Advancing translational applications of human organotypic thyroid assays

Chad Deisenroth, Ph.D.
Center for Computational Toxicology and Exposure
U.S. EPA
deisenroth.chad@epa.gov

International Akademie Fresenius Conference - Assessment of Thyroid Disrupting Chemicals
February 2, 2023

Disclaimer: The views expressed are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

EPA United States Environmental Protection Agency

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

Endocrine Disruptor Screening Program

Endocrine Pathway	Tier 1 Screening											Tier 2 Testing			
	In vitro					In vivo						In vivo			
	ER Binding	AR Binding	ER Transcriptional Activation*	Aromatase Inhibition	Steroidogenesis*	Uterotrophic*	Hershberger*	Pubertal Male	Pubertal Female	Amphibian Metamorphosis*	Fish Short Term Reproduction*	Rat 2-gen/ EOGRT*	MEOGRT*	LAGDA*	JQTT
E+	■		■		■	■			■		■	■	■	■	■
E-	■			■	■				■		■	■	■	■	■
A+		■			■		■	■			■	■	■	■	■
A-		■			■		■	■			■	■	■	■	■
HPT Axis								■	■	■		■		■	■

The current EDSP assay battery evaluates effects of chemical exposures on estrogen, androgen, and thyroid endocrine pathways

- No *in vitro* tests for thyroid endpoints
- No human representation for thyroid
- Too reliant on animal tests

***In vivo* endpoints for thyroid-related endocrine testing in guideline studies**

- Serum T3, T4 and TSH
- Thyroid and Pituitary weights
- Thyroid Histopathology

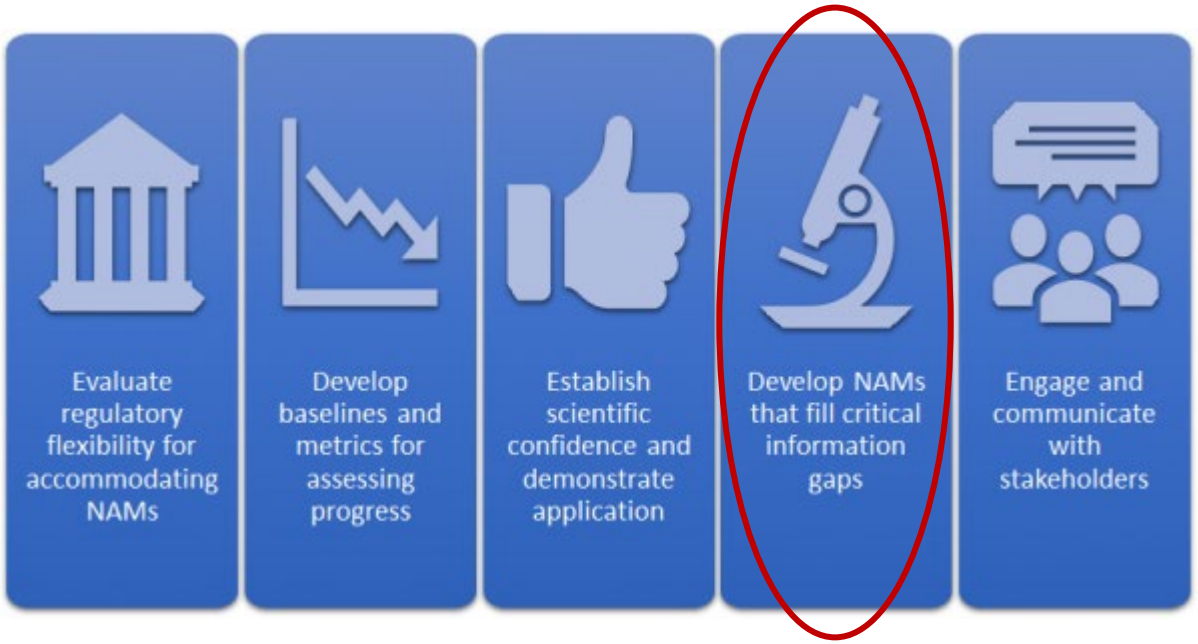
Screening Assay	Thyroid weight	Pituitary weight	Thyroid Histopathology	Serum TH levels
OECD TG 407	+	+	+	+ (optional)
OECD TG 408	-	-	+	-
OECD TG 416	+	+	-	-
OECD TG 422	-	-	+	-
OECD TG 441	-	-	-	+ (T3 and T4, optional)
OECD TG 443	+	+	+ (optional)	+ (T4 and TSH)
OECD TG 451			+	
OECD TG 452	+		+	
OECD TG 453	+		+	
EPA 15-day intact adult male rat assay	+	-	+	+
EPA Pubertal male	+	+	+	+ (T4 and TSH)
EPA Pubertal female	+	+	+	+ (T4 and TSH)

Jomaa, B. (2015).

DOI: 10.1289/EHP1304

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.



