

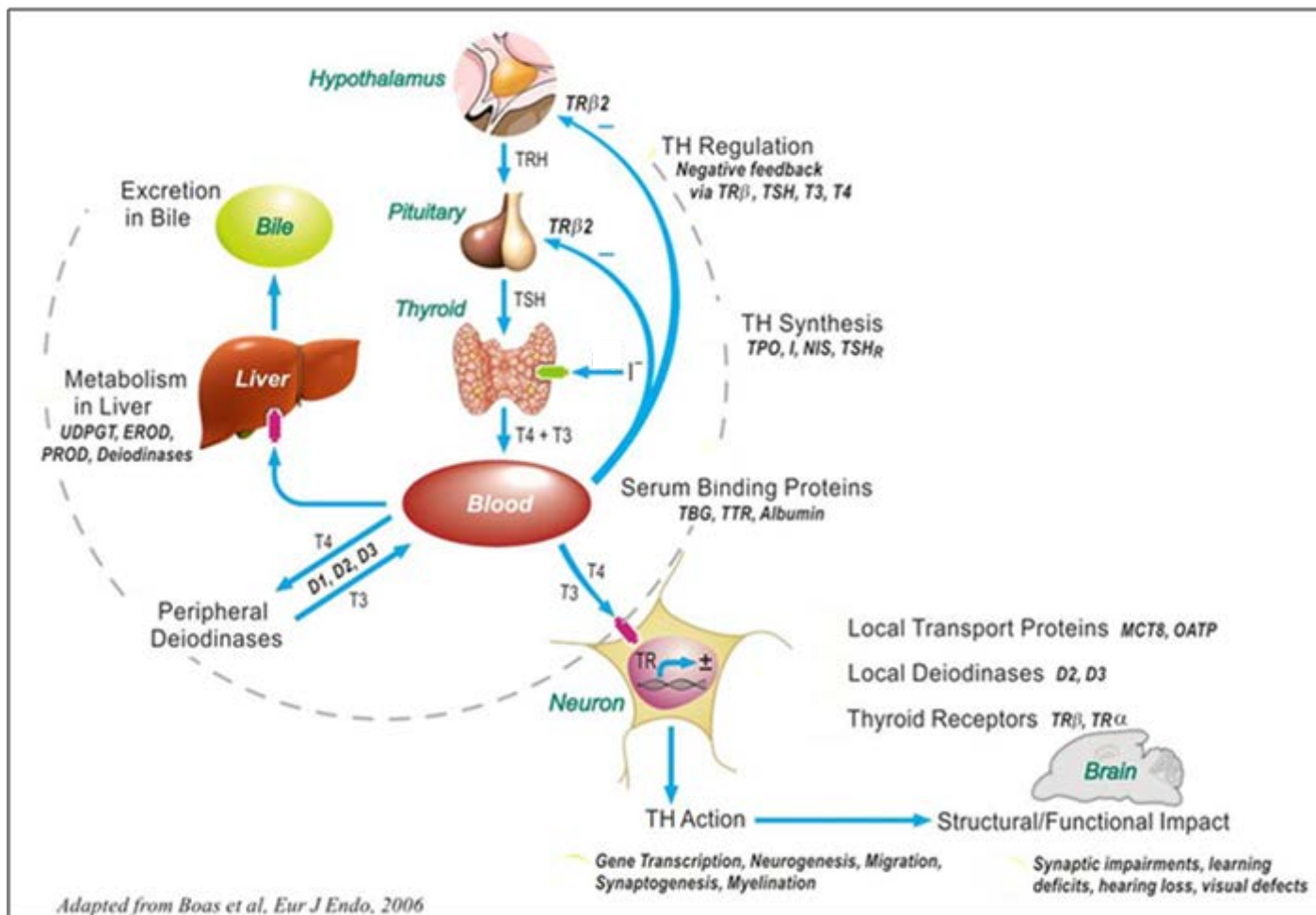
Development of a Human Thyroid Microtissue Model for Evaluation of Thyroid Hormone Synthesis

*Chad Deisenroth (NCCT)
RIVM Meeting*

4/18/2018

*The views expressed in this presentation are those of the author and do not
necessarily reflect the views or policies of the U.S. EPA*

Thyroid Disruption: Why Do We Care?

