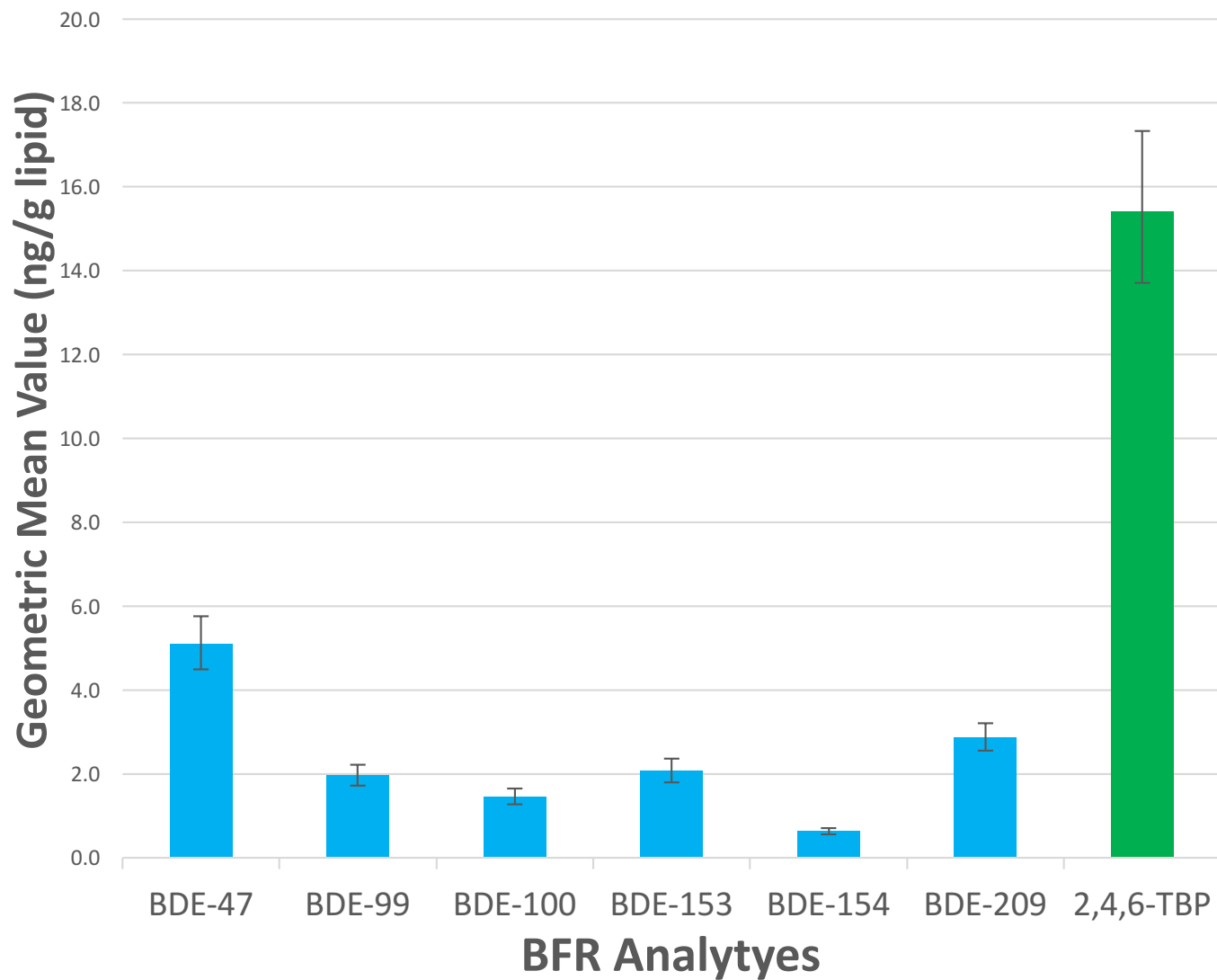
Experimental Approach



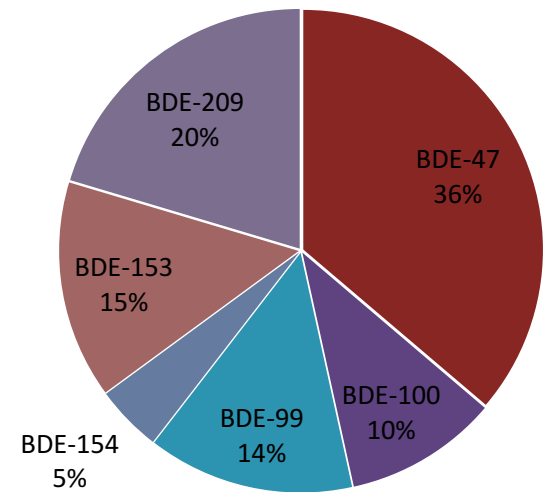
Thyroid hormone levels (ng/g ww) and BFR concentrations (ng/g lipid) measured in term placenta tissue

