# Advancing Translational Application and Acceptance of the Human Thyroid Microtissue Assay

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ASCCT-ESTIV Webinar: Progress in in vitro Thyroid Disruption Approaches
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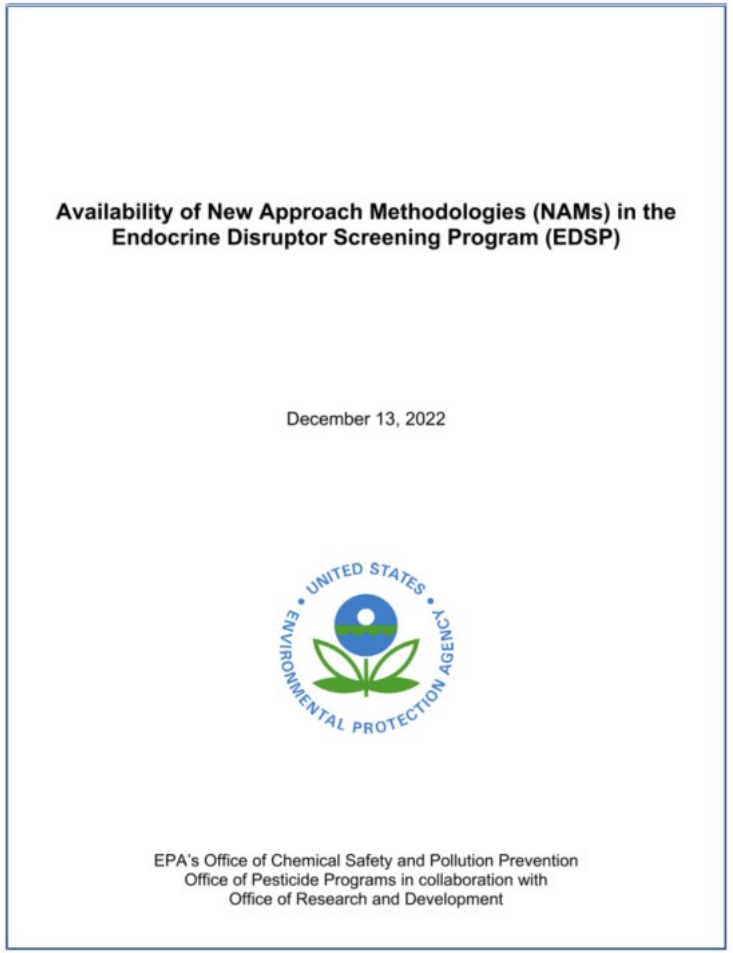
*Disclaimer: The views expressed are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. There are no conflicts to declare.*

# Adoption of New Approach Methods in the Endocrine Disruptor Screening Program

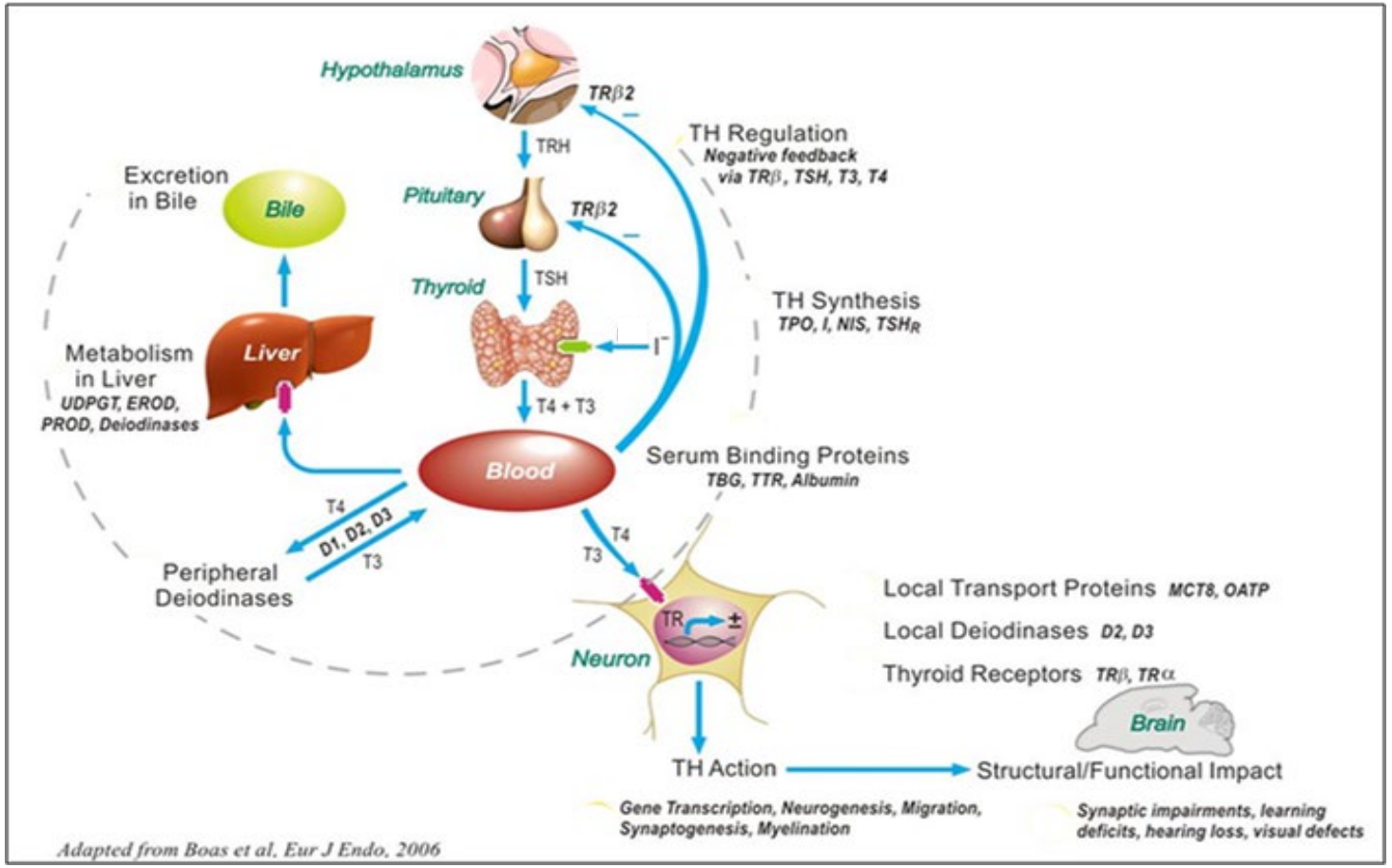

Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)

December 13, 2022

EPA's Office of Chemical Safety and Pollution Prevention
Office of Pesticide Programs in collaboration with
Office of Research and Development

- The EDSP evaluates chemical effects on estrogen, androgen, and thyroid endocrine pathways.
- The validated Estrogen Receptor (ER) and Androgen Receptor (AR) pathway models may be used as an alternative to the Tier 1 screening assays.
- **Continue development of a Thyroid Pathway Framework that includes *in vitro* assays for thyroid-relevant targets to produce an integrated prediction model.**

