Advancing Translational Applications of Human Organotypic Thyroid Assays

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Risk Assessment Specialty Section (RASS)
Monthly Webinar
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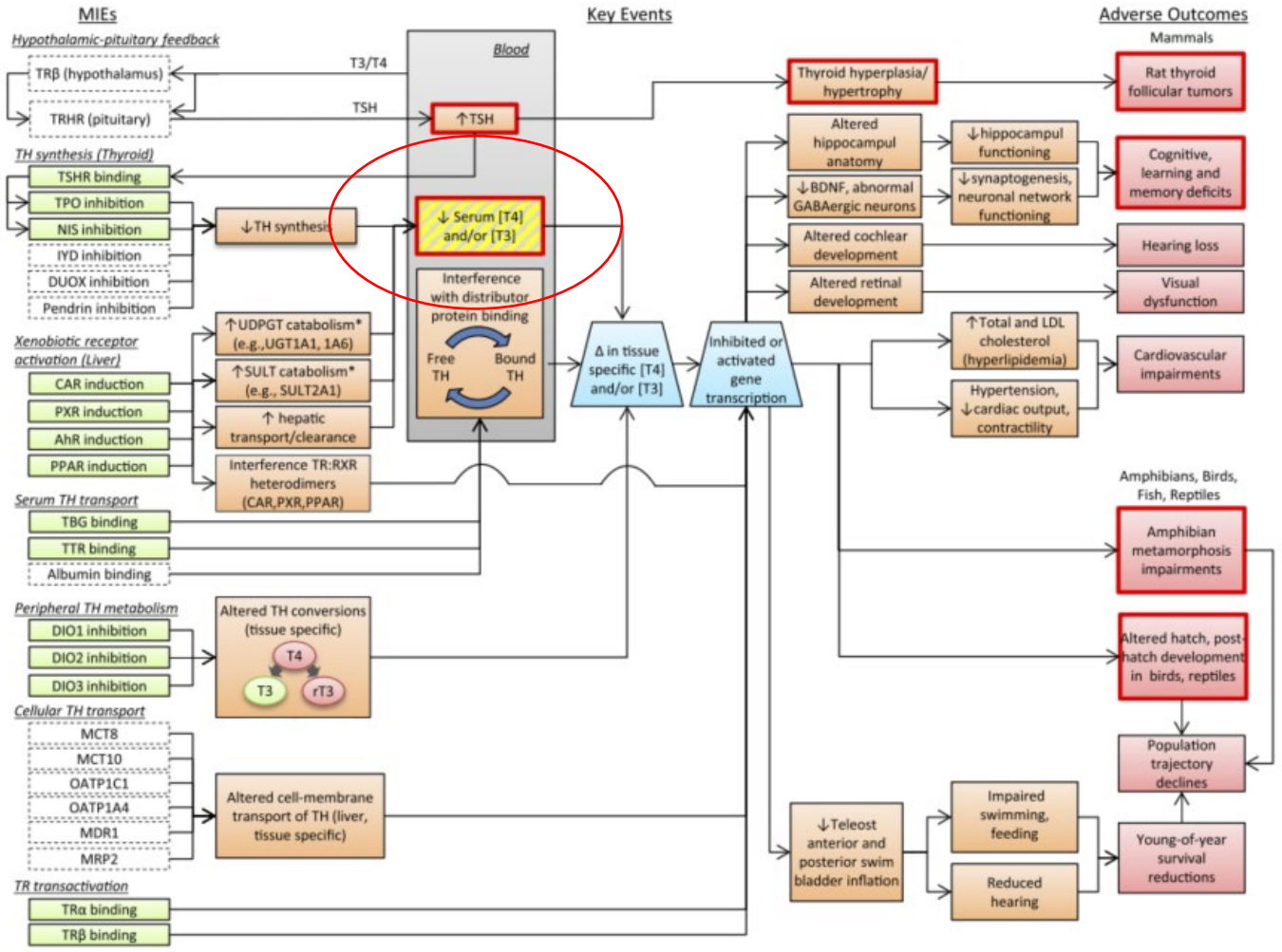
Outline

- Development of a human thyroid organotypic culture model to address data gaps in screening and prioritization of thyroid disrupting chemicals
- Establishing confidence with an inter-laboratory prevalidation study of the human thyroid microtissue assay
- Orthogonal screening of prioritized chemicals in human thyroid microtissues for functional and mechanistic relevance

Thyroid AOP Network: Broad Coverage of Mechanistic MIE-based Thyroid Assays



2013 Murk, A. J. *et al.* Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. *Toxicology in vitro*.

2014 OECD. New Scoping Document on in vitro and ex vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling. *OECD Series on Testing and Assessment, No. 207*

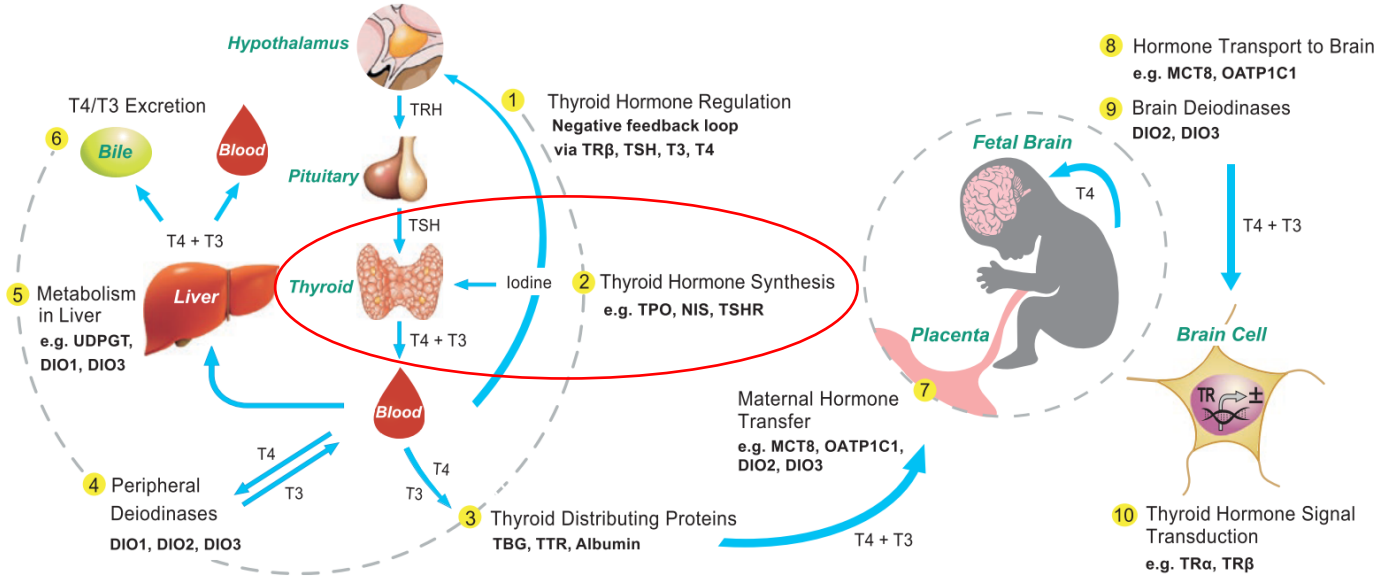
2019 Noyes, P.D. *et al*. Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. *Environ Health Perspect.*

How can the human thyroid gland be represented *in vitro* to provide 'key event' coverage?

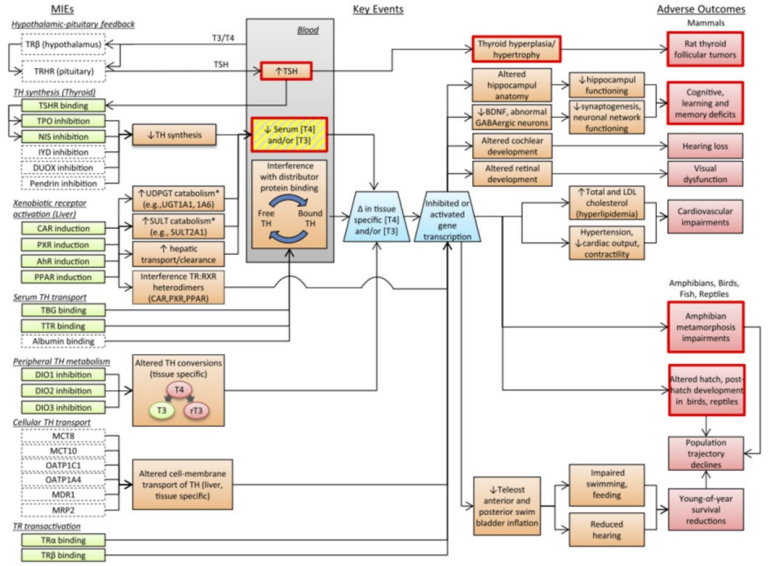
DOI: 10.1289/EHP5297

Challenges with *In Vitro* Thyroid Testing: Thyroid HTS Assays Do Not Directly Measure Thyroid Hormone Disruption

Sites of Interference for Thyroid Disrupting Chemicals



Thyroid AOP Network

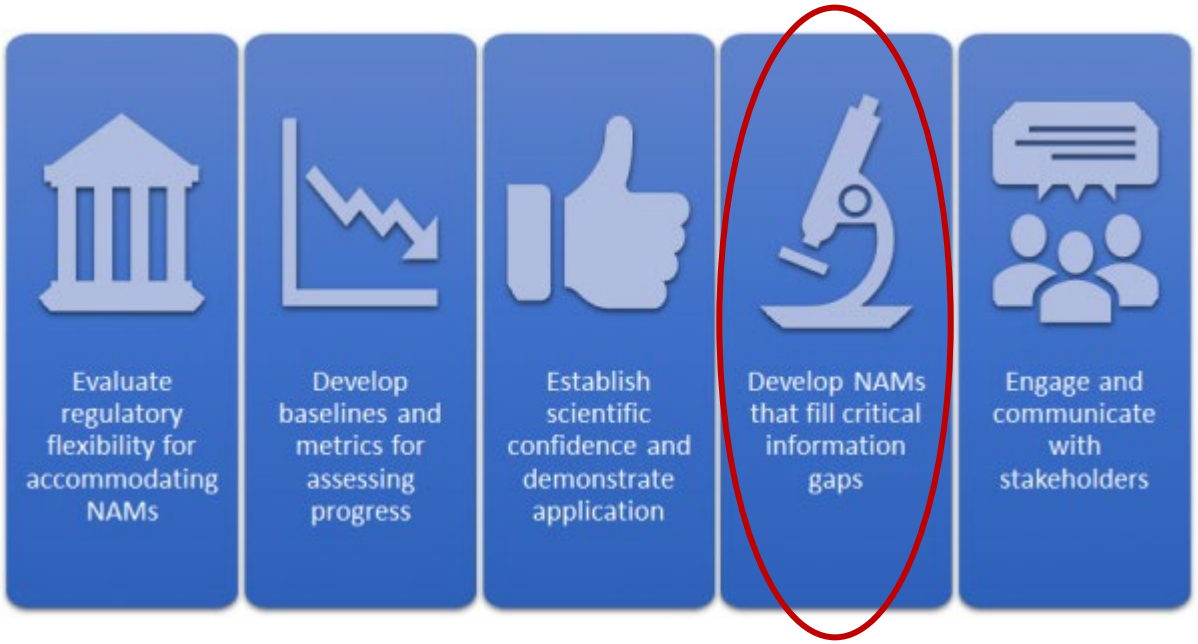


Thyroid MIE	Assay	Environmental Chemicals Screened	Active Chemicals	% Active	Reference
TSHR	Engineered Cell Line	7871	825	10	TCPL: TOX21_TSHR_Agonist, TOX21_TSHR_Antagonist
TPO	Microsomal Enzyme	1074	150	14	K. Paul Friedman et al, ToxSci, 151(1), 2016, 160-180
NIS	Engineered Cell Line	293	137	47	J. Wang et al, EnvironSciTechn, 52, 2018, 5417-5426
NIS	Engineered Cell Line	768	167	22	J. Wang et al, Environment International, 126, 2019, 377-386
DIO 1	Recombinant Enzyme	292	18	6	M. Hornung et al, ToxSci, 162(2), 2018, 570–581
DIO 1	Recombinant Enzyme	1819	139	8	J. Olker et al, ToxSci, 168(2), 2019, 430-442
IYD	Recombinant Enzyme	1825	148	8	J. Olker et al, Toxicol In Vitro. 2021 Mar;71:105073.

DOI: 10.1210/endocr/bqaa106; DOI: 10.1289/EHP5297

EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing

New Approach Methods – any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment to avoid the use of animal testing.



