Inferring *in vivo* thyroid impacts from high-throughput *in vitro* data

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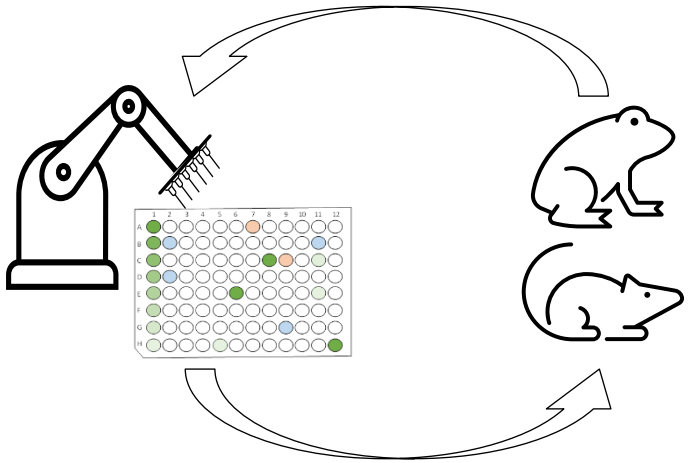
Overview

- Endocrine Disruptor Screening Program
- *in vitro* to *in vivo* framework
- High throughput screening at GLTED



History of the EDSP

1996	Food Quality Protection Act
1996	EPA Forms the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)
1998	EPA Publishes Endocrine Disruptor Screening Program Description
2000	EPA Submits Progress Report to Congress summarizing the program
2002	EPA Submits a Progress Report to Congress describing validation processes
2005	EPA Publishes the Final Approach for Initial Screening
2007	EPA Publishes a Draft First List of Chemicals for Screening
2009	EPA Publishes the Final List of Initial Pesticide Active Ingredients and Pesticide Inert Ingredients for Screening
2015	EPA Releases Screening Results of Endocrine Disruptor Screening for 52 Pesticide Chemicals
2015	EPA Announces the Use of Cutting-Edge Technology to Screen Chemicals
2023	Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program

https://www.epa.gov/endocrine-disruption

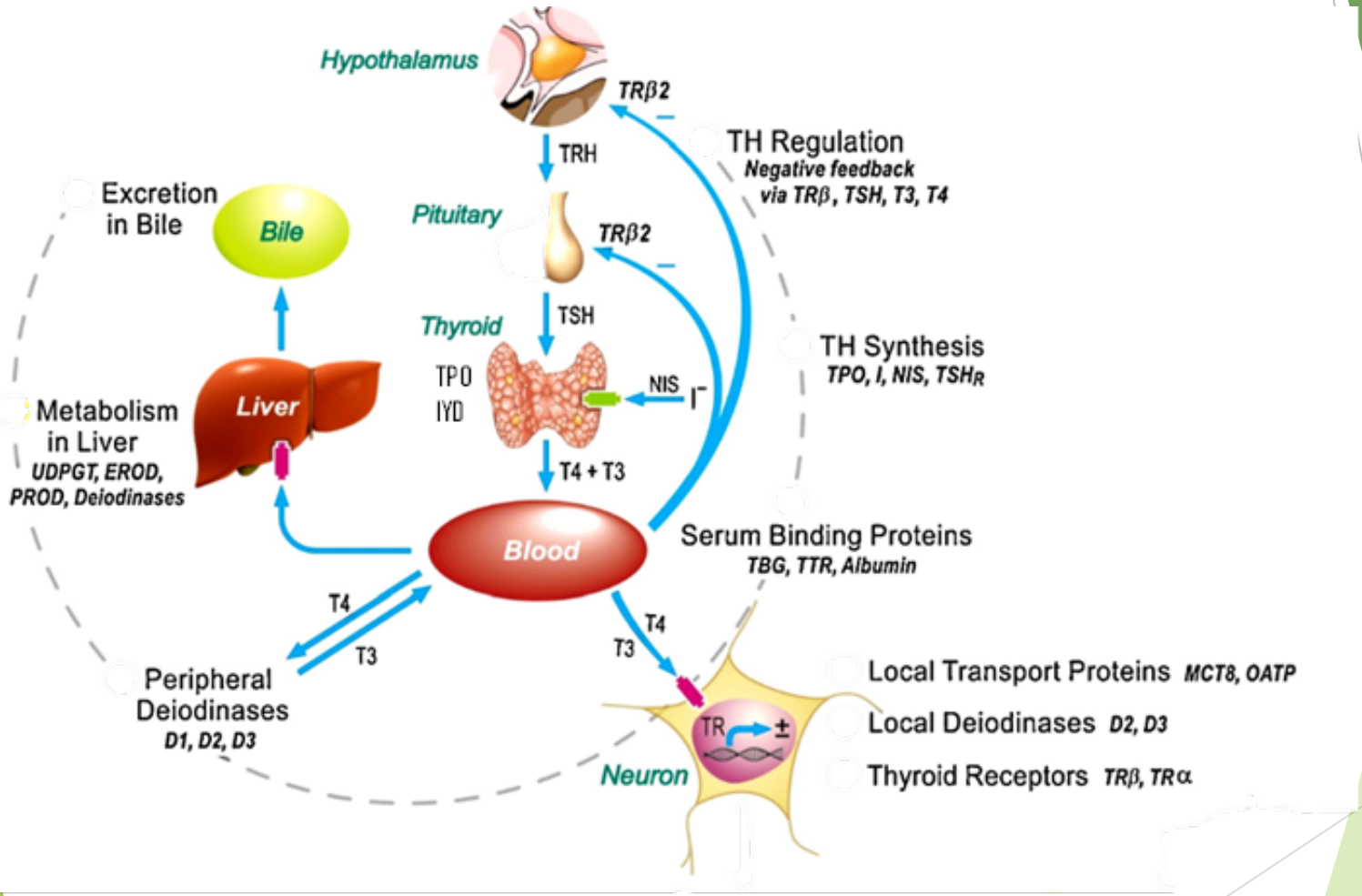
EDSP

- Two-tiered approach
  - Identify chemicals with the potential to interact with the endocrine system
  - Identify adverse endocrine effects and determine dose-effect relationship
- 52 chemicals on the first list
- Tier 1 testing
  - Five *in vitro* assays
  - Six *in vivo* assays

OECD Framework for Testing and Assessment of EDCs

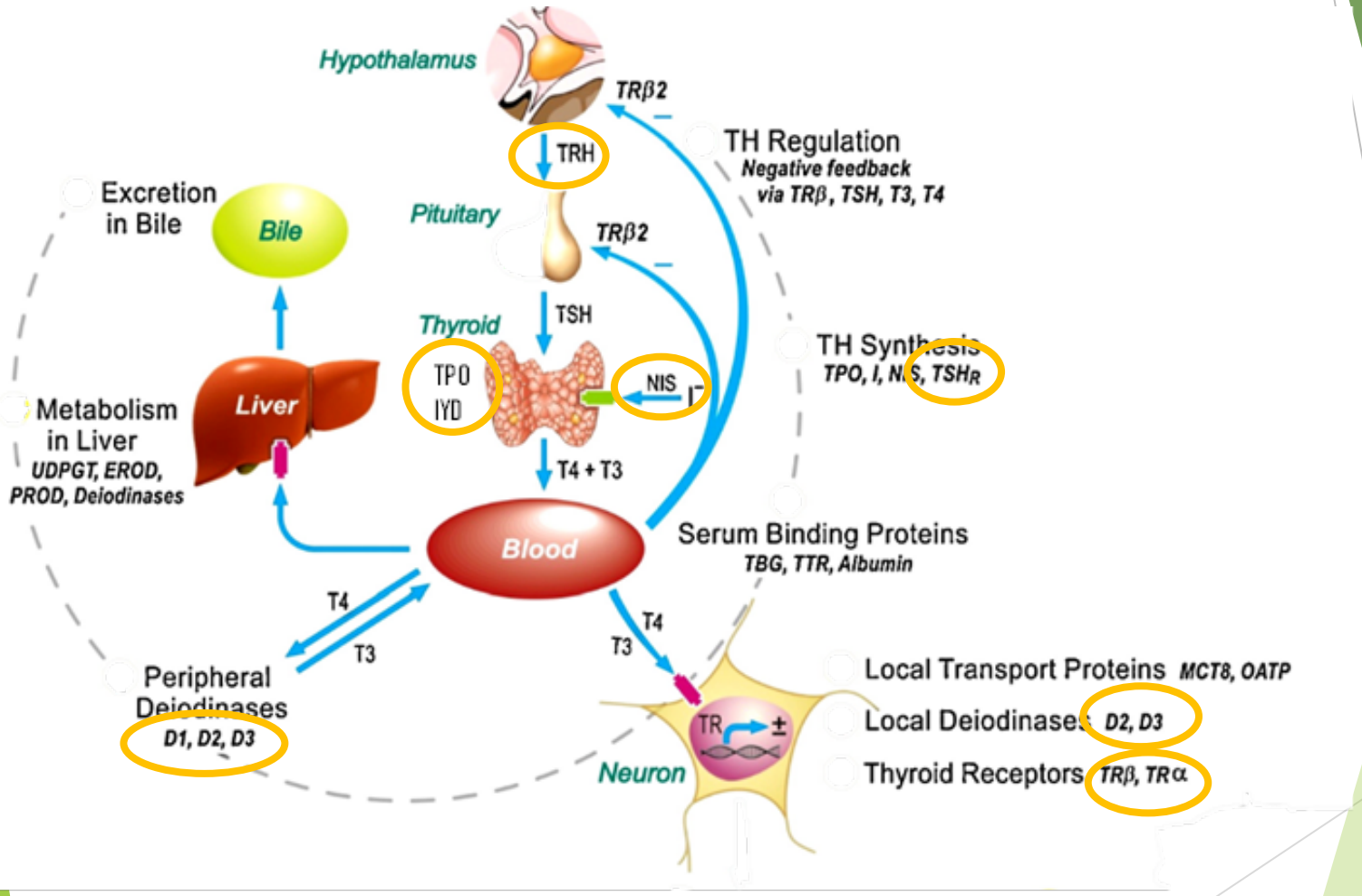
Level 1	Existing data and existing or new non-test information	-Physical/chemical properties -All available (eco)toxicological data -Read-across, chemical categories, QSAR/in silico predictions, ADME predictions
Level 2	In vitro assays providing data about selected endocrine mechanisms/pathways	-**Estrogen/androgen receptor binding affinity** (EDSP Tier 1) -**Estrogen**/androgen **receptor transactivation** (EDSP Tier 1) -**Steroidogenesis** (EDSP Tier 1) -**Aromatase assay** (EDSP Tier 1) -Thyroid disruption assays -Other hormone receptor assays as appropriate -High-throughput screens
Level 3	In vivo assays providing data about selected endocrine mechanisms/pathways	-**Uterotrophic assay** (EDSP Tier 1) -**Hershberger assay** (EDSP Tier 1) -**Amphibian metamorphosis assay** (AMA) (EDSP Tier 1) -**Fish short-term reproduction assay** (EDSP Tier1)
Level 4	In vivo assays providing data on adverse effects on endocrine-relevant endpoints	-**Male/female pubertal assays** (EDSP Tier 1) -Larval amphibian growth and development assay (EDSP Tier 2)
Level 5	In vivo assays providing more comprehensive data on adverse effects over more extensive parts of the life cycle of the organism	-Extended one-generation reproductive toxicity study -Two-generation reproduction toxicity study