Variable	MDL	Detection Frequency (%)	Geometric Mean	Min	Max
Thyroid hormones (n=102)					
T4	0.002	100	27.8	11.8	53.6
T3	0.002	100	0.38	0.10	0.90
rT3	0.002	100	2.66	0.73	7.59
PBDEs (n=102)					
BDE-47	0.07	91.2	5.09	0.12	141
BDE-99	0.07	68.6	1.95	0.09	223
BDE-100	0.02	88.2	1.45	0.03	50.1
BDE-153	0.01	93.1	2.06	0.02	513
BDE-154	0.01	83.3	0.63	0.01	20.2
BDE-209	0.17	52.9	2.86	0.18	50.4
ΣPBDEs			17.6	0.54	528
Phenolic compound (n=102)					
2,4,6-TBP	0.05	100	15.4	1.31	316
ΣBFRs			37.3	2.18	568

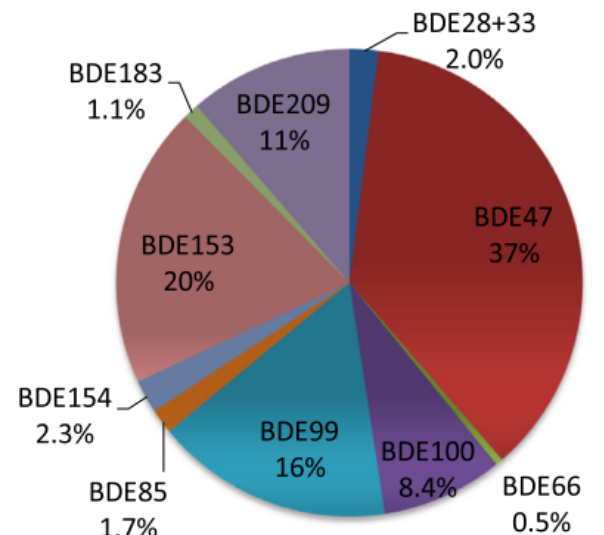
Geometric Mean Values of BFRs



PBDE Congener Distribution



This study



PBDEs

Nanes et al., 2014