# Endocrine Toxicology: Why Do We Care About Thyroid?

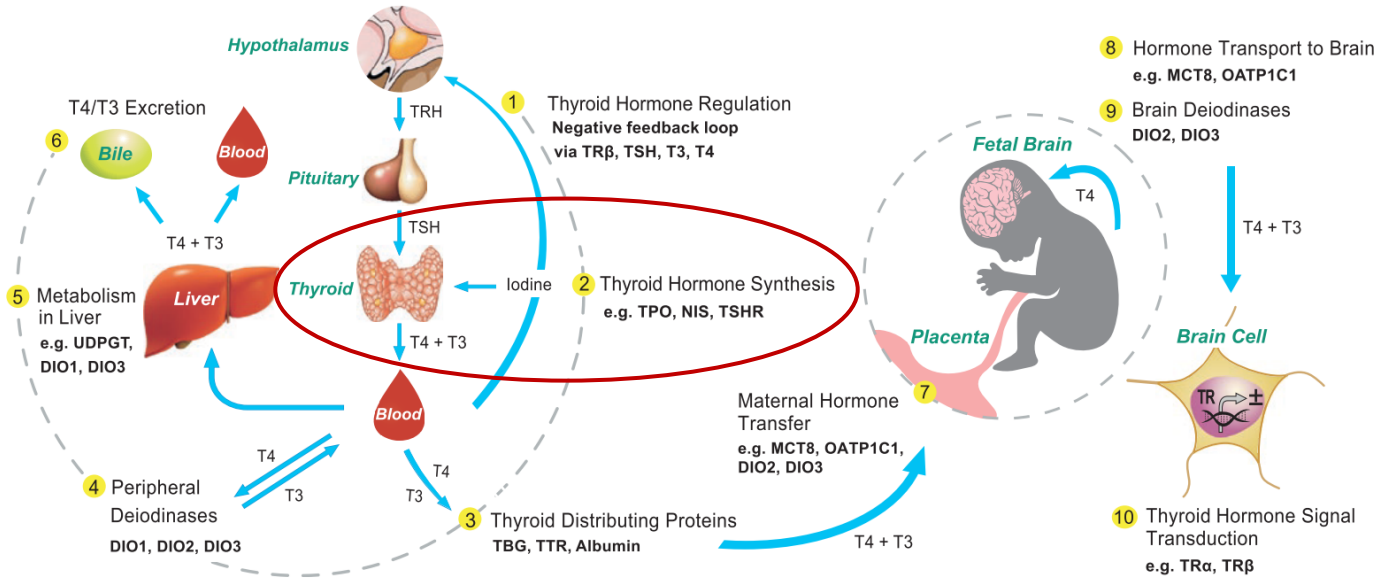



- Thyroid hormones are essential for normal growth, development, cell differentiation, and energy homeostasis.
- Thyroid dysfunction is characterized by under- (hypothyroidism) or over- (hyperthyroidism) activity of the gland, impacting:
  - Neurodevelopment and function
  - Cardiovascular function
  - Energy metabolism
  - Cancer
- Environmental chemical exposures are associated with thyroid dysfunction.