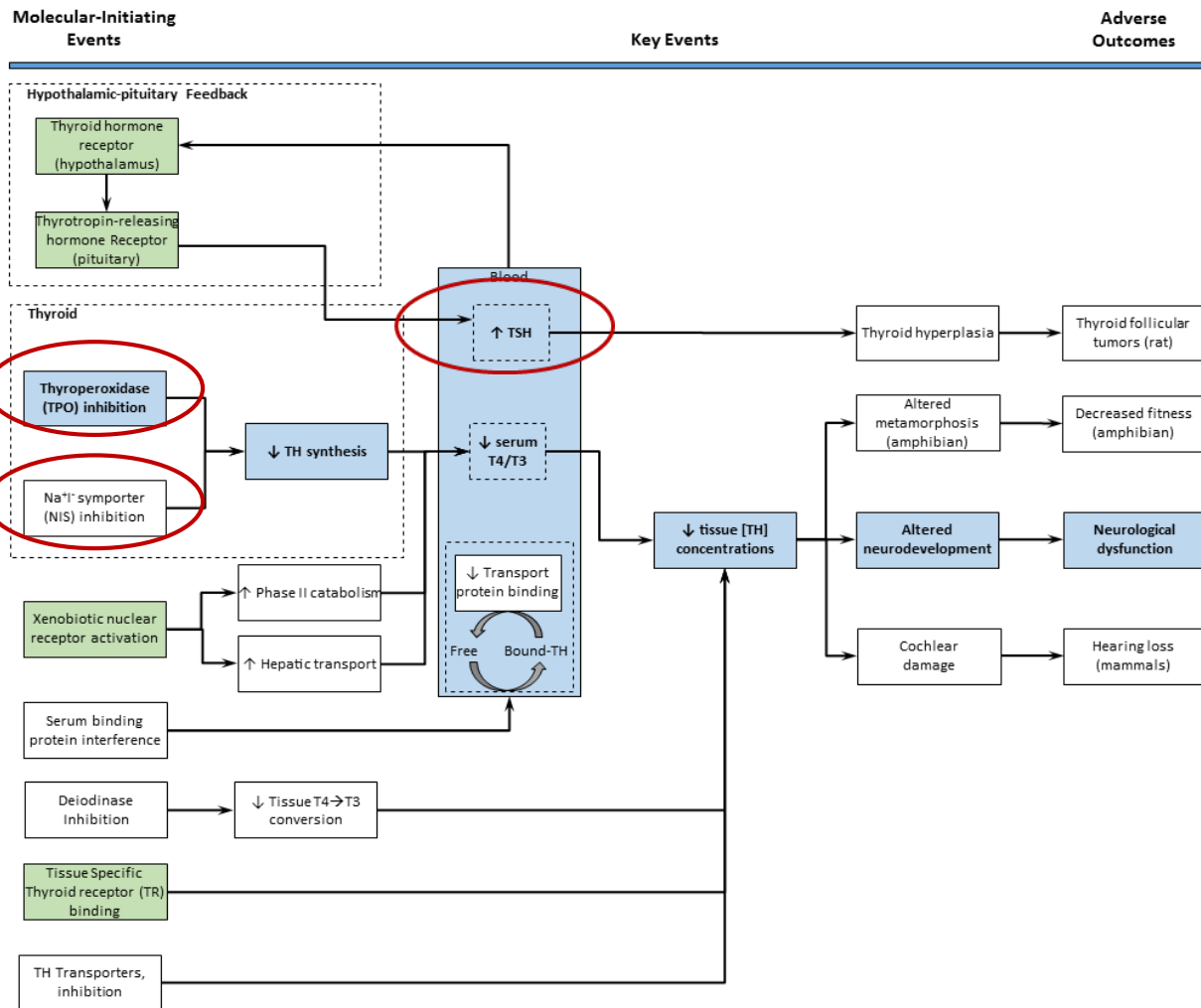


Endocrine Disruptor Screening Program (EDSP): Current Approach to Hazard Identification

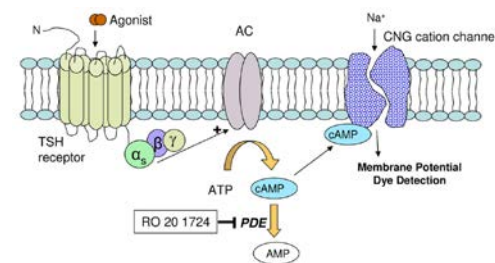
- EPA guideline screening batteries evaluate effects of chemical exposure on estrogen, androgen, and **thyroid** endocrine pathways
- Problems
 - Too reliant on animal tests
 - No *in vitro* tests for thyroid disruption
 - Possibly low human relevance to testing

Endocrine Pathway	Tier 1 Screening Battery										Tier 2 Testing Assays				
	ER Binding	ERα Transcriptional Activation*	AR Binding	Aromatase Inhibition	Steroidogenesis*	Uterotrophic*	Hershberger*	Pubertal Male	Pubertal Female	Amphibian Metamorphosis*	Fish Short Term Reproduction*	Rat 2-gen/Extended One-Gen*	Medaka Extended One-Gen Repro Test*	Amphibian Growth and Dev Assay*	Japanese Quail Two Gen Toxicity Test
E+	■	■			■	■			■		■	■	■	■	■
E-	■			■	■				■		■	■	■	■	■
A+			■		■		■	■			■	■	■	■	■
A-			■		■		■	■			■	■	■	■	■
HPT Axis								■	■	■		■		■	■

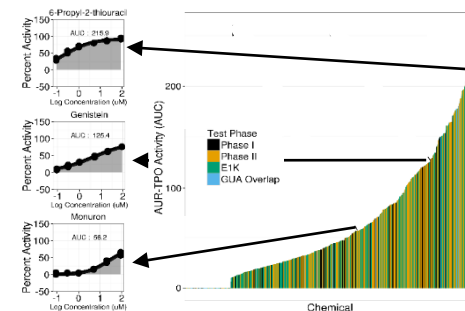
Thyroid Toxicity Testing: High Throughput Assays