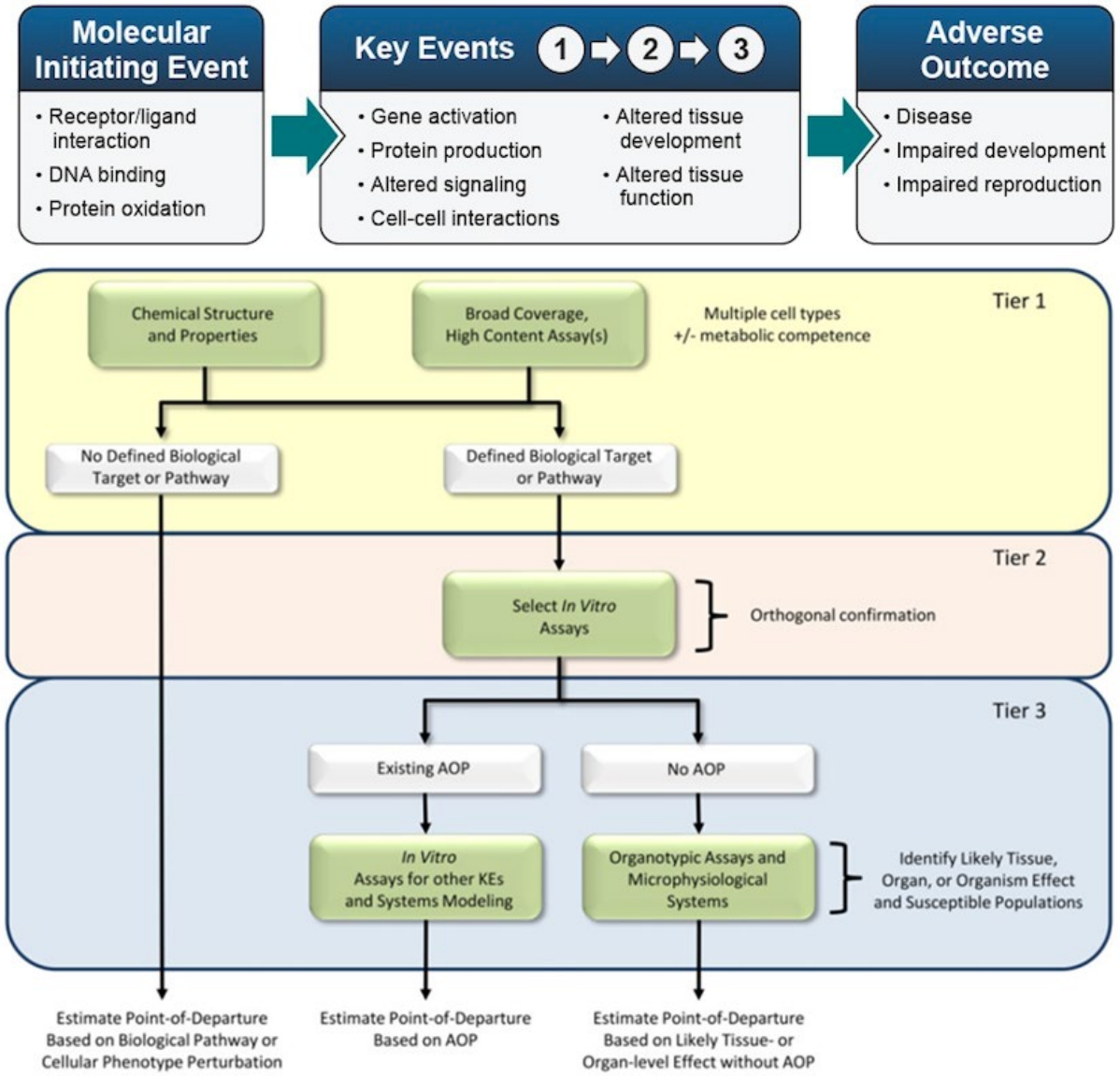
Five work plan objectives

Examples of information gaps

- Inadequate coverage of biological targets.
- Minimal capacity for addressing xenobiotic metabolism in *in vitro* test systems.
- Limited capability to address tissue- and organ-level effects.
- Lack of robust integrated approaches to testing and assessment (IATAs) for complex biology.

https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf

EPA Computational Toxicology Blueprint: Tiered Hazard Screening and Prioritization



https://ntp.niehs.nih.gov/go/niceatm-aop; DOI: 10.1093/toxsci/kfz058

Tier 3 Experimental Approaches

- **Tier 1/2 Prioritized Chemicals**: Reduce HTS data uncertainty and provide more physiologically relevant insight into spatial and temporal toxicodynamics.
- **Organotypic Culture Models (OCMs)**: Primary cells or tissues in complex culture systems that more closely mimic organ structure and function.
- **Complex In Vitro Model Platforms:** 3D microtissues or organoids, micropatterned co-culture systems, microphysiological systems, 3D bioprinted tissues

Filling Technology Gaps for *In Vitro* Thyroid Testing

United States Environmental Protection Agency

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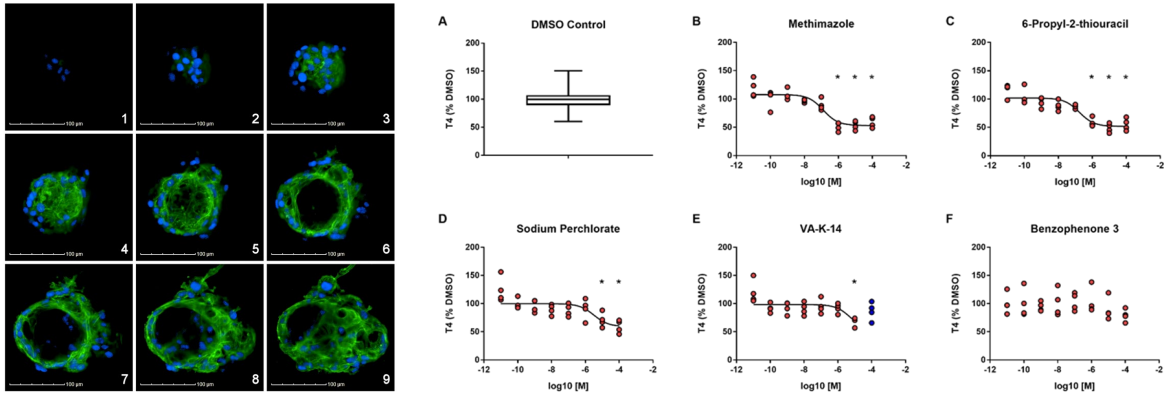
INSCREENEX

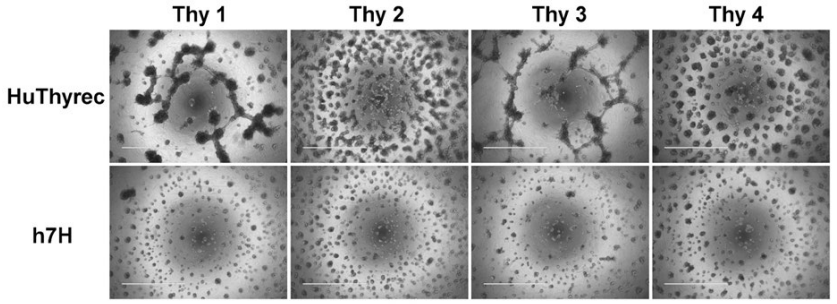
Development of an *In Vitro* Human Thyroid Microtissue Model for Chemical Screening

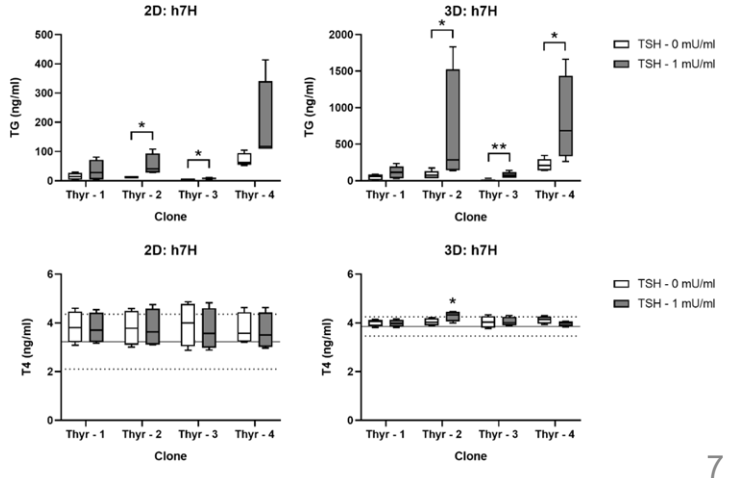
Chad Deisenroth ,*,[1] Valerie Y. Soldatow,[†] Jermaine Ford,[‡] Wendy Stewart,* Cassandra Brinkman,* Edward L. LeCluyse,[†] Denise K. MacMillan,[‡] and Russell S. Thomas *



Characterization of Novel Human Immortalized Thyroid Follicular Epithelial Cell Lines

Kristen Hopperstad,[1,*] Theresa Truschel,[2,*] Tom Wahlicht,[2] Wendy Stewart,[1] Andrew Eicher,[1] Tobias May,[2] and Chad Deisenroth[1,†]



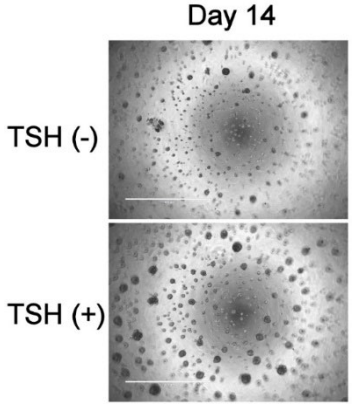


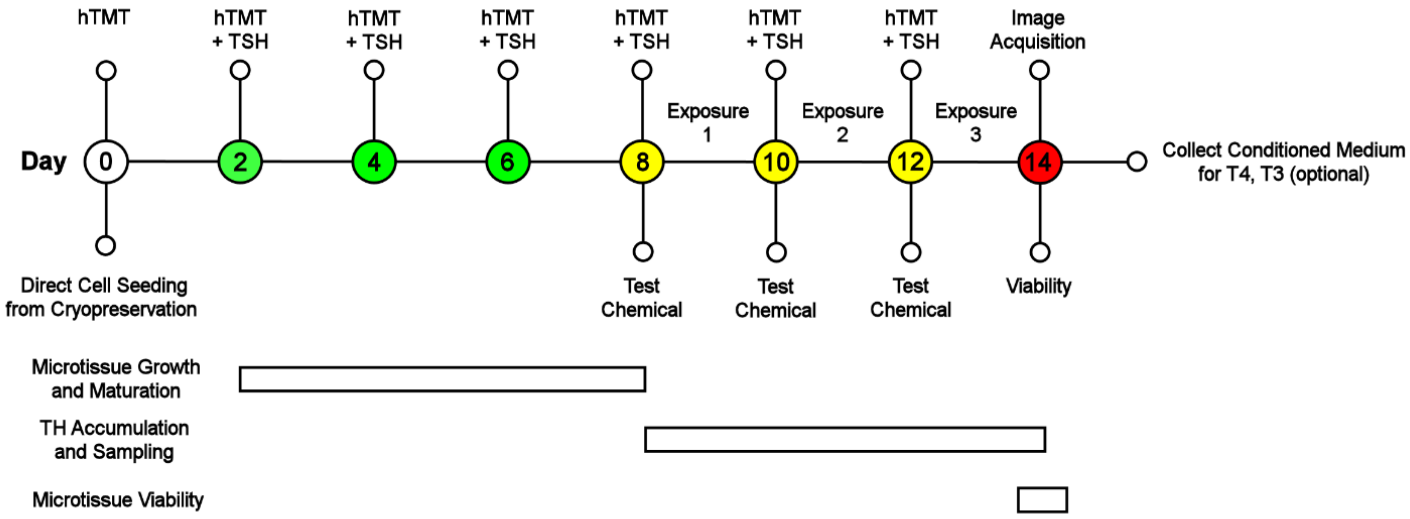
- A “Tier 3” assay designed to evaluate thyroid hormone disruption as a mode-of-action for endocrine-related hazard screening.
- Established commercial sources of primary human thyrocytes and immortalized cell lines.