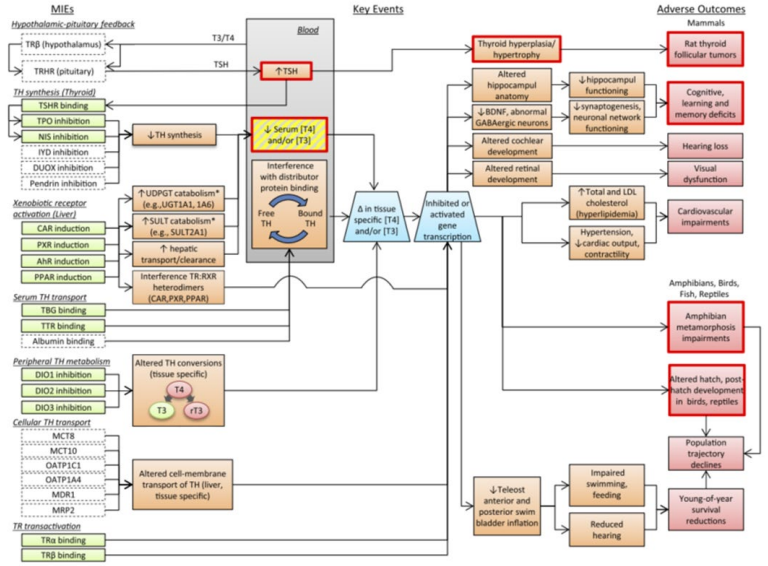
doi: 10.2779/921523

# Thyroid HTS Assays Do Not Directly Measure Thyroid Hormone Synthesis

Sites of Interference for Thyroid Disrupting Chemicals



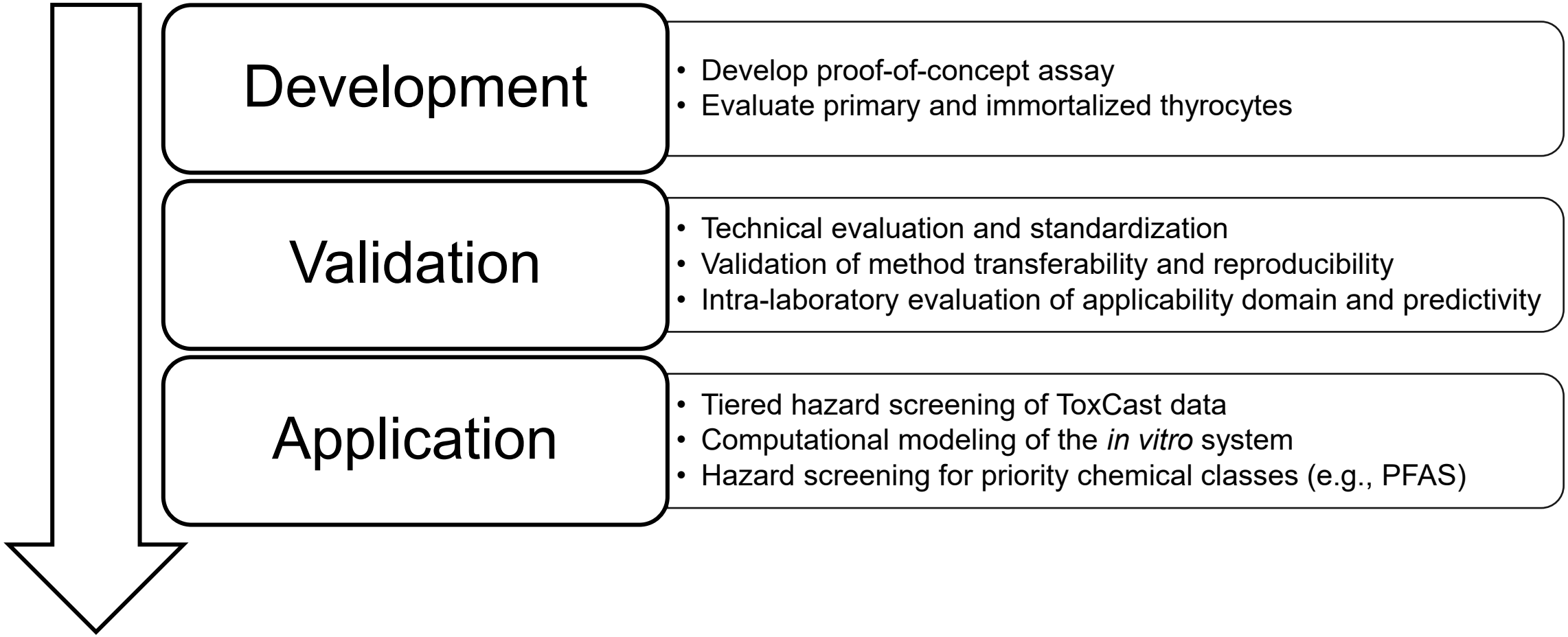

Thyroid AOP Network




| 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. |

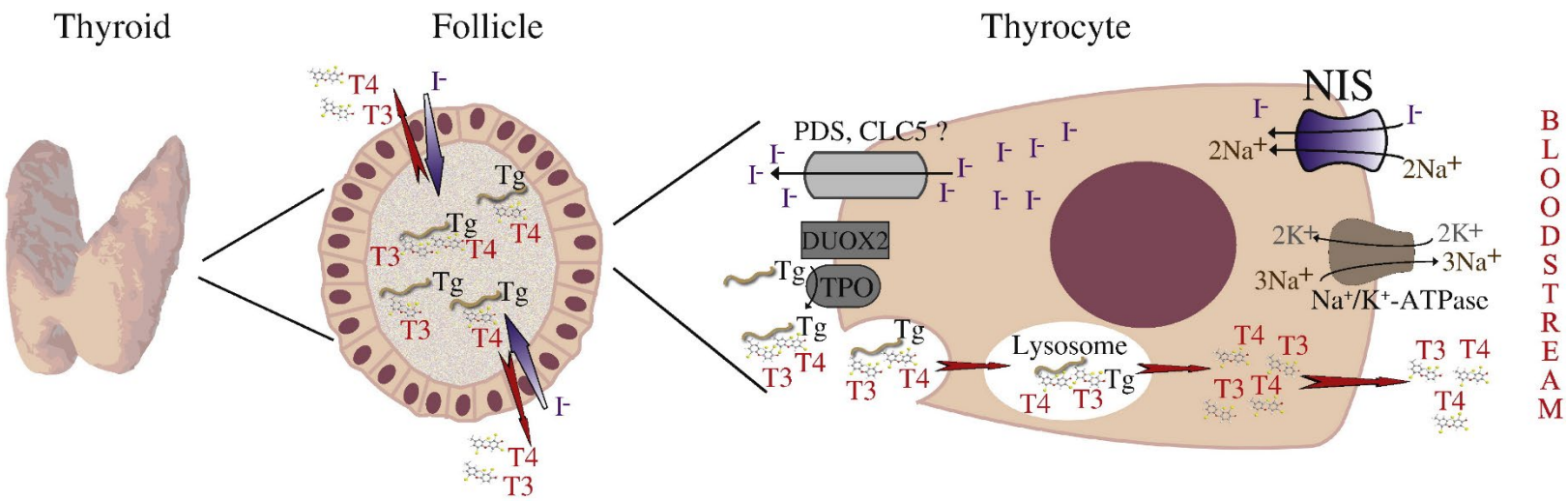
*DOI: 10.1210/endocr/bqaa106; DOI: 10.1289/EHP5297*

# Human Thyroid Microtissue Assay




**Goal**: Establish a validated test method for human thyroid hormone disruption.

# 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*

# Human Thyroid Microtissue Model: Proof of Concept

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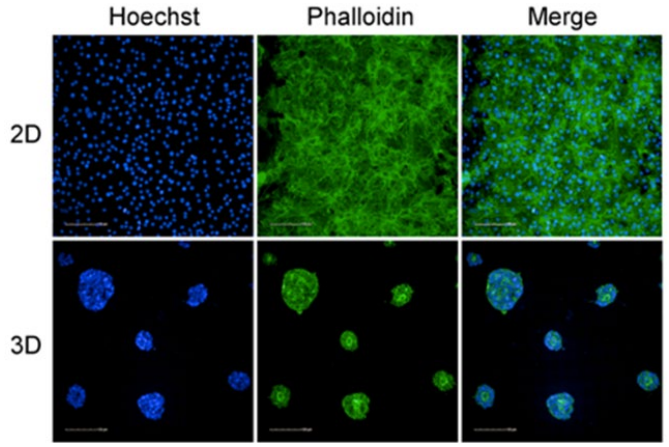
SOT | Society of Toxicology
academic.oup.com/toxsci

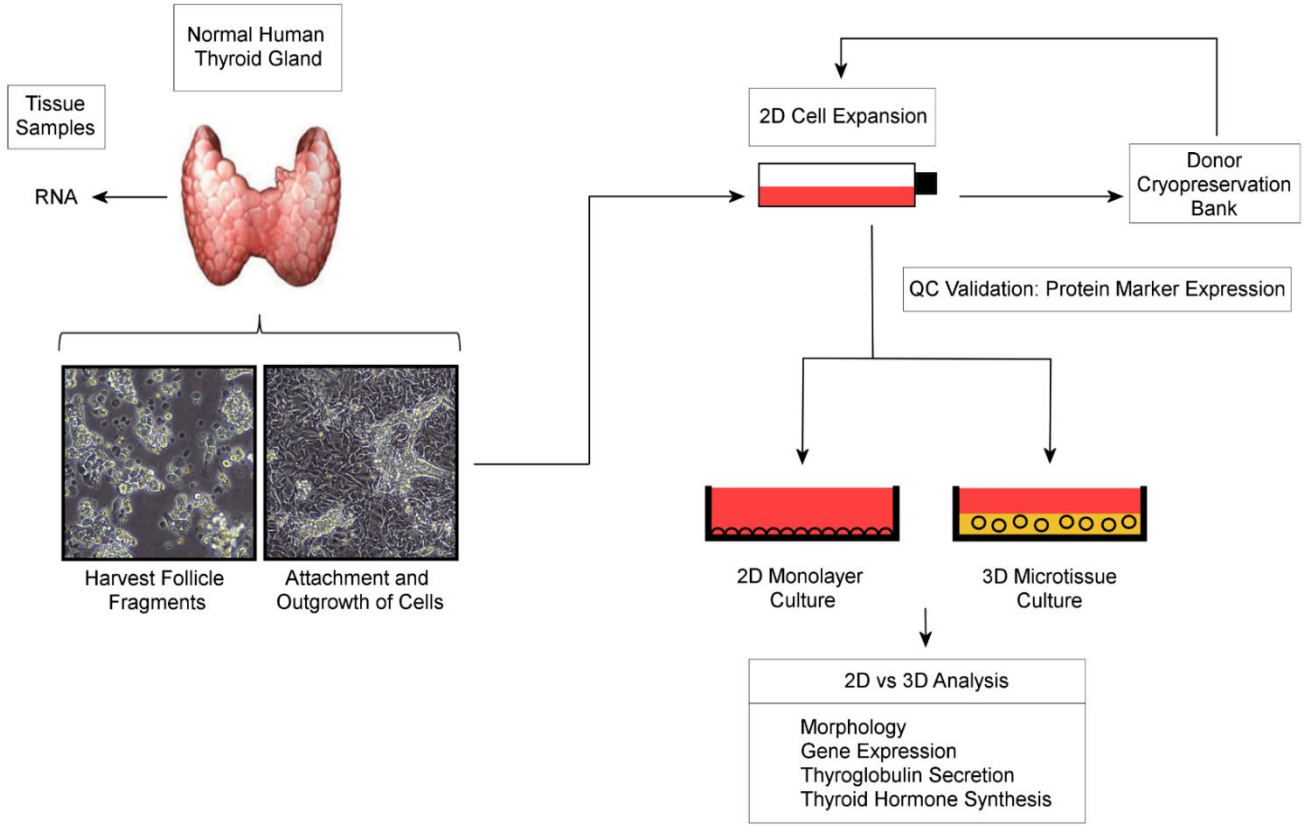
TOXICOLOGICAL SCIENCES, 174(1), 2020, 63–78