Thyroid system



Gilbert et al. (2012) "Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences." Neurotoxicology **33**(4): 842-852. doi: 10.1016/j.neuro.2011.11.005

Thyroid system



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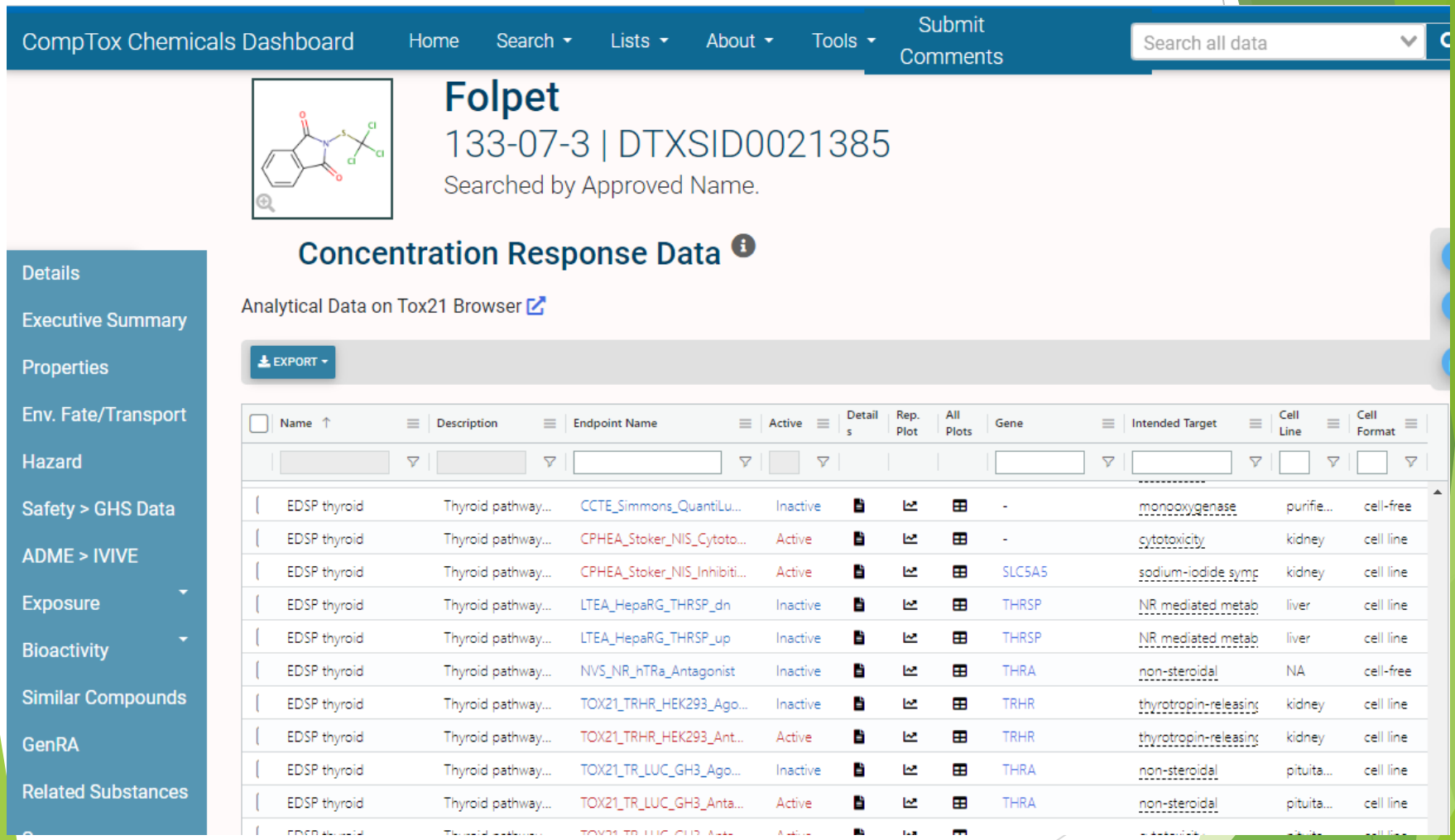
Objective

- Determine whether the currently available thyroid relevant HTS assay provide sufficient lines of evidence supporting direct action on receptors and proteins in the thyroid system

Approach

- Step 1
  - Pull available in vitro data from the CompTox Chemicals Dashboard for the 52 chemicals
  - 3 chemicals unavailable
    - Not amenable to aqueous, cell-based screening (acetone, fenbutatin oxide)
    - Not amenable to using DMSO as the sample diluent (glyphosate)