Outline

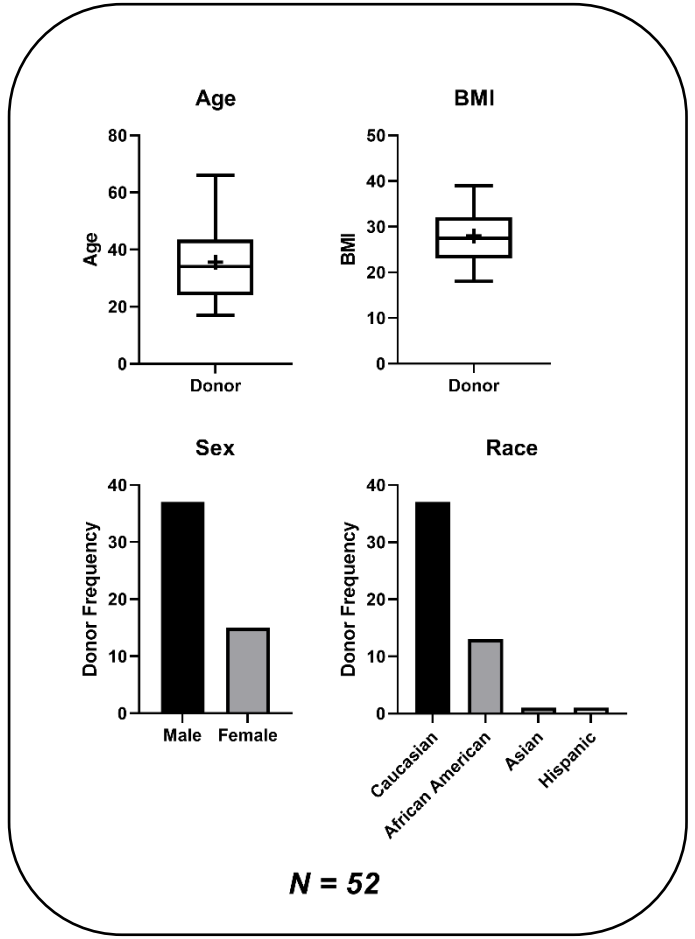
- Development of a human thyroid organotypic culture model to address data gaps in screening and prioritization of thyroid disrupting chemicals
- Establishing confidence with an inter-laboratory prevalidation study of the human thyroid microtissue assay
- Orthogonal screening of prioritized chemicals in human thyroid microtissues for functional and mechanistic relevance

Improving the Platform: Human Thyroid Microtissue Assay v2.0



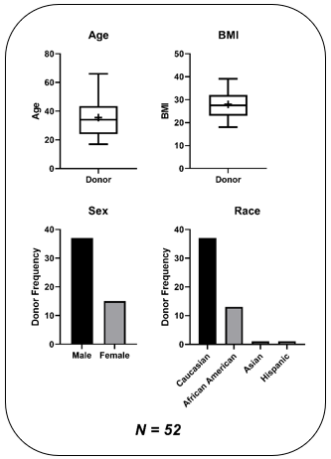


Method modifications for improved performance, accessibility, and implementation.

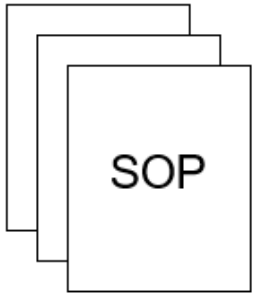


Expansion of the EPA Thyroid Donor Biobank

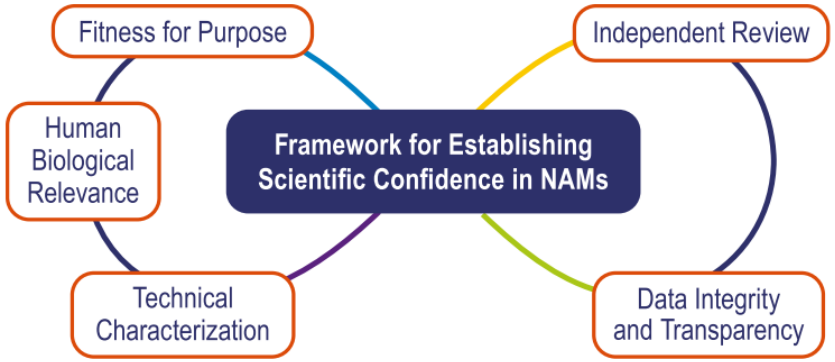
Increasing Confidence in a Human Thyroid Microtissue New Approach Method (NAM)

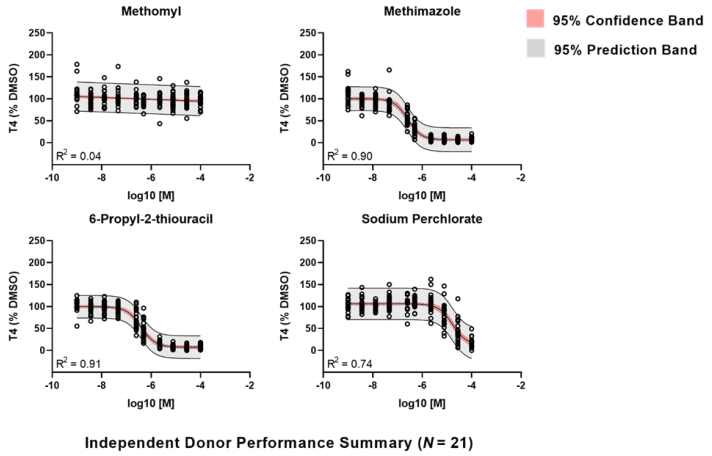


Characterizing the EPA Donor Biobank

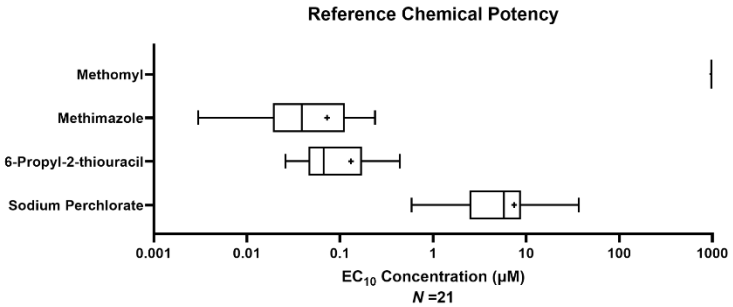


Independent Peer Review by Validation Management Team

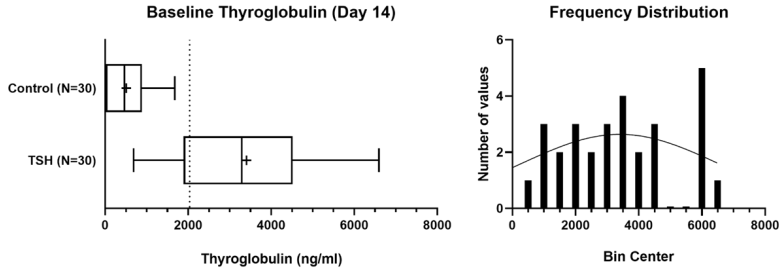




Evaluating Human Donor Variability



Selecting Reference Chemicals



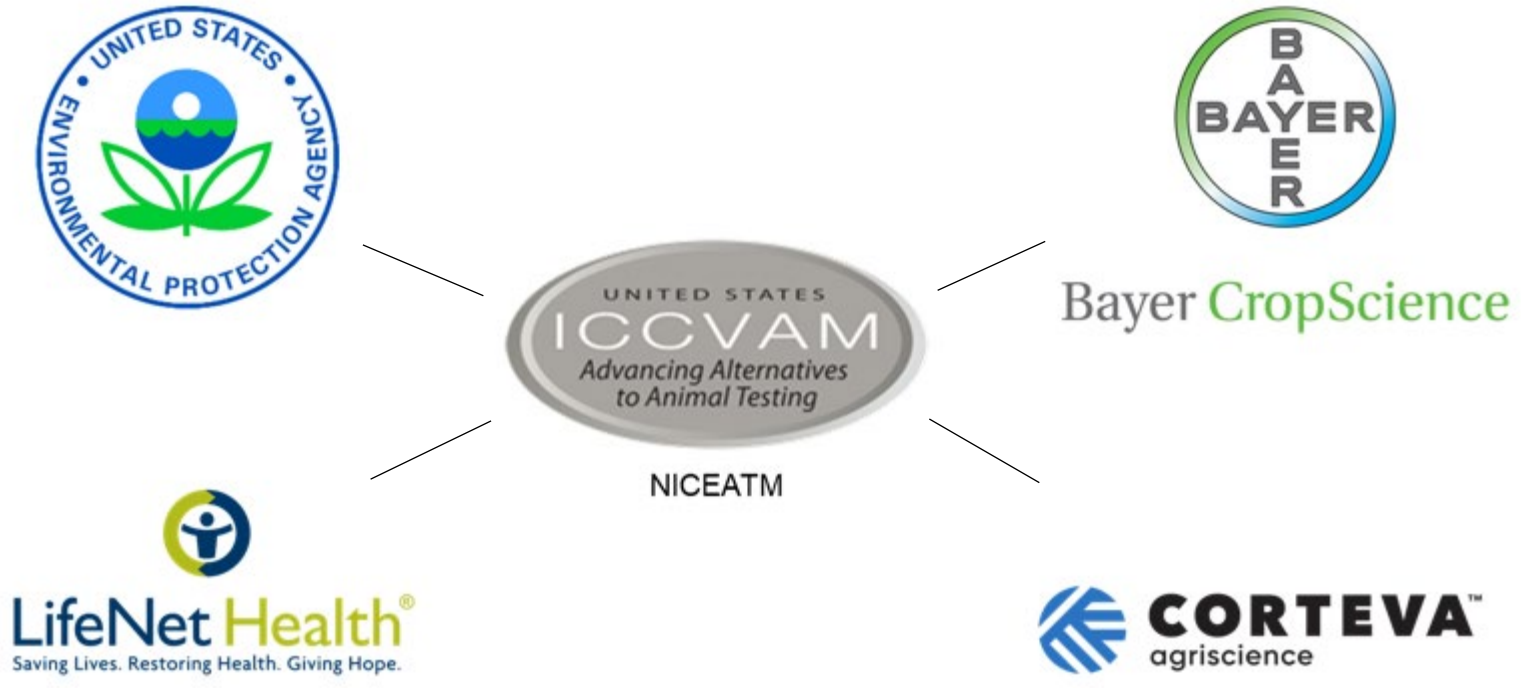
Establishing Donor Acceptance Criteria

Long Range Goal: Establish a validated test method for human thyroid hormone disruption

doi.org/10.1007/s00204-022-03365-4

Inter-laboratory Prevalidation of the Human Thyroid Microtissue Assay

Goal: To structure and support a preliminary assessment of the test method reliability and relevance.

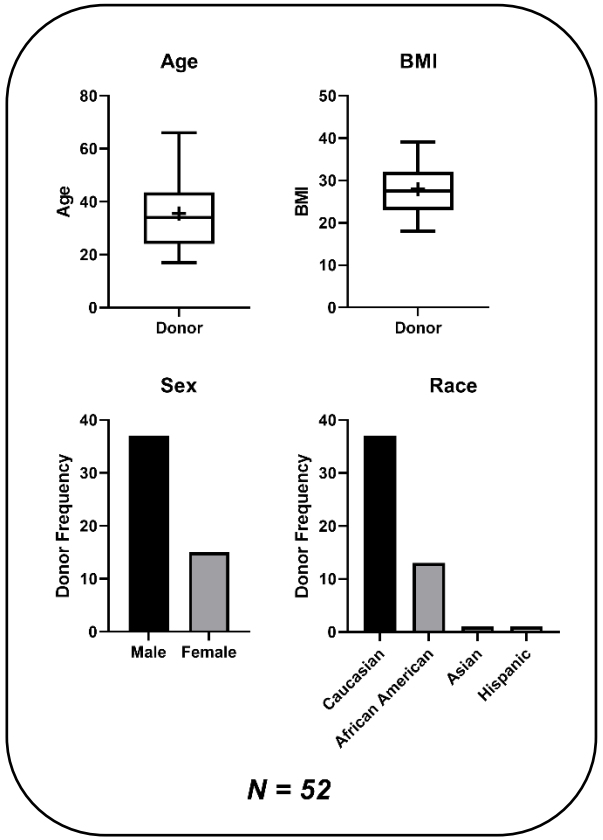


Independent Validation Management Team

Objectives

1. Test method standardization.
2. Test method transfer, training and intra-laboratory model performance evaluation.
3. Limited inter-laboratory reference chemical testing and assay performance evaluation.

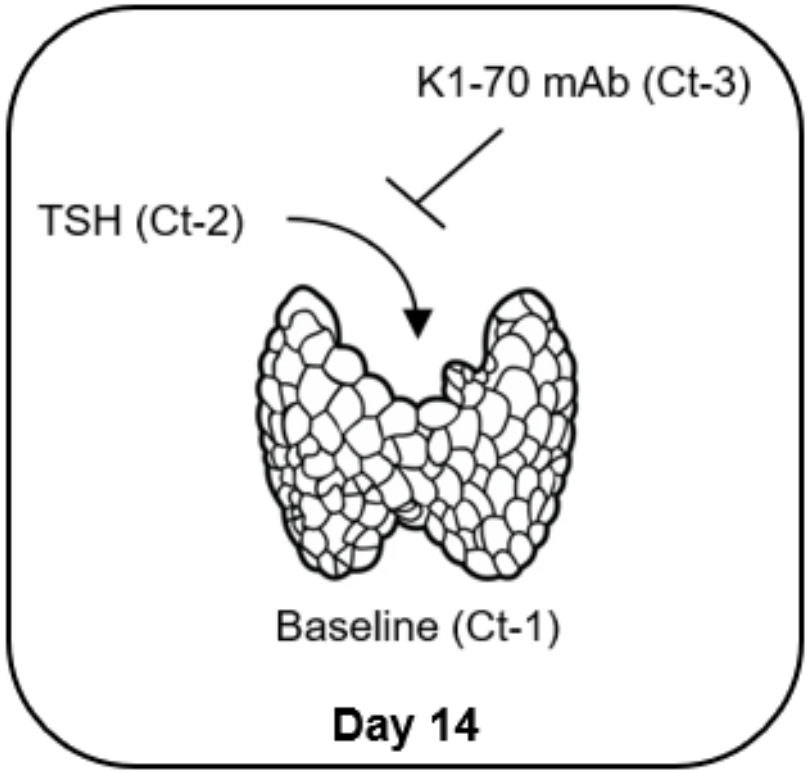
EPA Thyroid Donor Biobank – Donor Characterization and Qualification



Biobank Summary (August 2017 – Present)	
Donors	52
Median Age	34 (17-66)
Sex	Male (37); Female (15)
Race	Caucasian (37), African American (13), Hispanic (1), Asian (1)
Median BMI	28 (19-39)
Serologies	CMV, EBV
Euthyroid	51/52

What donors should be tested for the model and performance validation phases?