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

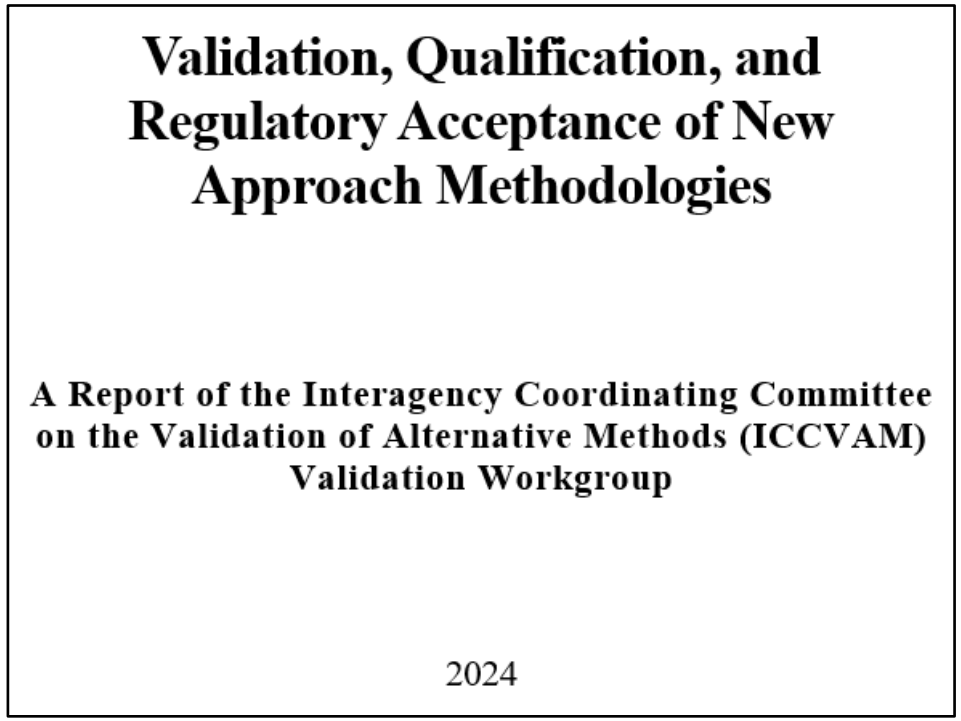
Chad Deisenroth ,*,[1] Valerie Y. Soldatow,[†] Jermaine Ford,[‡] Wendy Stewart,* Cassandra Brinkman,* Edward L. LeCluyse,[†] Denise K. MacMillan,[‡] and Russell S. Thomas *

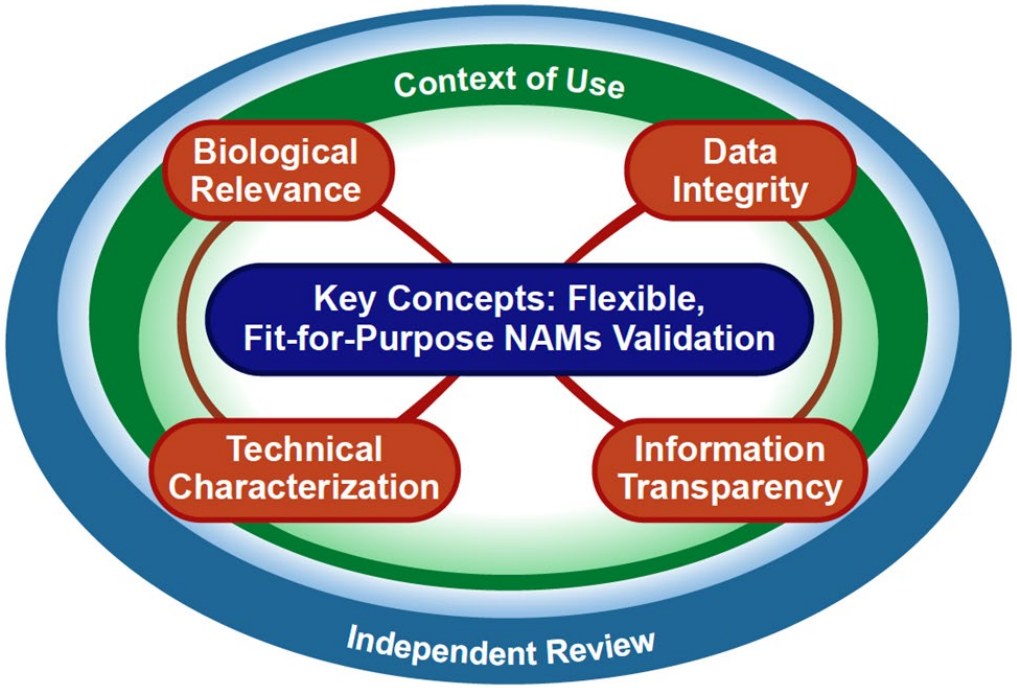



Developed a medium-throughput organotypic screening assay comprised of reconstructed human thyroid microtissues to quantitatively evaluate the disruptive effects of chemicals on thyroid hormone synthesis and secretion.

# Increasing Confidence in the Human Thyroid Microtissue Assay as a New Approach Method



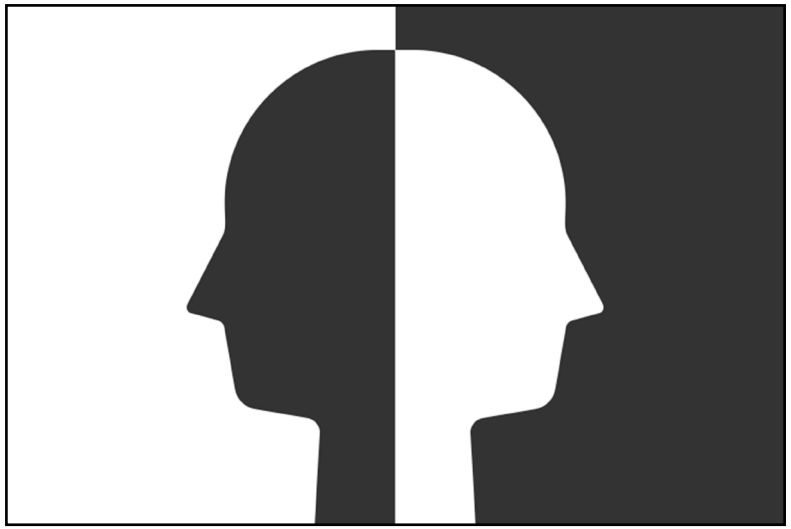

- Guided by underlying principles of OECD GD 34, a framework to validate NAMs that are fit-for-purpose, reliable, and relevant to the species of interest.
- Intended to be a modular and flexible approach to test method validation that accommodates shifting trends in assay technologies and applications.
- Reduce the time and cost of validation to accelerate regulatory adoption and implementation.



https://doi.org/10.22427/NICEATM-2

# Standardizing Organotypic Assays is Challenging

"I want an assay that is reproducible"



"I want an assay that predicts a range of human responses"

How do technical precision and biological variability co-exist?

**Goal**: Establish minimum acceptance criteria for donor qualification and quantitative performance guidelines to increase confidence in the 'true' biological performance variability.

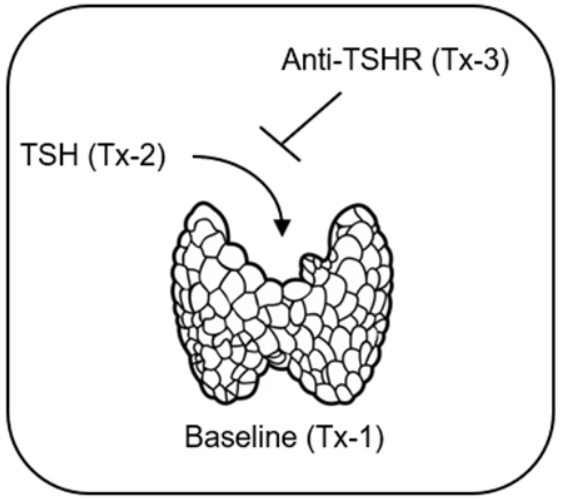
# Standardization of the Human Thyroid Microtissue Assay

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

*Toxicological Sciences*, 2024, 1–19
https://doi.org/10.1093/toxsci/kfae014
Advance Access Publication Date: February 4, 2024
Research article