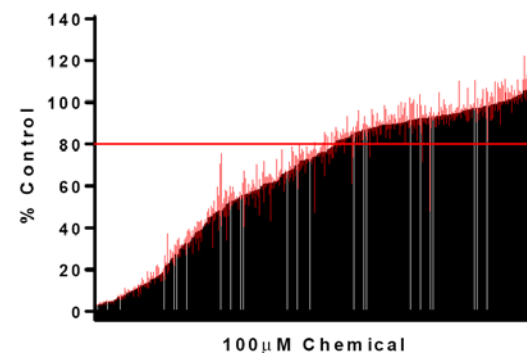
TSH Receptor (TSHR) Screen



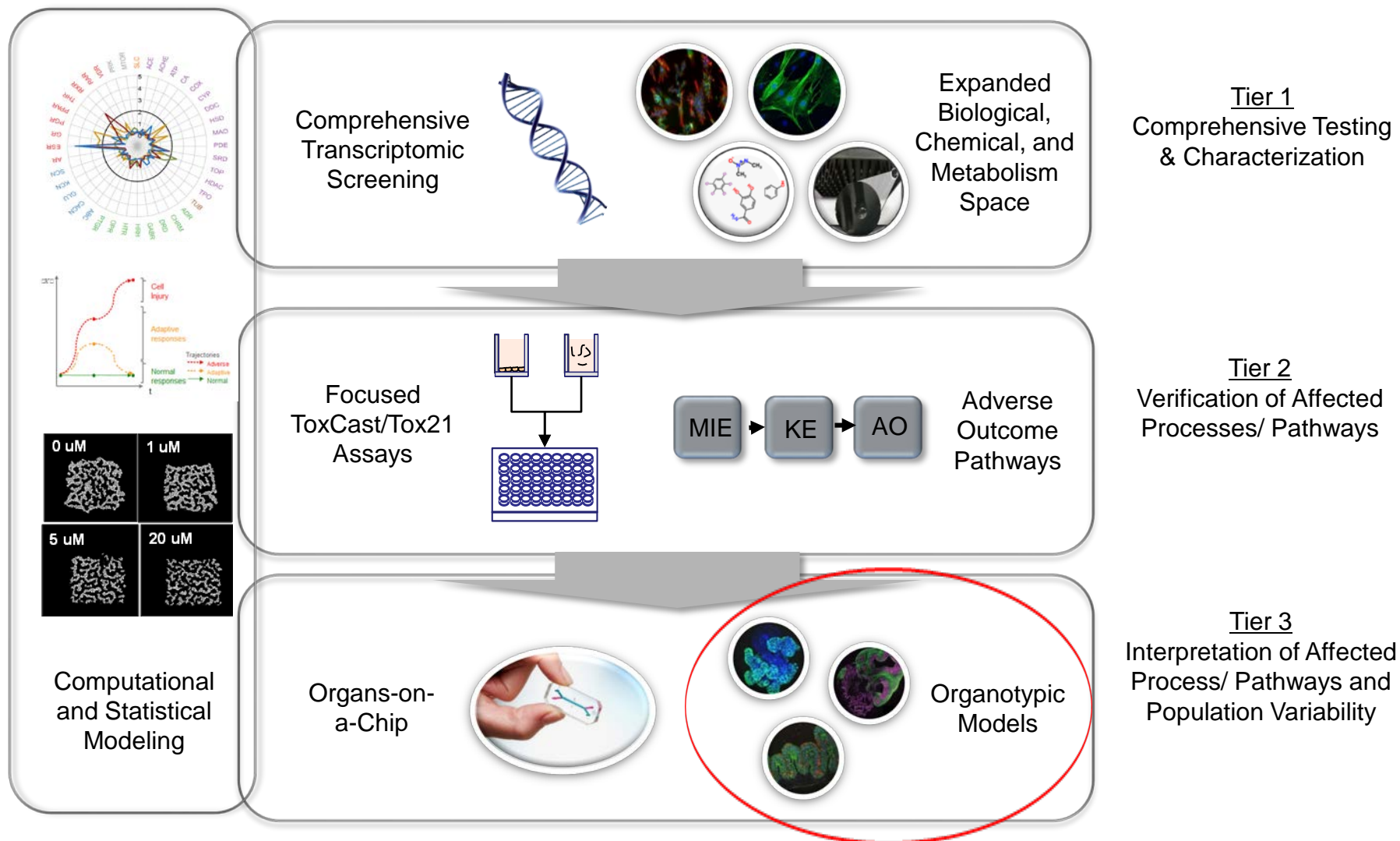
Thyroid Peroxidase (TPO) Screen



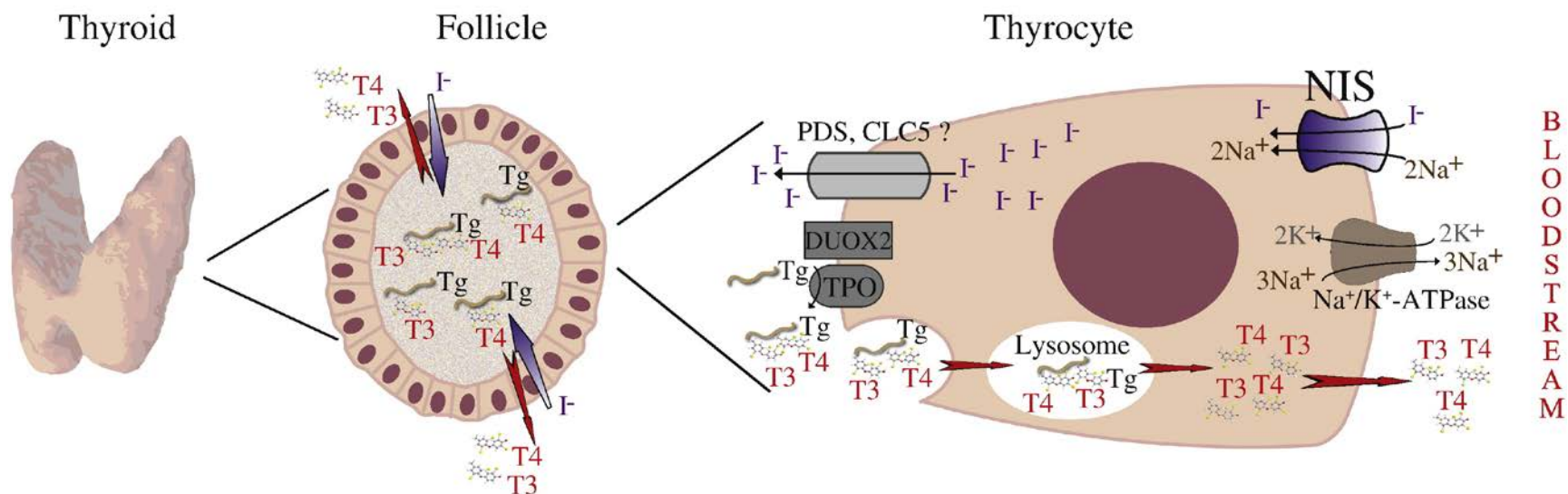
Sodium-Iodide Symporter (NIS) Screen



National Center for Computational Toxicology: A Strategy for Integrated Tiered Testing Approaches