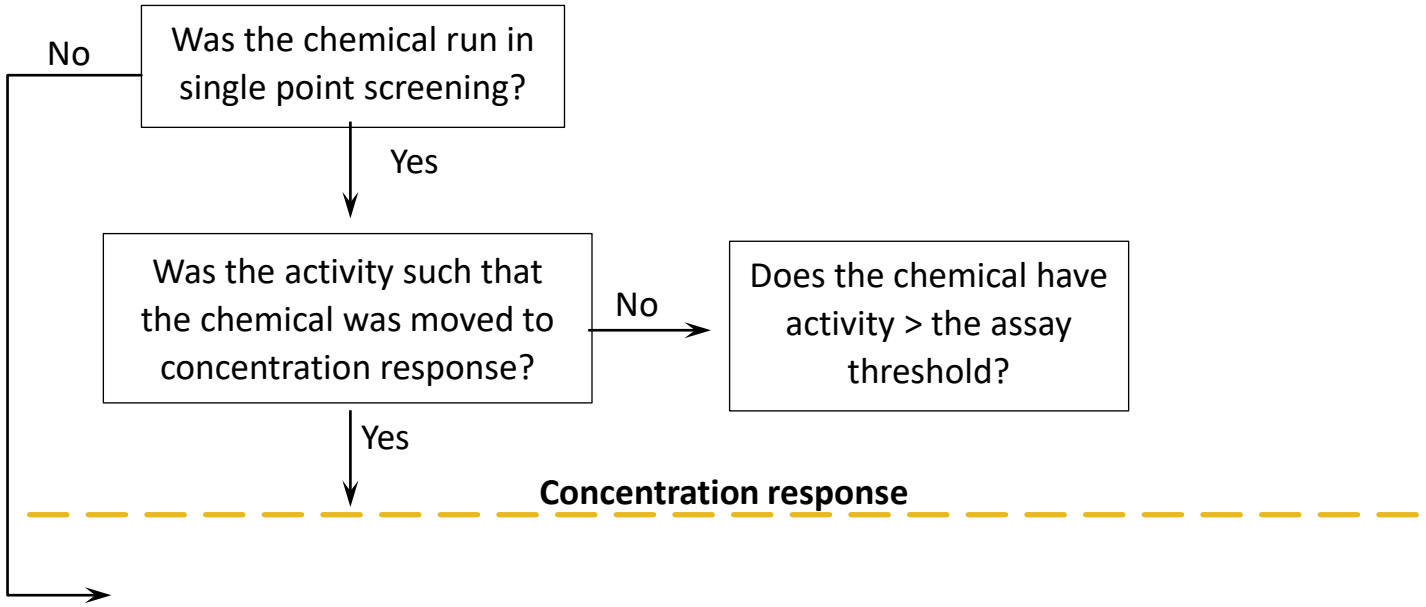
in vitro data



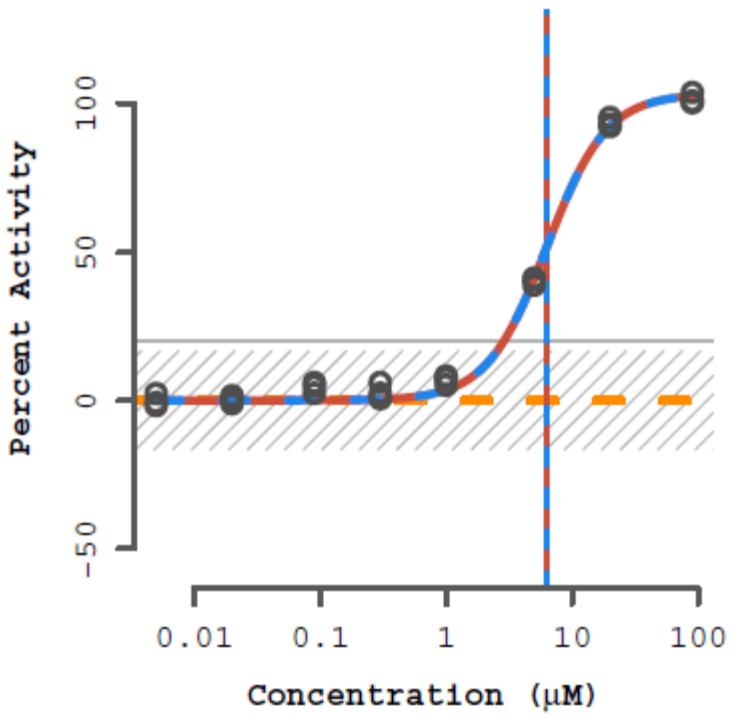
in vitro thyroid assays

Target	Assays
thyroid hormone receptor, alpha	NVS_NR_hTRa_Antagonist ATG_THRa1_TRANS_up ATG_THRa1_TRANS_dn TOX21_TR_LUC_GH3_Agonist
thyroid hormone receptor, beta	TOX21_TR_LUC_GH3_Agonist TOX21_TR_LUC_GH3_Antagonist
thyroid stimulating hormone receptor	TOX21_TSHR_HTRF_Agonist_ratio TOX21_TSHR_HTRF_Antagonist_ratio
background activity for TSHR assays	TOX21_TSHR_HTRF_wt_ratio
thyrotropin releasing hormone receptor	NVS_GPCR_rTRH
thyroid peroxidase	CCTE_Simmons_GUA_TPO_dn CCTE_Simmons_AUR_TPO_dn
nonspecific enzyme inhibition	CCTE_Simmons_QuantiLum_inhib_2_dn
sodium/iodide symporter	CPHEA_Stoker_NIS_Inhibition_RAIU
iodothyronine deiodinase	CCTE_GLTED_hDIO1_dn CCTE_GLTED_hDIO2_dn CCTE_GLTED_hDIO3_dn
iodotyrosine deiodinase	CCTE_GLTED_hIYD_dn
cytotoxicity	CCTE_Simmons_CellTiterGLO_HEK293T TOX21_TR_LUC_GH3_Antagonist _viability CPHEA_Stoker_NIS_Cytotoxicity

in vitro decision tree



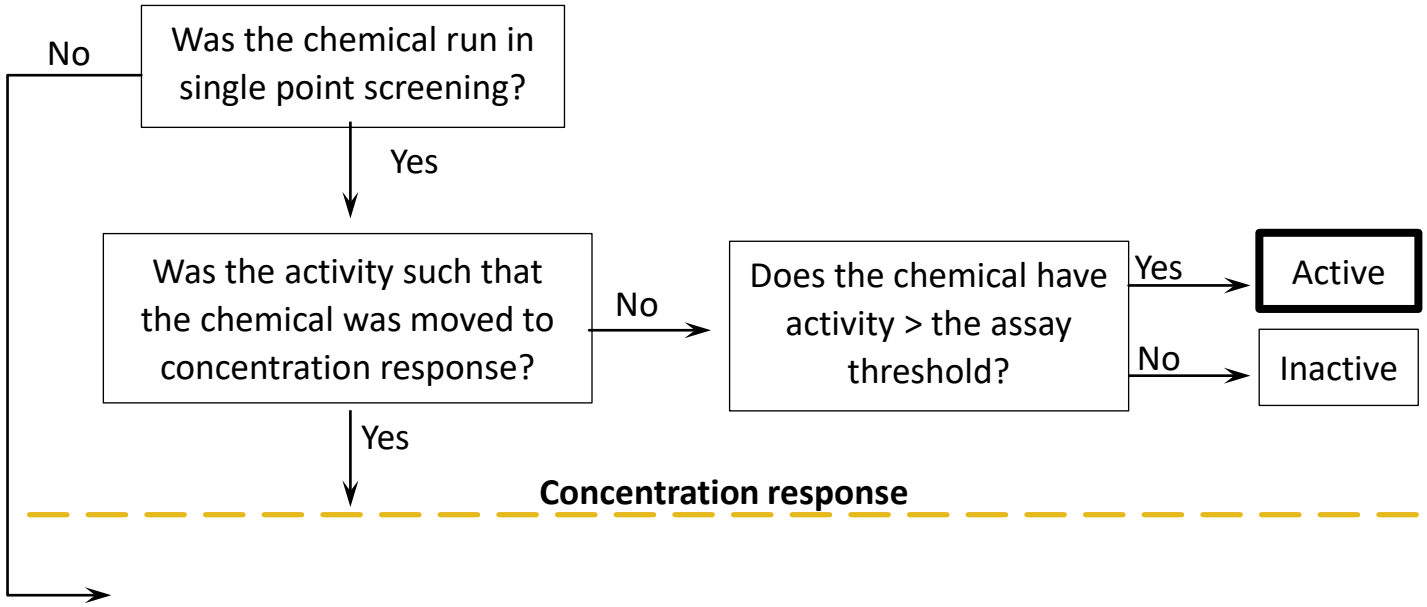
Activity threshold



ASSAY: AEID1508 (CCTE_Simmons_AUR_TPO_dn)

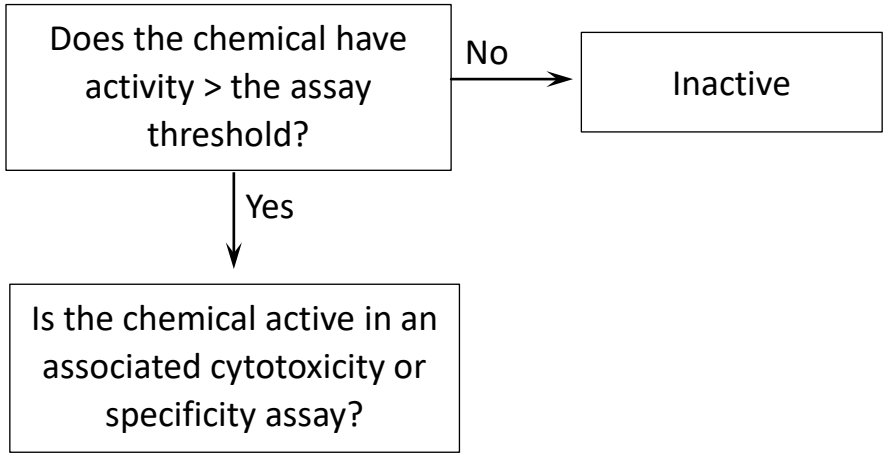
NAME: Dimethoate
CHID: 20479 CASRN: 60-51-5
SPID(S): TP0001194H18
M4ID: 9148681

in vitro decision tree



in vitro decision tree

Concentration response



Cytotoxicity or Specificity Assay

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NAME: Dimethoate
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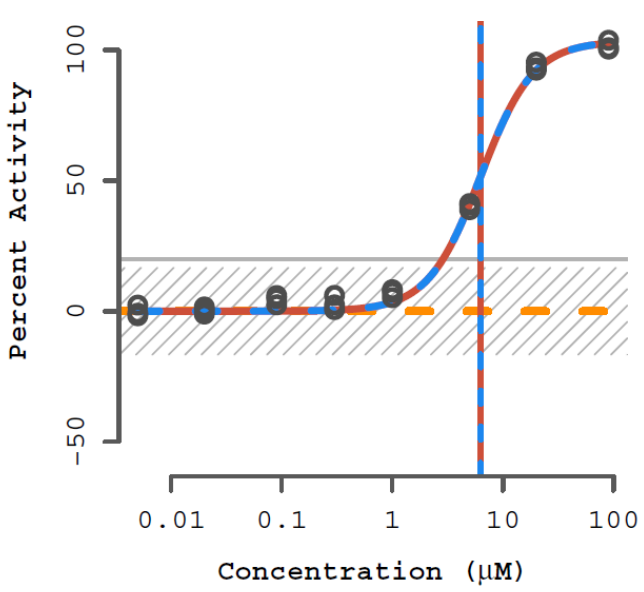
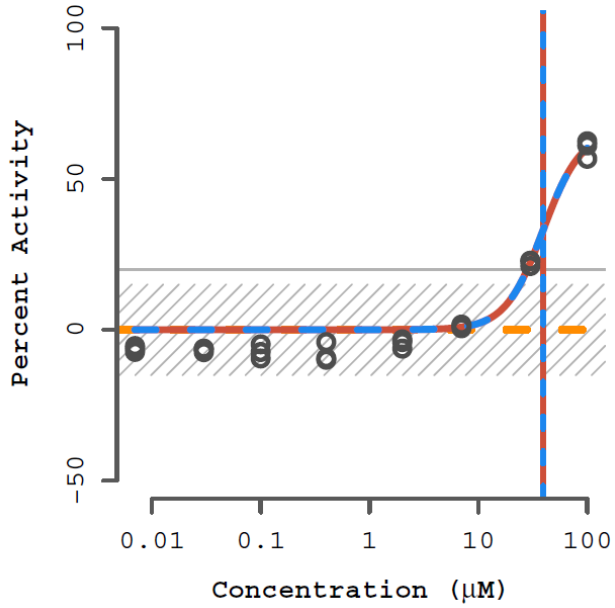
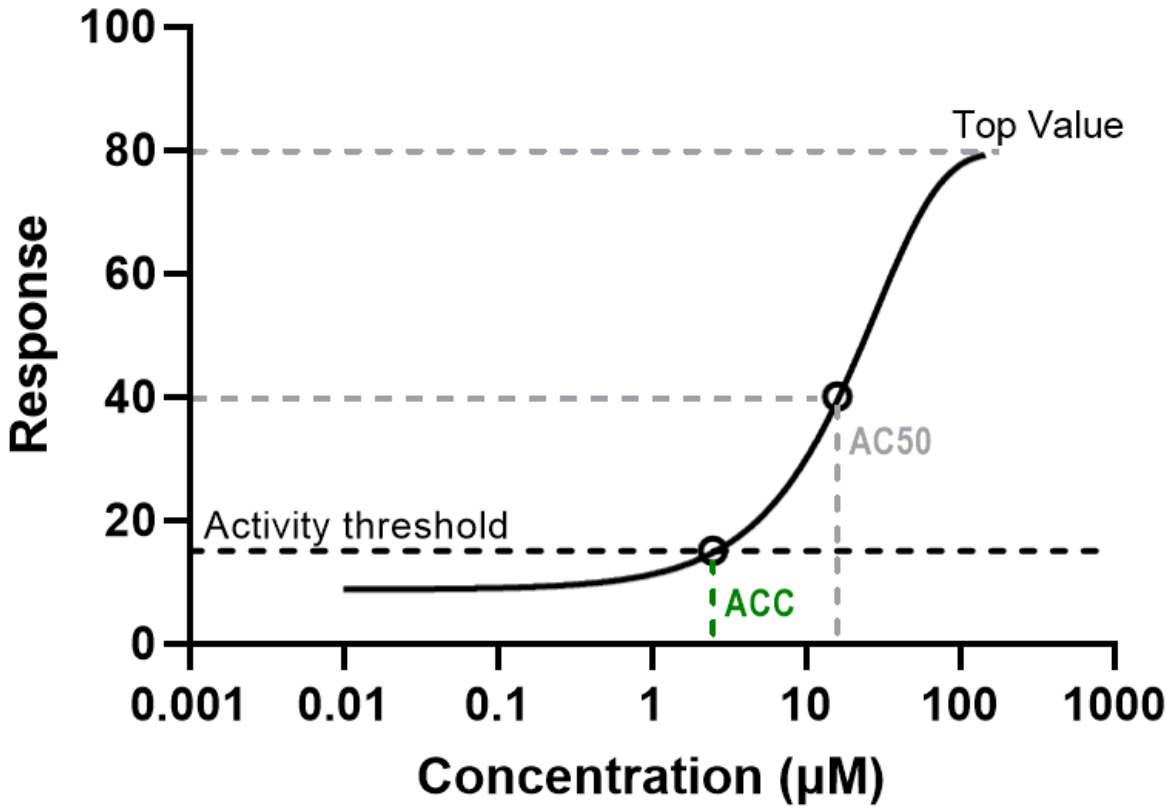