## Technical evaluation and standardization of the human thyroid microtissue assay

Briana Foley,[1] Kristen Hopperstad,[1] John Gamble,[1,2] Scott G. Lynn,[3] Russell S. Thomas,[1] Chad Deisenroth[1,*]



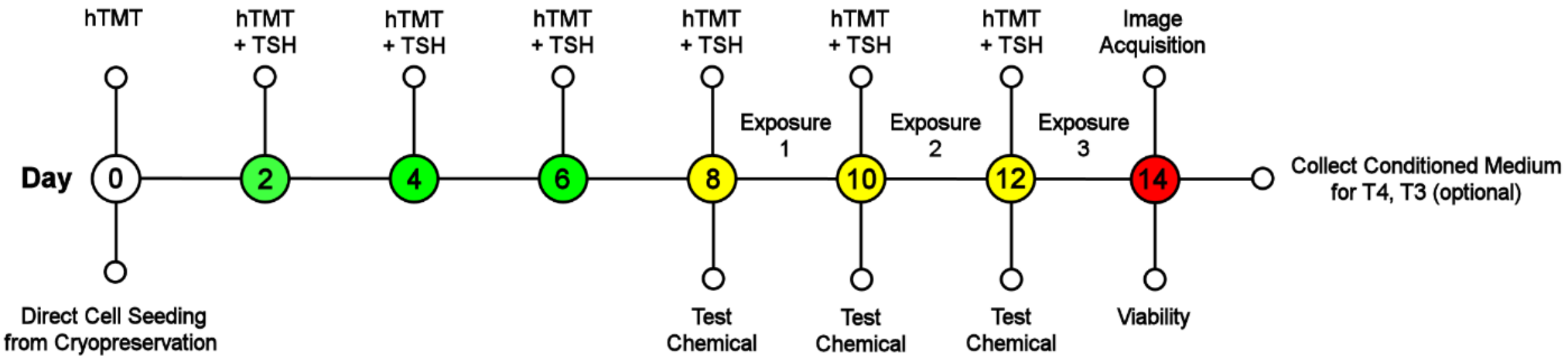

**Objectives**: 1) Define technical parameters for donor procurement, thyrocyte qualification, and assay performance, 2) Set benchmark ranges for reference chemical responses.

### Donor Cohort Demographic Summary

| | |
|---|---|
| **Donors** | 32 |
| **Age** | 34 (17-61) |
| **Sex** | Male (24), Female (8) |
| **Race** | Caucasian (25), African American (7) |
| **BMI** | 28 (18-37) |

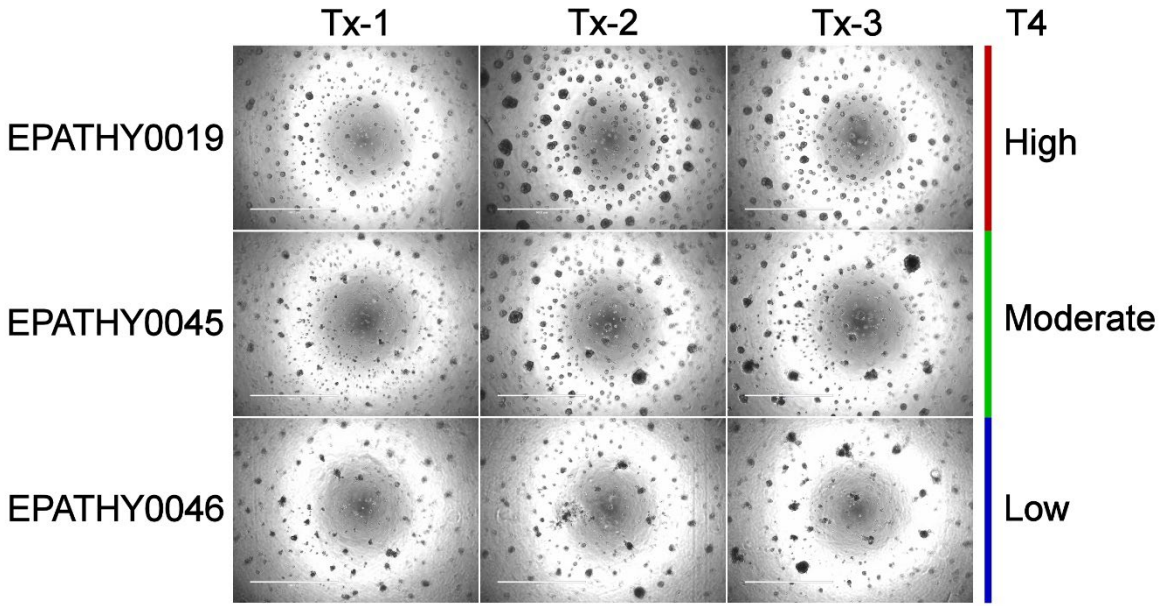
- Microtissue Morphology
- Microtissue Biomass
- TSH Receptor Sensitivity
- Thyroglobulin Synthesis
- Hormone Synthesis
- Reference Chemical Response

# Human Thyroid Microtissue Assay v2.0

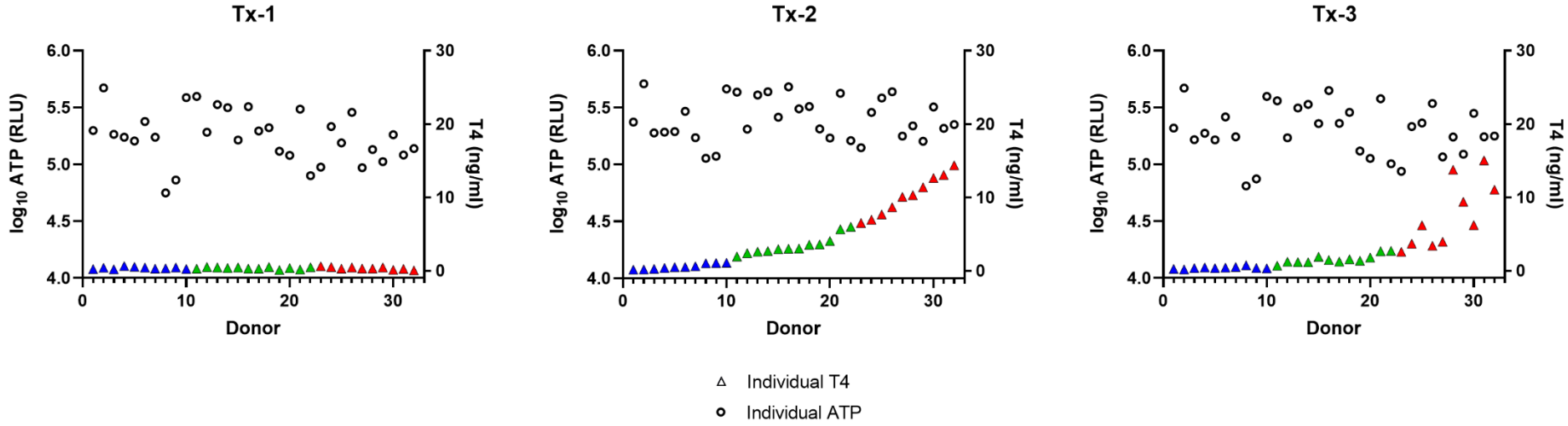



Protocol modified to enhance performance and improve durability for method transfer.