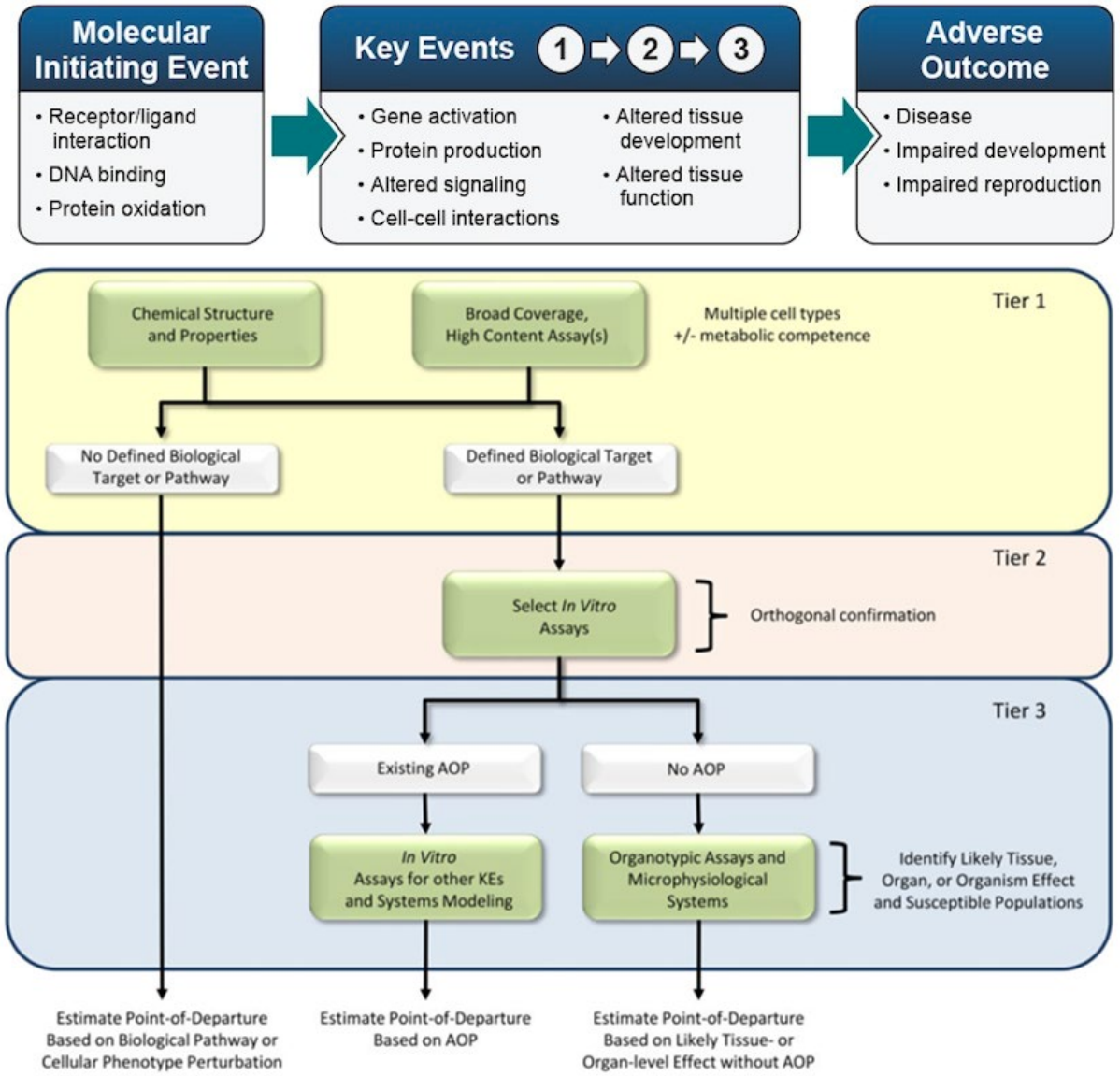
Five work plan objectives

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

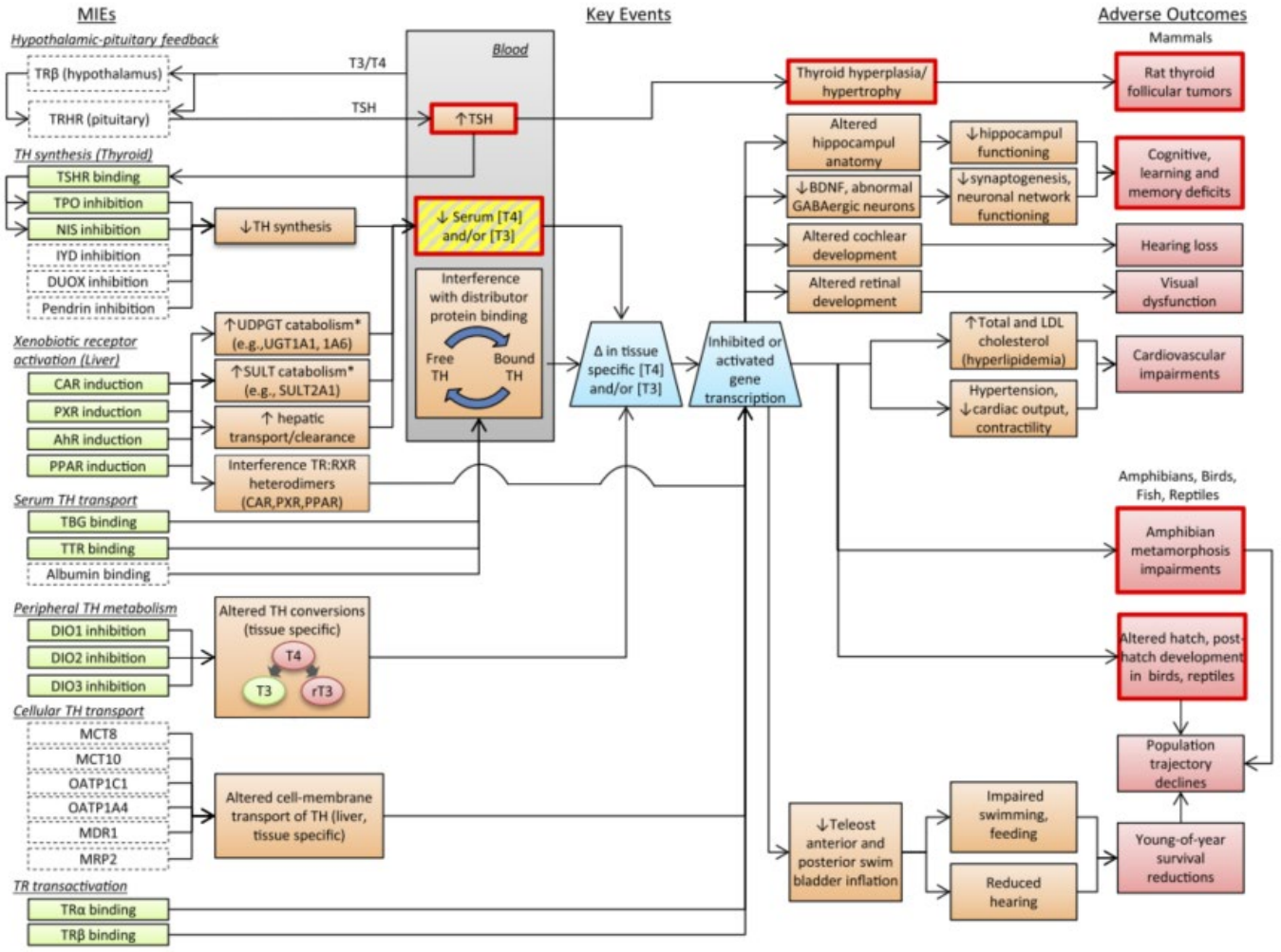


https://ntp.niehs.nih.gov/go/niceatm-aop; DOI: 10.1093/toxsci/kfz058

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.
- **Microphysiological Systems**: Microfluidic device containing OCMs in a controlled microenvironment.

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*.

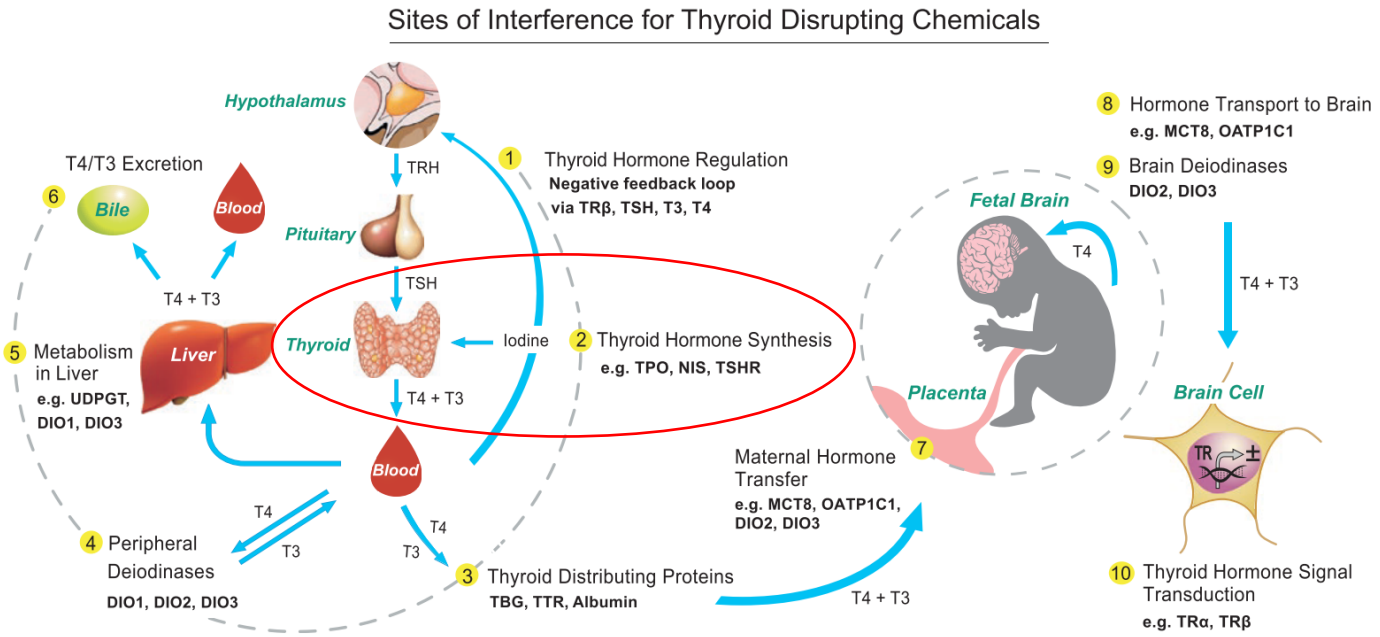
2014 OECD. New Scoping Document on in vitro and ex vivo Assays for the Identification of Modulators of Thyroid Hormone Signalling. *OECD Series on Testing and Assessment, No. 207*

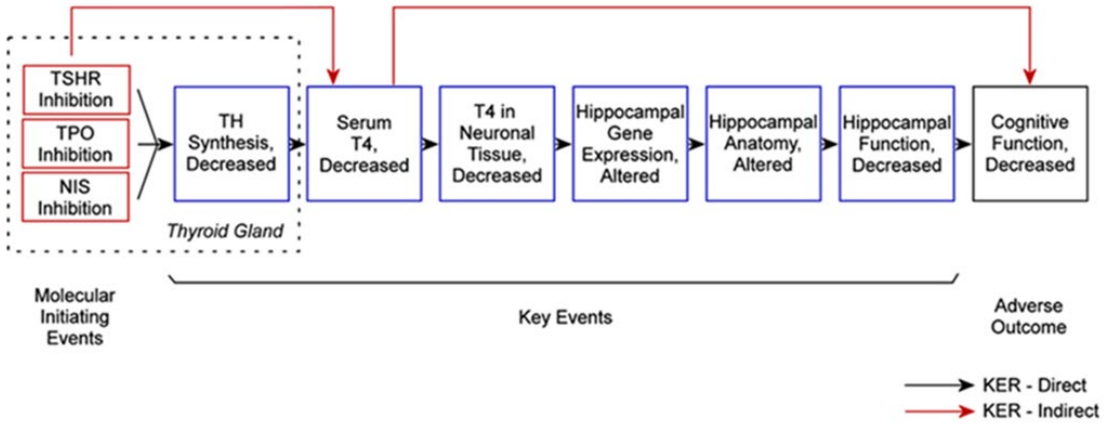
2019 Noyes, P.D. *et al*. Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. *Environ Health Perspect.*

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

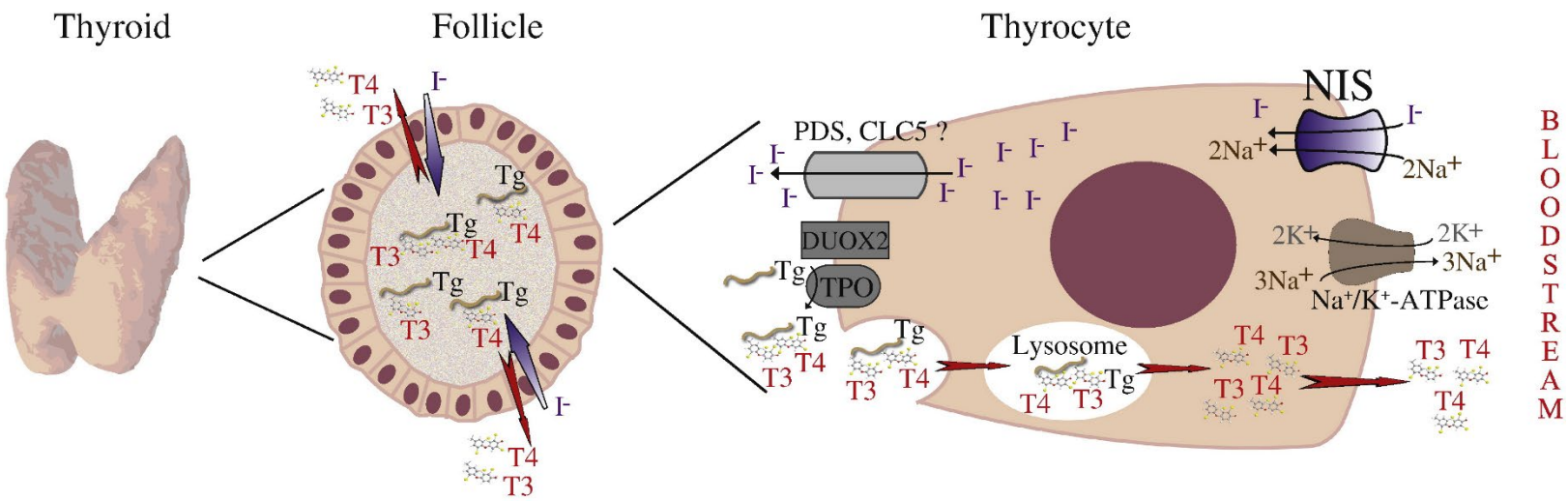




Thyroid MIE	Assay	Environmental Chemicals Screened	Active Chemicals	% Active	Reference
TSHR	Engineered Cell Line	7871	825	10	TCPL: TOX21_TSHR_Agonist, TOX21_TSHR_Antagonist
TPO	Microsomal Enzyme	1074	150	14	K. Paul Friedman et al, ToxSci, 151(1), 2016, 160-180
NIS	Engineered Cell Line	293	137	47	J. Wang 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

Challenges with *In Vitro* Thyroid Testing: Cell Type and Architecture are Critical Determinants for Hormone Synthesis



Cell Type

- No primary or thyroid cell lines, of any species, demonstrate appreciable capacity for thyroid hormone synthesis in 2D models
- Primary thyrocytes lose essential functions when cultured in conventional monolayer systems

Cell Architecture

- Follicular morphology is a critical feature for retaining hormone synthesis dynamics

DOI: 10.1016/j.bbamem.2013.08.013

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

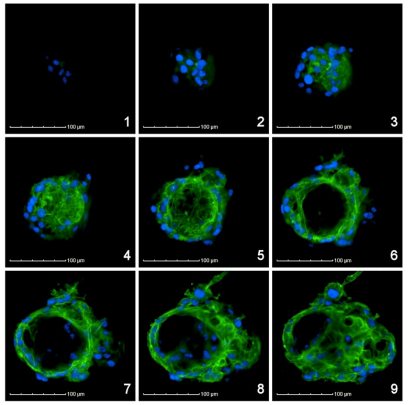
OXFORD SOT Society of Toxicology academic.oup.com/toxsci

