

Inter-laboratory Prevalidation Study of the Human Thyroid Microtissue Assay

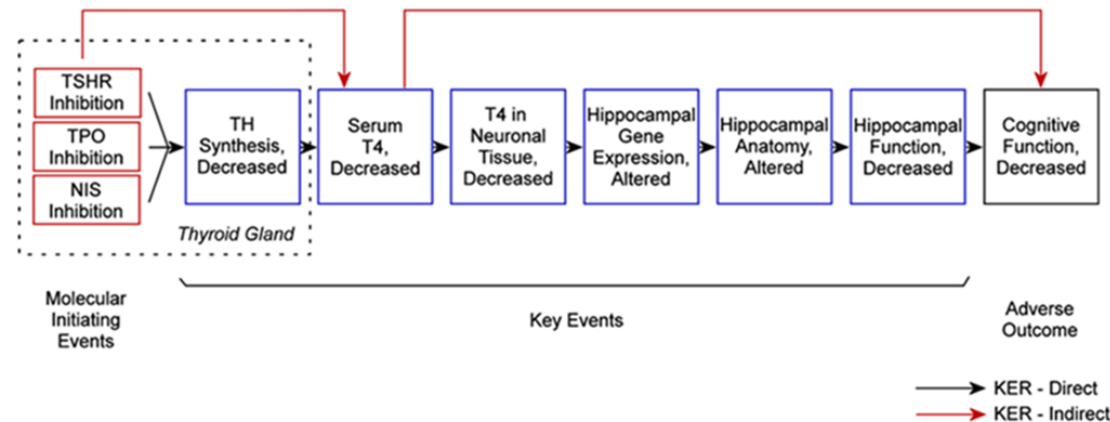
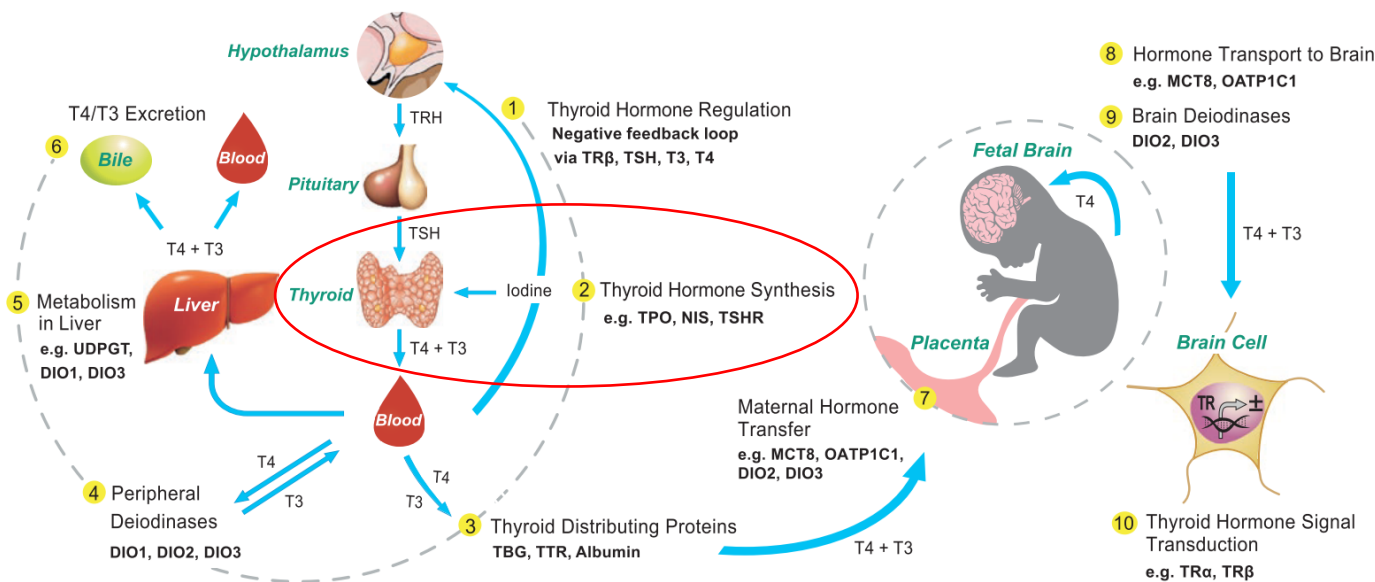
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Challenges with *In Vitro* Thyroid Testing: Thyroid HTS Assays Do Not Directly Measure Thyroid Hormone Disruption

Sites of Interference for Thyroid Disrupting Chemicals



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.

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



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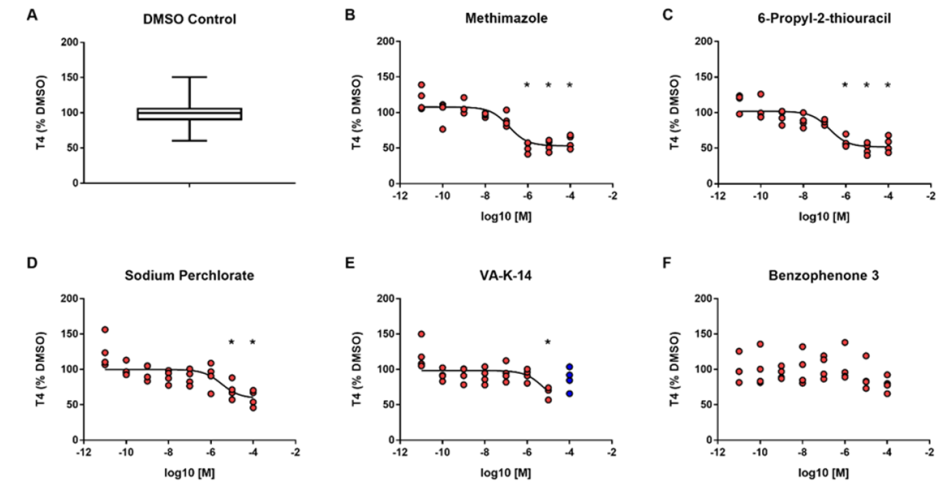
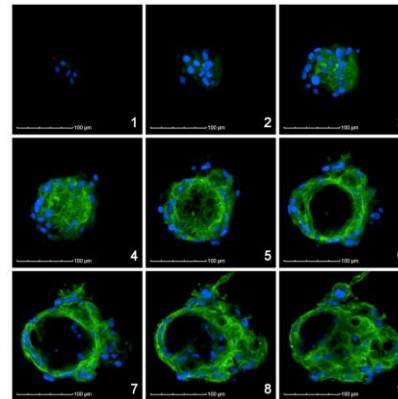
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Research Article