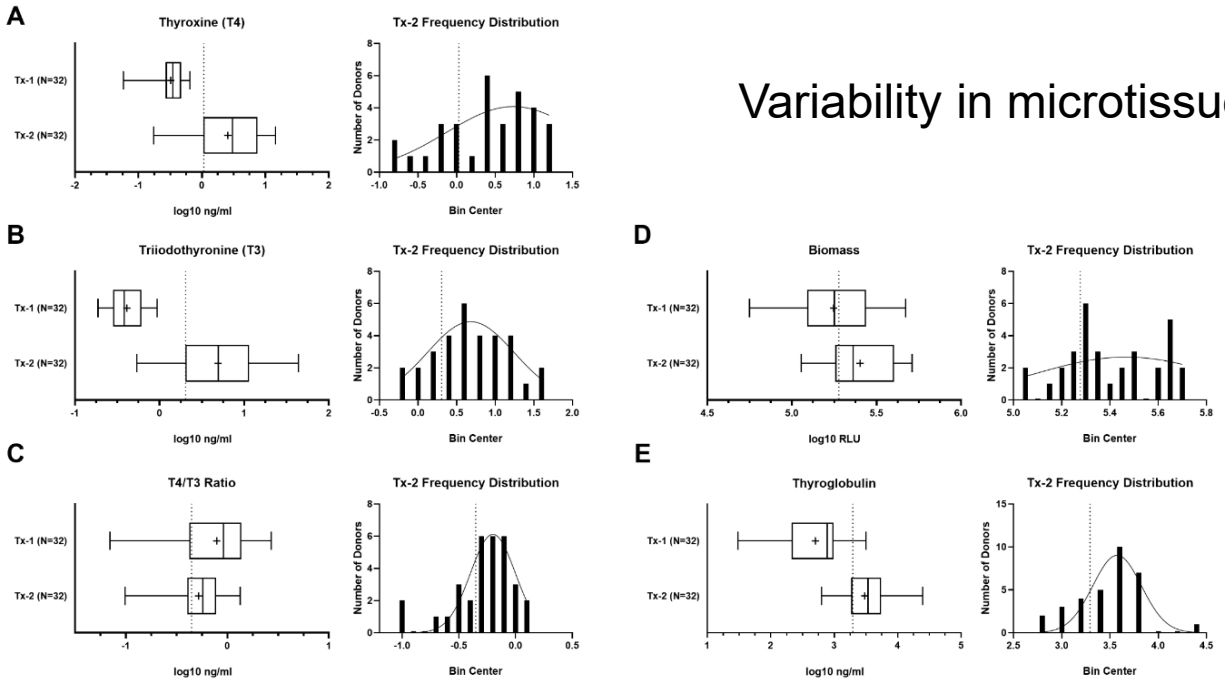
# Microtissue Morphology and Biomass




- Donors exhibit a wide range of hormonogenic competence.
- No clear relationship between microtissue size or morphology and hormone synthesis.



# Donor Qualification – Setting Minimum Acceptance Criteria for Hormonogenic Competence

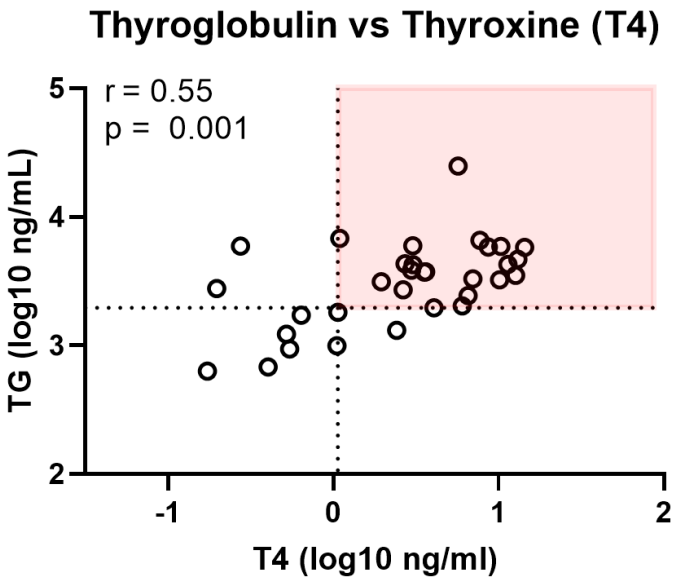


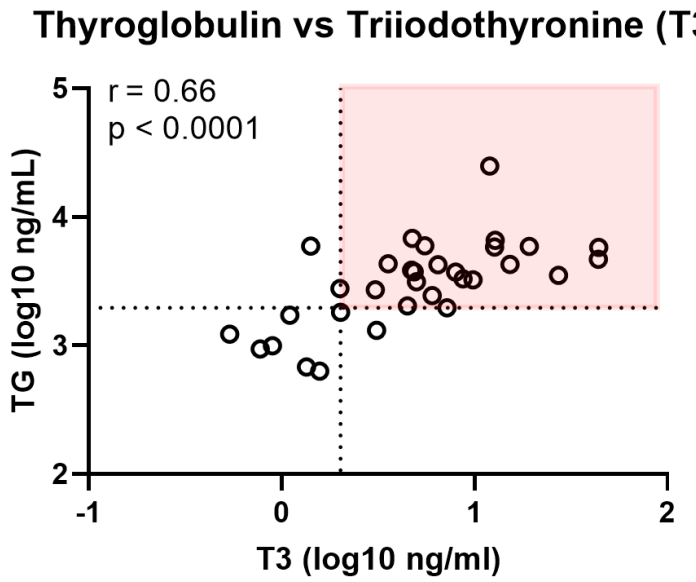

Variability in microtissue performance evaluated.

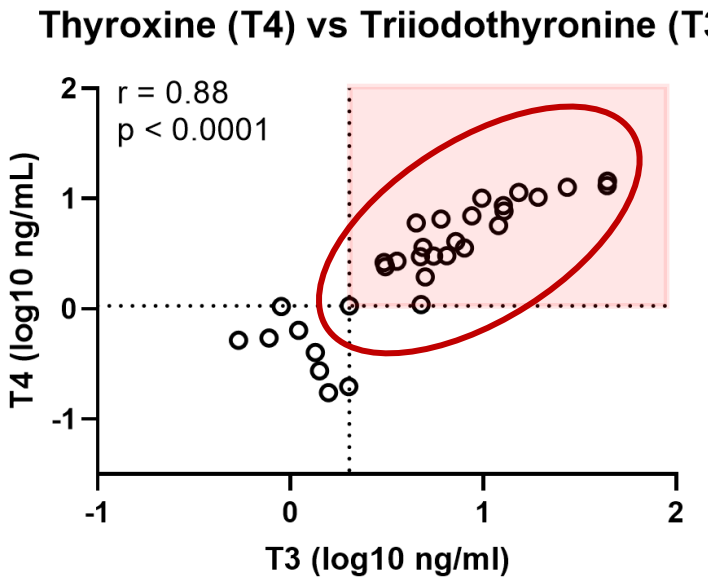
| | Donor thyrocyte qualification | | | | |
|---|---|---|---|---|---|
| **Tx-2 99% CI** | **Biomass (RLU)** | **Thyroglobulin (ng/ml)** | **T4 (ng/ml)** | **T3 (ng/ml)** | **T4/T3 ratio (ng/ml)** |
| Median | 230 197 | 3405 | 3.03 | 4.94 | 0.57 |
| Lower confidence limit | 189 321 | 1961 | 1.07 | 2.02 | 0.45 |
| Criteria | ≥180 000 | ≥1900 | ≥1.0 | ≥2.0 | ≥0.4 |
| Priority | Optional | Optional | Required | Recommended | Optional |

Lower confidence limits used to establish minimum donor acceptance criteria.

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




- Thyroxine (T4) vs Triiodothyronine (T3) exhibit the cleanest binning for donor-based performance.
- Data suggests up to 25% of donors would not qualify for use in the assay.