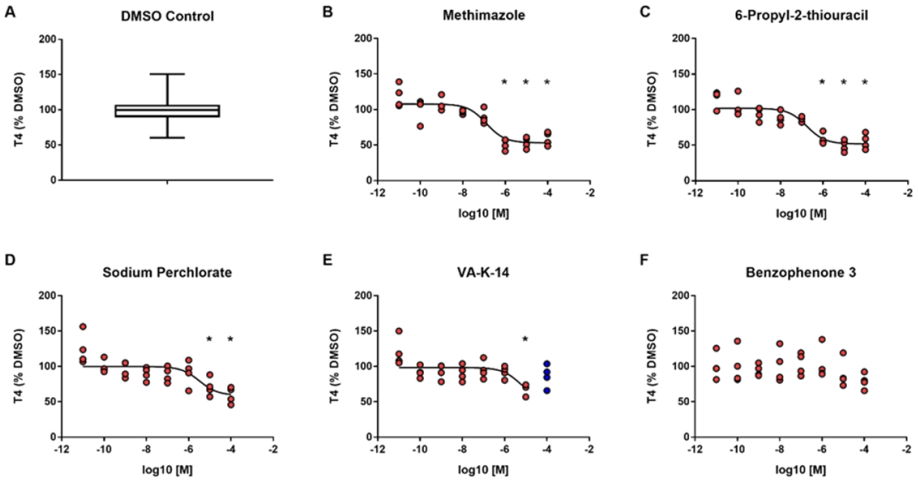
TOXICOLOGICAL SCIENCES, 174(1), 2020, 63–78

doi: 10.1093/toxsci/kfz238
Advance Access Publication Date: December 6, 2019
Research Article

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 *





Challenge

- Thyroid high-throughput screening (HTS) assays do not directly measure thyroid hormone disruption.
- Many HTS prioritized chemicals need orthogonal confirmation for biological and mechanistic relevance.
- Regulatory decisions for chemical safety currently use *in vivo* apical endpoints like serum thyroid hormone levels as indicators of thyroid disruption.

Impact

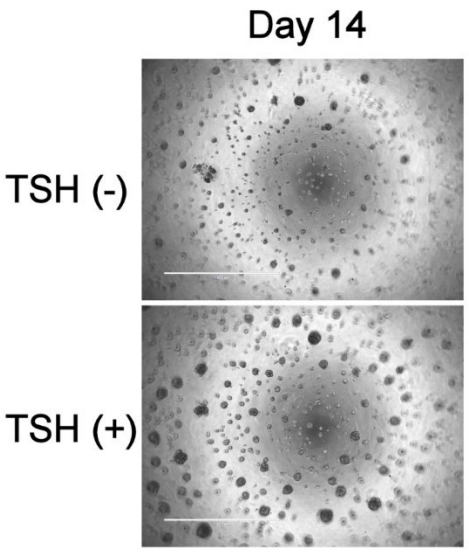
- May enable chemical regulatory bodies to apply human *in vitro* data for identifying thyroid as a mode-of-action for endocrine disruption.
- Chemical manufacturers could benefit from insight into thyroid toxicity early in the development process.

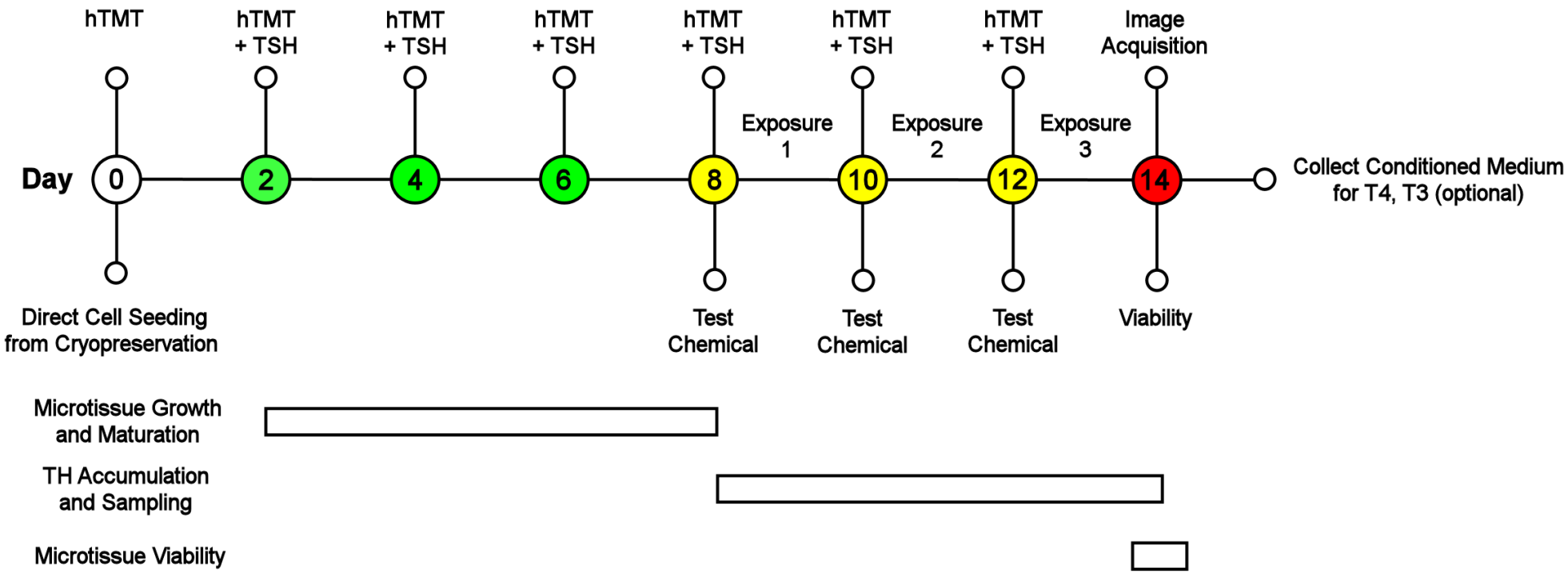
EPA United States Environmental Protection Agency

Outline

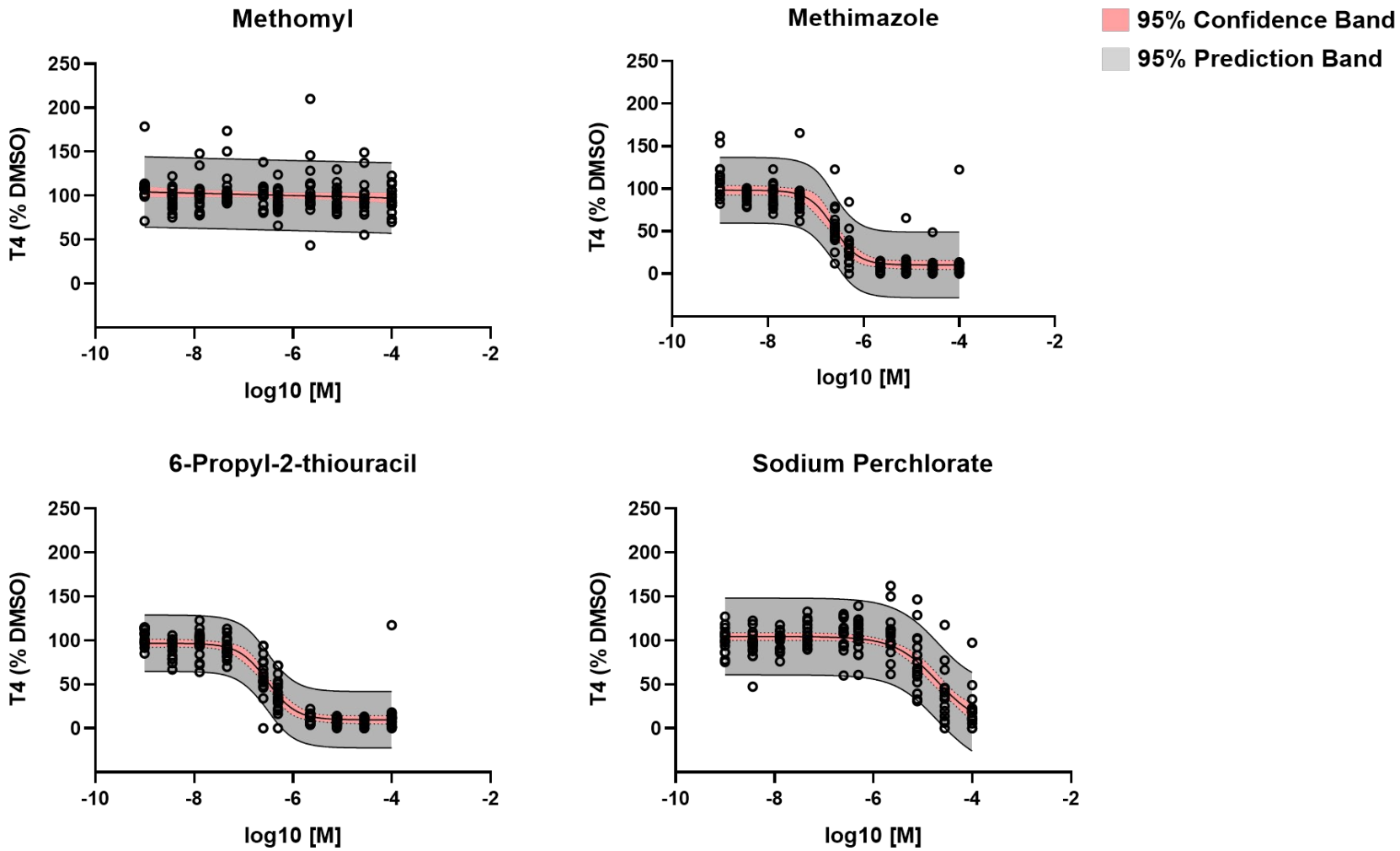
- Development of a human thyroid organotypic culture model to address data gaps in screening and prioritization of thyroid disrupting chemicals
- Establishing confidence with an inter-laboratory prevalidation study of the human thyroid microtissue assay
- Orthogonal screening of prioritized chemicals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