ASSAY: AEID1848 (CCTE_Simmons_QuantiLum_inhib_2_dn)

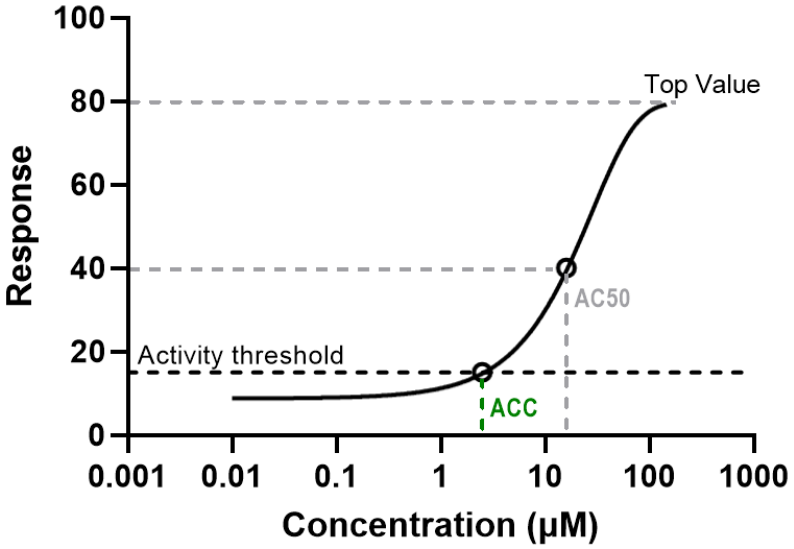
NAME: Dimethoate
CHID: 20479 CASRN: 60-51-5
SPID(S): TP0001194H18
M4ID: 9150226