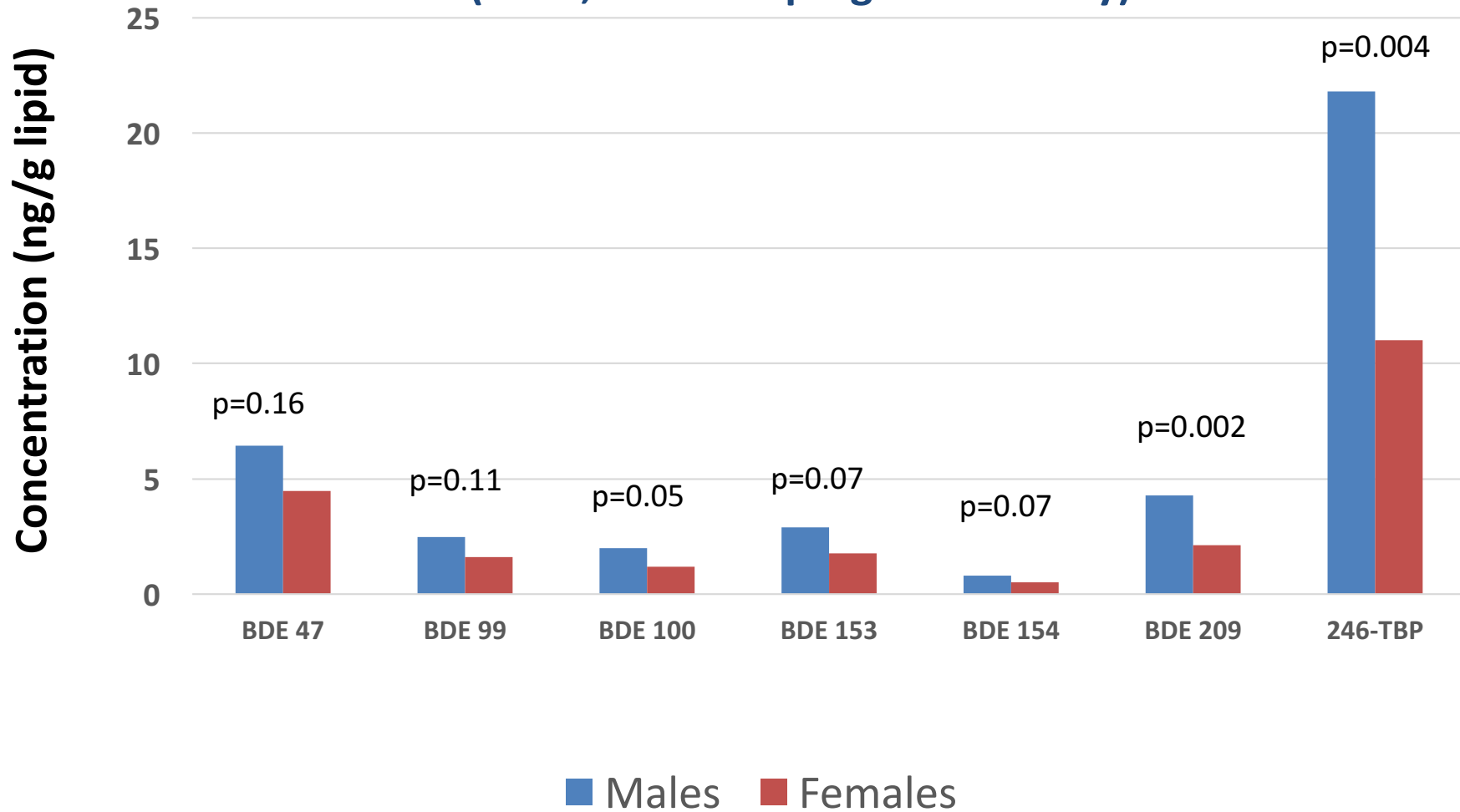
Spearman correlation matrix for BFRs (ng/g lipid)

Analyte	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	2,4,6-TBP	ΣPBDEs	ΣBFRs
BDE-47	1.00	0.48 [#]	0.88 [#]	0.58 [#]	0.61 [#]	0.49 [#]	0.50 [#]	0.84 [#]	0.73 [#]
BDE-99		1.00	0.52 [#]	0.43 [#]	0.52 [#]	0.60 [#]	0.66 [#]	0.68 [#]	0.72 [#]
BDE-100			1.00	0.71 [#]	0.71 [#]	0.50 [#]	0.48 [#]	0.89 [#]	0.77 [#]
BDE-153				1.00	0.71 [#]	0.50 [#]	0.38 [#]	0.77 [#]	0.66 [#]
BDE-154					1.00	0.54 [#]	0.50 [#]	0.77 [#]	0.72 [#]
BDE-209						1.00	0.58 [#]	0.73 [#]	0.72 [#]
2,4,6-TBP							1.00	0.58 [#]	0.85 [#]
ΣPBDEs								1.00	0.89 [#]
ΣBFRs									1.00