Ensuring High Quality Cells - Donor Characterization and Qualification Parameters

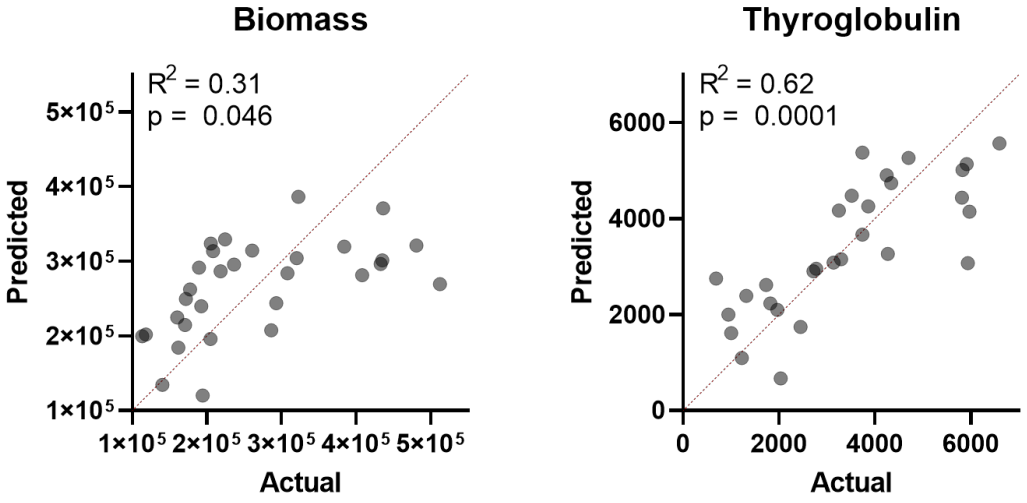


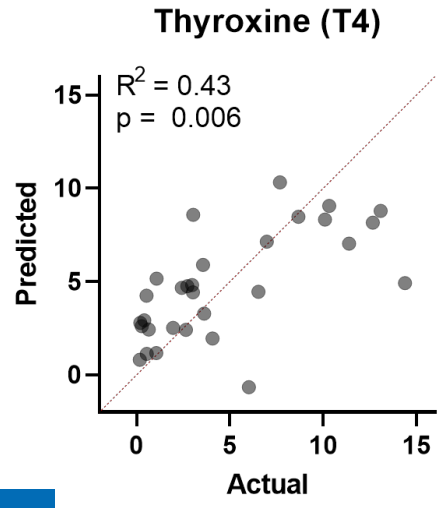
Donor Cohort Demographic Summary

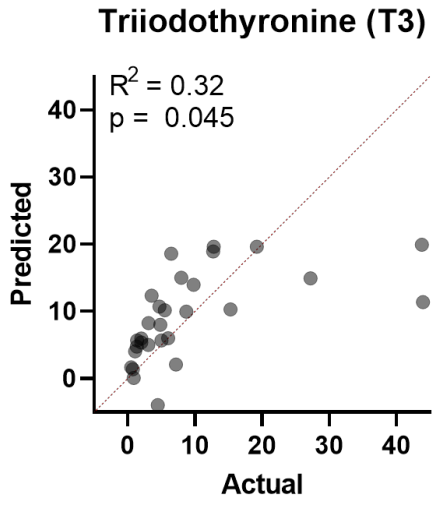
Donors	30
Age	35 (17-61)
Sex	Male (23), Female (7)
Race	Caucasian (24), African American (6)
BMI	28 (20-37)

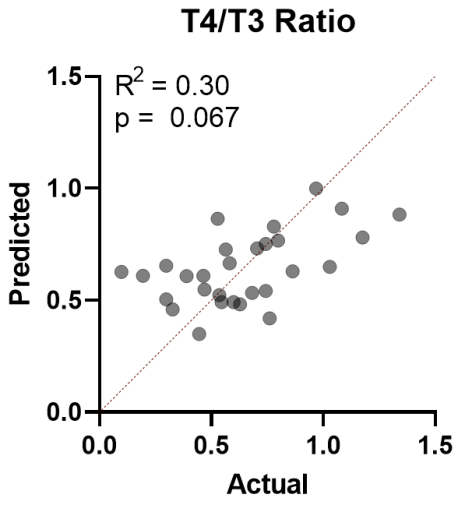
- Microtissue Morphology
- Microtissue Biomass
- TSH Receptor Sensitivity
- Thyroglobulin Synthesis
- Hormone Synthesis
- Reference Chemical Response

Identifying TSH-dependent Performance Parameters Influenced by Donor Demographics

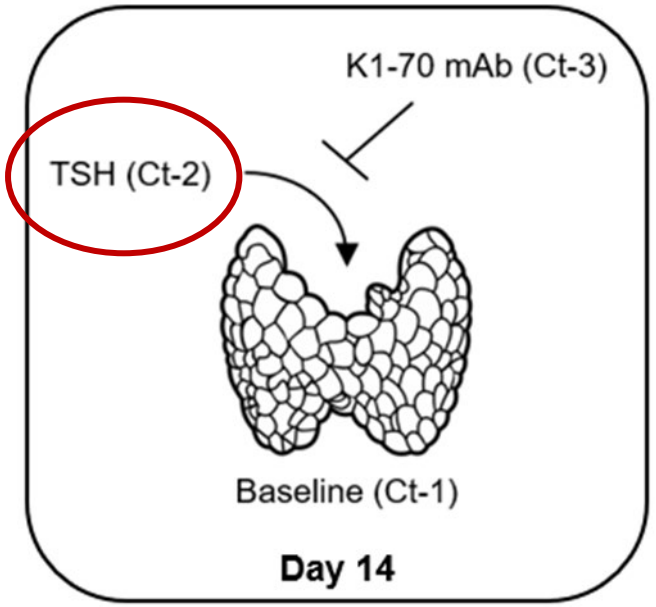




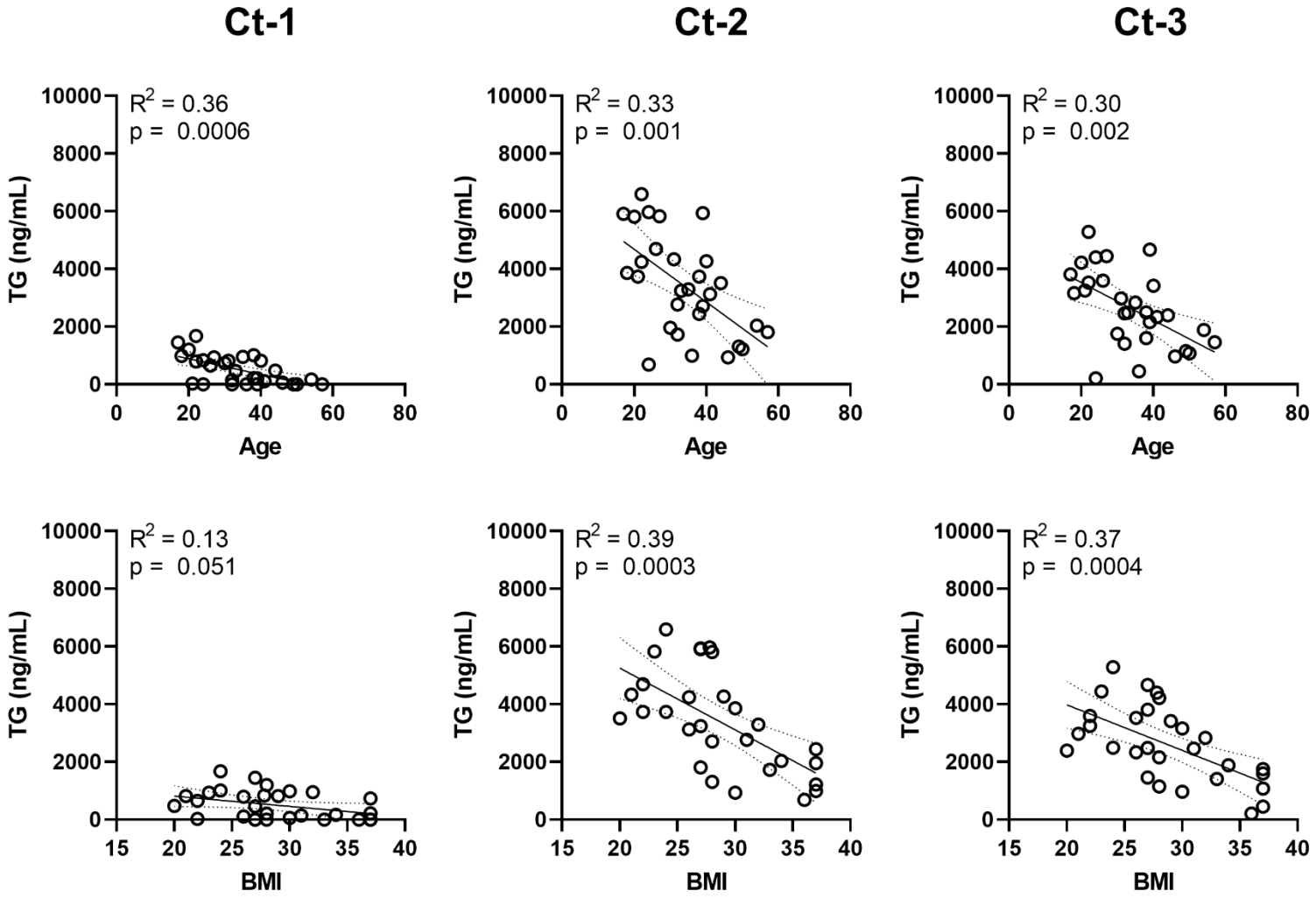




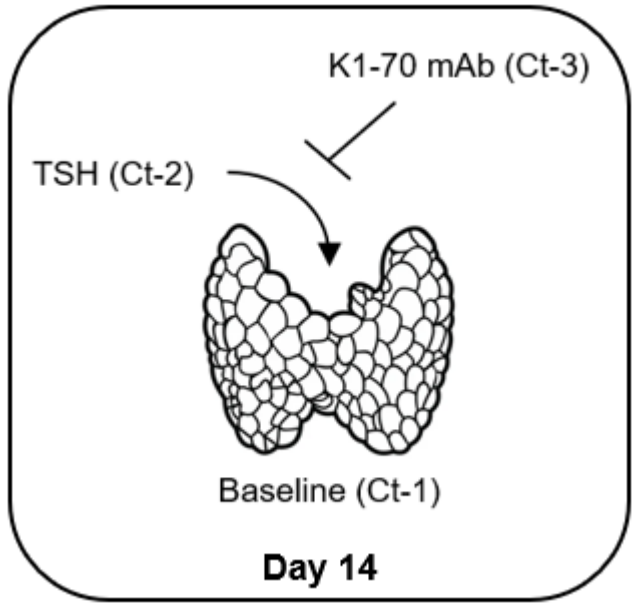
- **Thyroglobulin** and **Thyroxine (T4)** synthesis are most impacted by donor demographics.
- 30-62% of the total variance explained by age, sex, race, and BMI.



What Parameters Predict Thyroglobulin Synthesis?