Human Thyroid Microtissue Assay v2.0



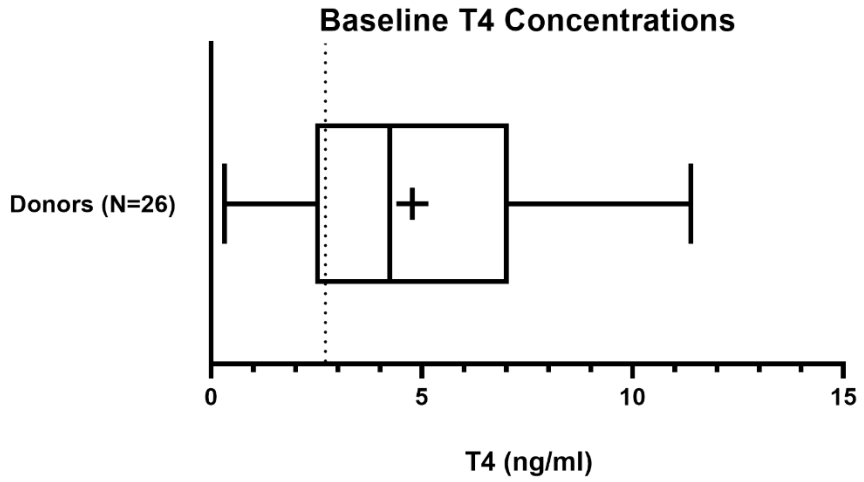


Historical Data Supports Reproducibility in a Variable-Donor Assay Platform



Independent Donor Performance Summary (*N* = 16)

Historical Data Supports Reproducibility in a Variable-Donor Assay Platform



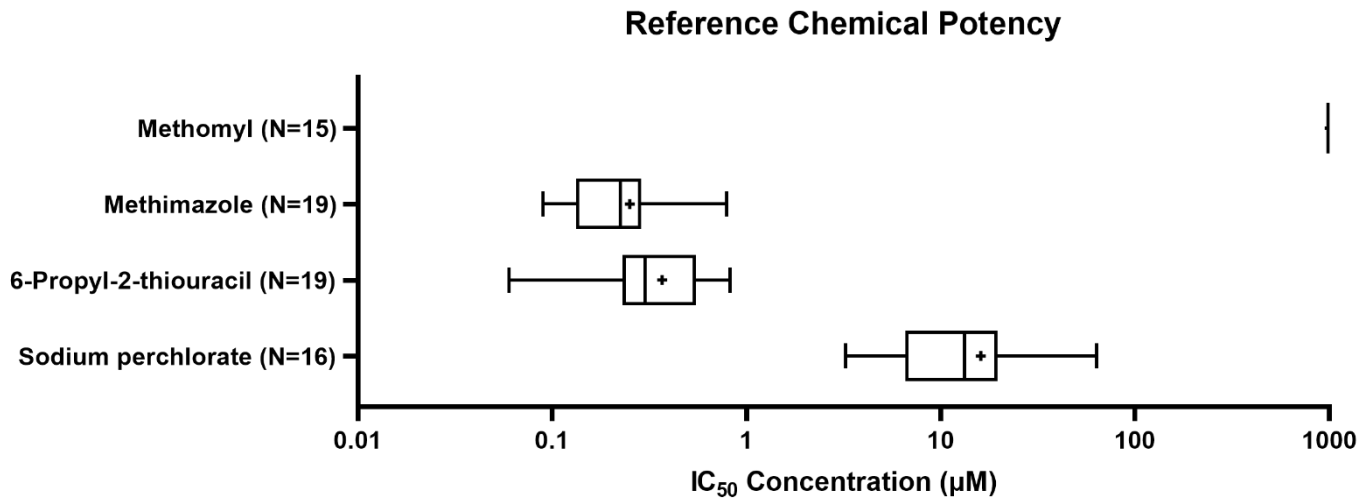
95% CI of median	
Actual confidence level	97.10%
Lower confidence limit	2.710
Upper confidence limit	6.520

Establishing Donor Acceptance Criteria

- Independent donors evaluated
- Minimum T4 synthesis threshold

Selecting Reference Chemicals

- Time Span: 2018 – 2022
- T4 synthesis inhibition



Test Method Validation Principles

- Validation provides confidence to regulatory stakeholders that a test method is **reliable**, **relevant**, and can be used for decision-making in a defined regulatory application.
    - **Reliability (Reproducibility)**: “Measures of the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability.”
    - **Relevance**: “Description of relationship of the test to the effect of interest and whether it is meaningful and useful for a particular purpose. It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (concordance) of a test method.”

OECD GD 34 (ENV/JM/MONO(2005)14)

Inter-laboratory Prevalidation of the Human Thyroid Microtissue Assay

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

NICEATM

Objectives

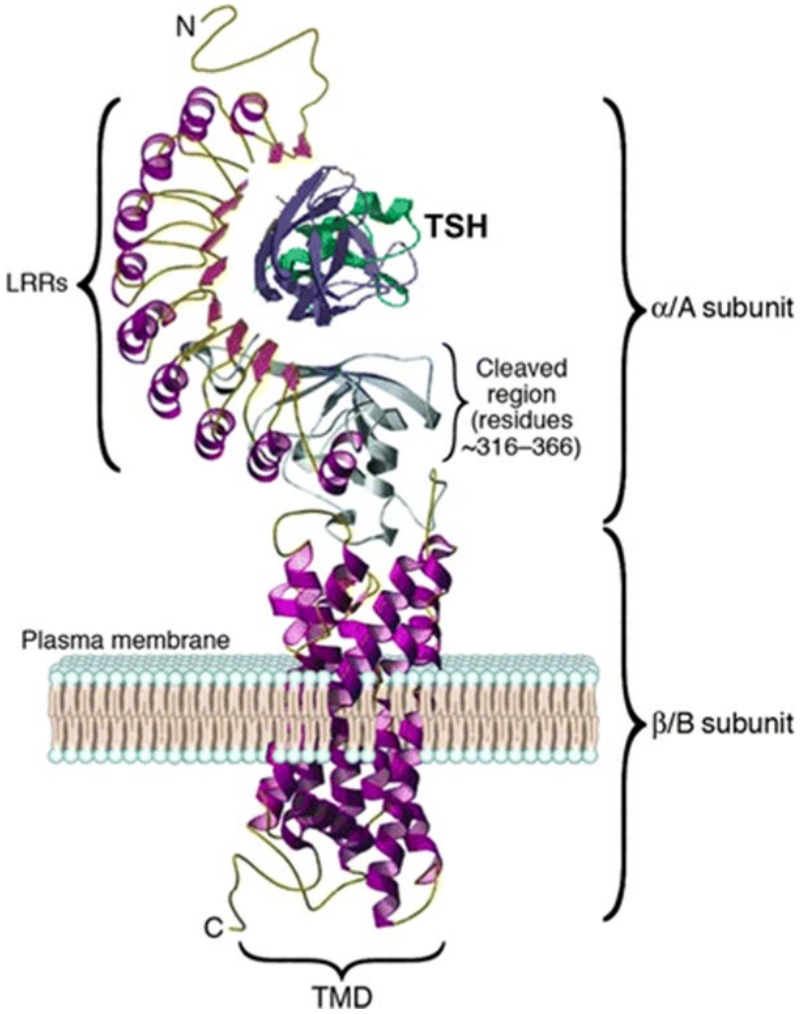
1. Collaborative effort on the study design, analytical approaches, chemical selection, and data interpretation.
2. Test method standardization.
3. Test method transfer, training and intra-laboratory model performance evaluation.
4. Limited inter-laboratory reference chemical testing and assay performance evaluation.

EPA United States Environmental Protection Agency

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

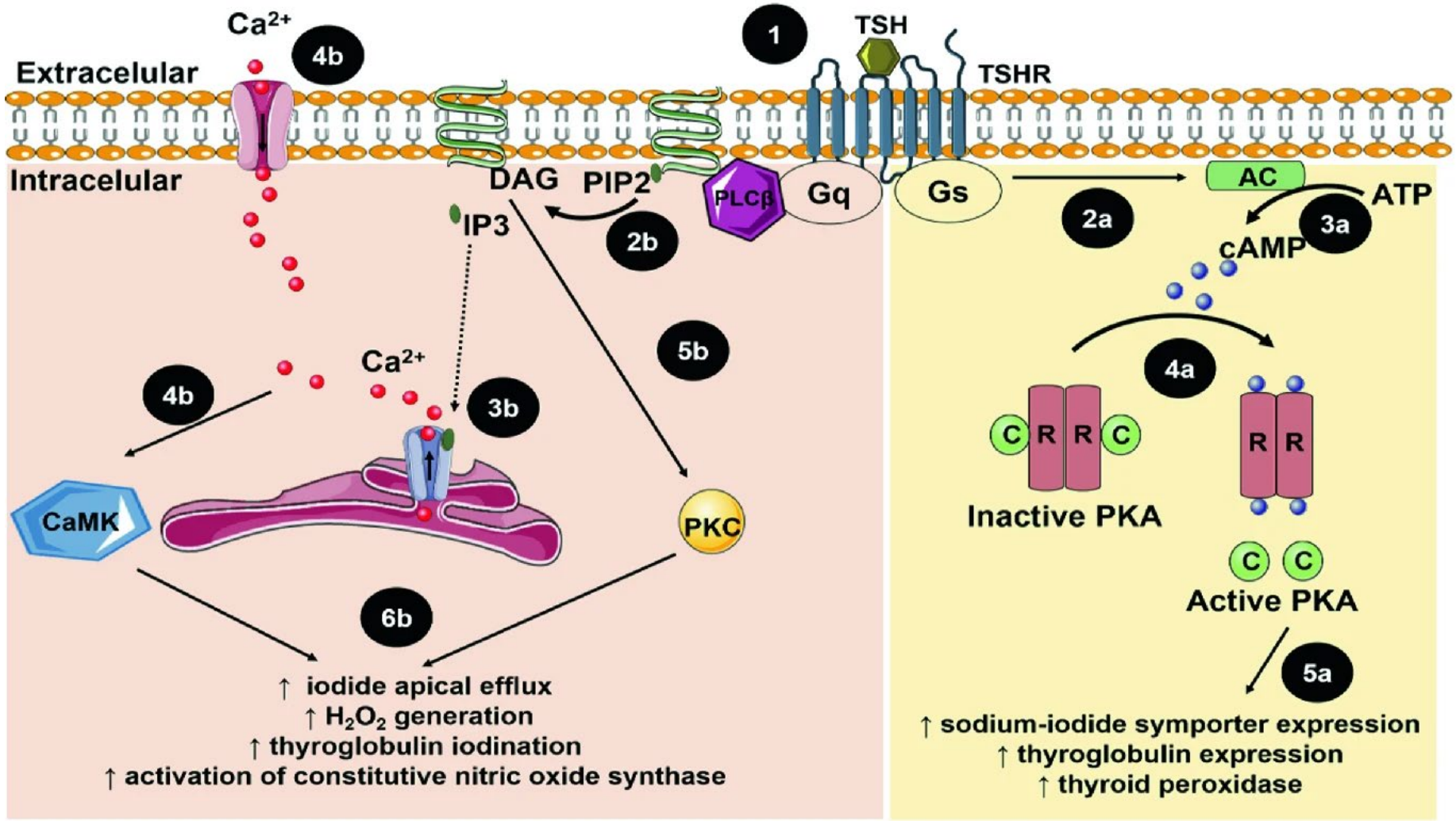
Is the Thyroid Stimulating Hormone Receptor (TSHR) a Target for Environmental Chemicals?