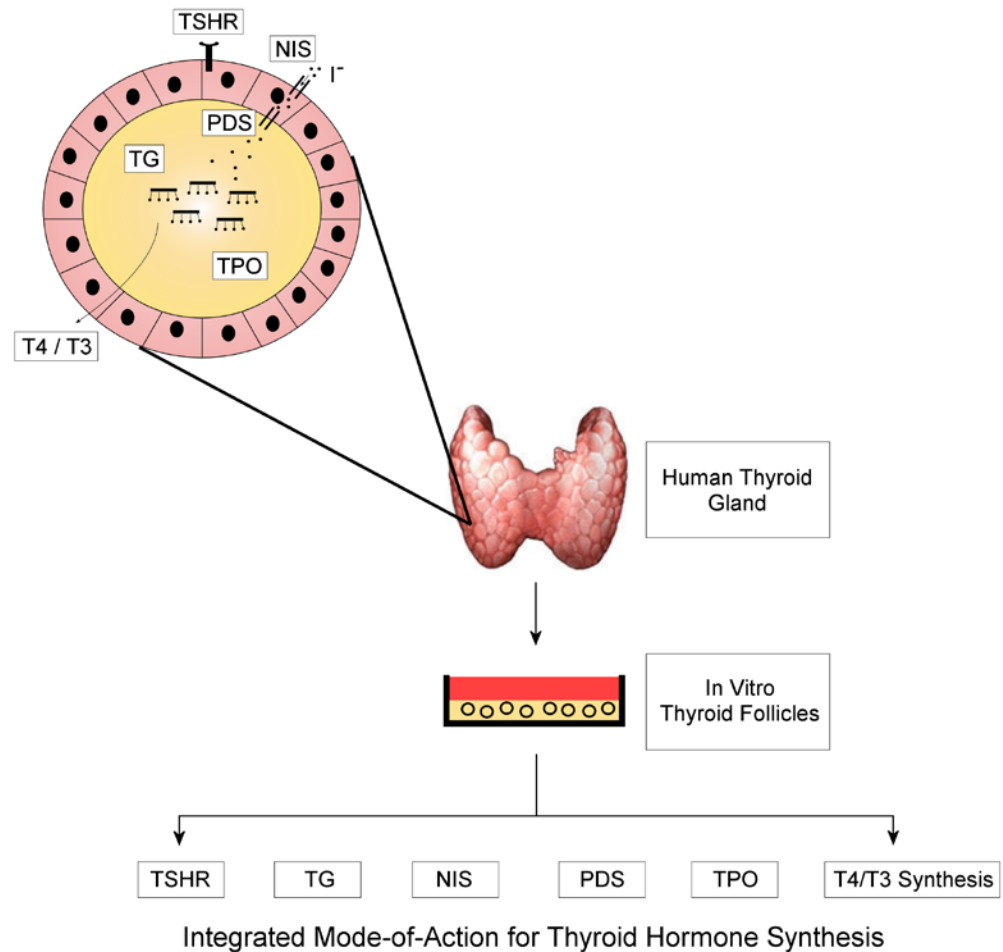


Thyroid Hormone Synthesis: Cell Type and Architecture are Critical for Hormone Synthesis



Assay Concept: Develop an In Vitro Human Thyroid Microtissue Model for Evaluation of Thyroid Hormone Synthesis

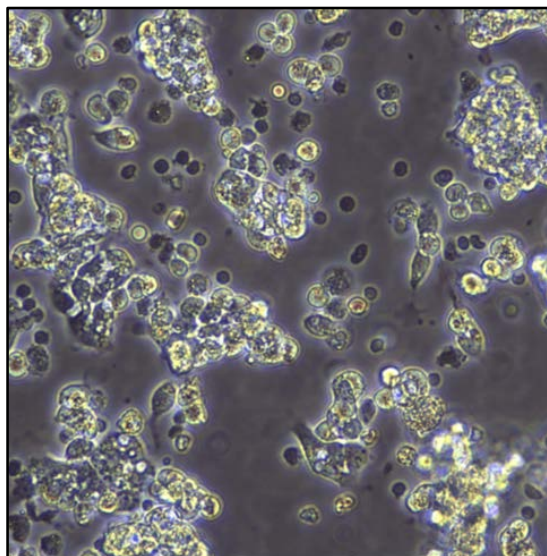
- **Tier 3 Assay**
 - 96-well medium throughput
- **Scope**
 - Functional evaluation of physiological thyroid hormone synthesis
- **Cell type:**
 - Primary human thyroid epithelial
- **Features**
 - Human-derived cells
 - Maintenance of biochemical and morphological features
 - Integrated MOA
 - Increased culture longevity
 - Suitable for concentration-response screening
- **Assay readout**
 - Integrated assay endpoints for key events with initial focus on T4/T3 secretion as an “apical” outcome



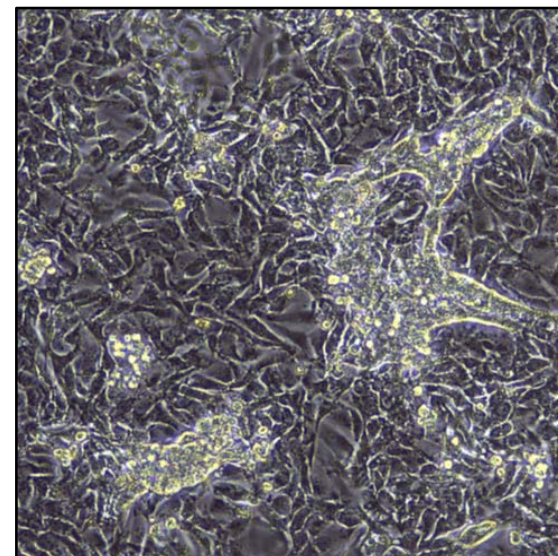
Thyroid Tissue Procurement and Cell Isolation

Key Considerations

1. Tissue clearance and stabilization
2. Tissue transport and timing
3. Tissue preparation
4. Digestion cocktail and timing
5. Viability enrichment
6. Cell counting, plating and passaging
7. Cell culture medium formulation
8. Live cell transport
9. Cryopreservation formulation
10. Quality control criteria



Follicle Fragments

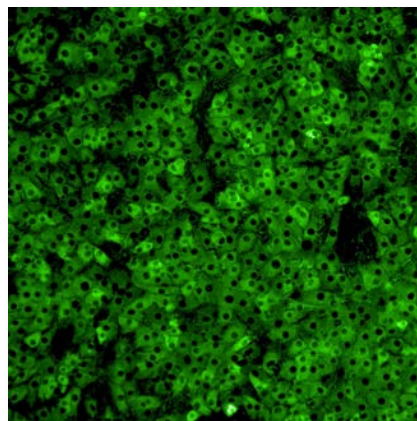


Attachment and Outgrowth

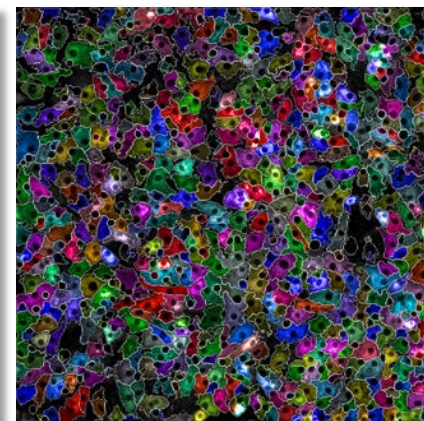
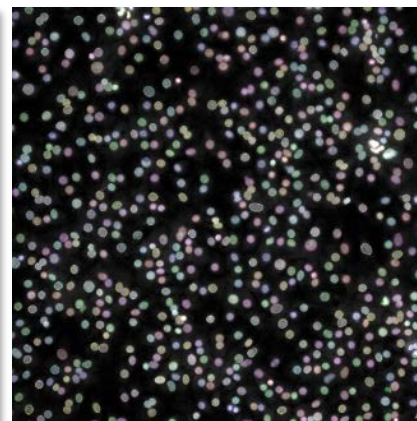
Thyroid Cell Characterization: QC for Population Purity