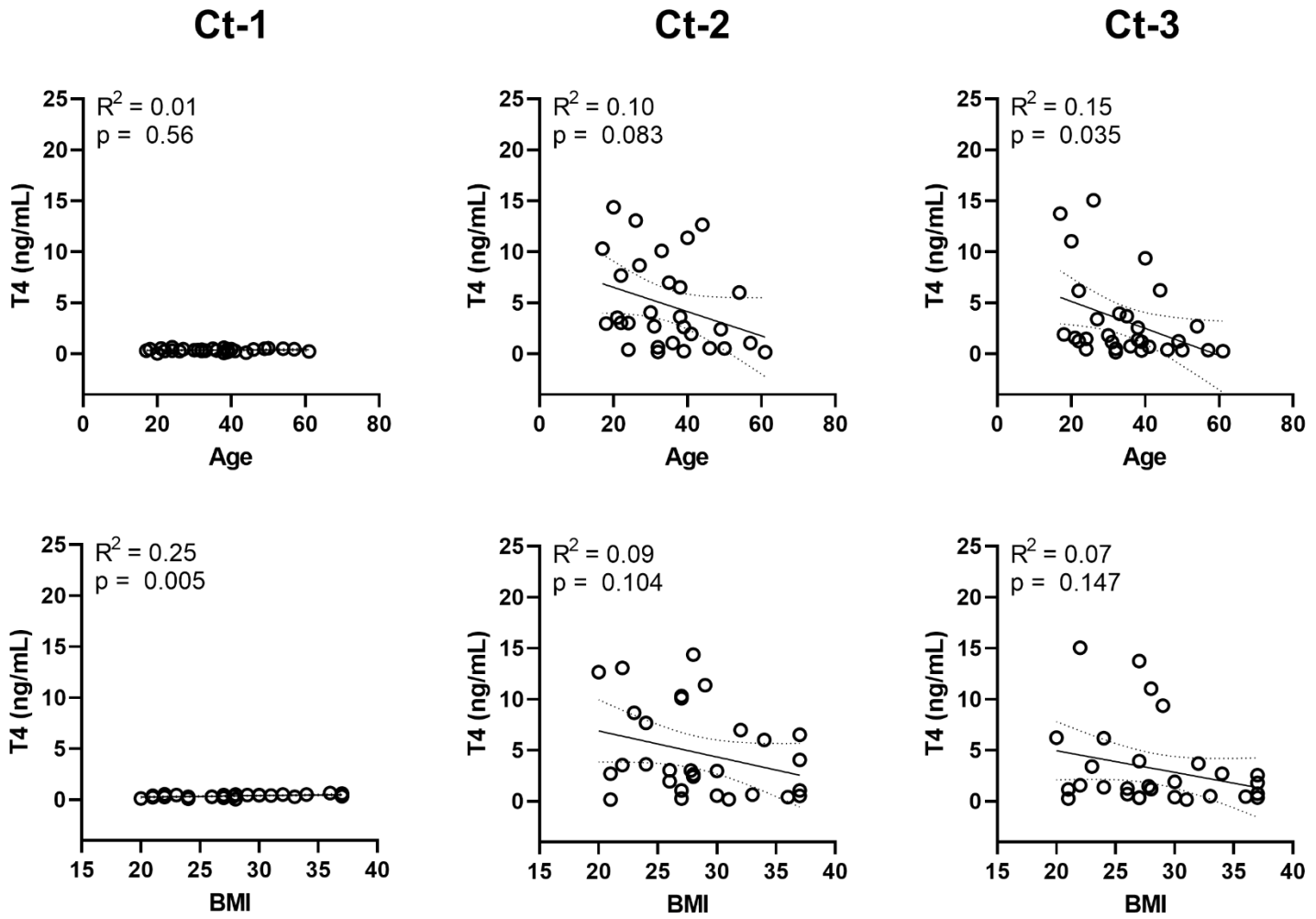


N=30

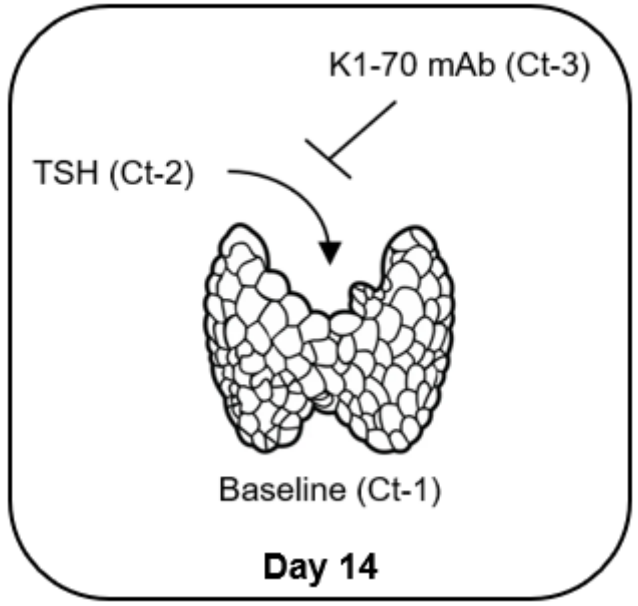


Generally, there is an inverse relationship between *in vitro* performance and age or BMI.

What Parameters Predict Thyroxine Synthesis?

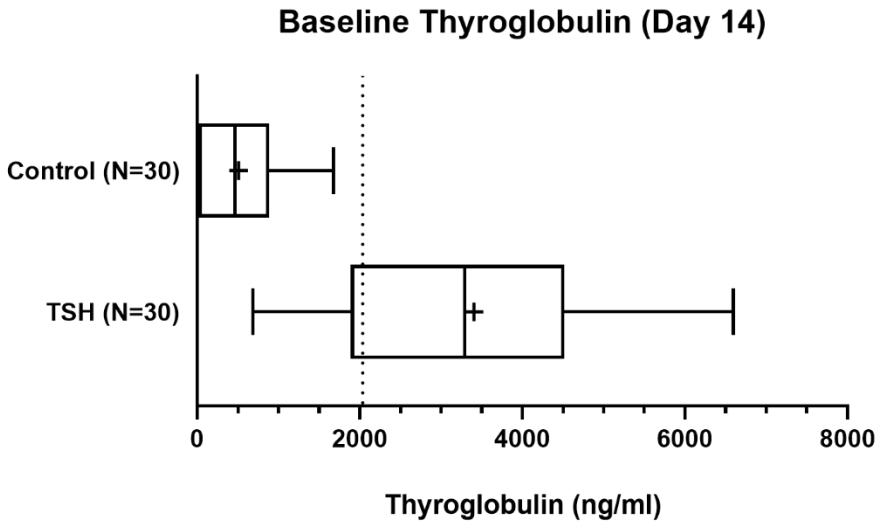


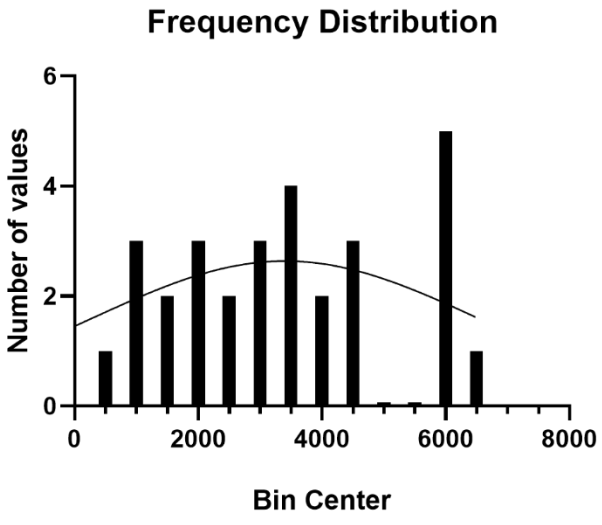
N=30

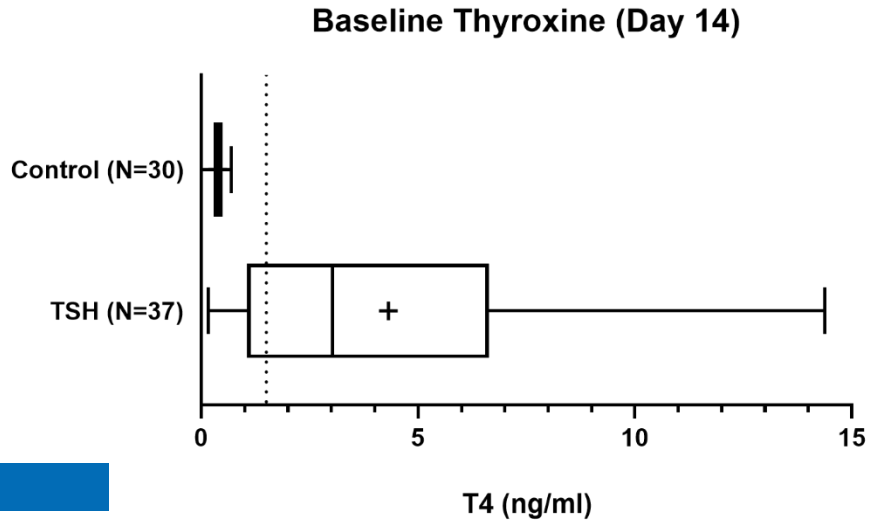


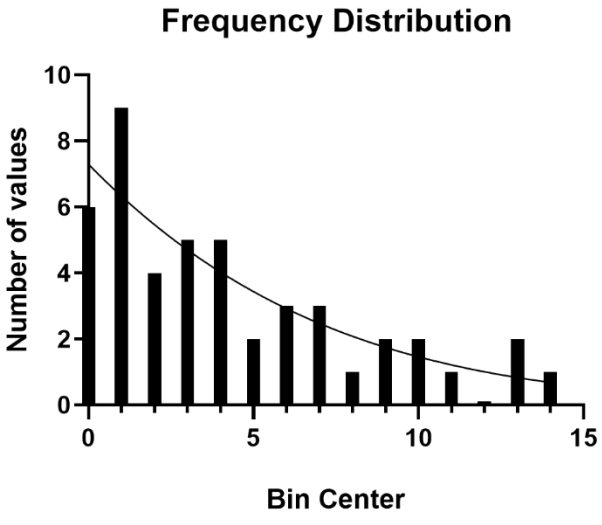
Generally, there is an inverse relationship between *in vitro* performance and age or BMI.

Donor Qualification – Setting Minimum Acceptance Criteria for Hormonogenic Competence





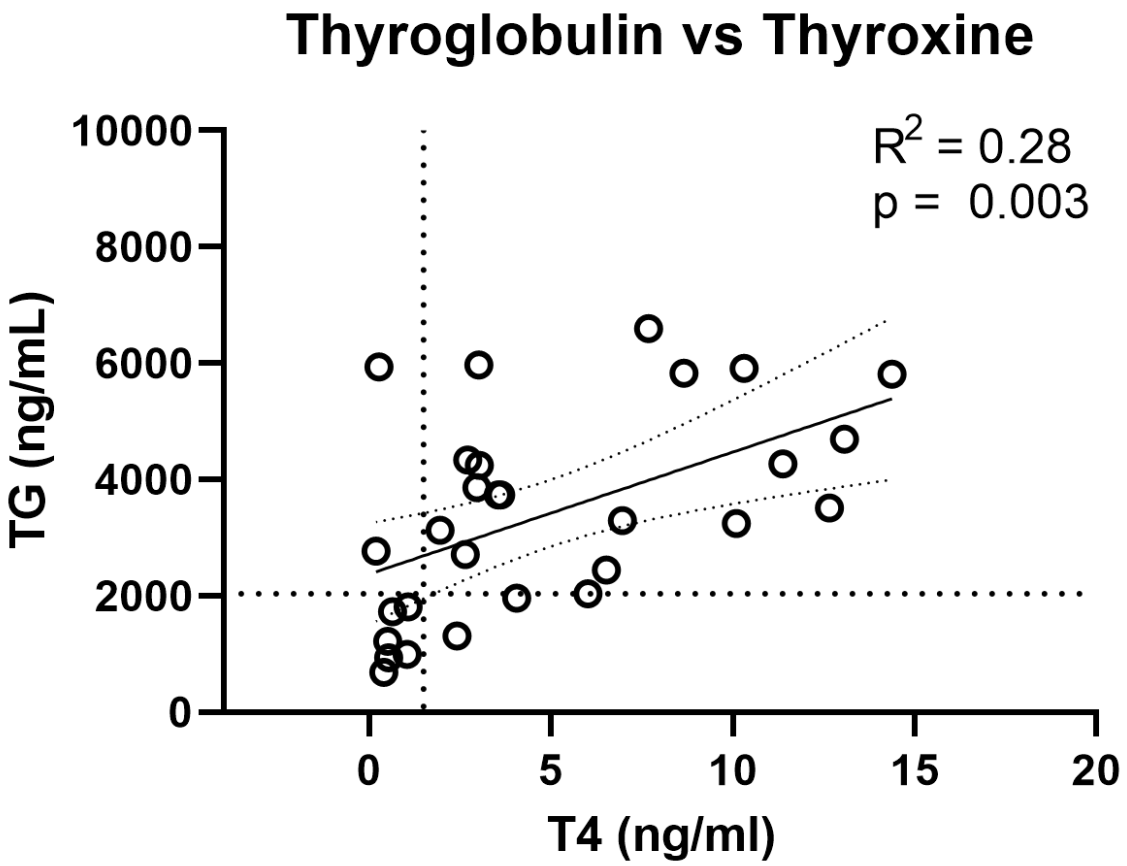




- Minimum Thyroglobulin and Thyroxine (T4) synthesis thresholds set for TSH sensitivity.