- TSHR is a G-protein-coupled receptor expressed primarily in thyrocytes.
- The primary ligand is Thyroid Stimulating Hormone (TSH), a pituitary hormone that regulates thyrocyte growth and hormone synthesis.
- 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

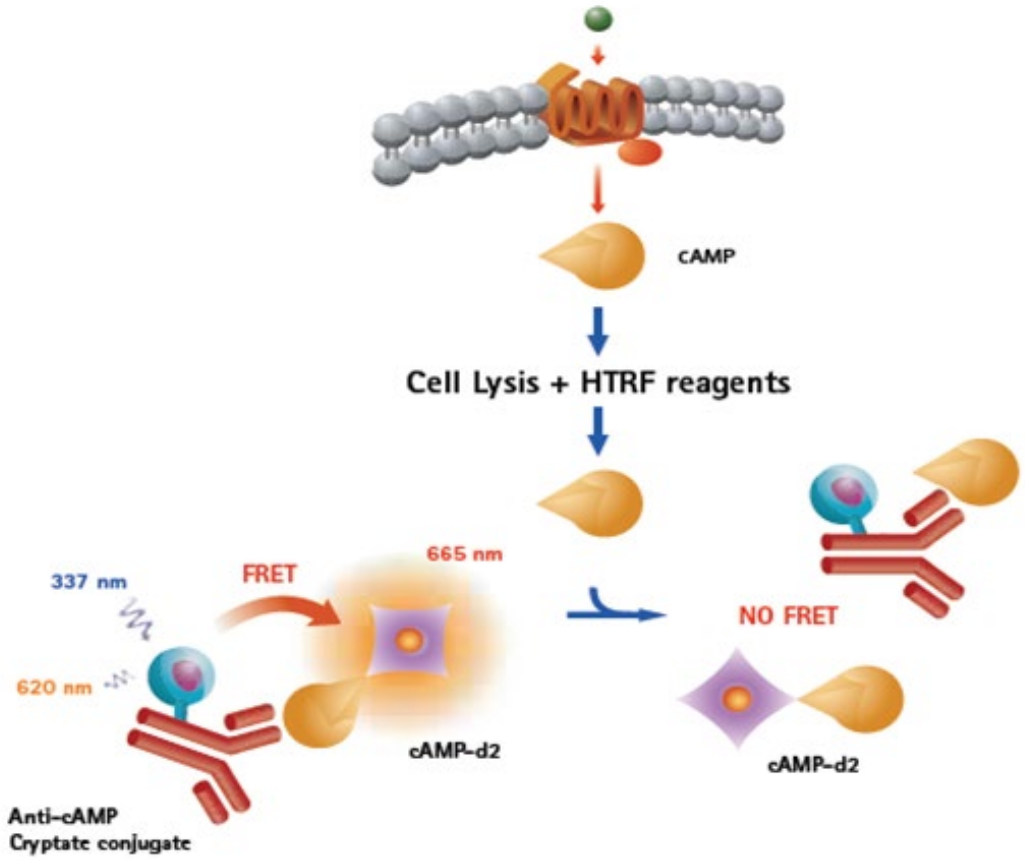


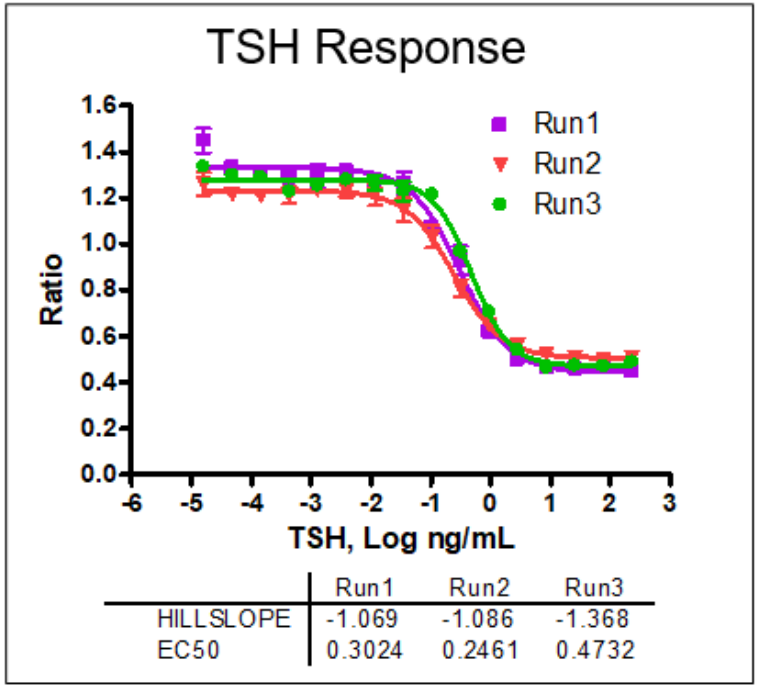
Non-Canonical

Canonical

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

Tox21 TSHR Assay – Screening the Tox21 Chemical Library





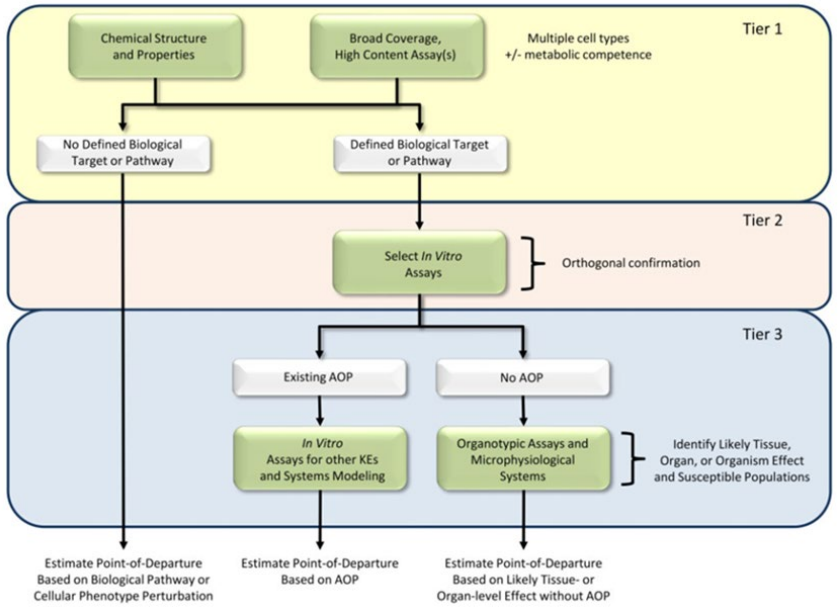
	Run1	Run2	Run3
HILLSLOPE	-1.069	-1.086	-1.368
EC50	0.3024	0.2461	0.4732

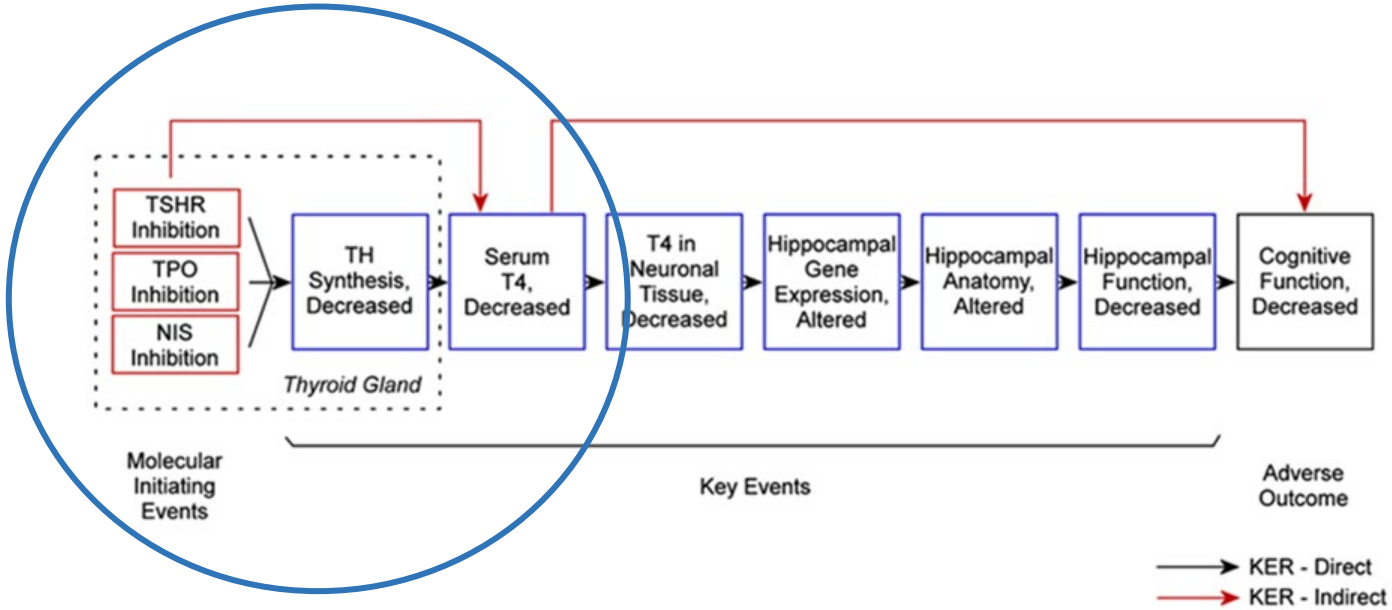
Bioactivity hit rate: 825 of 7871 chemicals (10%)

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 Induction	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, HTS assay specificity and promiscuity
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 apical 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