Cytotoxicity or Specificity Assay



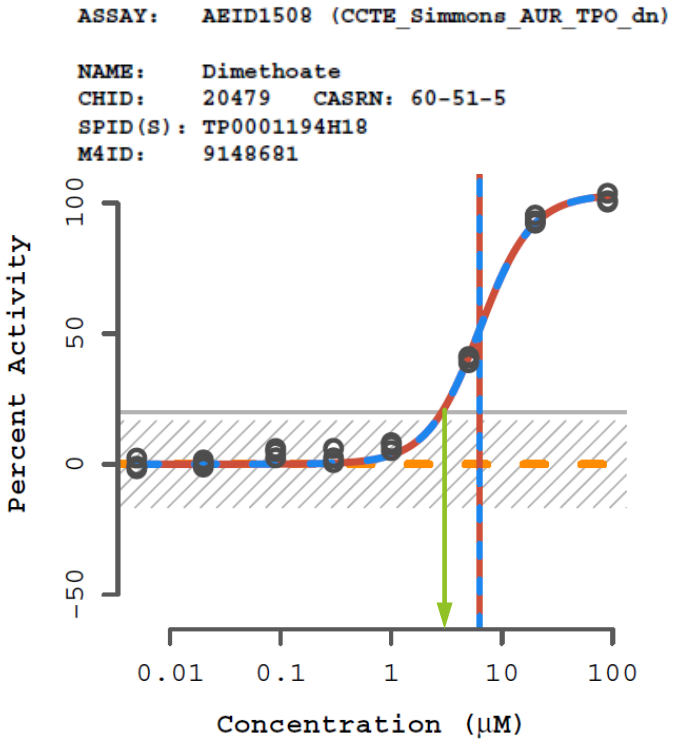
Cytotoxicity or Specificity Assay

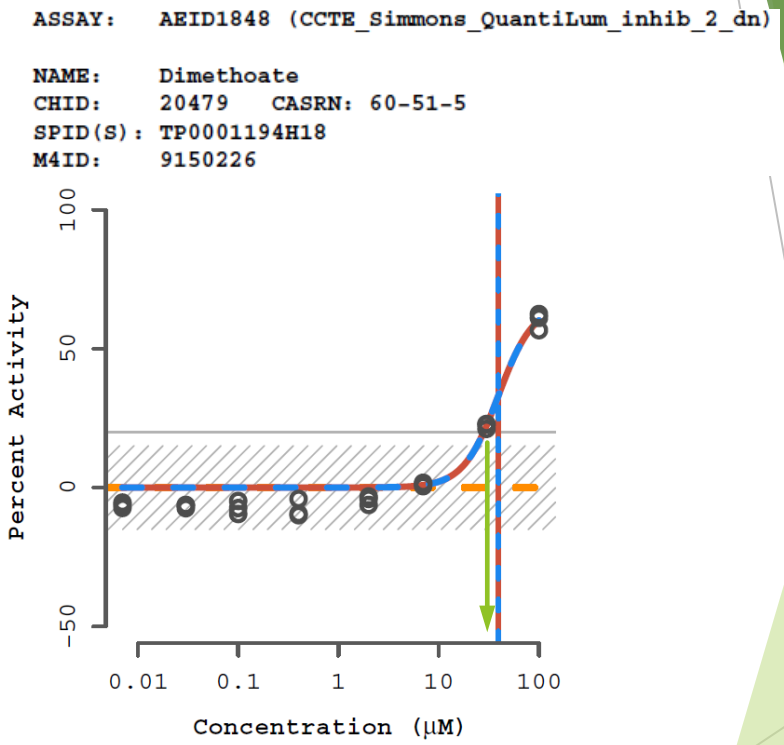


$$Selectivity\ score = \frac{ACC_{target}}{ACC_{parallel}}$$

SS < 1 = target
SS >1 = cytotoxic or not specific

Cytotoxicity or Specificity Assay

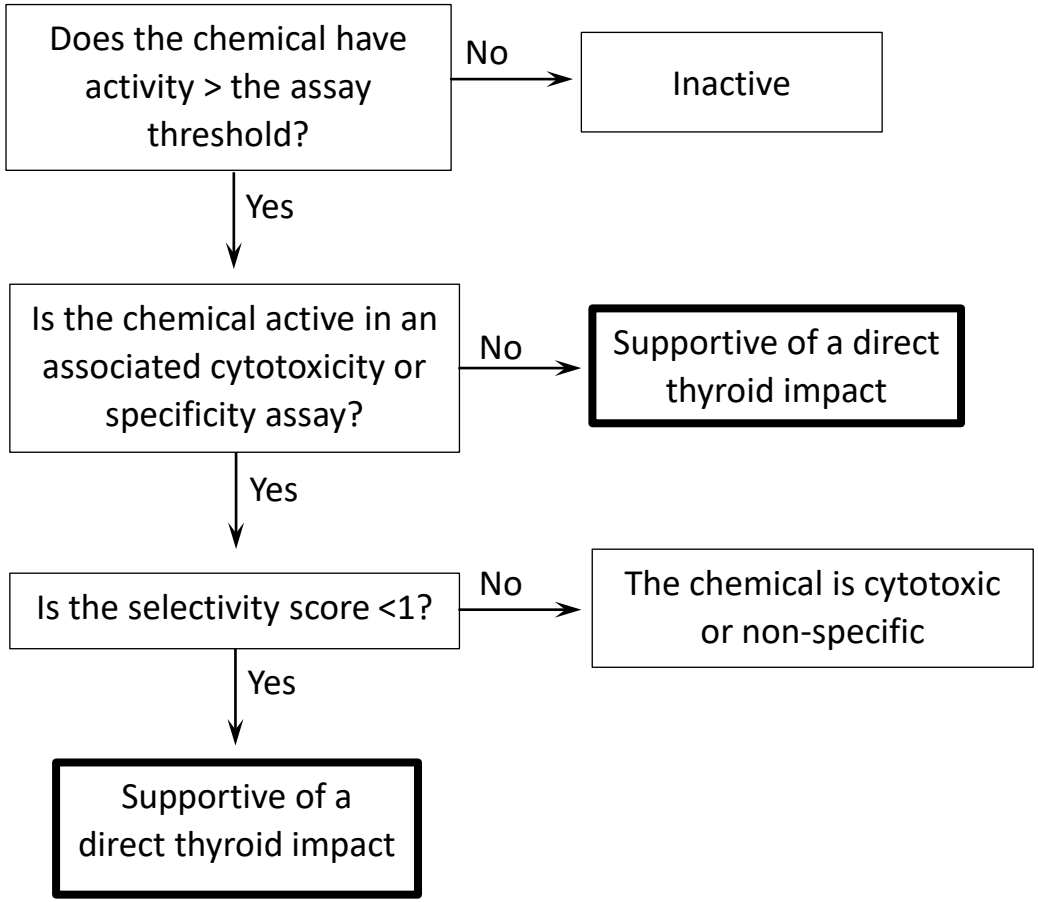




$$\frac{2.87}{28.13} = 0.102$$

in vitro decision tree

Concentration response



Approach

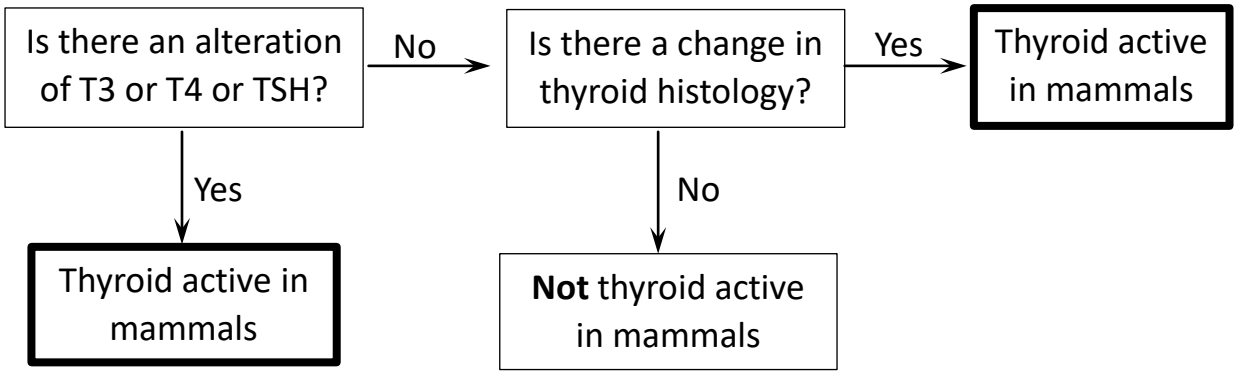
- Step 2
  - Pull available in vivo data from the weight of evidence documents
    - Tests that were ordered for Tier 1
    - 40 CFR Part 158
    - Other scientifically relevant information (OSRI)

in vivo data

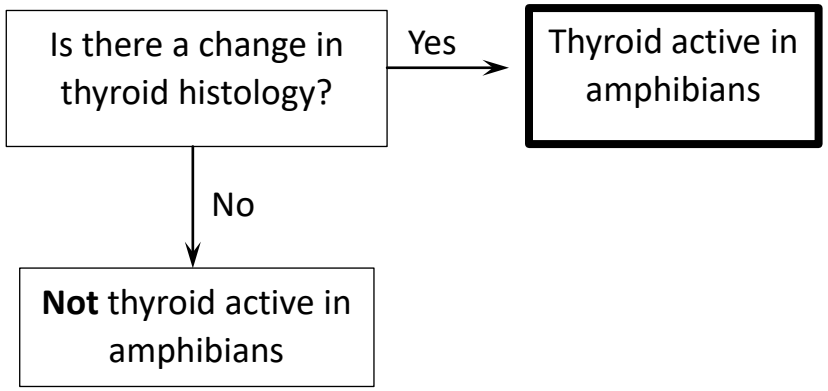
Assay	Guideline	Thyroid-specific endpoints
Pubertal Male (rat)	OCSPP 890.1500	Thyroid gland weight and histology, serum concentrations of TSH and T4
Pubertal Female (rat)	OCSPP 890.1450	Thyroid gland weight and histology, serum concentrations of TSH and T4
Amphibian metamorphosis (frog)	OCSPP 890.1100 / OECD 231	Development stage, histology of thyroid gland
90-day rodent (rat/mouse)	40 CFR Part 158	Thyroid histopathology
90-day dog (dog)	40 CFR Part 158	Thyroid organ weight and histopathology
One-year chronic dog (dog)	40 CFR Part 158	Thyroid histopathology, thyroid weight, and thyroid hormones
Chronic mouse study (mouse)	40 CFR Part 158	Thyroid histopathology, thyroid weight, and thyroid hormones
Chronic rat study (rat)	40 CFR Part 158	Thyroid histopathology, thyroid weight, and thyroid hormones

in vivo decision tree

Male/female pubertal assays, Part 158, OSRI



Amphibian Metamorphosis Assay



in vivo results

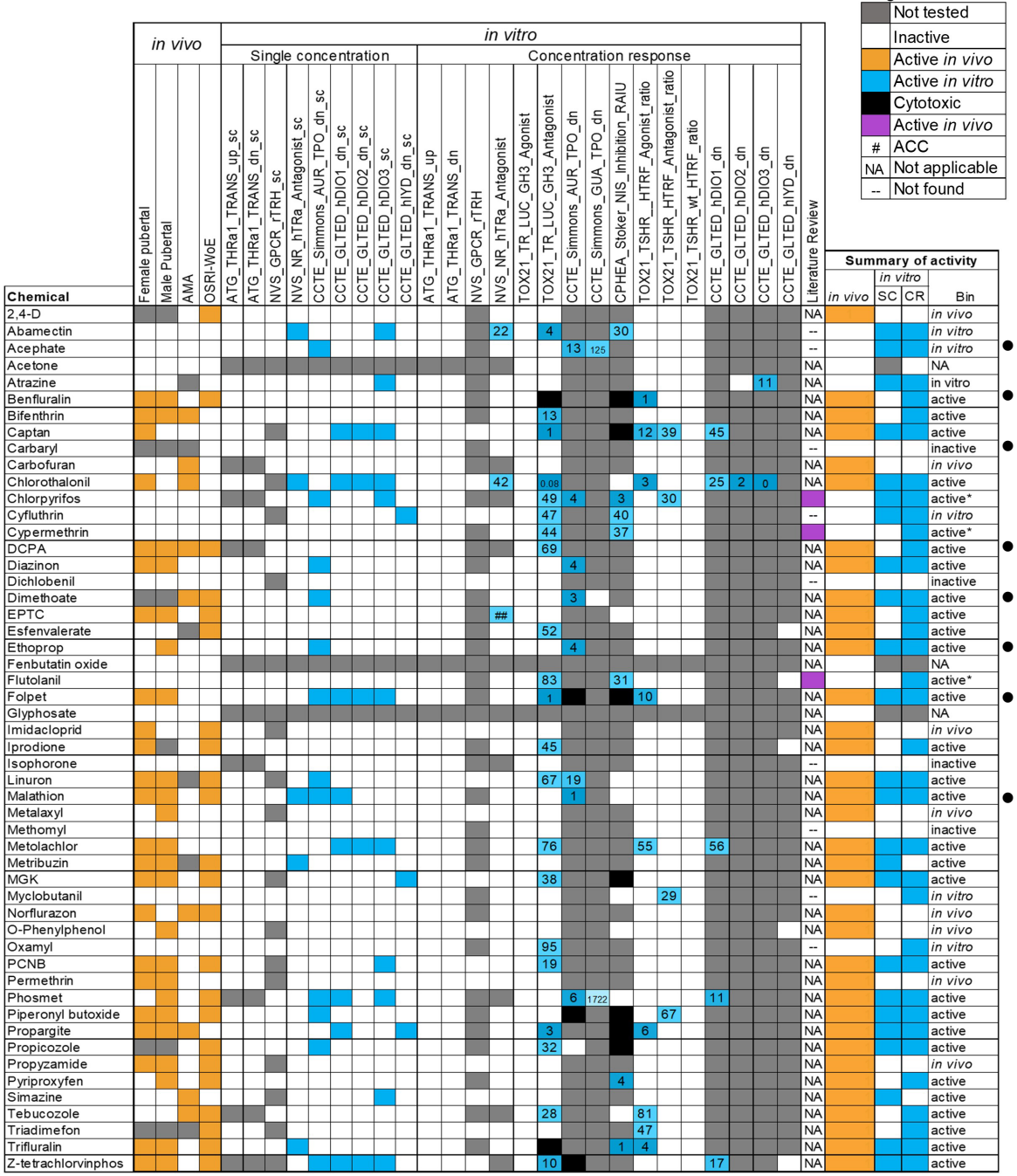
- 36 chemicals active *in vivo* based on WoE
  - 27 active in mammals
  - 2 active in AMA only
  - 7 active in both

Approach

- Step 3
  - Literature review
    - Chemicals that were active in vitro or inactive
    - Recent studies demonstrating thyroid impacts of these chemicals in vivo?

Literature review

- Review of literature for *in vivo* effects
  - *in vitro* only
    - Found *in vivo* impacts for 3 of 9 chemicals
  - Inactive
    - No studies found meeting our data quality requirements



- 49 chemicals screened *in vitro*
- 37 active *in vitro*
- 39 active in vivo

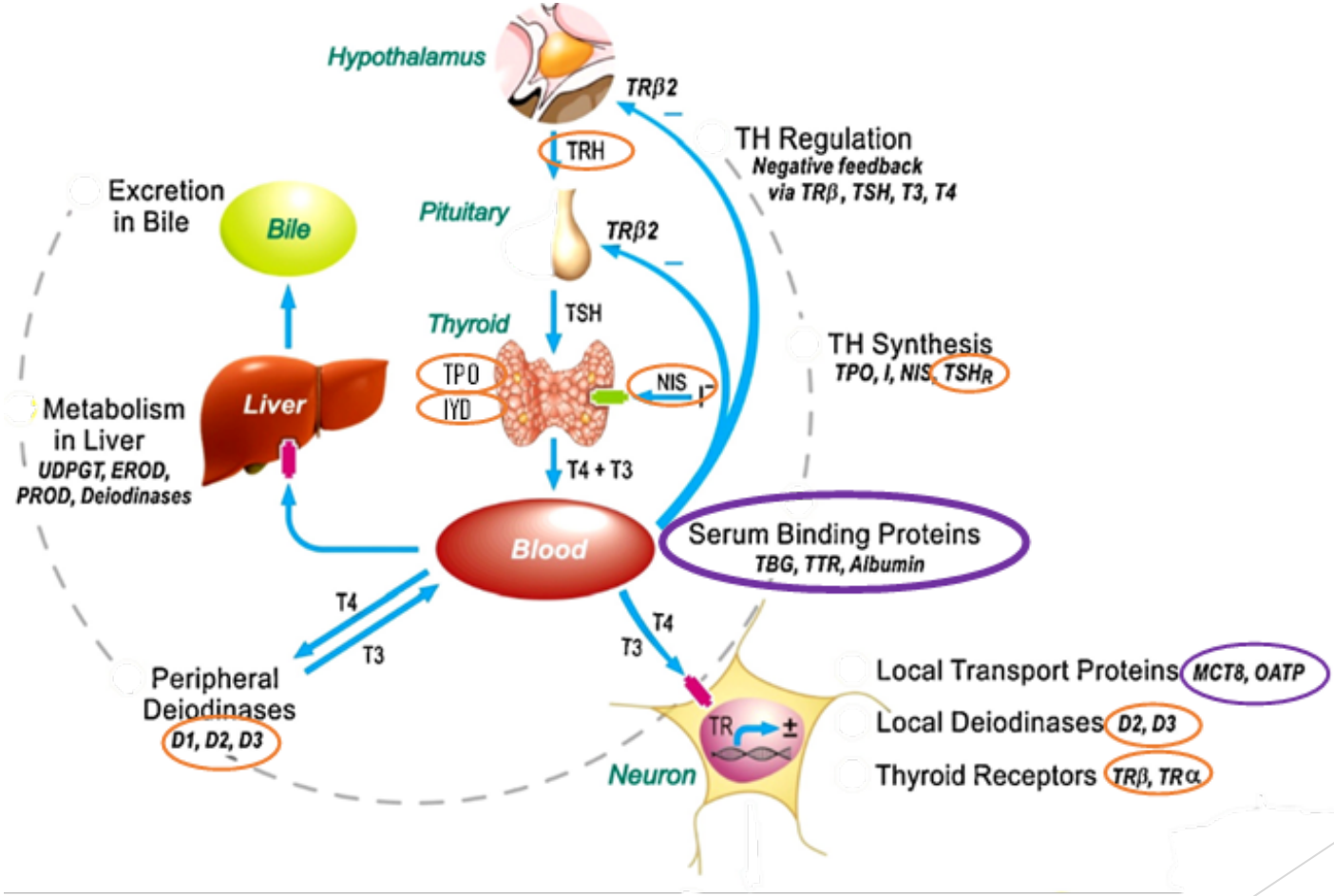
- 31 chemicals overlap
- 6 active only in vitro (12%)
- 8 active only in vivo (16%)
- 4 inactive