Nuclear Mask



Cytosol Mask

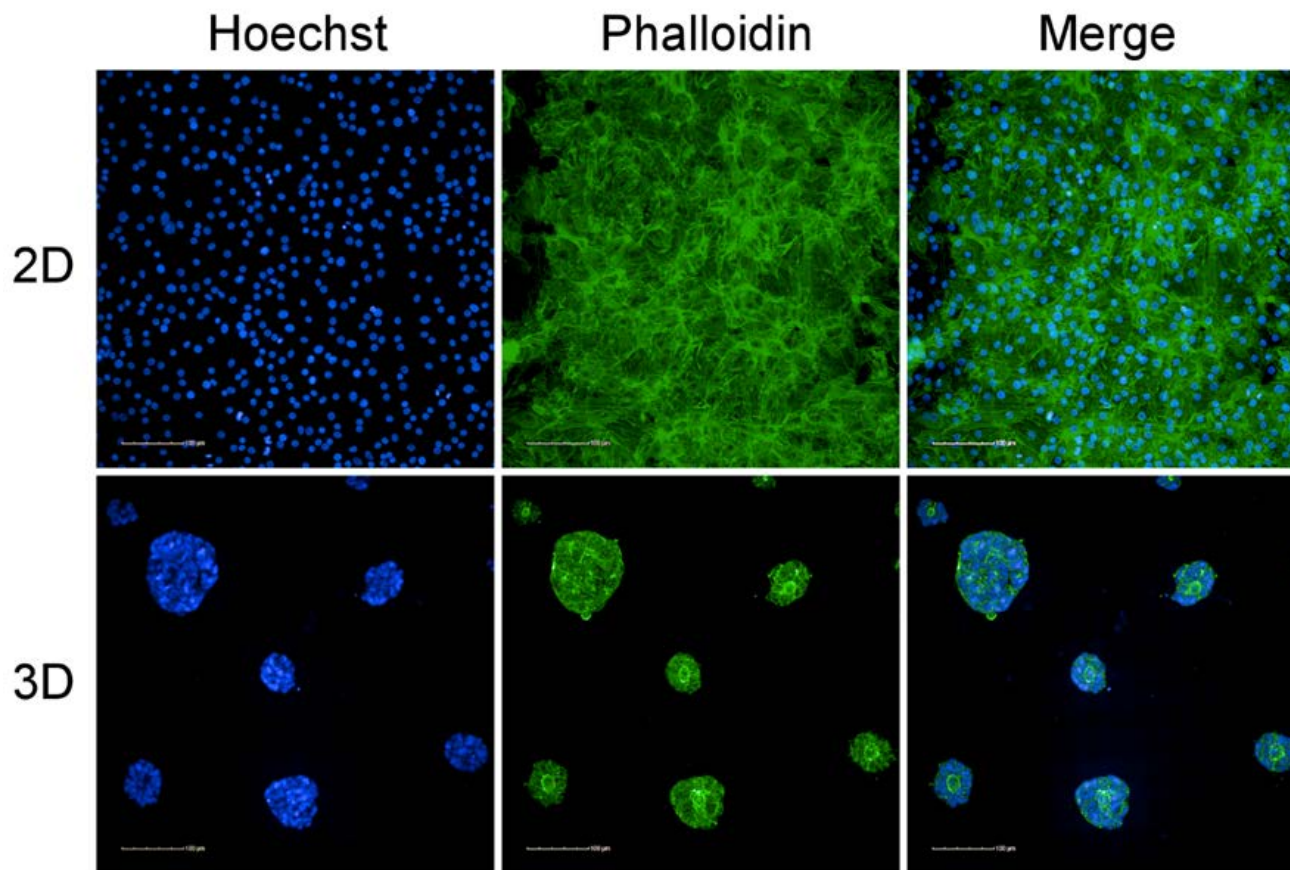


Thyroglobulin immunostaining in donor LNH 1722161 at passage 1

	IgG			IgG, kappa			NKX2-1			KRT7			TG		
	% POS	SD	N	% POS	SD	N	% POS	SD	N	% POS	SD	N	% POS	SD	N
NKX2-1	0.00	0.00	3	0.02	0.00	3	94.70	0.60	3	-	-	-	-	-	-
KRT7	1.00	0.01	3	1.00	0.06	3	-	-	-	94.23	0.42	3	-	-	-
TG	1.08	0.09	3	1.00	0.09	3	-	-	-	-	-	-	99.90	0.10	3