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

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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.

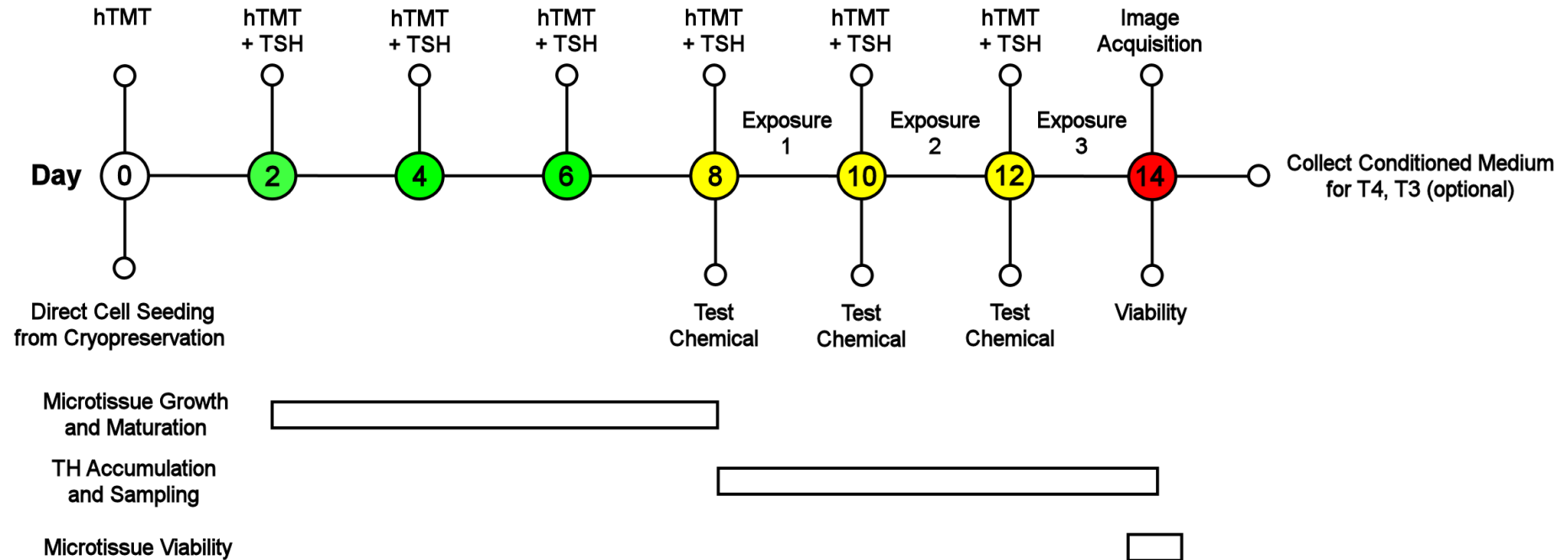
Innovation

- Developed a human thyroid microtissue assay to evaluate chemical effects on thyroid hormone synthesis, secretion, and tissue viability.

Impact

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

Revised Assay Workflow



Technical Modifications – Intended to streamline workflow efficiency for optimal model performance, minimize end-user learning curve, maintain apical endpoints that bridge *in vivo* observations.

- Developed a COA design for donor characterization and selection.
- Direct plating of cryopreserved cells.
- Optimized human Thyroid Microtissue (hTMT) medium.
- Increased test chemical exposure frequency and cumulative duration.
- Demonstration of thyroid epithelial cell pooling.

Team Members



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Proposed Inter-laboratory Prevalidation of the Human Thyroid Microtissue Assay

Goal: To structure and support a preliminary assessment of the test method reliability and relevance for submission to a larger validation study.

Objectives:

- 1) Collaborate on the overall study design, analytical approaches, chemical selection, and data interpretation for the human thyrocyte microtissue assay within respective capabilities and expertise.
- 2) Test method harmonization and standardization.
- 3) Test method transfer, training and intra-laboratory model performance evaluation.
- 4) Limited inter-laboratory reference chemical testing and assay performance evaluation.

Phase 1 - *Study Preparation*: Coordinate communication plan, establish experimental design, define lab reagents for distribution, characterize and select lots of human primary thyrocytes for distribution, reference chemical selection, draft and review standard operating protocols (SOPs).

Phase 2 - *Model Validation*: Conduct intra-laboratory performance evaluation (Lab 1-2 and VMT).

Phase 3 - *Method Transfer and Model Validation*: Method training, intra-laboratory performance evaluation (Lab 3-4 and VMT), SOP review (Lab 1-4 and VMT).

Phase 4 - *Assay Performance Evaluation*: Reference chemical procurement, inter-laboratory chemical testing and performance evaluation (Lab 1-4 and VMT).

Phase 5 – *Study Report*: Draft a joint manuscript for peer-reviewed publication (Lab 1-4 and VMT).