CI (95%)	Thyroglobulin (ng/ml)	Thyroxine (T4) (ng/ml)
Lower Confidence Limit	**2035**	**1.50**
Upper Confidence Limit	4270	5.32

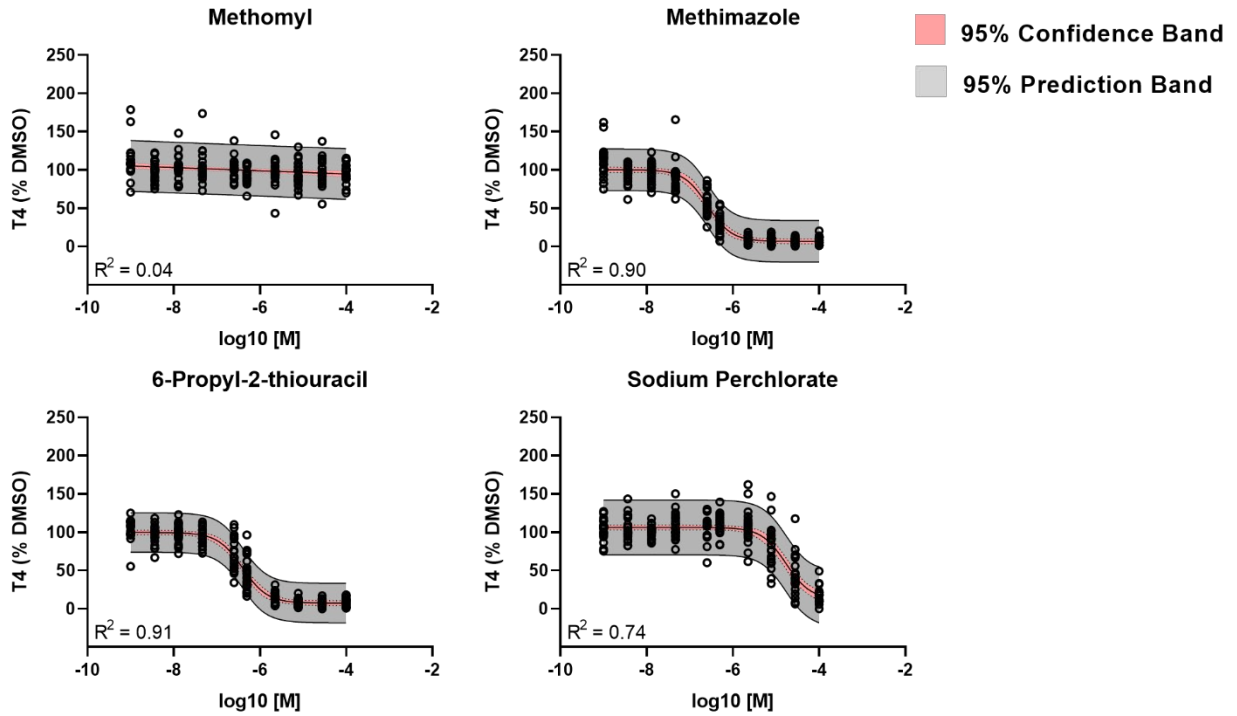
Donor Qualification - Curation of the Donor Cohort to Emphasize Hormonogenic Competence



- Thyroglobulin (TG) and Thyroxine (T4) synthesis are positively correlated.
- 21/30 donors qualify based on T4 synthesis threshold.
- Data suggests up to 30% of donors would not qualify for use in the assay.

Reproducibility is Supported in a Variable-Donor Assay Platform with Qualified Donors

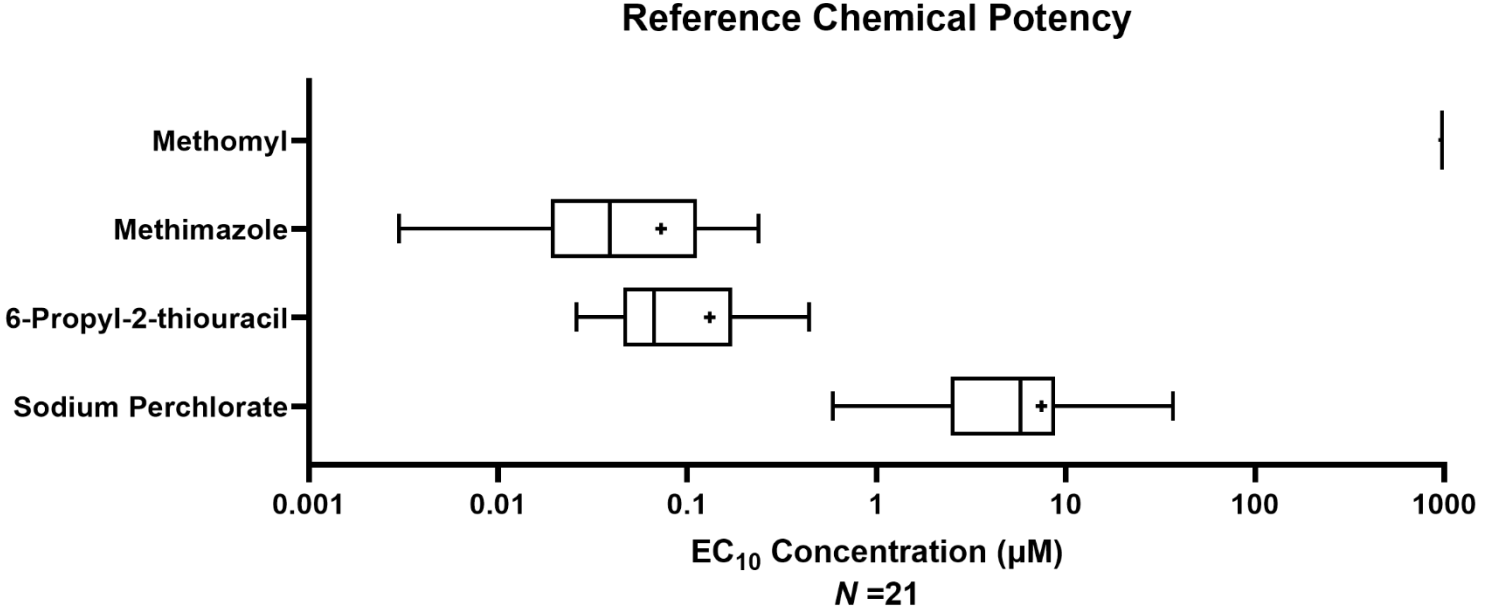
- Reference chemicals provide coverage for key mechanistic targets and exhibit expected responses.
- Population-level data modeling defines the EC10 as the POD.

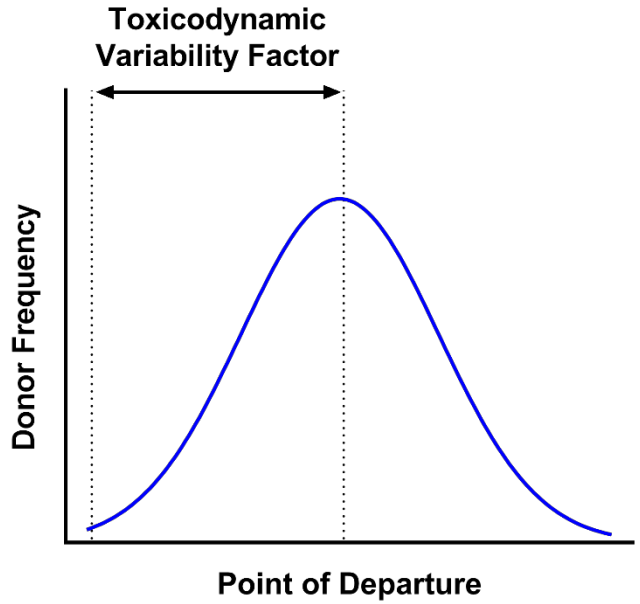


Independent Donor Performance Summary (*N* = 21)

	Methomyl		Methimazole		6-Propyl-2-thiouracil		Sodium Perchlorate	
	Best-fit values	CI (95%)	Best-fit values	CI (95%)	Best-fit values	CI (95%)	Best-fit values	CI (95%)
Log EC10 (M)	NA	NA	-7.2	-7.5 to -7.0	-7.0	-7.2 to -6.9	-5.4	-5.6 to -5.2
EC10 (µM)	NA	NA	0.06	0.04 to 0.11	0.09	0.06 to 0.13	4.0	2.6 to 5.9

Evaluating Inter-individual Human Toxicodynamic Variability to Benchmark Expectations in a Variable-Donor Assay Platform





EC_{10} (µM)	Methomyl	Methimazole	6-Propyl-2-thiouracil	Sodium Perchlorate
Range	NA	0.003 – 0.24	0.026 – 0.44	0.59 – 36.97
Median	1000	0.039	0.067	5.79
$TDVF_{01}$	**1**	**13**	**2.6**	**9.8**

IVIVE in Human Thyroid Microtissues

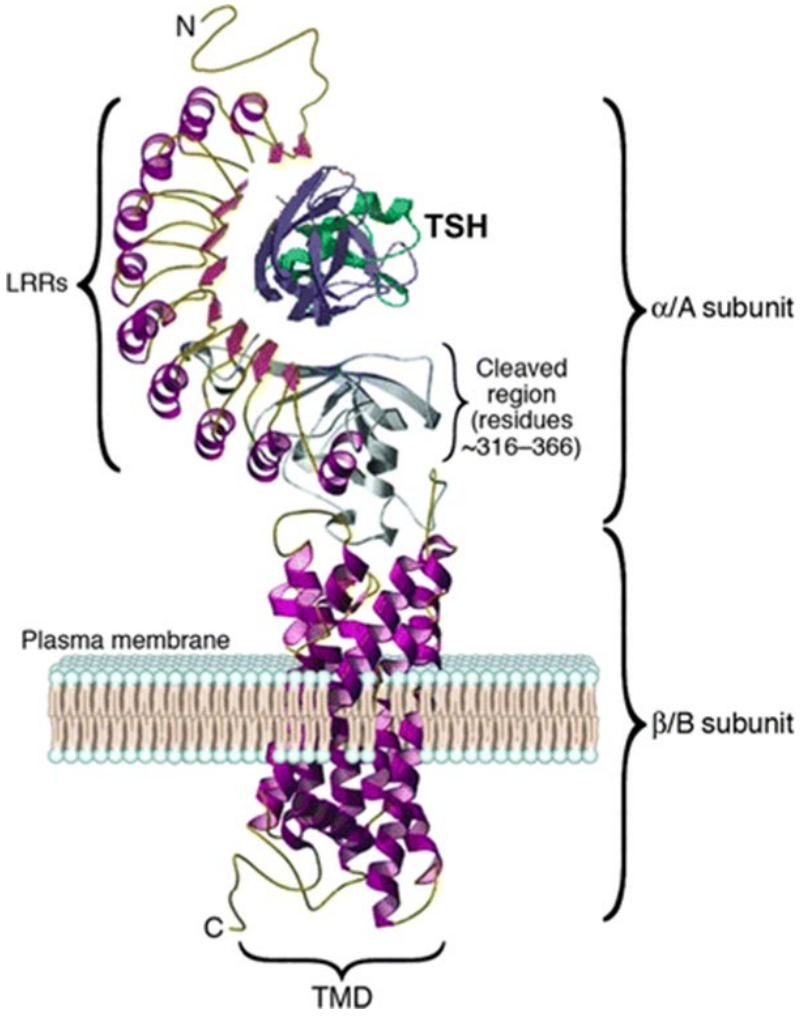
N=21

Equivalent administered dose (median Cmax) of multi-donor thyroid hormone disruption can be compared to exposure predictions to evaluate margins of exposure.

Outline

- Development of a human thyroid organotypic culture model to address data gaps in screening and prioritization of thyroid disrupting chemicals
- Establishing confidence with an inter-laboratory prevalidation study of the human thyroid microtissue assay
- Orthogonal screening of prioritized chemicals in human thyroid microtissues for functional and mechanistic relevance

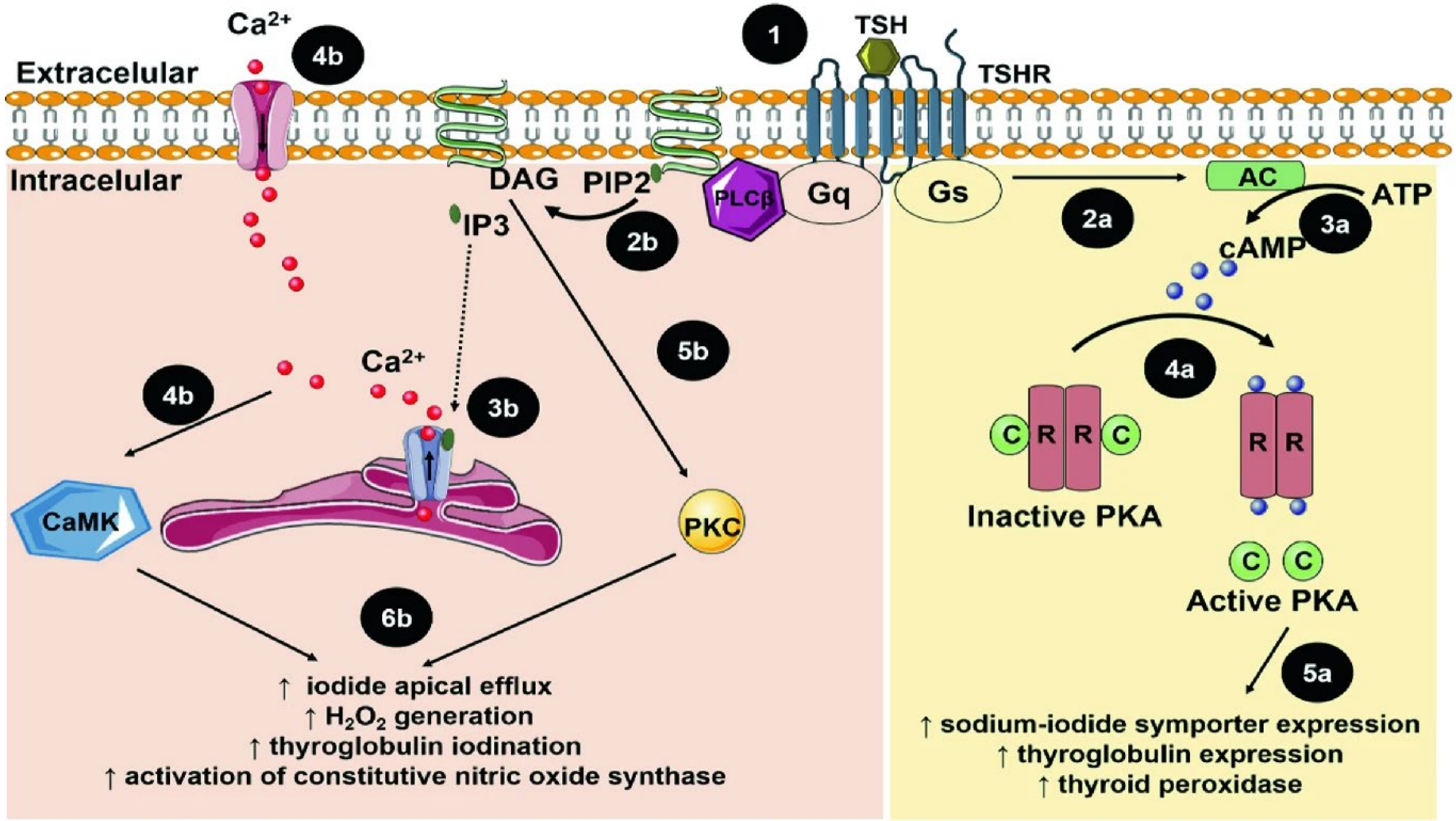
Is the Thyroid Stimulating Hormone Receptor (TSHR) a Target for Environmental Chemicals?