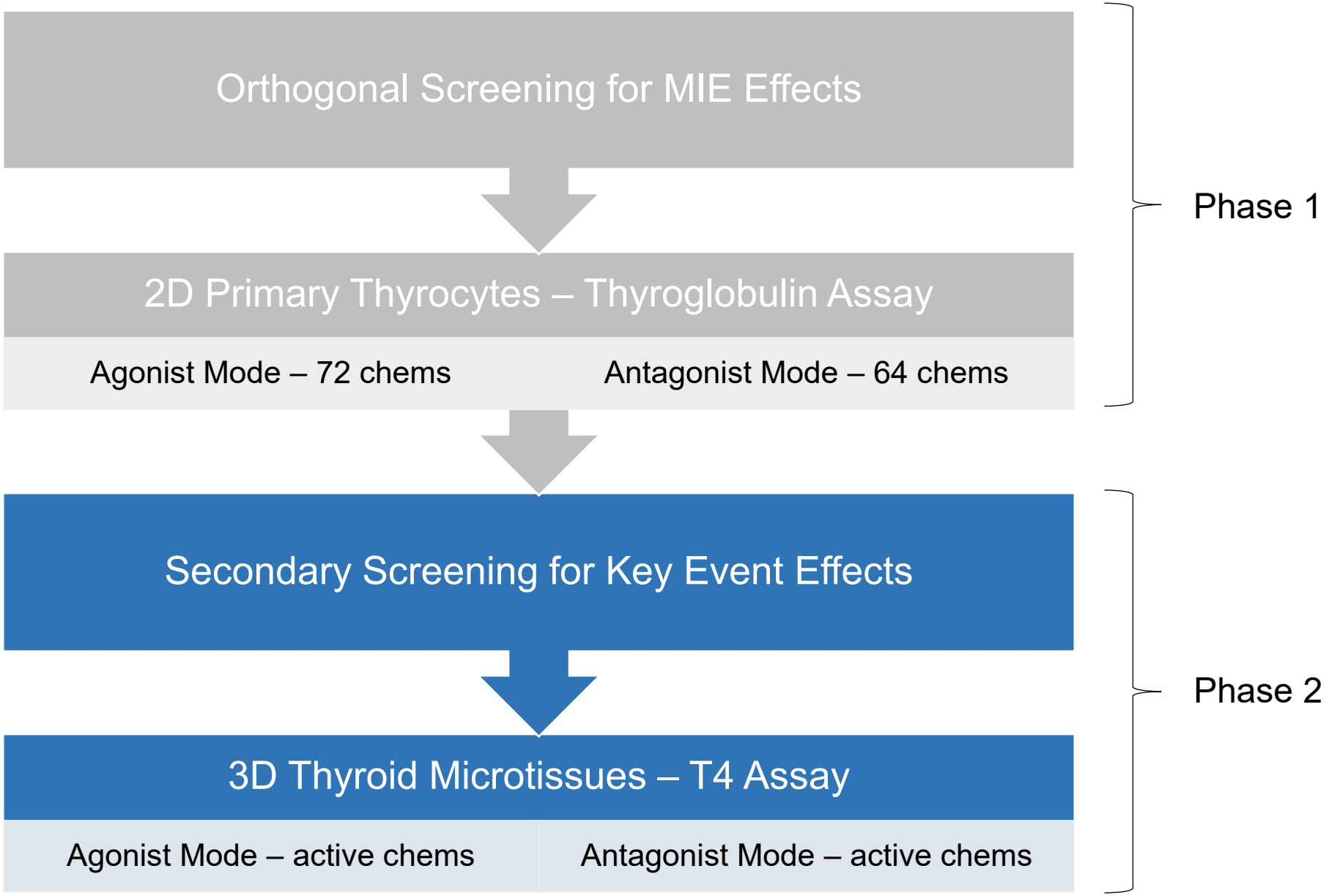
- High Bioactivity Signal
- Non-cytotoxic
- Structural Diversity

Prioritized Chemicals

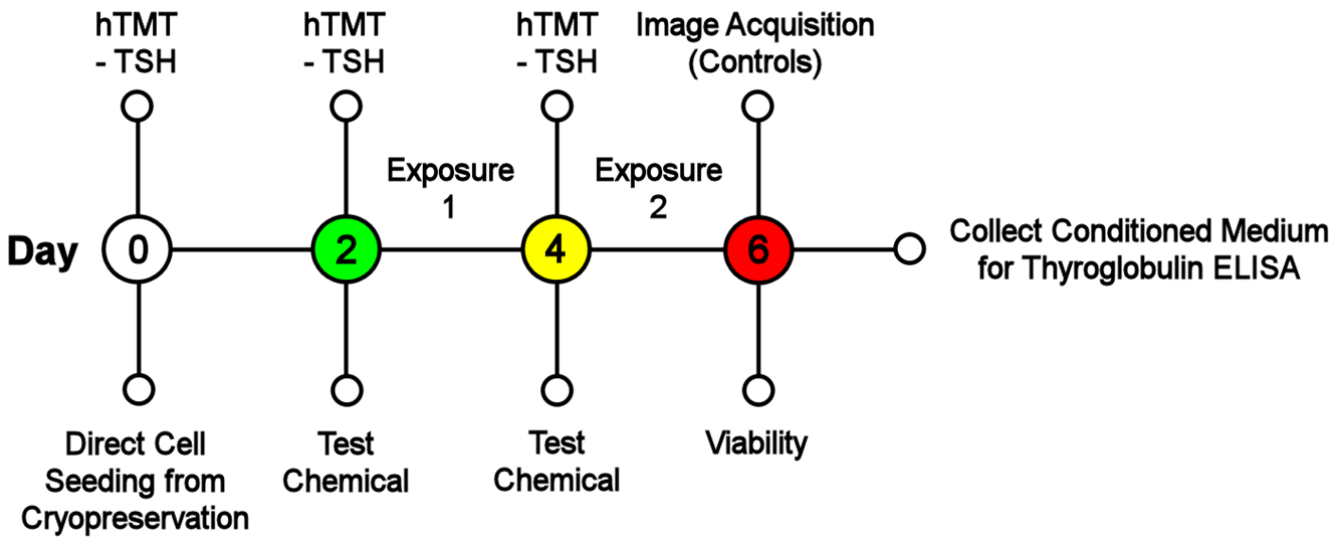
	Chemicals	Agonist Mode	Antagonist Mode
Agonists	54	54	-
Agonist-Antagonist	8	8	8
Antagonist	46	-	46
Inactive	10	10	10
Total	**118**	**72**	**64**

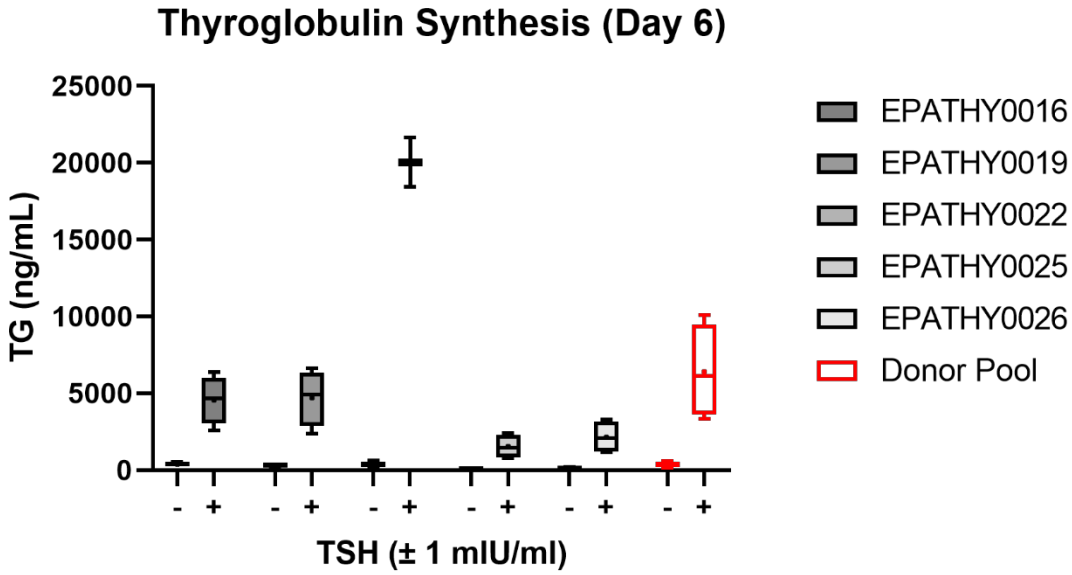
Prioritized chemicals: 108 of 825 (~13%)