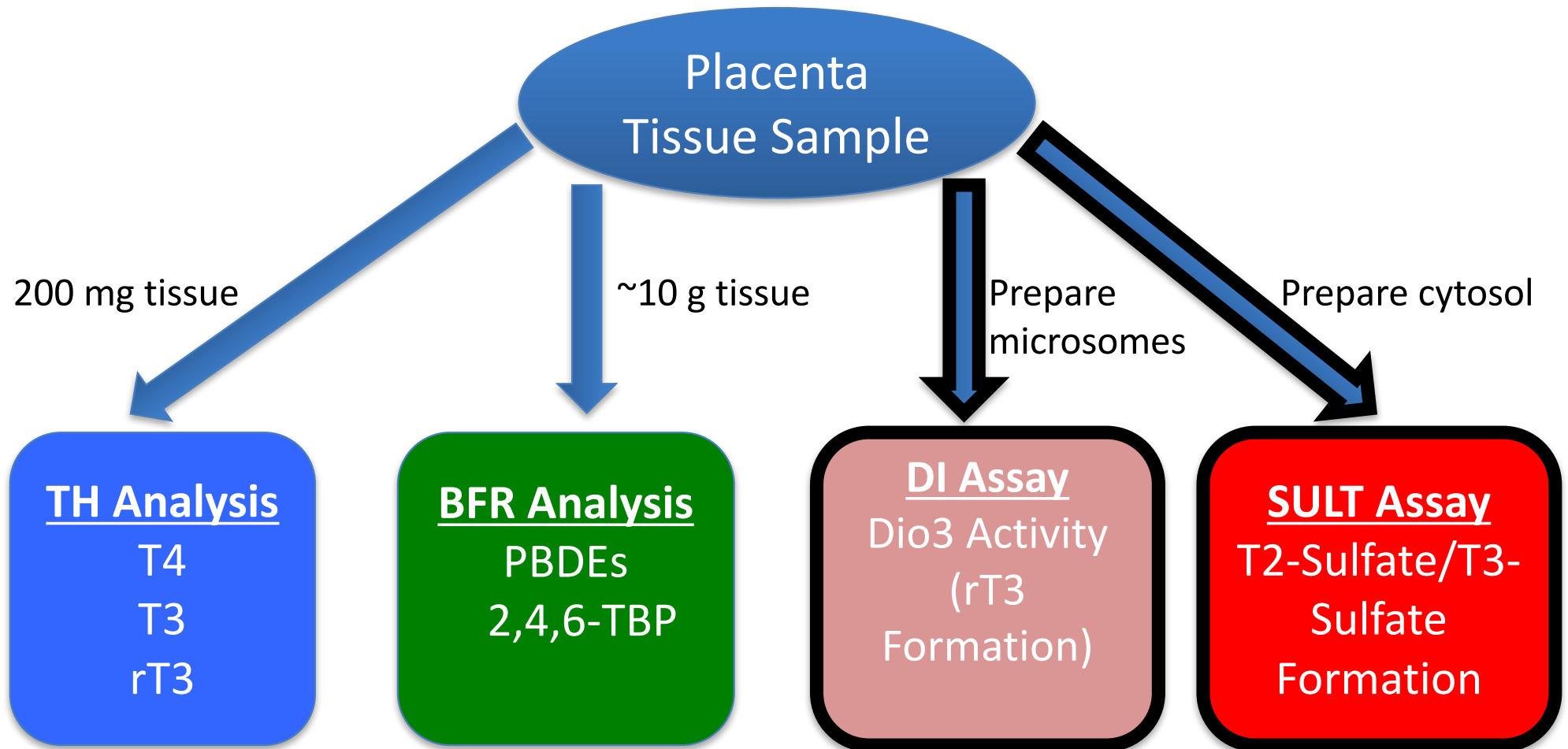
[#] p<0.001

Geomean BFR Placental Levels by Infant Sex

(n=94; full term pregnancies only)