- TSHR is a G-protein-coupled receptor expressed primarily in thyrocytes.
- The primary ligand is Thyroid Stimulating Hormone (TSH).
- Biological and chemical modulators
  - TSH and TSHR autoimmune antibodies bind to the ectodomain (α subunit)
  - Small molecule ligands bind to the transmembrane domain (β subunit)
- Modulator classifications
  - Agonist – Activation from basal state
  - Antagonist – Inhibition of activated state
  - Inverse Agonist – Inhibition of basal state (constitutive activity)
- Toxicological outcomes
  - May contribute to hyperthyroidism (TSHR agonism) or hypothyroidism (TSHR antagonism) and associated adverse effects.

DOI: 10.1007/s12026-012-8312-8.

TSHR Signaling Pathways in Thyroid Follicular Epithelial Cells

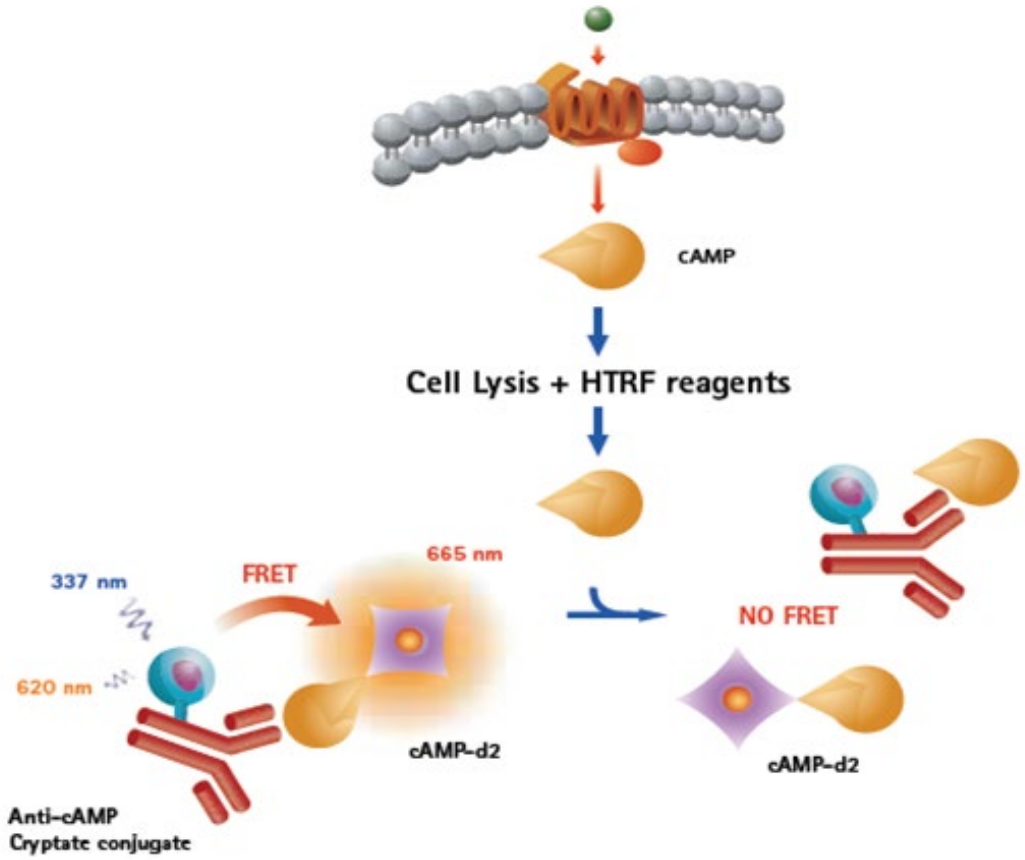


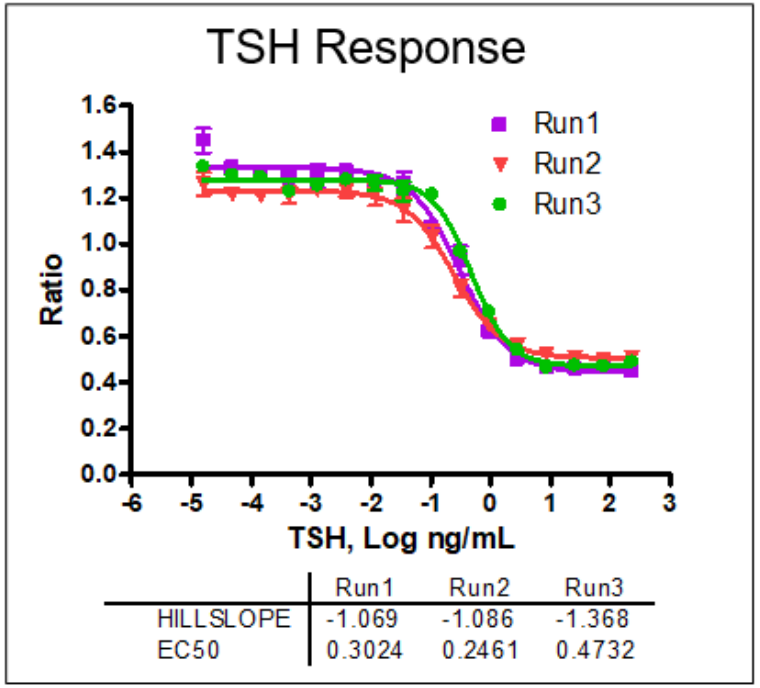
Non-Canonical

Canonical

DOI: 10.1007/978-3-030-44436-5_3.

Tox21 TSHR Assay – Screening the Tox21 Chemical Library





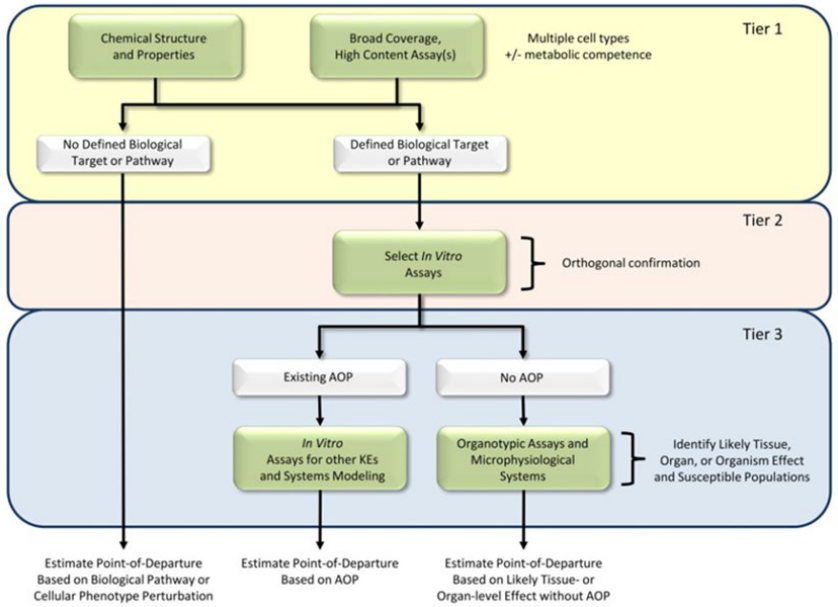
	Run1	Run2	Run3
HILLSLOPE	-1.069	-1.086	-1.368
EC50	0.3024	0.2461	0.4732

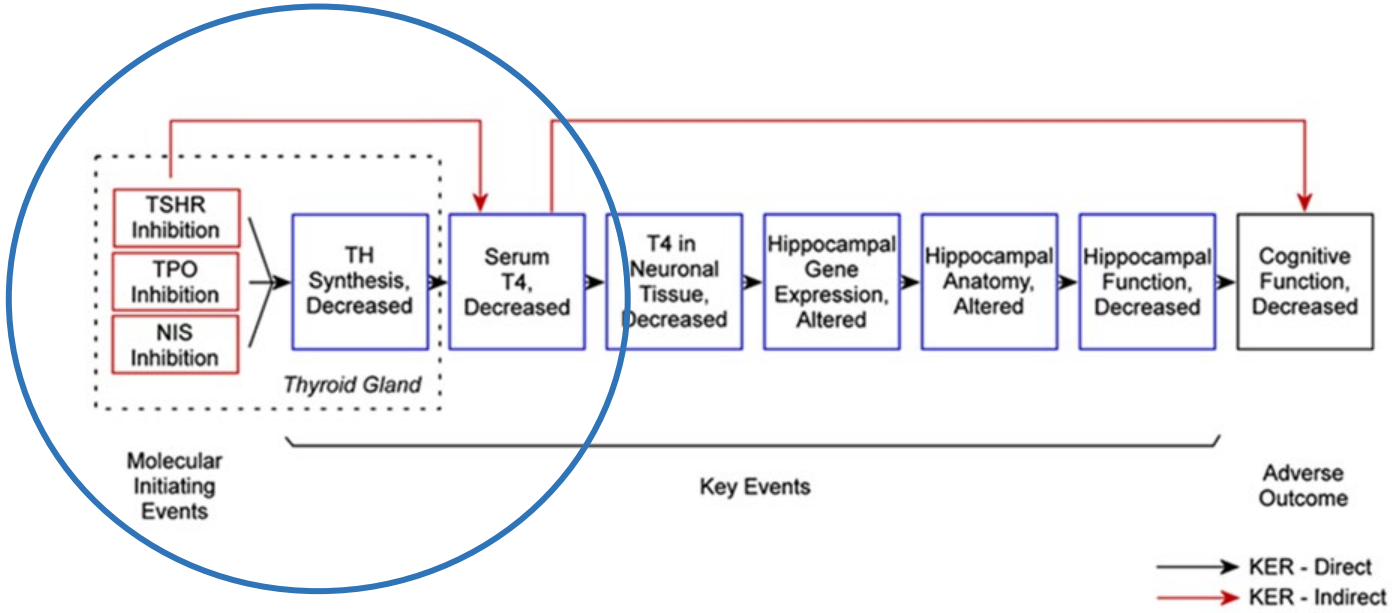
Bioactivity hit rate: 825 of 7871 chemicals (10%)

Assay	Cell Type	TSHR Expression	Test Chemical Exposure	Endpoint	Detection Technology
ACTOne-Gs TSHR GPCR HEK293	Human Embryonic Kidney Cell Line	Recombinant	30 min	cAMP	HTRF

https://www.cisbio.eu/

Tier 3 Screening of TSHR-Prioritized Chemicals in Human Thyrocyte Assays





TSHR Hit Prioritization Workflow	• Chemical selection based on bioactivity, structural diversity, and HTS specificity
Orthogonal Screening for MIE Effects	• Confirmation of TSHR bioactivity in normal human thyrocytes using a native protein biomarker
Secondary Screening for Key Event Effects	• Extension of MIE perturbation (TSHR) to key event (T4 synthesis)

Tox21 TSHR Assay – Active Chemicals Prioritization Workflow

Tox21_TSHR Assay

- TOX21_TSHR_Agonist_ratio - 489
- TOX21_TSHR_Antagonist_ratio - 336
- Dual Agonist/Antagonist - 49

Tox21 Screened Inventory: 7871 Chemicals

Quality Control

- DMSO solution availability
- Purity and degradation

Filters

- Remove mixtures, unavailable chemicals, flagged inventory, QC fails, dyes, and detergents
- Remove ToxPrints associated with fluorescence artifacts
- Remove promiscuous compounds

Selection Parameters

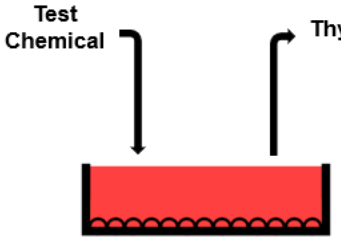
- High Bioactivity Signal
- Non-cytotoxic
- Structural Diversity