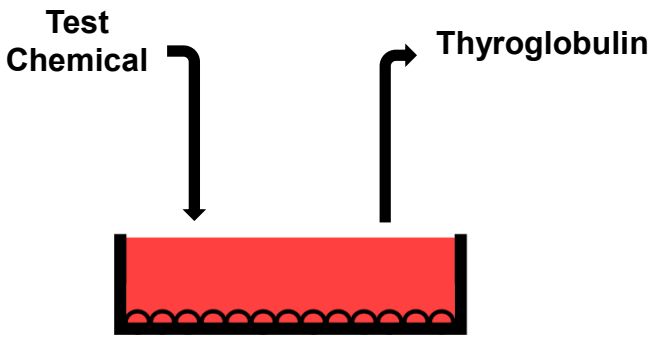
TSHR Screening Workflow



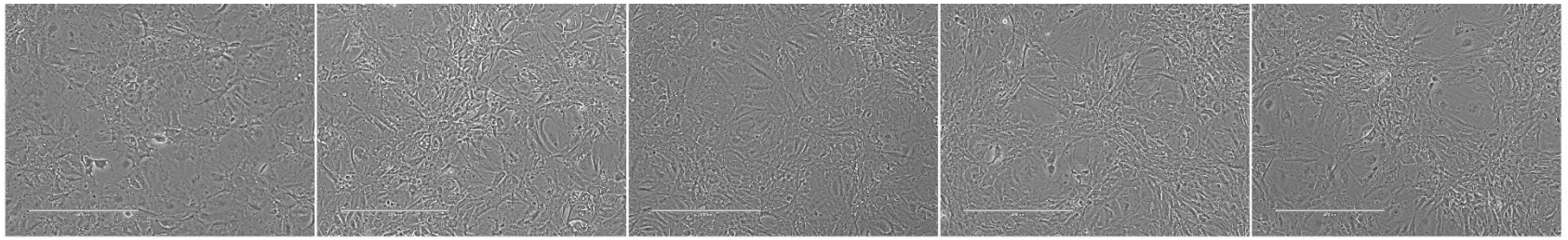
2D Thyroglobulin Assay - Workflow



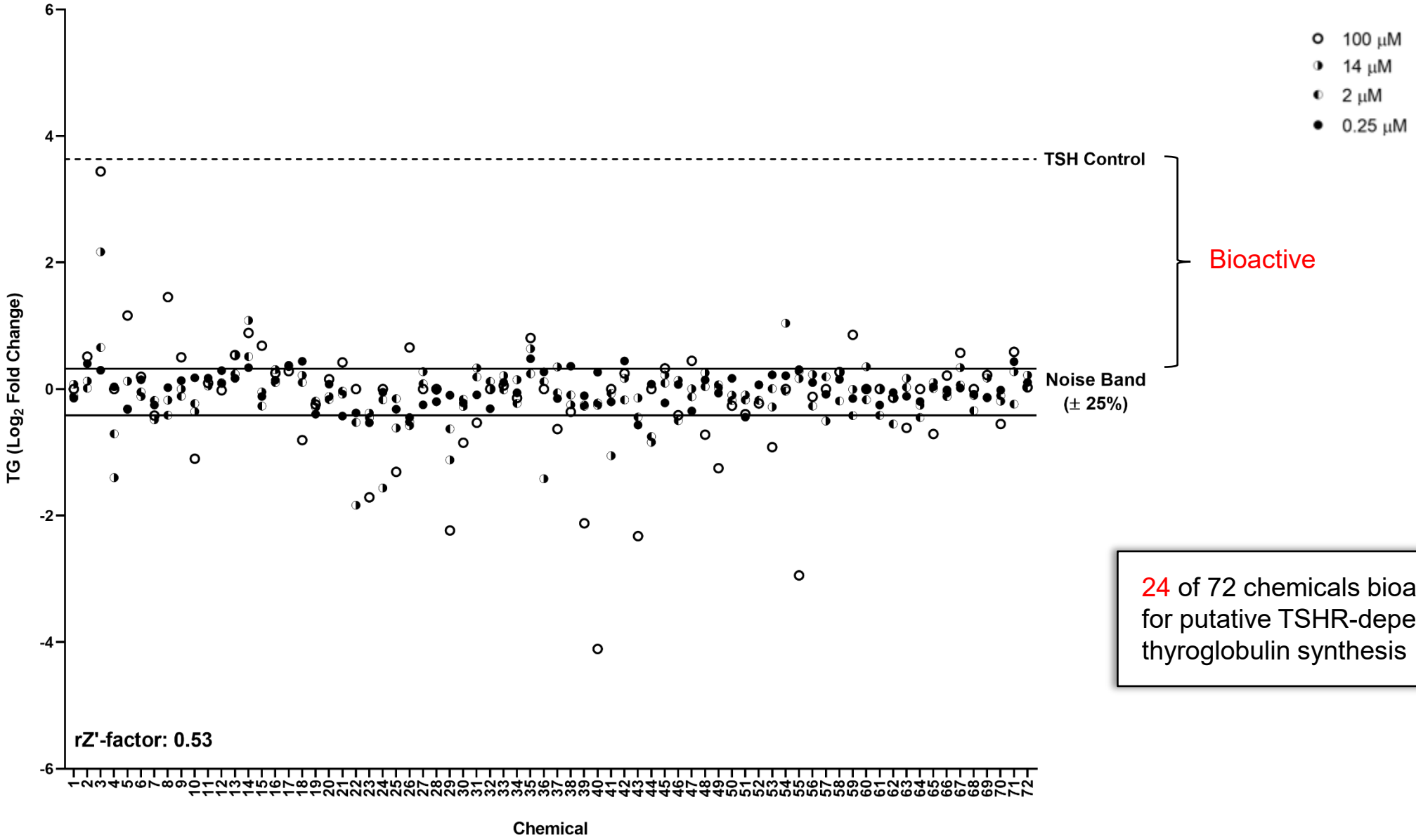




Donor ID	Sample	Age	Sex	Race	BMI	Euthyroid (Y/N)	Passage
EPATHY0016	Human Thyroid	23	M	Asian	36	Y	P0
EPATHY0019	Human Thyroid	20	M	Caucasian	28	Y	P0
EPATHY0022	Human Thyroid	34	F	African American	29	Y	P0
EPATHY0025	Human Thyroid	44	F	Caucasian	20	Y	P0
EPATHY0026	Human Thyroid	24	M	Hispanic	26	Y	P0



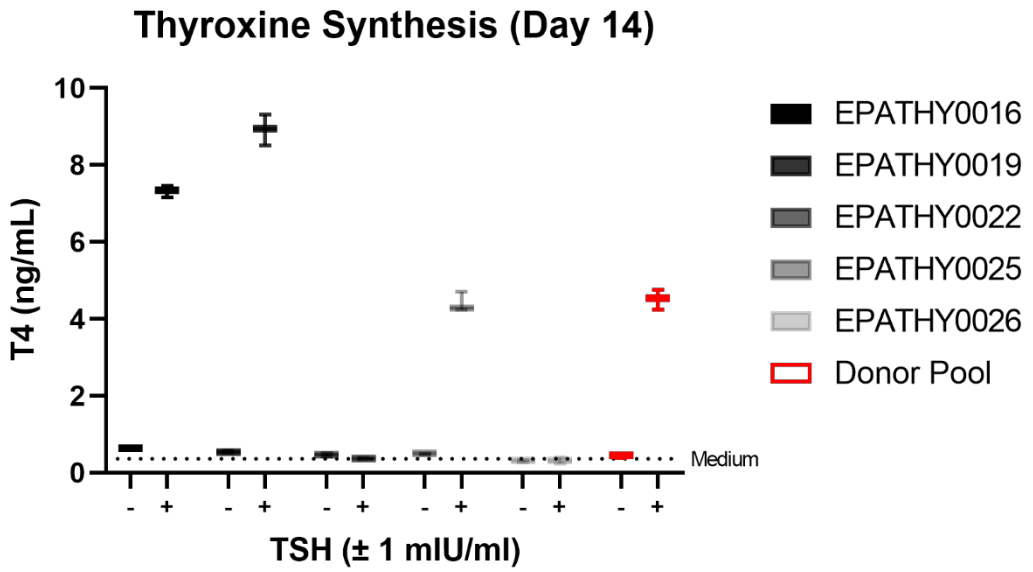
2D Thyroglobulin Assay - Screen Results

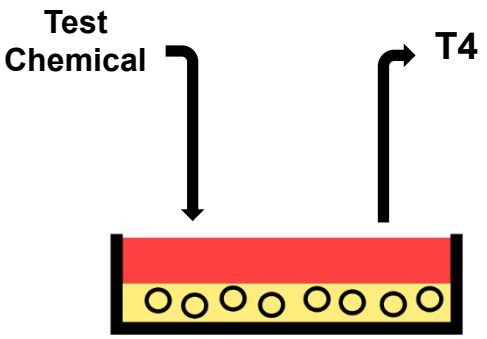


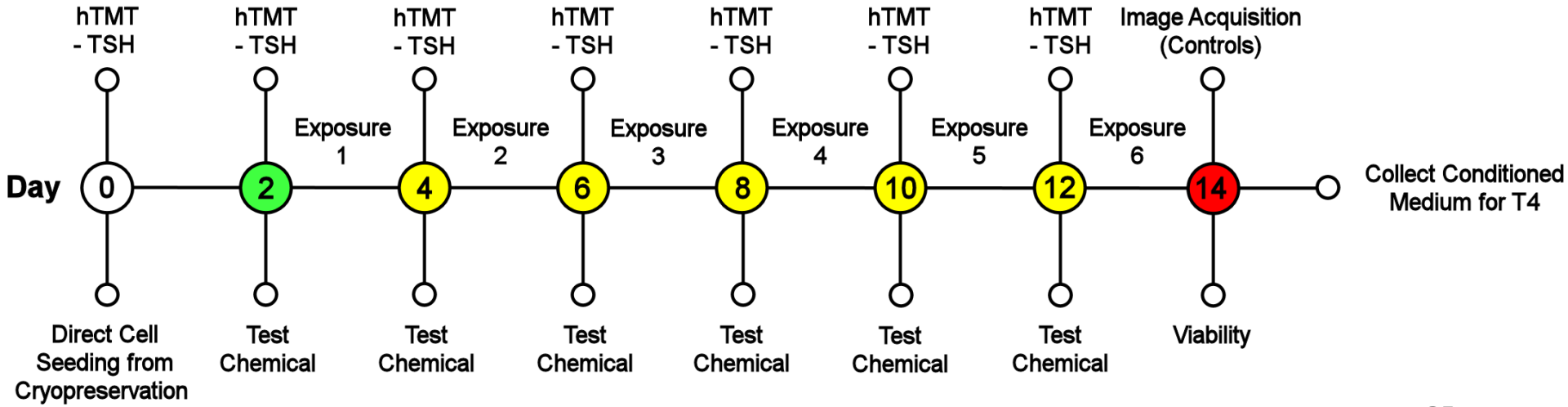
24 of 72 chemicals bioactive for putative TSHR-dependent thyroglobulin synthesis

3D Thyroid Microtissue Assay (TSHR Agonist Variant) - Workflow

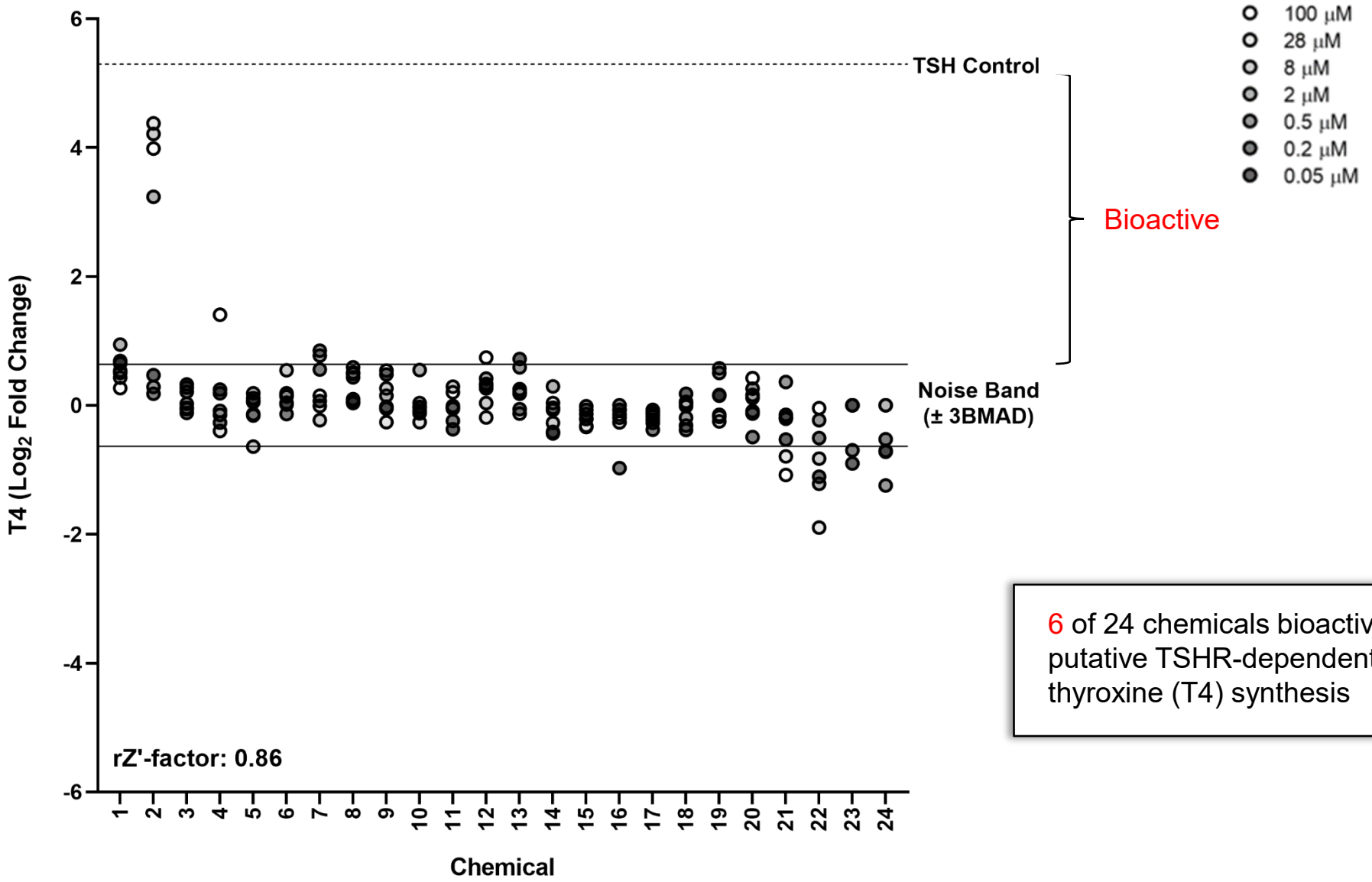
Donor ID	Sample	Age	Sex	Race	BMI	TG	T4
EPATHY0016	Human Thyroid	23	M	Asian	36	+	+
EPATHY0019	Human Thyroid	20	M	Caucasian	28	+	+
EPATHY0022	Human Thyroid	34	F	African American	29	+	-
EPATHY0025	Human Thyroid	44	F	Caucasian	20	+	+
EPATHY0026	Human Thyroid	24	M	Hispanic	26	+	-