- 71% concordance (35/49)

Limitations

- Sensitivity in HTS
- Lack of metabolic capability
  - May be protective (elimination)
  - Metabolites may be more toxic
- Research is newer than for ER/AR
  - Lacking redundancy/confirmatory assays
  - Lack of reference chemicals
- Thyroid system is very complex
  - feedback
  - **multiple targets**

Gaps in *in vitro* assays



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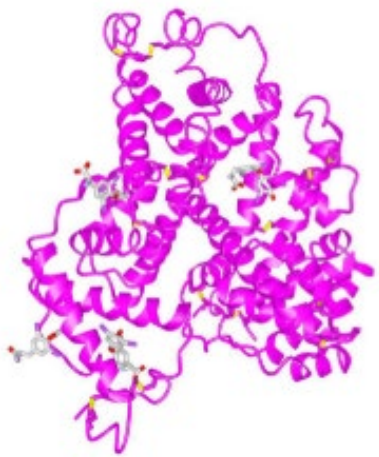
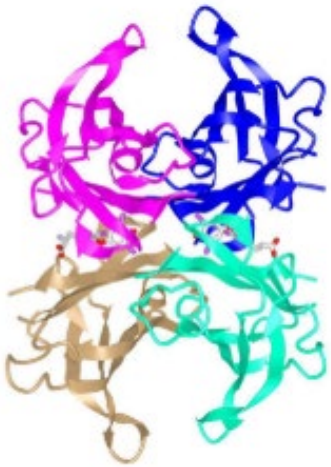
Thyroid hormone distributor proteins



Albumin

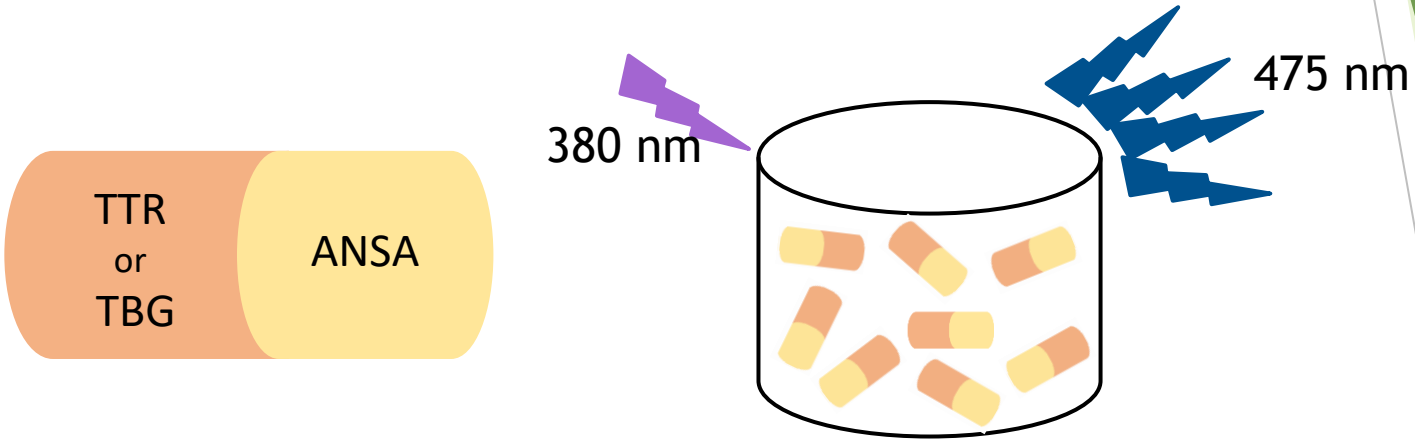


Transthyretin

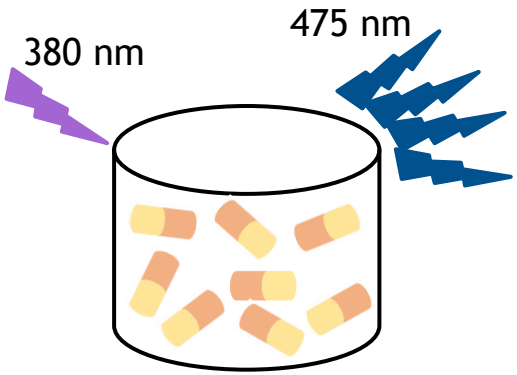
Thyroxine-binding globulin

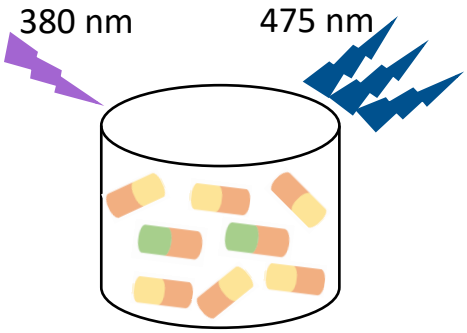
Rabah, S. A., et al. (2019). "Thyroid Hormone Distributor Proteins During Development in Vertebrates." Front Endocrinol (Lausanne) **10**: 506. doi: 10.3389/fendo.2019.00506

Fluorescence assays

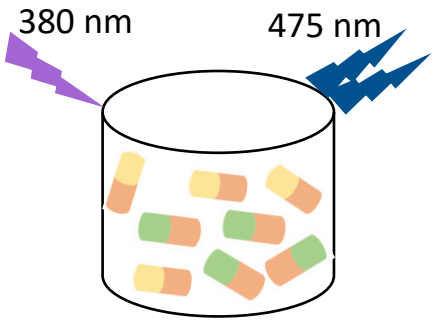


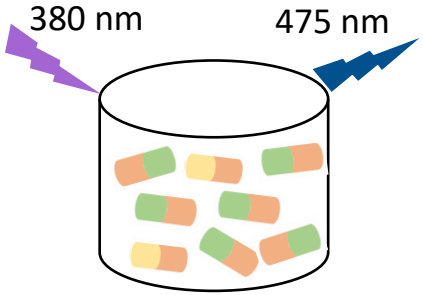
T4 curve





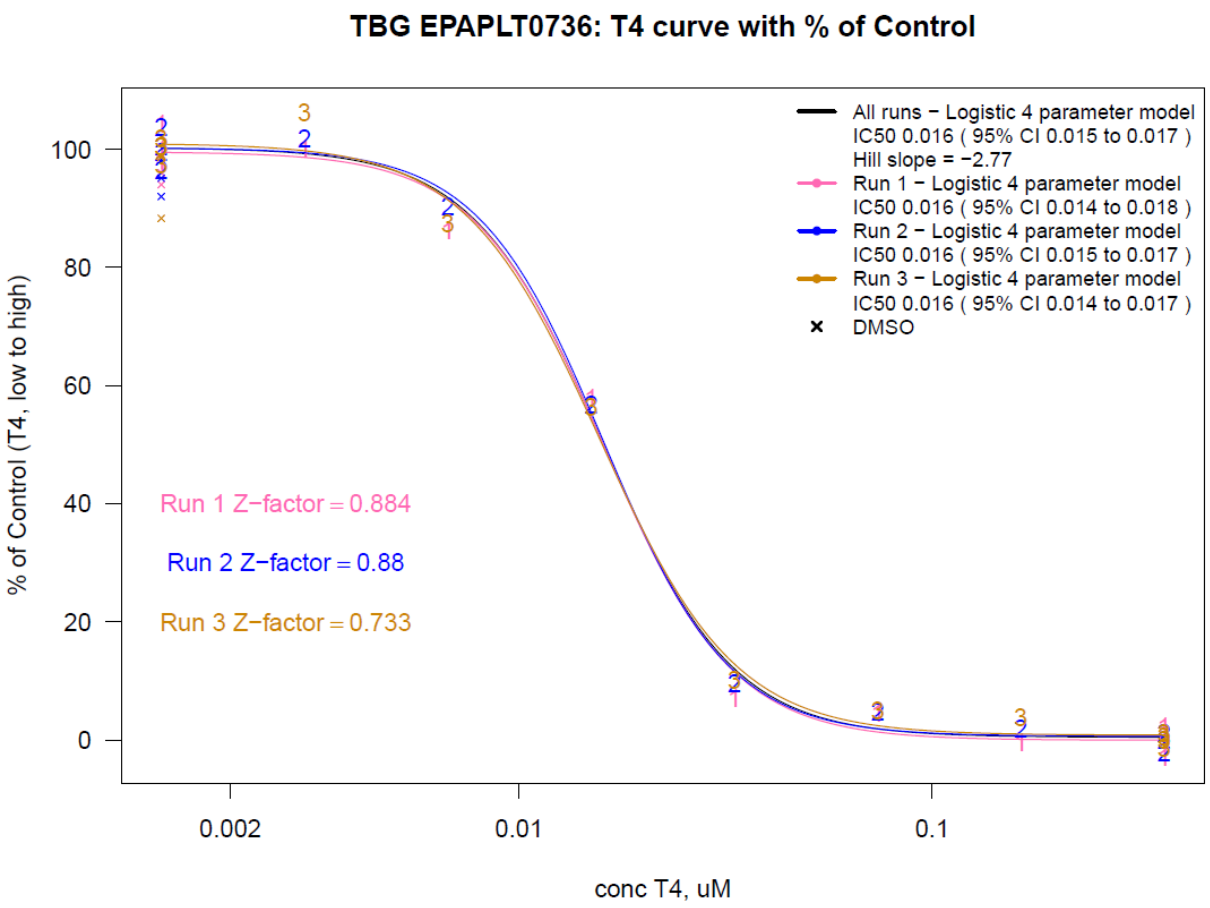
low [T4]