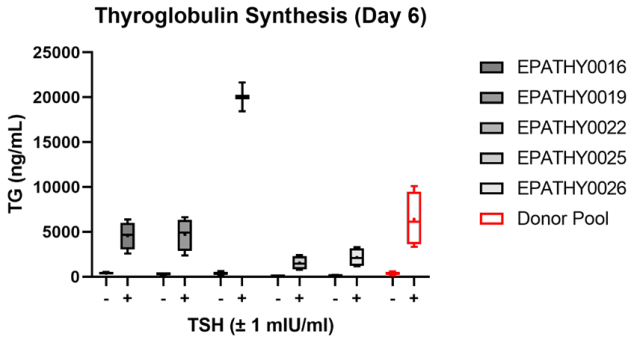
Prioritized Chemicals

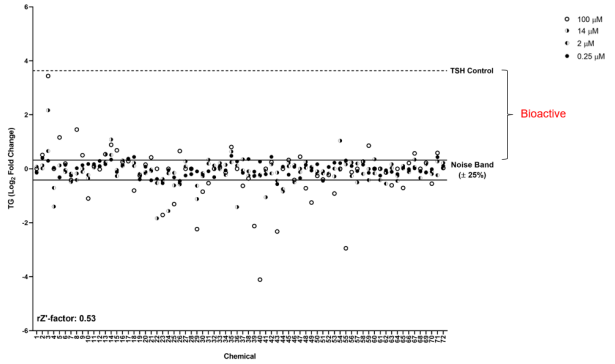
	Chemicals	Agonist Mode	Antagonist Mode
Agonists	54	54	-
Agonist-Antagonist	8	8	8
Antagonist	46	-	46
Inactive	10	10	10
Total	**118**	**72**	**64**

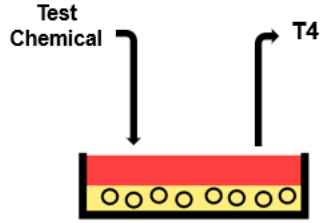
Prioritized chemicals: 108 of 825 (~13%)

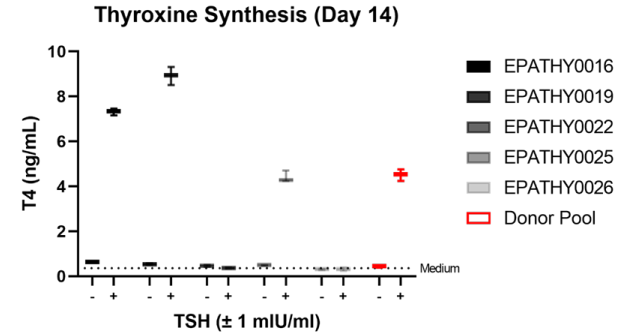
Thyroid Organotypic Culture Models – TSHR Hazard Screening Workflow

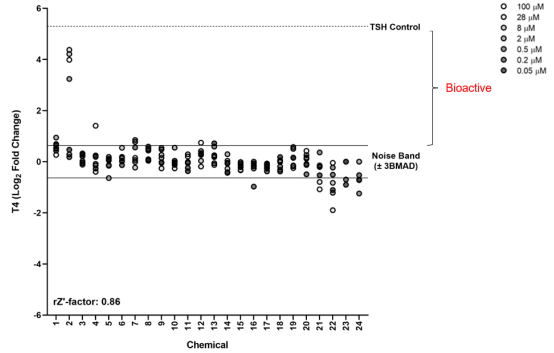




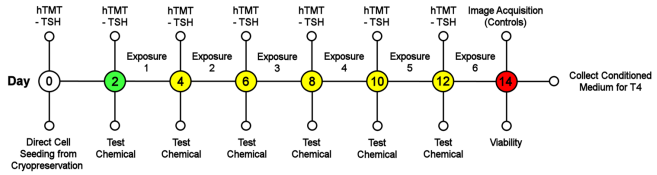


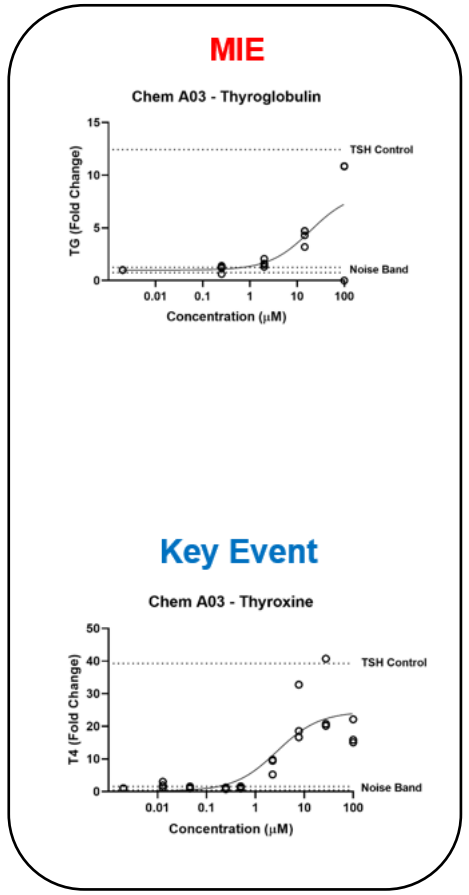






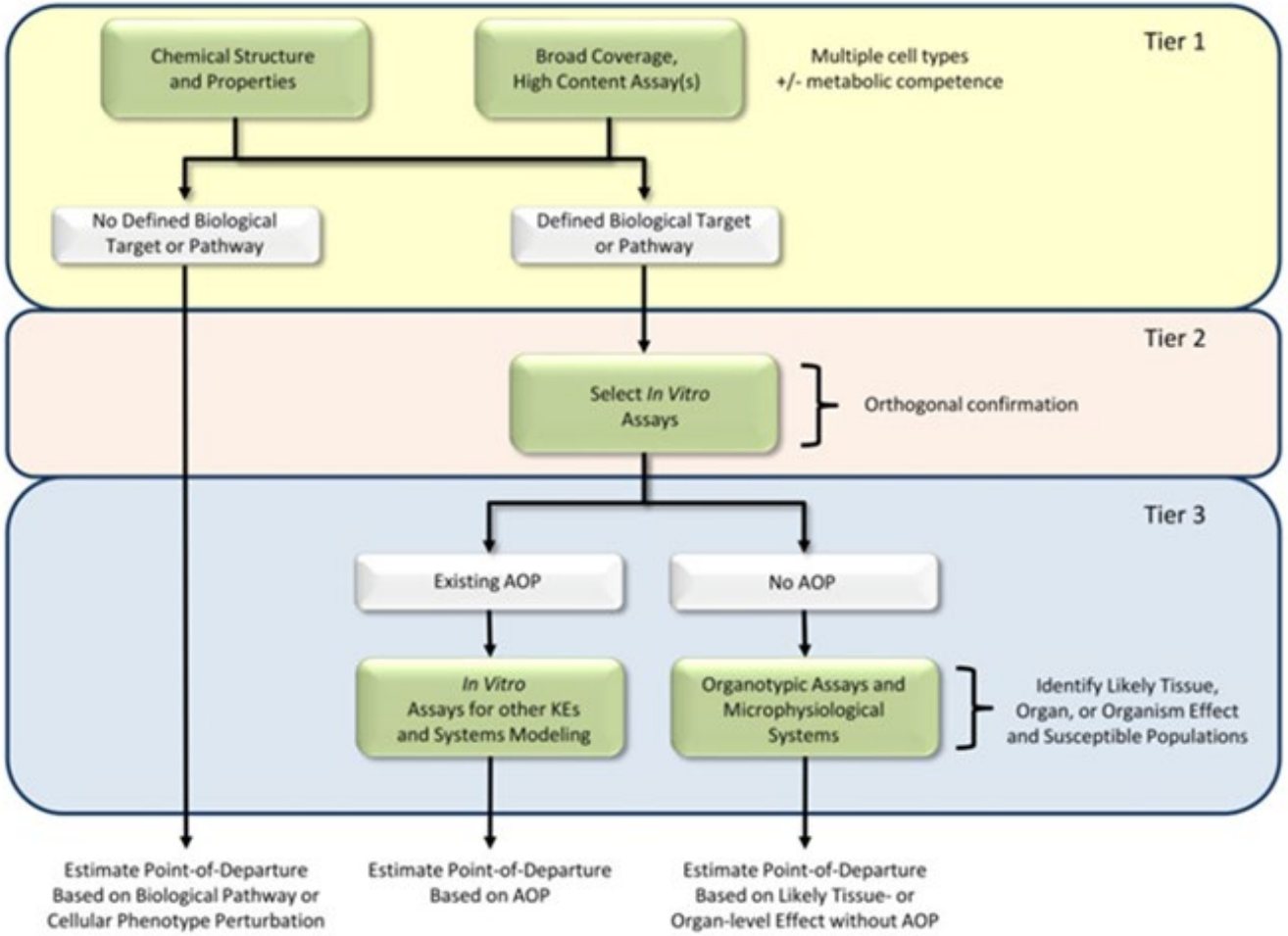
Donor ID	Sample	Age	Sex	Race	BMI	Euthyroid (Y/N)	Passage
EPATHY0016	Human Thyroid	23	M	Asian	36	Y	P0
EPATHY0019	Human Thyroid	20	M	Caucasian	28	Y	P0
EPATHY0022	Human Thyroid	34	F	African American	29	Y	P0
EPATHY0025	Human Thyroid	44	F	Caucasian	20	Y	P0
EPATHY0026	Human Thyroid	24	M	Hispanic	26	Y	P0





- Decreasing uncertainty in prioritized HTS data using longer-term 2D and 3D human thyroid assays.
- Consideration of general population effects using validated sample pools.
- Collecting quantitative data in a thyroid AOP context to evaluate MIE and Key Event relationships.

A Tiered Testing Paradigm to Identify Potential TSHR-dependent Human Thyroid Disruptors



Tox21 Screening Library	• 7871 Chemicals
Tier 2: TSHR Screening Assay Bioactivity	• 825 Chemicals
TSHR Hit Prioritization Workflow (Agonist)	• 72 Chemicals
Tier 3: Orthogonal Screening for MIE Effects	• 24 Chemicals
Tier 3: Secondary Screening for Key Event Effects	• 6 Chemicals

6 chemicals identified as potential human thyroid agonists

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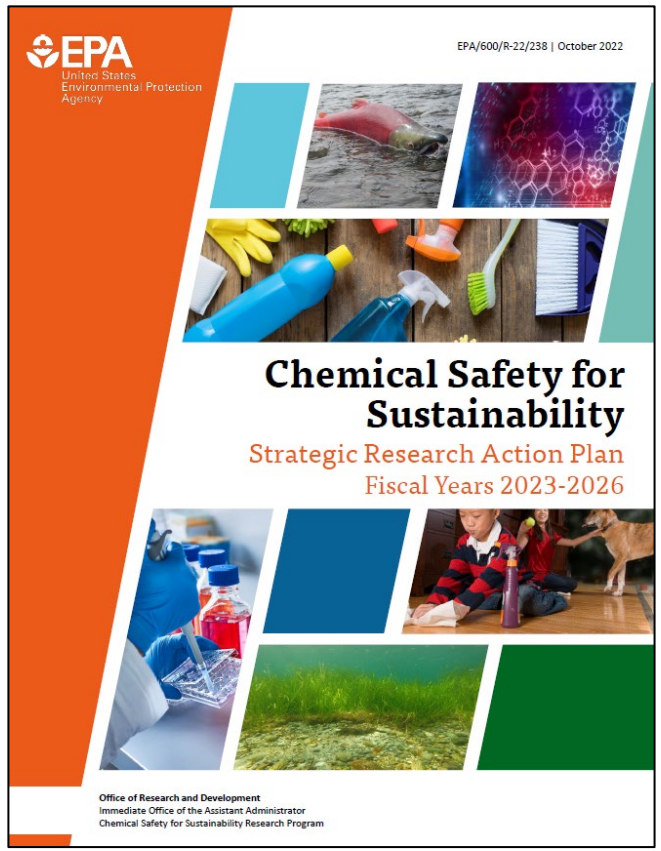
Parting Thoughts - Virtual and Complex Tissue Models Play a Pivotal Role in Chemical Hazard Identification and Characterization.



- **Complex Biology and Lifestage Sensitivity:** Assay batteries to evaluate neurodevelopment, human pregnancy, and early embryonic development.
- **Target Organ Toxicity:** Cellular composition and architectures that better represent *in vivo* systems biology with phenotypes not captured in many conventional high-throughput assays.
- **Tiered Hazard Screening:** Medium-throughput organotypic culture models fit into a tiered hazard screening paradigm to provide critical context to mechanistic high-throughput screening assays.
- **Human Relevance and Toxicological Variability:** Primary cell types derived from human donors enable evaluation of human inter-individual toxicodynamics that cannot be addressed with inbred animal models or isogenic cell lines.
- **More Flexible Exposure Paradigms**: Long-term culture models enable repeat exposure paradigms simulating acute and sub-acute exposure scenarios.
- **Computational Modeling:** Quantitative data generation useful for establishing virtual tissue model simulations, and MIE and KE data for qAOPs.

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