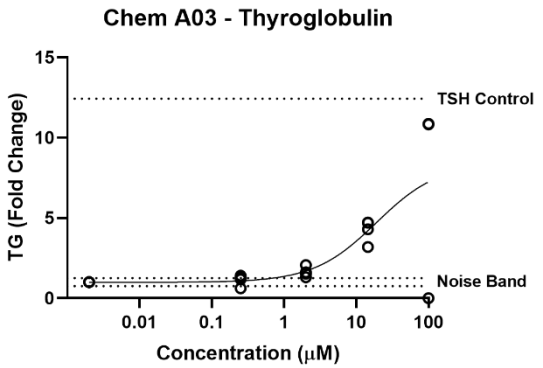
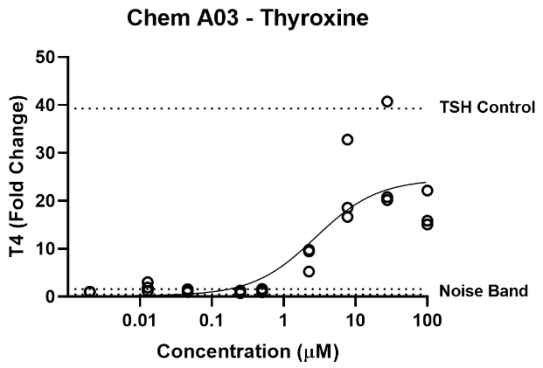
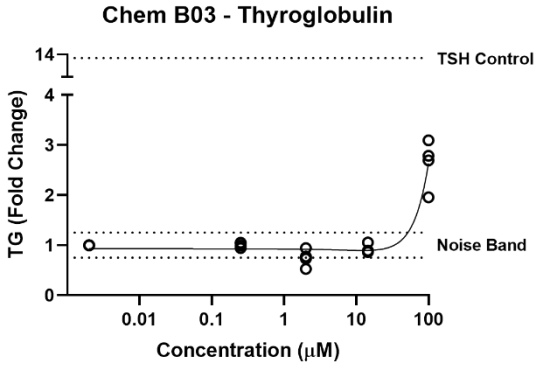
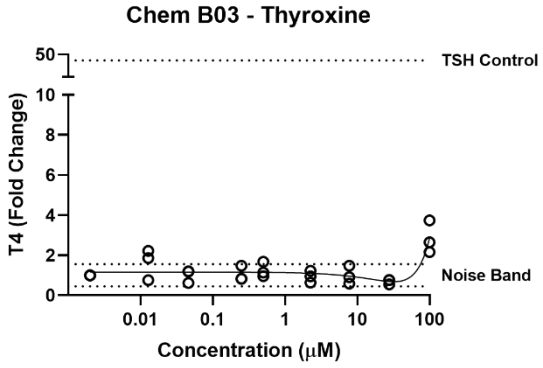
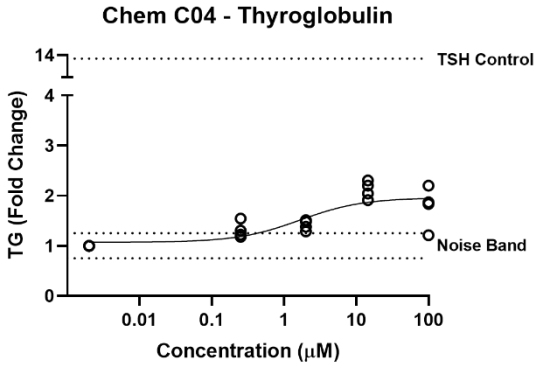
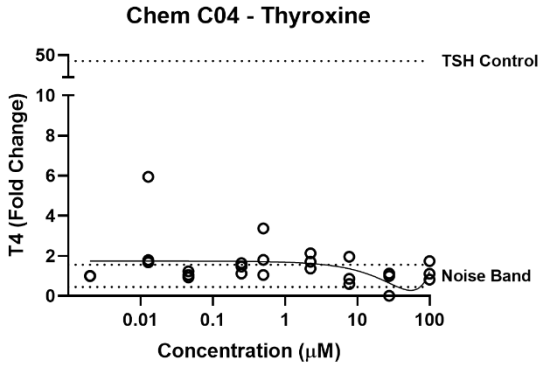


3D Thyroid Microtissue Assay (TSHR Agonist Variant) – Screen Results



6 of 24 chemicals bioactive for putative TSHR-dependent thyroxine (T4) synthesis

3D Thyroid Microtissue Assay (TSHR Agonist Variant) – Representative Effects

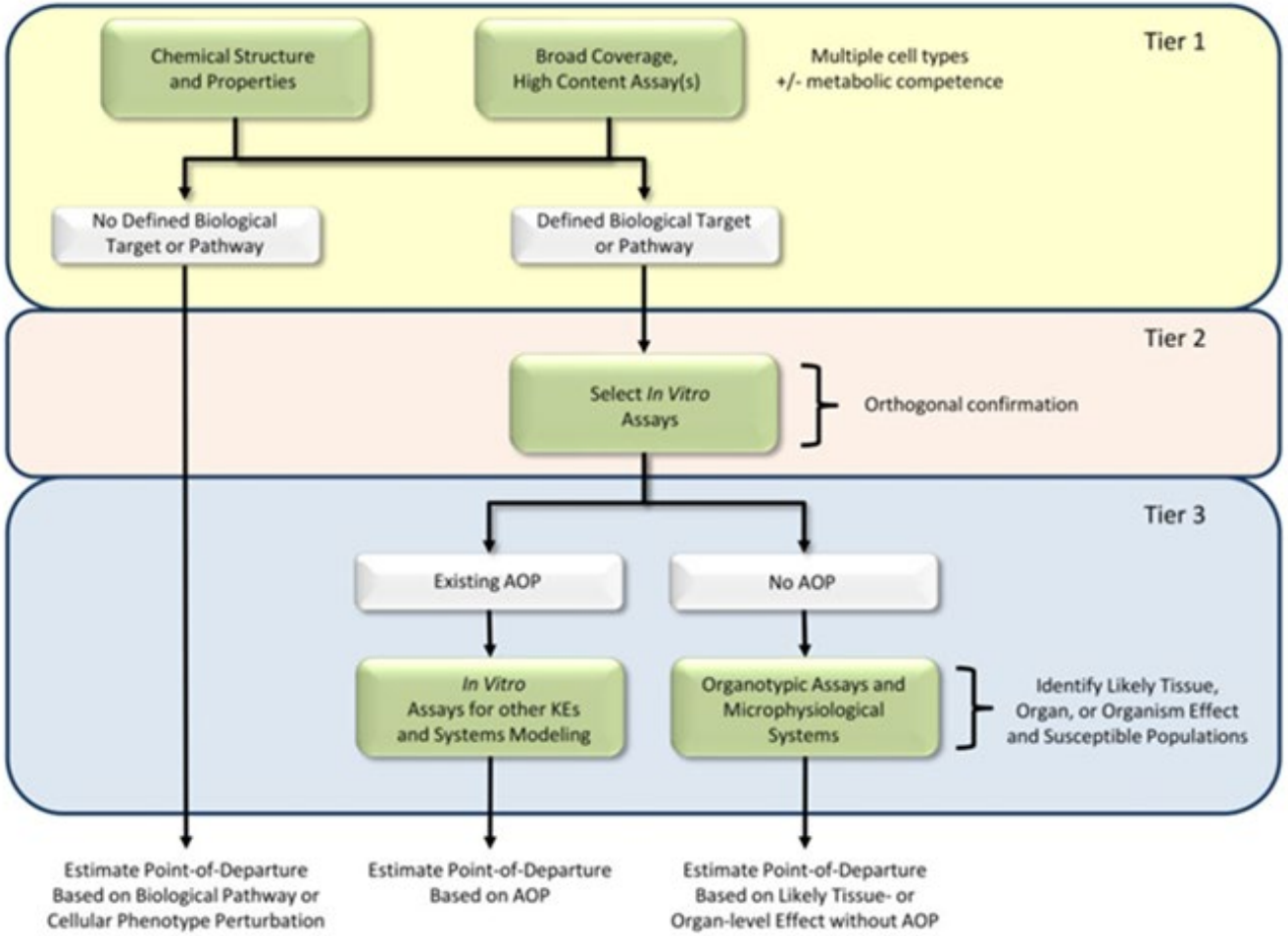
	MIE	Key Event
Strong		
Moderate		
Weak		

TSHR Hit Prioritization Workflow

Orthogonal Screening for MIE Effects

Secondary Screening for Key Event Effects

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

DOI: 10.1093/toxsci/kfz058

Acknowledgements

Briana Foley
Jermaine Ford
Wendy Stewart
Kristen Hopperstad
Cassandra Brinkman
Mahmoud Shobair
Grace Patlewicz
Katie Paul Friedman
Ann Richard
Rusty Thomas

Ed LeCluyse
Eda Rogers
Valerie Soldatow
Jingsong Chen
Sharon Presnell
Paul Gallant
Lindsay Whaley

Nicole Kleinstreuer
Helena Hogberg-Durdock
Agnes Karmaus
Dave Allen
Michaela Blaylock

Helen Tinwell
Julia Kuehnlenz
Frederic Schorsch
Olivier Blanck

Jessica LaRocca
Enrica Bianchi
Mercedes Biven
Wei Chen

Contact

deisenroth.chad@epa.gov