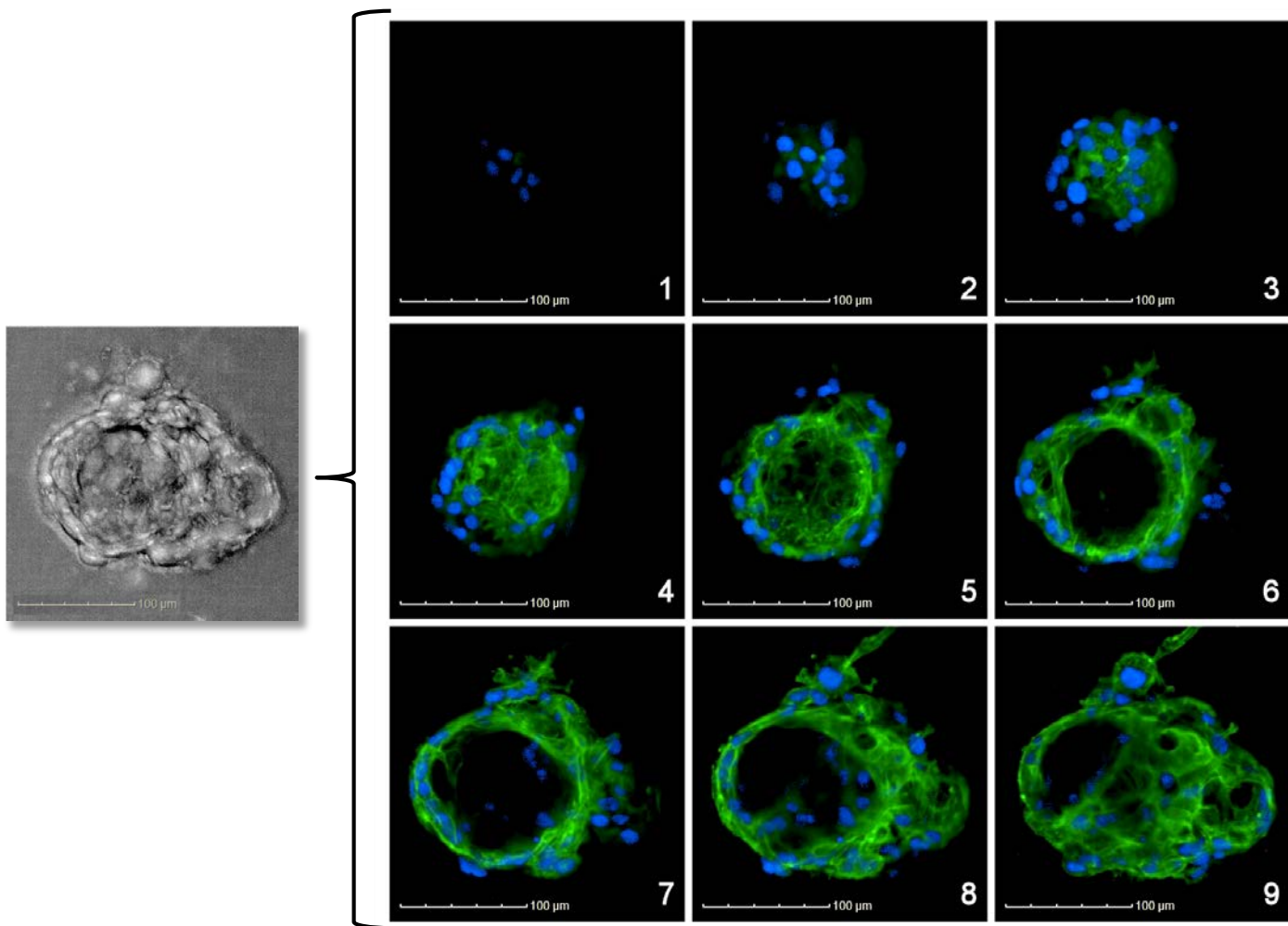
- **Image Cytometry:** Opera Phenix HCl used to acquire and analyze images
- **Analysis:** Total cell counts and % Positive for specified marker
 - NKX2-1: Nuclear transcription factor expressed in thyroid epithelial cells
 - KRT7: Cytokeratin enriched in thyroid epithelial cells
 - TG: Functional marker for thyroid-dependent gene expression

Thyroid Cell Characterization: 2D vs 3D



Donor LNH 1722161: High-density 2D and 3D sandwich model stained with Hoechst 33342 and Phalloidin-AF488 at Day 10 of culture.

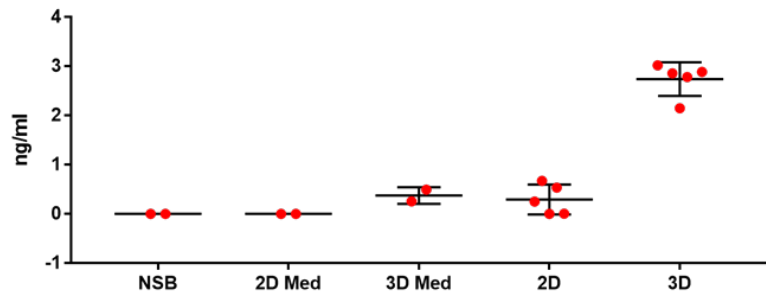
Thyroid Microtissue Morphology: The “Bird’s Nest”



Donor LNH 1722161: Confocal series of 3D sandwich model.

Hormone Analysis: More T4 and T3 is Secreted in 3D than 2D Culture

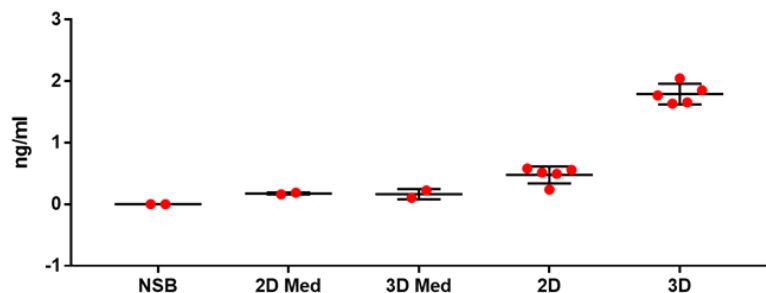
Thyroxine (T4)



LNH1722161 (Day 9; 48 Hr h7H CS-FBS + 1 mIU/ml TSH)

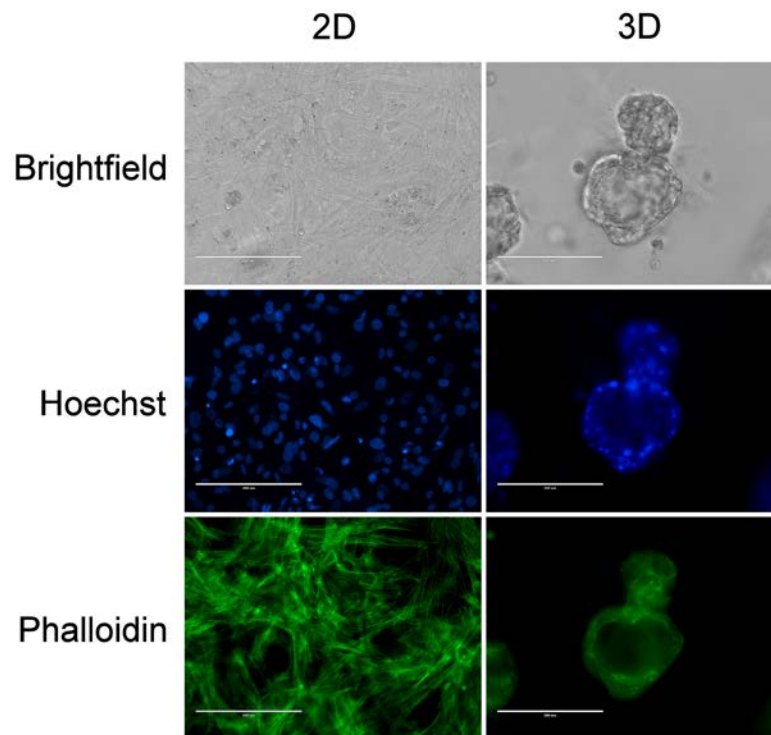
Human Serum Total T4 Reference Range: 50-125 ng/ml