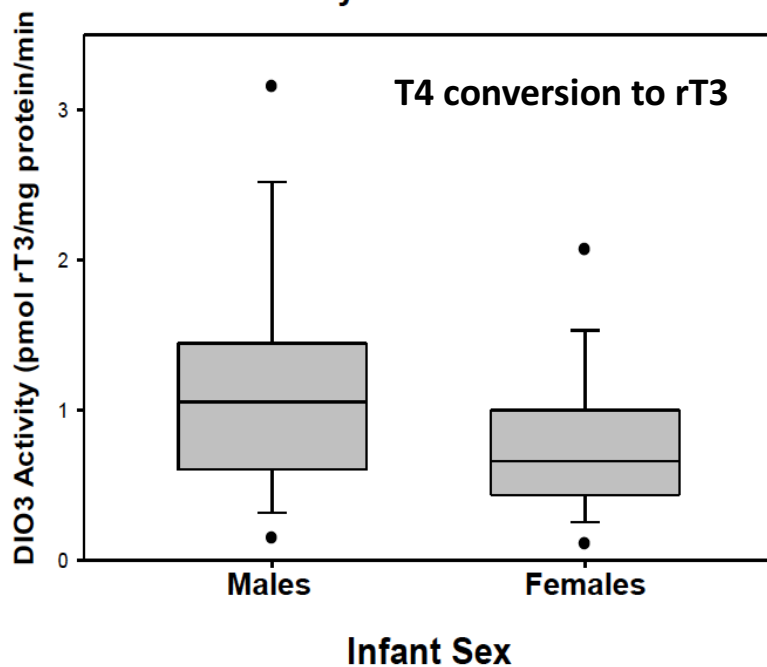


Experimental Approach

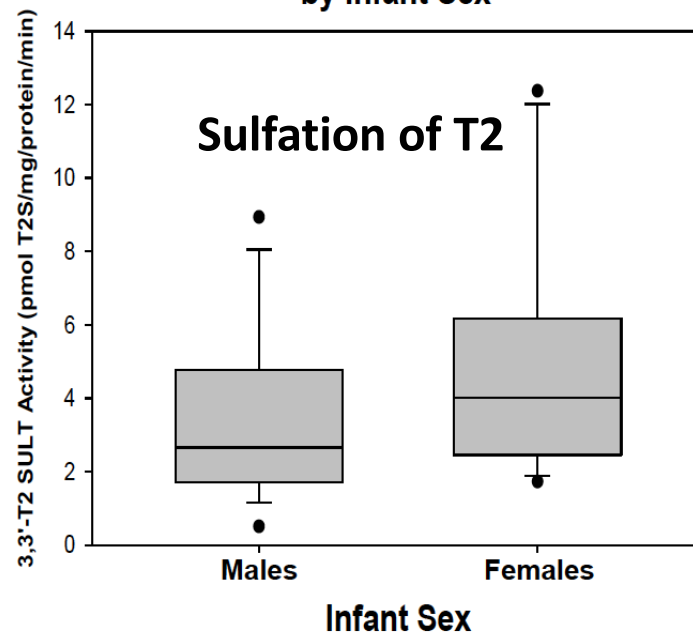


Enzyme Activities by Infant Sex

Endogenous DIO3 Activity in Placenta Tissue by Infant Sex



Endogenous 3,3'-T2 SULT Activity in Placenta Tissue by Infant Sex



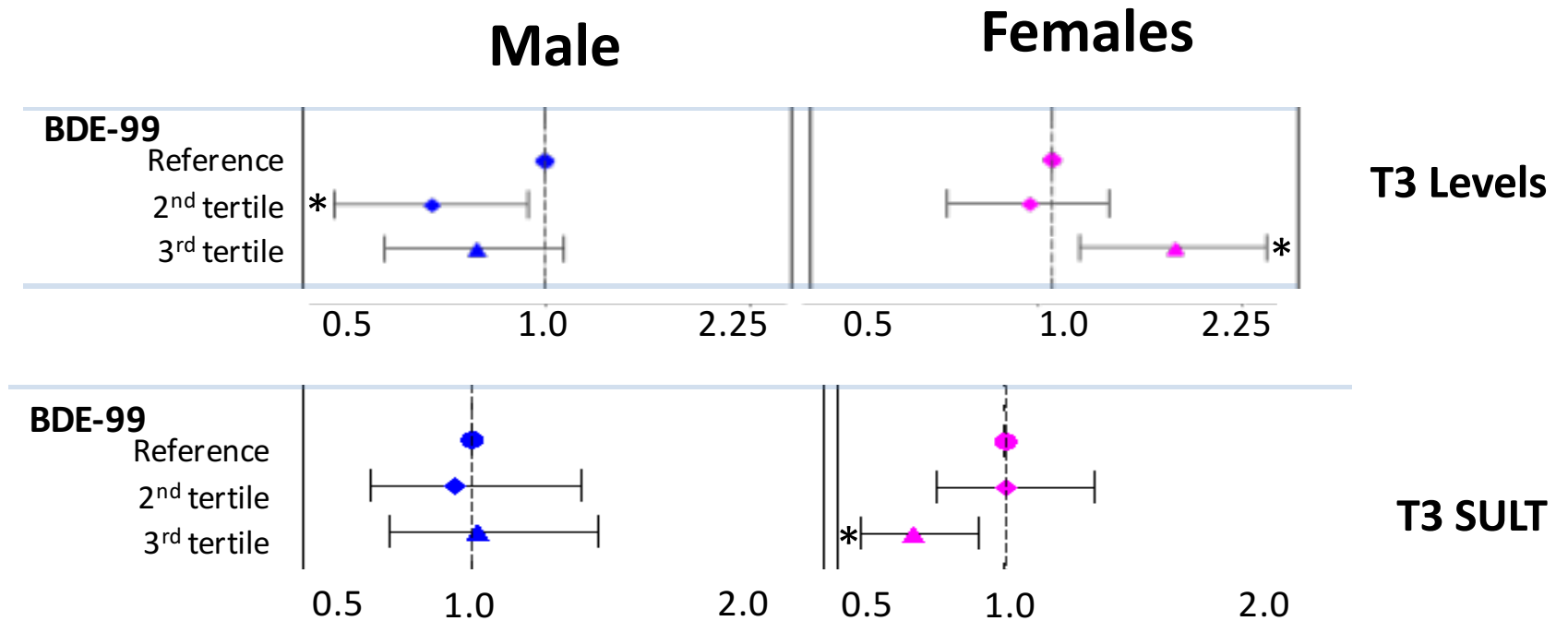
- DIO3 Activity Significantly Higher in Placental Samples from Males (1.8 X on average; $p < 0.01$)
- T2 Sulfation Significantly Higher in Placental Samples from Females (1.5 X on average; $p < 0.05$)