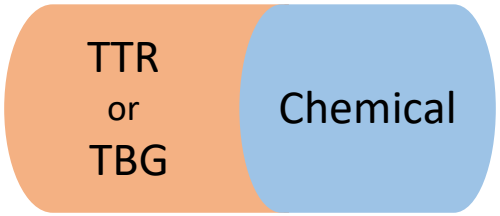


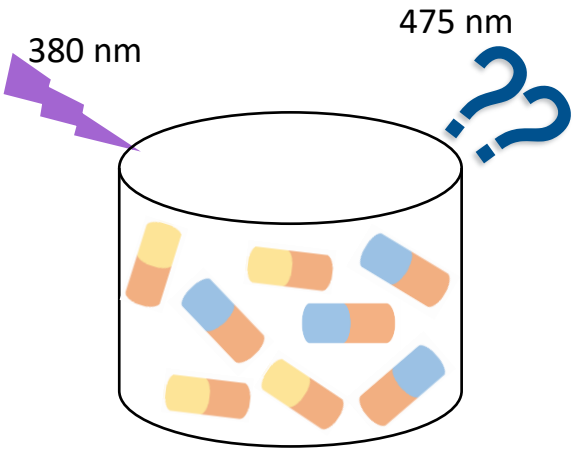
high [T4]

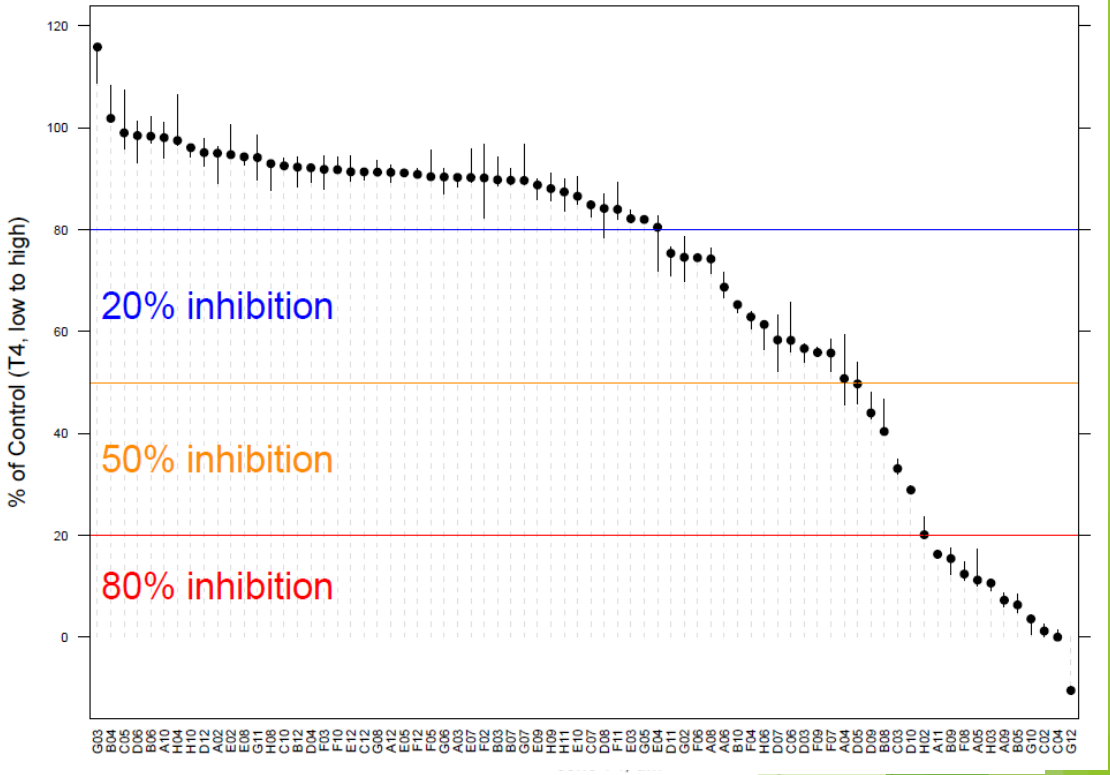
T4 curve


TBG EPAPLT0736: T4 curve with % of Control
All runs − Logistic 4 parameter model
IC50 0.016 (95% CI 0.015 to 0.017)
Hill slope = −2.77
Run 1 − Logistic 4 parameter model
IC50 0.016 (95% CI 0.014 to 0.018)
Run 2 − Logistic 4 parameter model
IC50 0.016 (95% CI 0.015 to 0.017)
Run 3 − Logistic 4 parameter model
IC50 0.016 (95% CI 0.014 to 0.017)
DMSO
Run 1 Z−factor = 0.884
Run 2 Z−factor = 0.88
Run 3 Z−factor = 0.733
% of Control (T4, low to high)
100
80
60
40
20
0
0.002
0.01
0.1
conc T4, uM