Triiodothyronine (T3)



LNH1722161 (Day 9; 48 Hr h7H CS-FBS + 1 mIU/ml TSH)

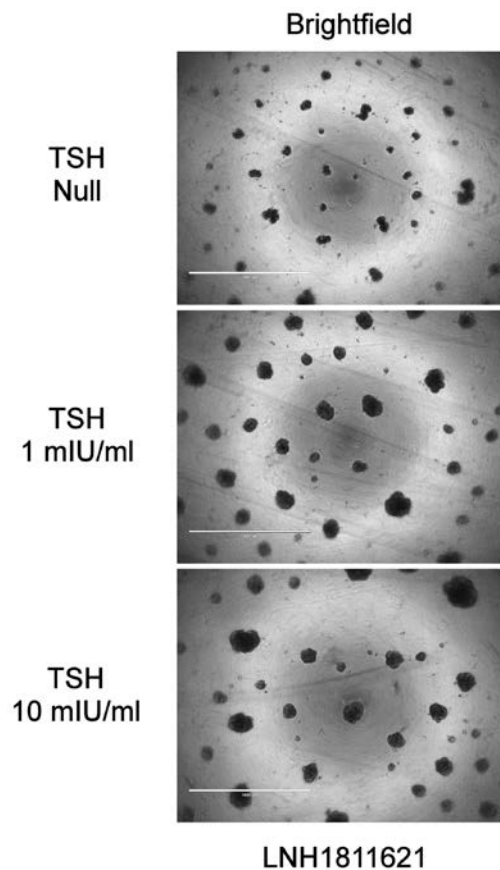
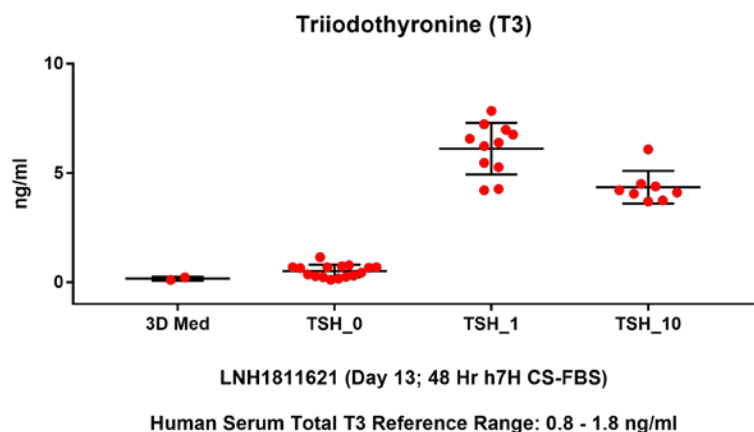
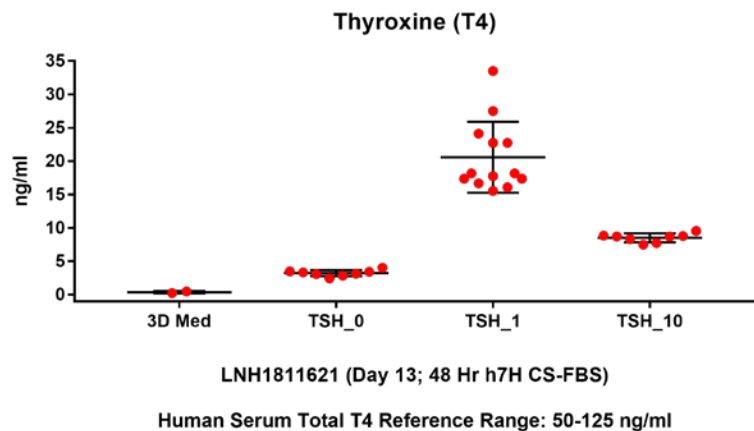
Human Serum Total T3 Reference Range: 0.80 - 1.8 ng/ml



LNH1722161

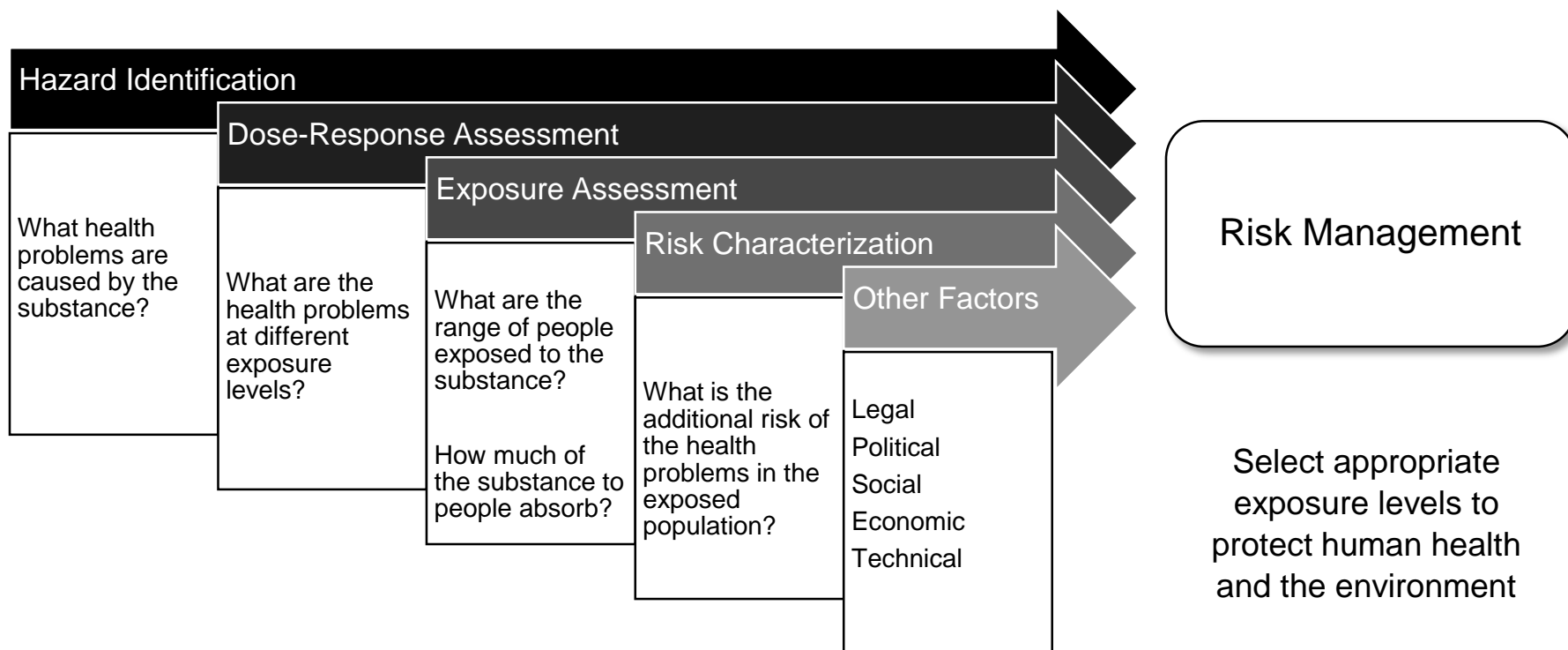
	T4				T3			
	Mean	SD	% CV	% TH	Mean	SD	% CV	% TH
NSB	0	0	0	0	0	0	0	0
2D Med	0	0	0	0	0.18	0.02	9.7	100
3D Med	0.38	0.17	45.1	69	0.17	0.08	51.0	31
2D Sup	0.29	0.30	104.2	38	0.48	0.14	29.0	62
3D Sup	2.74	0.34	12.5	60	1.79	0.17	9.36	40