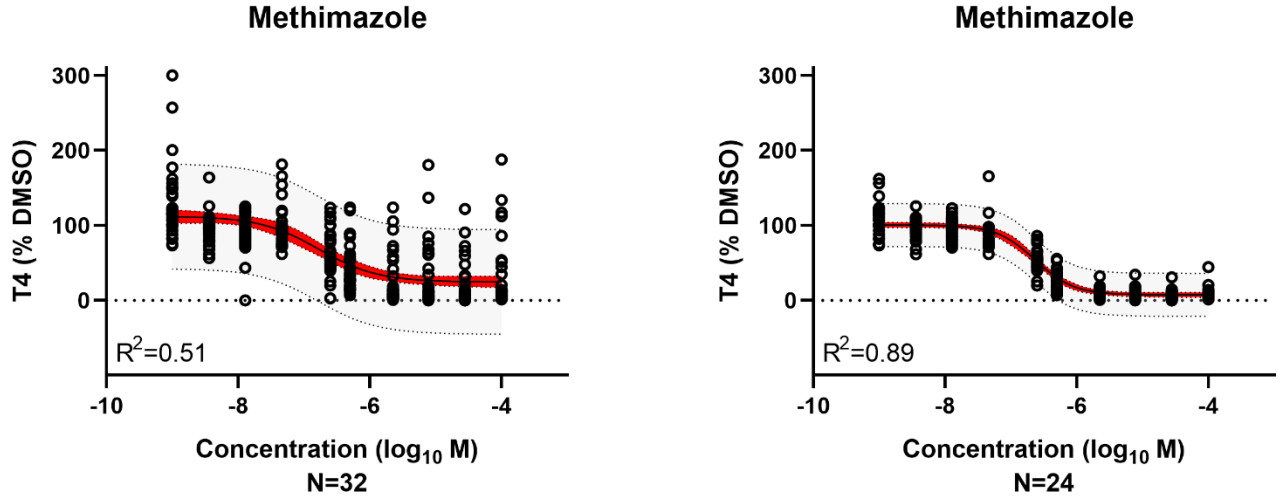
# Assay Technical Performance Metrics

| | | Total cohort ($N = 32$) | | Qualified cohort ($N = 24$) | |
|---|---|---|---|---|---|
| Solvent (DMSO) | Performance metrics | T4 median (±MAD) | T4 range | T4 median (±MAD) | T4 range |
| 0% | Dynamic range (rS/B) | 7.0 (±8.7) | 0.5–211.8 | 9.5 (±6.3) | 3.9–211.8 |
| | Precision (rCV) | 8.3 (±6.8) | 1.2–51.7 | 7.9 (±6.8) | 1.2–21.4 |
| | Screening quality (rZ′-factor) | 0.56 (±0.42) | −823.94–0.94 | 0.64 (±0.35) | −0.57–0.94 |
| 0.5% | Dynamic range (rS/B) | 9.9 (±8.5) | 0.5–508.8 | 11.6 (±7.0) | 2.6–508.8 |
| | Precision (rCV) | 11.8 (±6.1) | 0.6–60.3 | 10.6 (±5.4) | 0.6–24.0 |
| | Screening quality (rZ′-factor) | 0.53 (±0.37) | −17.50–0.94 | 0.61 (±0.25) | 0.00–0.94 |

Technical reproducibility is more strongly supported in the variable-donor platform when using qualified donors.

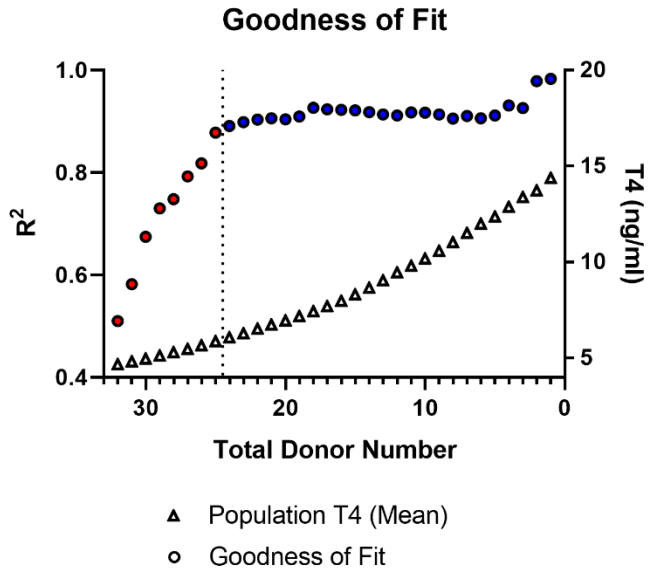
# Reference Chemical Benchmarks for Proficiency Testing

A




| Reference chemical | Proficiency testing benchmarks | | |
|---|---|---|---|
| | $IC_{10}$ (95% CI) | $IC_{50}$ (95% CI) | Units |
| Methimazole | 53 (29–94) | 234 (190–277) | nM |
| 6-Propyl-2-thiouracil | 76 (46–115) | 363 (311–422) | nM |
| Sodium perchlorate | 4 (2–6) | 18 (12–29) | μM |
| Methomyl | NA | NA | NA |

B

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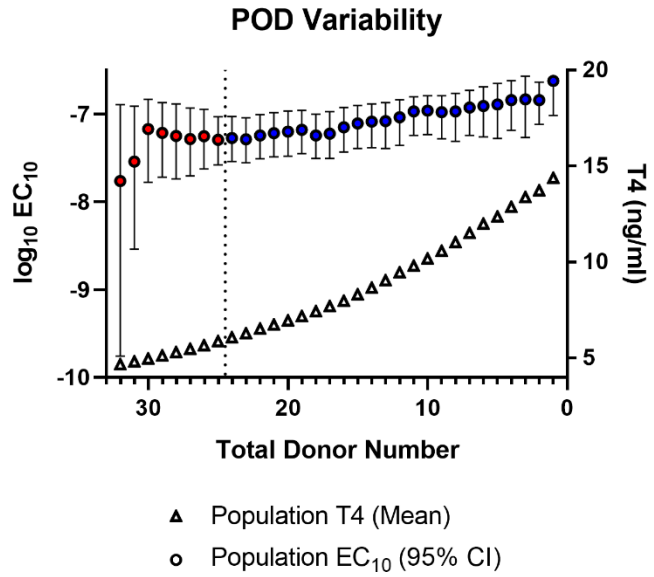
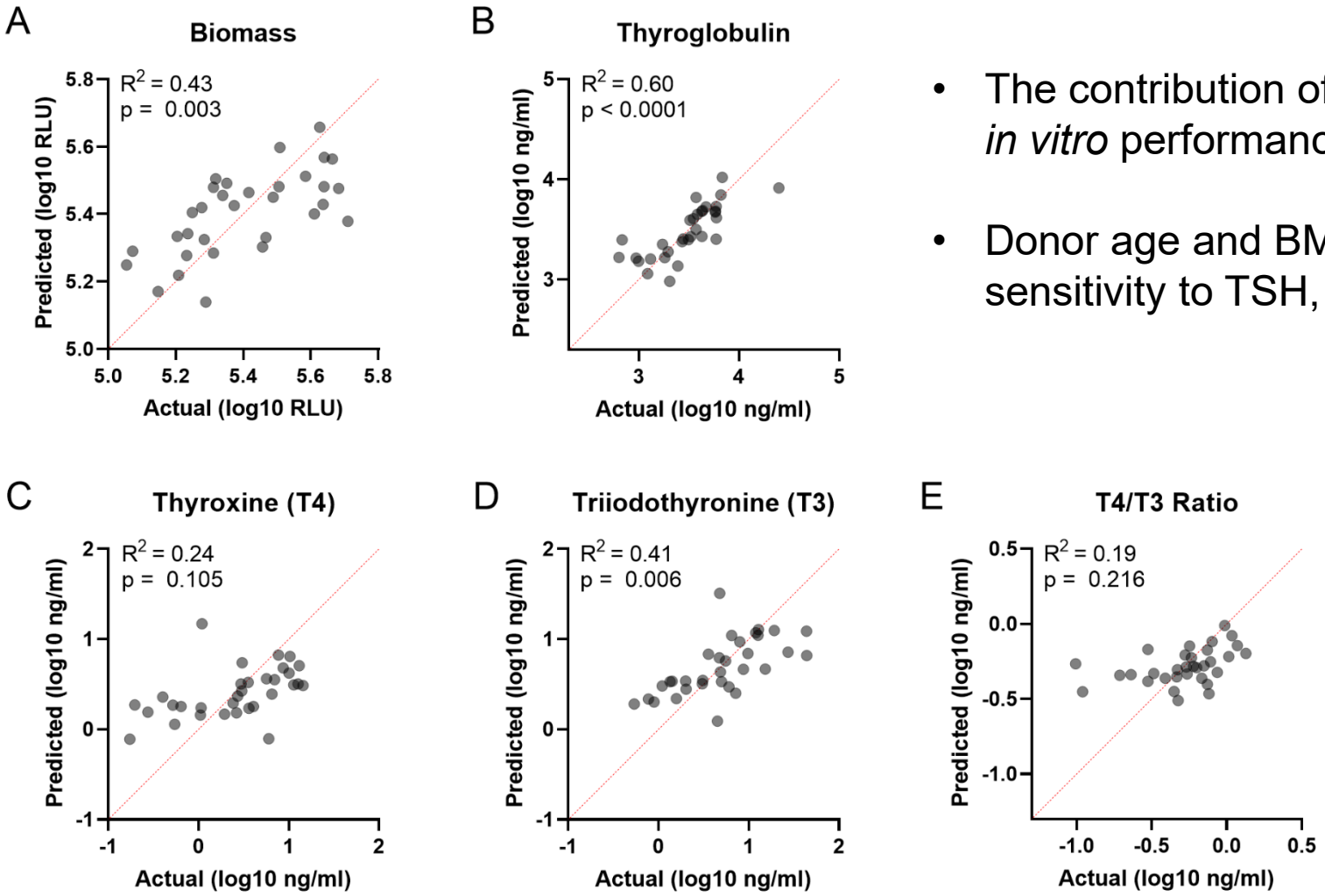