Spearman Correlation Analyses (n=94)

Spearman Correlation Coefficients by Infant Sex																
		BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	2,4,6-TBP	ΣBFR	ΣBDE	T3	rT3	T4	DI	T2S	
Males (n=48)	T3	-0.07	-0.21	-0.16	-0.03	-0.13	-0.10	-0.18	-0.22	-0.16	1.00					
	rT3	0.02	-0.34*	0.01	0.13	-0.04	-0.35*	-0.02	-0.13	-0.11	0.14	1.00				
	T4	-0.14	-0.21	-0.21	0.04	-0.07	-0.15	-0.11	-0.16	-0.15	0.53*	0.35*	1.00			
	DI	0.03	0.16	0.15	0.04	-0.05	-0.02	0.00	0.05	0.09	-0.05	0.05	-0.27	1.00		
	T2S	0.02	-0.14	0.07	-0.01	0.02	-0.14	-0.02	-0.05	-0.01	-0.13	0.29*	-0.15	0.11	1.00	
	T3S	0.29*	0.03	0.26 [#]	0.27 [#]	0.16	0.00	-0.11	-0.02	0.25 [#]	-0.15	0.10	-0.05	0.17	0.29	
Females (n=46)	T3	0.22	0.33*	0.19	0.23	0.08	0.17	0.36*	0.35*	0.25 [#]	1.00					
	rT3	-0.12	-0.34*	-0.08	-0.10	-0.07	-0.05	-0.14	-0.13	-0.19	-0.16	1.00				
	T4	0.08	-0.07	-0.02	-0.03	-0.06	0.06	0.06	0.10	0.03	0.45*	0.24	1.00			
	DI	0.10	0.00	0.18	0.11	0.24 [#]	0.22	0.02	0.08	0.09	-0.10	0.23	0.02	1.00		
	T2S	0.11	-0.06	0.05	0.13	0.11	-0.10	-0.11	0.02	0.13	-0.19	-0.08	-0.12	-0.16	1.00	
	T3S	0.08	-0.25 [#]	0.11	0.15	0.15	-0.02	0.02	0.05	0.05	-0.07	-0.06	0.05	0.17	0.19	
* < 0.05, # < 0.10																

Associations with Thyroid Hormones

- Spearman correlations among PBDEs and thyroid hormones revealed:
 - T3 positively correlated with BDEs in females (BDE 99 $r_s = 0.33$)
 - T3 negatively correlated with BDEs in males



(Adjusted for maternal and gestational age; n=94)

Mechanistic Pathways?

Observation:

- Higher BDE-99 exposure in placental tissues from infant females associated with higher T3, lower rT3, lower DIO3 and lower T3 SULT

Hypothesis:

- BDE-99/OH-BDEs inhibit DIO3 leading to lower rT3 (males and females)
- BDE-99/OH-BDEs inhibit SULT leading to higher T3 (females only?)

Why BDE-99?

In *in vitro* studies, BDE-99 was active, BDE-47 was not
Human glial cells for DIO2 activity (Roberts et al. 2015)
BeWO cells for T2 & T3 SULT activity (Leonetti et al. in draft)

What are the Consequences?

Affects on growth and development?
(Hoffman et al. 2016- PBDEs positively associated with higher growth rates in girls compared to boys).

Conclusions

- BFRs accumulation in the placenta appears to be sex-specific, and may have sex-specific effects on TH homeostasis
- BFRs may disrupt TH metabolism and alter T3 and rT3 levels in the placenta
 - Could this be driven by hydroxylated metabolites?
- More research is needed to understand sex specific effects of EDCs on placental function during pregnancy, and the consequences
- Next steps:
 - Are other POPs differentially accumulating in placenta?
 - What is the mechanism driving this differential accumulation?
 - Are placental BFR levels negatively associated with birth outcomes?

Acknowledgements

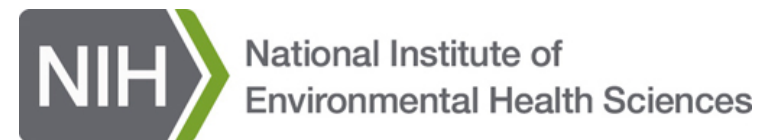
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