Hormone Analysis: TSH Modulates T4 and T3 Secretion in 3D Culture



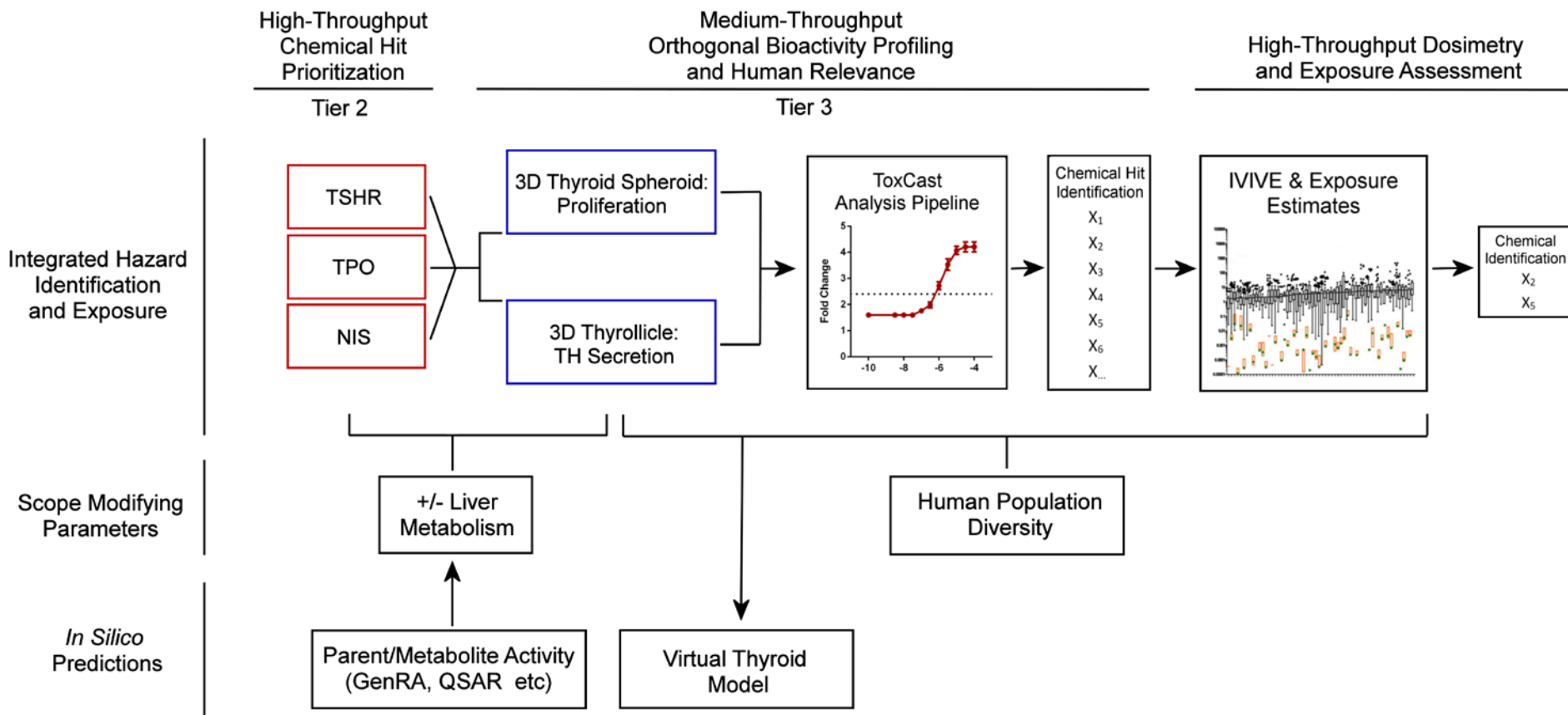
	T4				T3			
	Mean	SD	% CV	% TH	Mean	SD	% CV	% TH
3D Med	0.38	0.17	45.1	69	0.17	0.08	51.0	31
TSH 0	3.23	0.47	14.59	86	0.51	0.28	55.40	14
TSH 1	20.60	5.31	25.78	77	6.11	1.18	19.25	23
TSH 10	8.53	0.65	7.68	66	4.35	0.75	17.32	34

Phases of Chemical Risk Assessment and Management: Can an *In Vitro* Biologist Design Screening Workflows Useful for Risk Assessment?



- Risk assessment is a four step process that integrates hazard, dose-response, and exposure
- Risk management incorporates additional factors related to risk characterization to set appropriate response

In Vitro Screening for Thyroid Disruption: How Can Tier 3 Medium-Throughput Testing Fit In?



- **Hazard Identification:** Refinement of bioactivity hit calls and evaluation of variability in human populations
- **Toxicodynamics:** Increased comprehension of apical endpoint dosimetry and bioactivity/exposure margins
- **In Silico Predictions:** Data generation to enhance prediction of parent/metabolite activity and construct virtual simulations of thyroid perturbation