Chemical screening





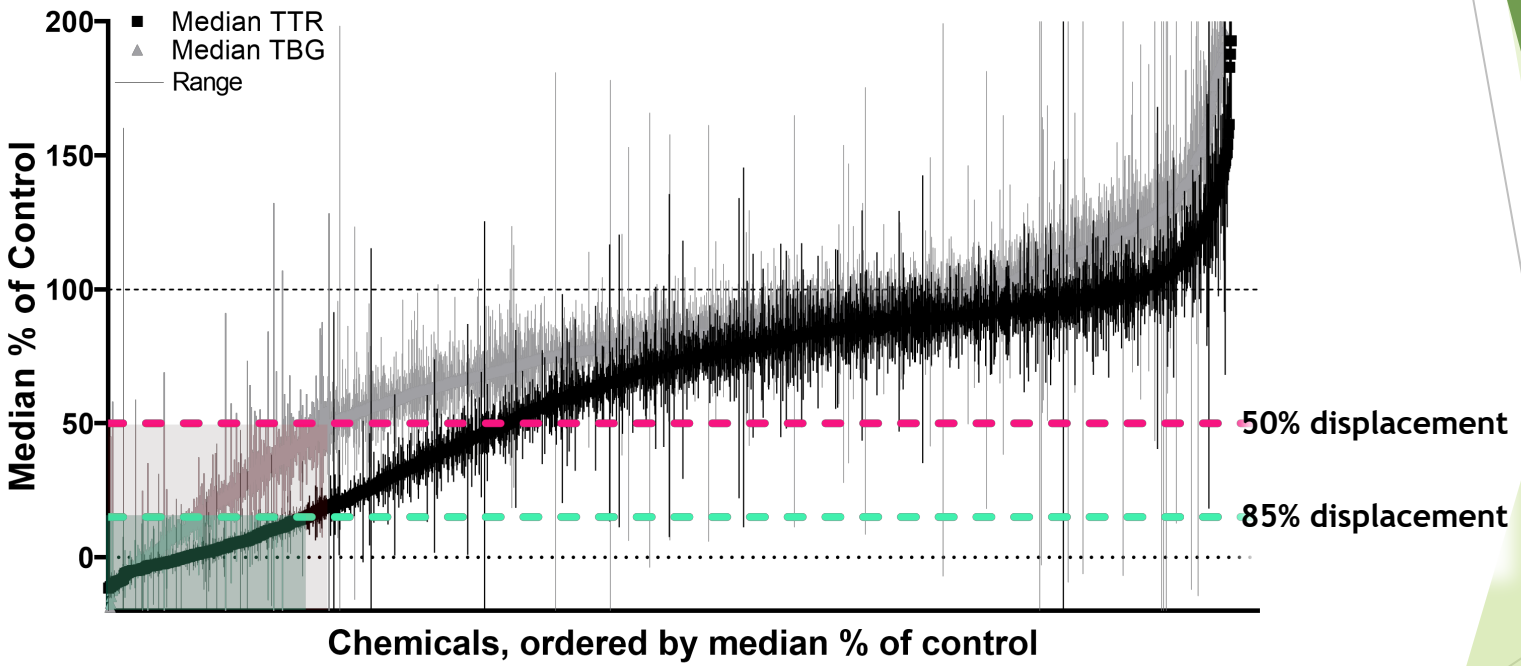


High throughput

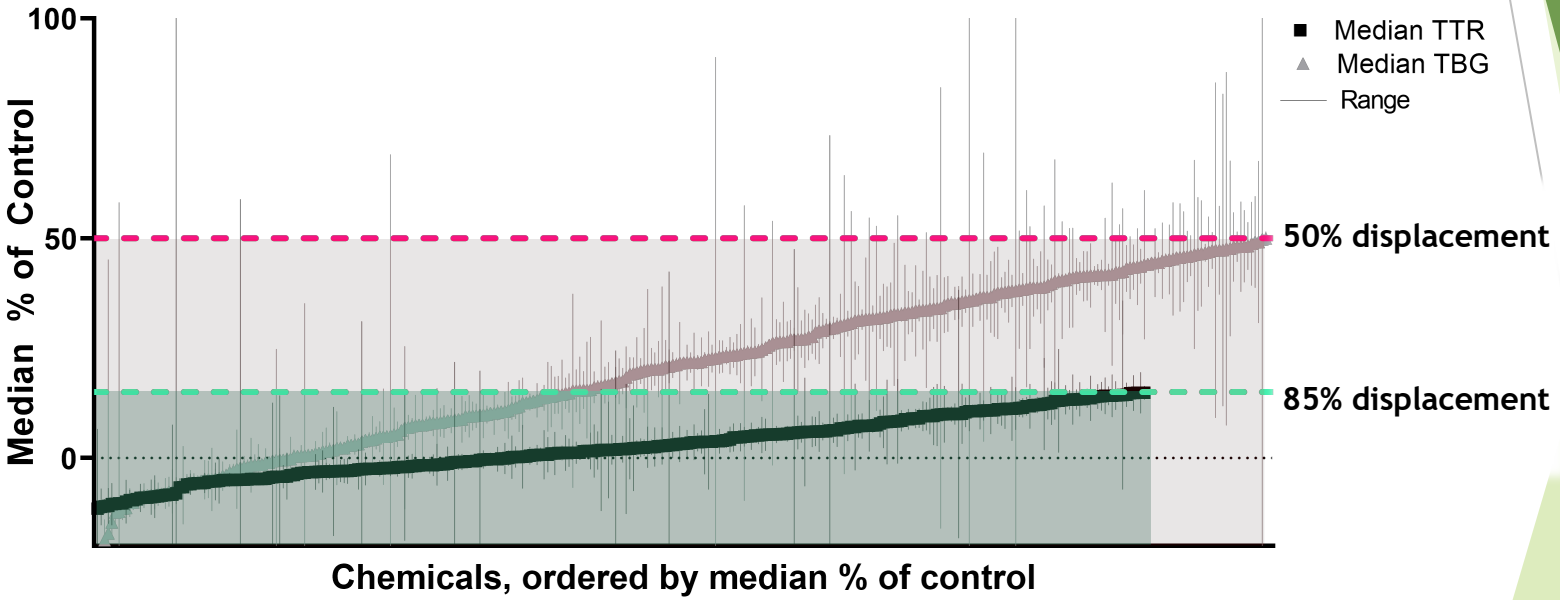
Screening Approach

- Single concentration
  - Ranged from 25 µM to 100 µM
  - Chemicals active above activity threshold moved onto concentration response
- Concentration response
  - 12 point or 8 point curves

Single concentration screening



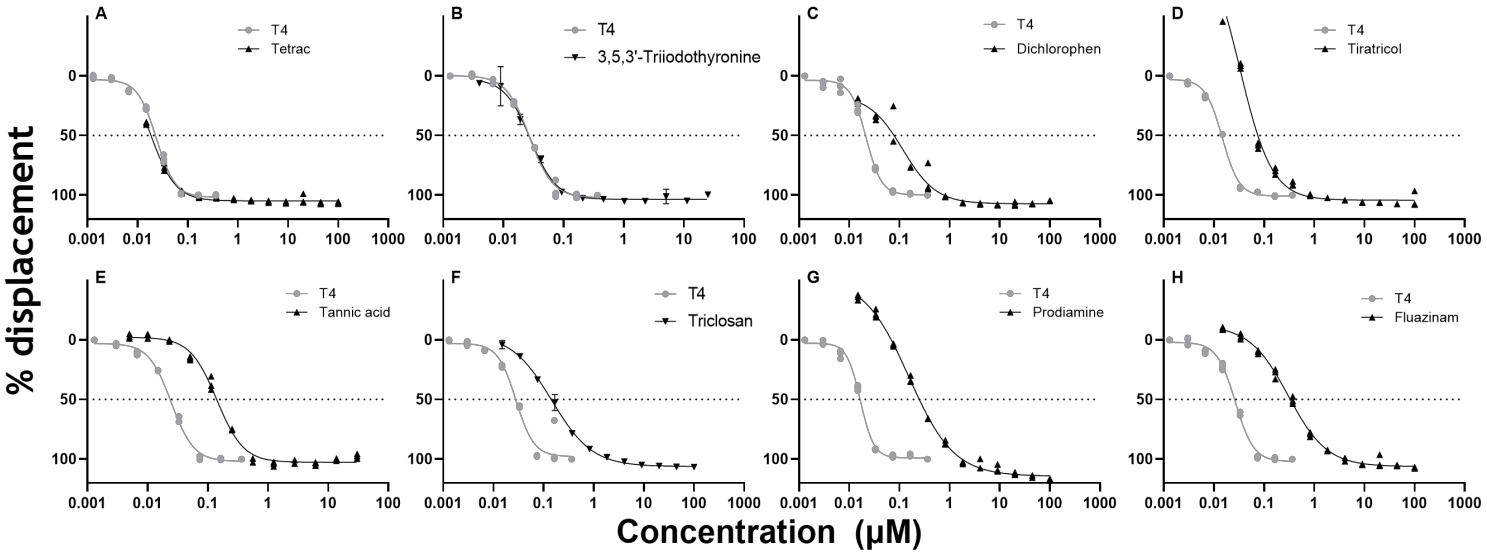
Single concentration screening



Single concentration screening

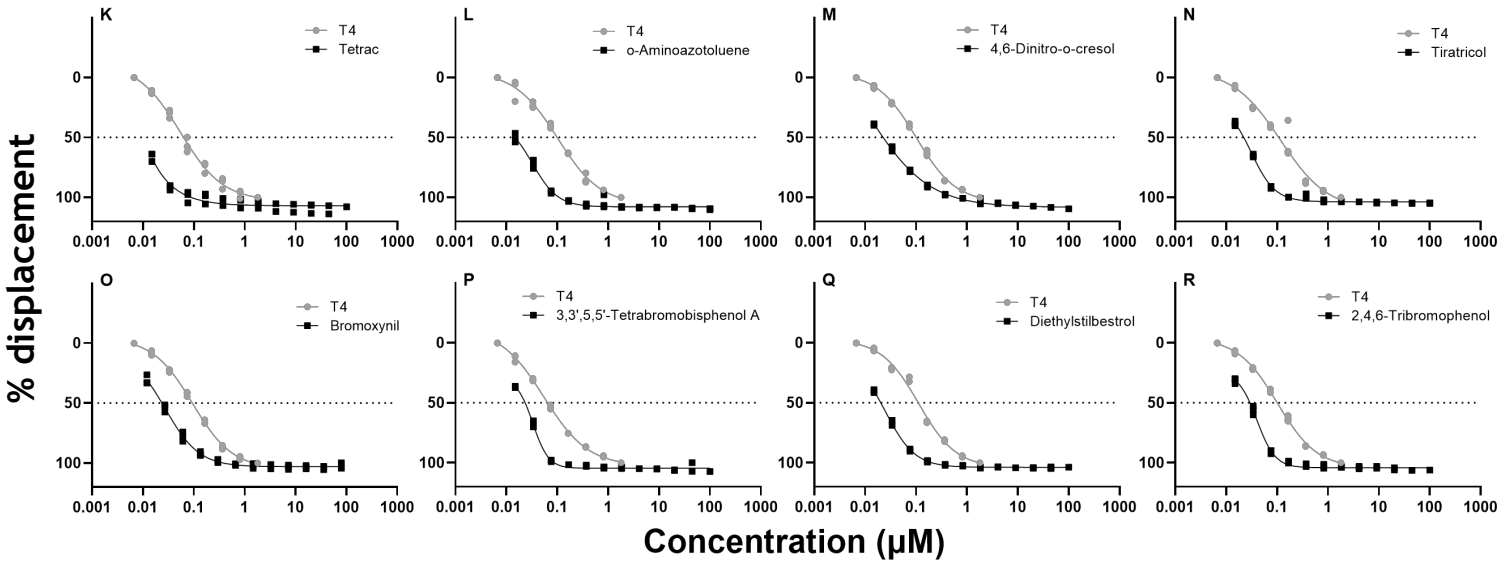
	TBG		TTR	
% displacement	Total # Chemicals	%	Total # Chemicals	%
NA	118	7	146	8
<20%	918	51	698	38
≥20%	777	43	969	53
≥50%	328	18	591	32
≥85%	133	7	294	16
Total	1813		1813	

Concentration response screening



Plot	Chemical name	Rank in SC	[max] (µM)	Median % activity SC at [max]	Median % activity CR at [max]	Conc Response IC50 (µM)	Conc Response Hill Slope
A	Tetrac	2	100	122.2	106.5	0.022	-3.589
B	3,5,3'-Triiodothyronine	96	25	92.0	104.6	0.026	-1.859
C	Dichlorophen	16	100	108.4	107.7	0.066	-1.037
D	Tiratricol	21	100	107.6	107.4	0.088	-1.754
E	Tannic acid	114	35	89.5	103.8	0.129	-1.939
F	Triclosan	12	100	109.5	106.6	0.153	-1.144
G	Prodiamine	11	100	110.1	118.0	0.307	-1.674
H	Fluazinam	9	100	111.3	106.9	0.382	-1.465

Concentration response screening



				Median % activity		Conc Response	
Plot	Chemical name	Rank in SC	[max] (µM)	SC at [max]	CR at [max]	IC50 (µM)	Hill Slope
K	Tetrac	36	100	105.0	107.7	0.017	-3.313
L	o-Aminoazotoluene	3	100	111.0	109.4	0.018	-1.445
M	4,6-Dinitro-o-cresol	9	100	109.9	109.5	0.020	-0.778
N	Tiratricol	40	100	105.0	104.7	0.021	-1.505
O	Bromoxynil	90	80	101.8	103.5	0.022	-1.209
P	3,3’,5,5’-Tetrabromobisphenol A	28	100	105.8	107.2	0.024	-2.744
Q	3,5,3-Triiodothyronine	61	100	103.2	104.3	0.025	-1.604
R	Acid Red 337	38	100	105.0	106.3	0.028	-1.961

Takeaways

- HTS can aid in identifying chemicals with the potential for endocrine disruption and are useful for ranking/prioritization for *in vivo* testing

- Developing HTS assays targeting additional MIEs or adding redundancy to the already available assays will benefit a WoE approach for identifying thyroid-disruptive chemicals

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Questions?