Donor qualification improves data modeling and decreases uncertainty in chemical potency determination.

# Performance Parameters Influenced by Donor Demographics




- The contribution of donor age, sex, race, and BMI to *in vitro* performance evaluated.
- Donor age and BMI influence microtissue formation, sensitivity to TSH, and thyroid hormone synthesis.

United States Environmental Protection Agency

# Key Points

**Advances AOP-based Thyroid Testing -** The human thyroid microtissue assay fills an important 'key event' gap in the context of the thyroid adverse outcome pathway network by enabling functional testing of potential thyroid toxicants on hormone synthesis.

**Standardizes Primary Human Thyrocyte Technology -** The establishment of guidelines for donor procurement and primary thyrocyte qualification move the technology toward standardization in a manner that directly addresses cell quality as a key vulnerability with the use of organotypic model systems.

**Sets Minimum Performance Guidelines -** Establishing minimum performance parameters introduces flexibility into the assay to enable evaluation of a range of human responses.

**Readiness for Method Transfer -** The benchmark reference chemical potency ranges establish quantitative parameters for evaluating proficiency of method transfer and reproducibility.

# Inter-laboratory Validation of the Human Thyroid Microtissue Assay

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

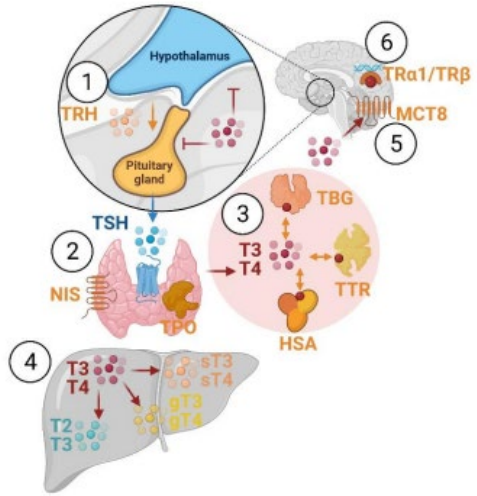

Independent Validation Management Team

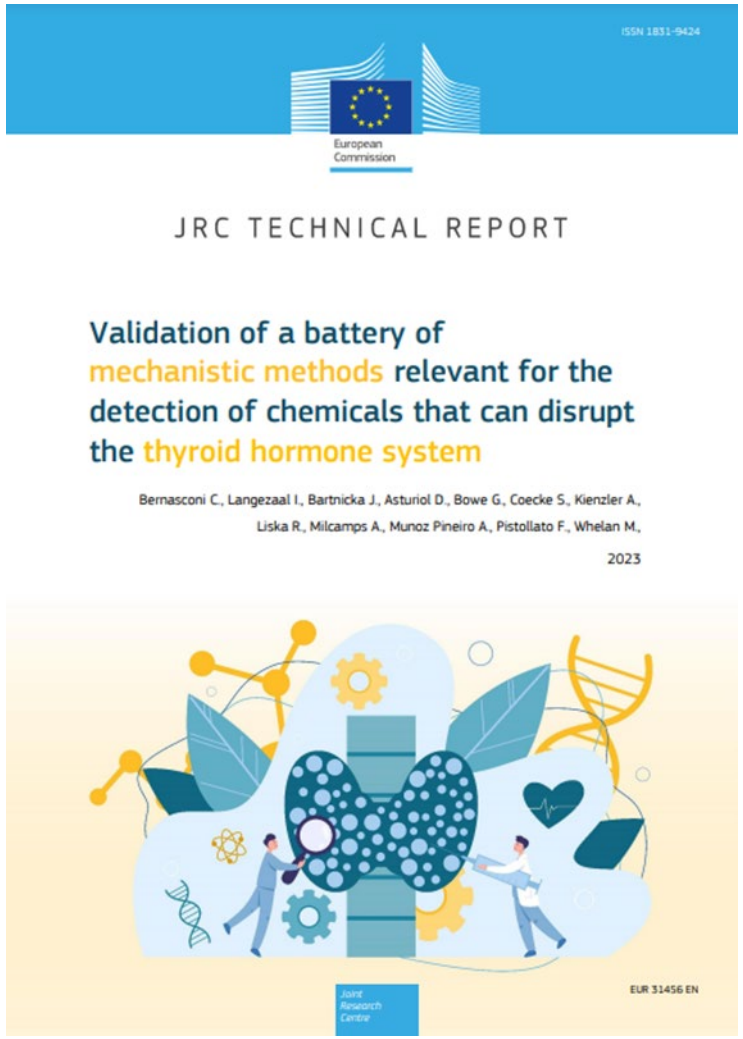
**Objectives**

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.

# International Thyroid Testing Landscape

| Block # | Description |
|---|---|
| 1 | Central regulation (HPT axis). |
| 2 | Thyroid Hormone (TH) synthesis. |
| 3 | Binding and transport in serum. |
| 4 | Metabolism and excretion (by hepatic deiodinases, glucuronidation and sulfation). |
| 5 | Local cellular concentrations (TH selective membrane transporters e.g. monocarboxylate transporter 8 (MCT8)). |
| 6 | Cellular responses (activation of specific nuclear receptors TRα and TRβ). |
| 7 | Relevant short term assays integrating multiple MoAs. |
| 8 | Integrative cellular assays |




EURL ECVAM Validation of Thyroid Test Methods

OECD

Working Party of the National Coordinators to the Test Guidelines Programme (WNT)

Thyroid Disruption Methods Expert Group



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