Finding Endocrine Bioactivity and Predictions in the CompTox Chemicals Dashboard

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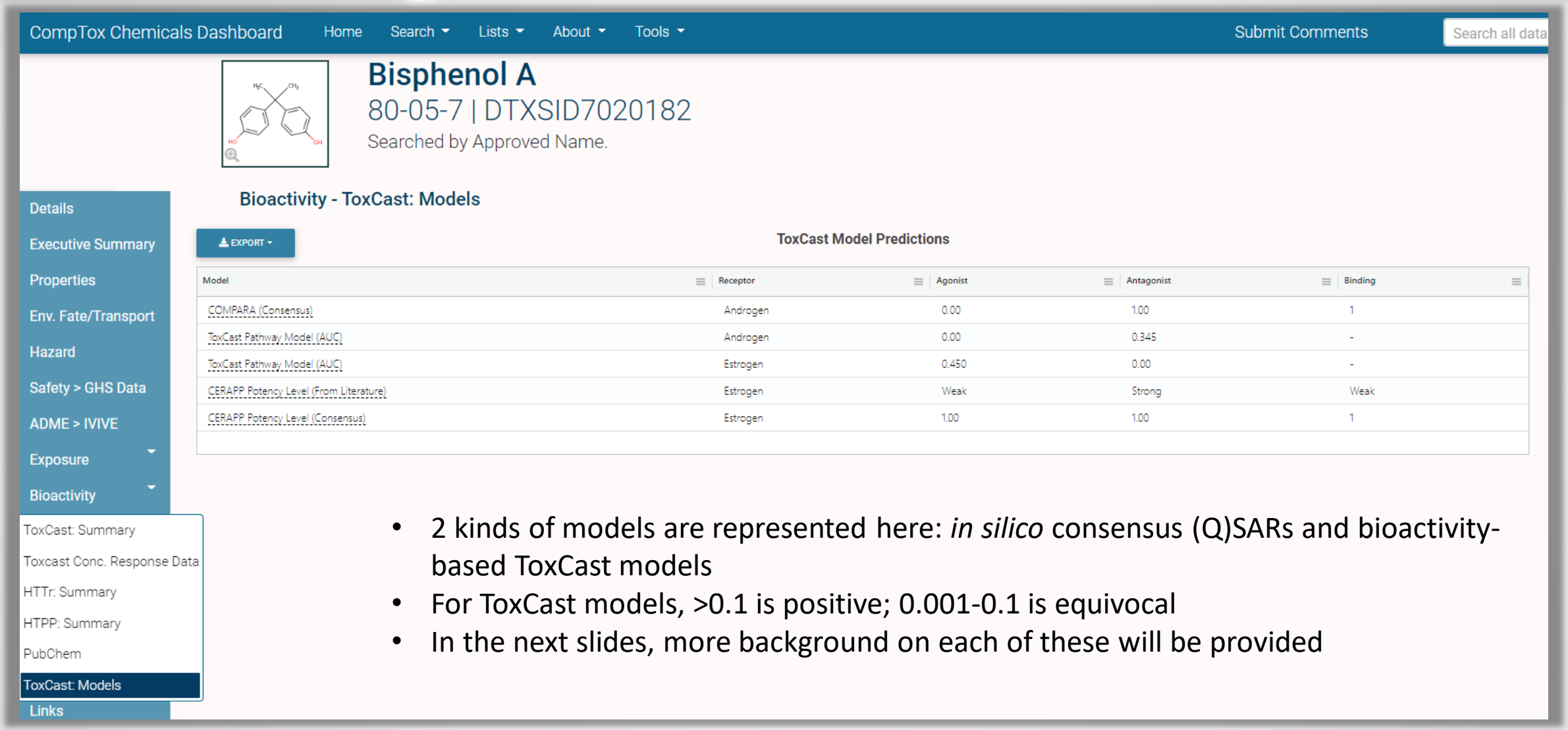
The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

Outline

- Models for estrogen and androgen receptors: CERAPP, COMPARA, ToxCast ER Pathway, ToxCast AR Pathway
  - In this section, I will briefly review the models and their availability
- Assay endpoints for thyroid
  - In this section, I will provide an overview of the type of information available
- Other endocrine-relevant models in publications (steroidogenesis)
  - In this section, due to time, I provide a brief overview of research available on the use of a high-throughput steroidogenesis assay

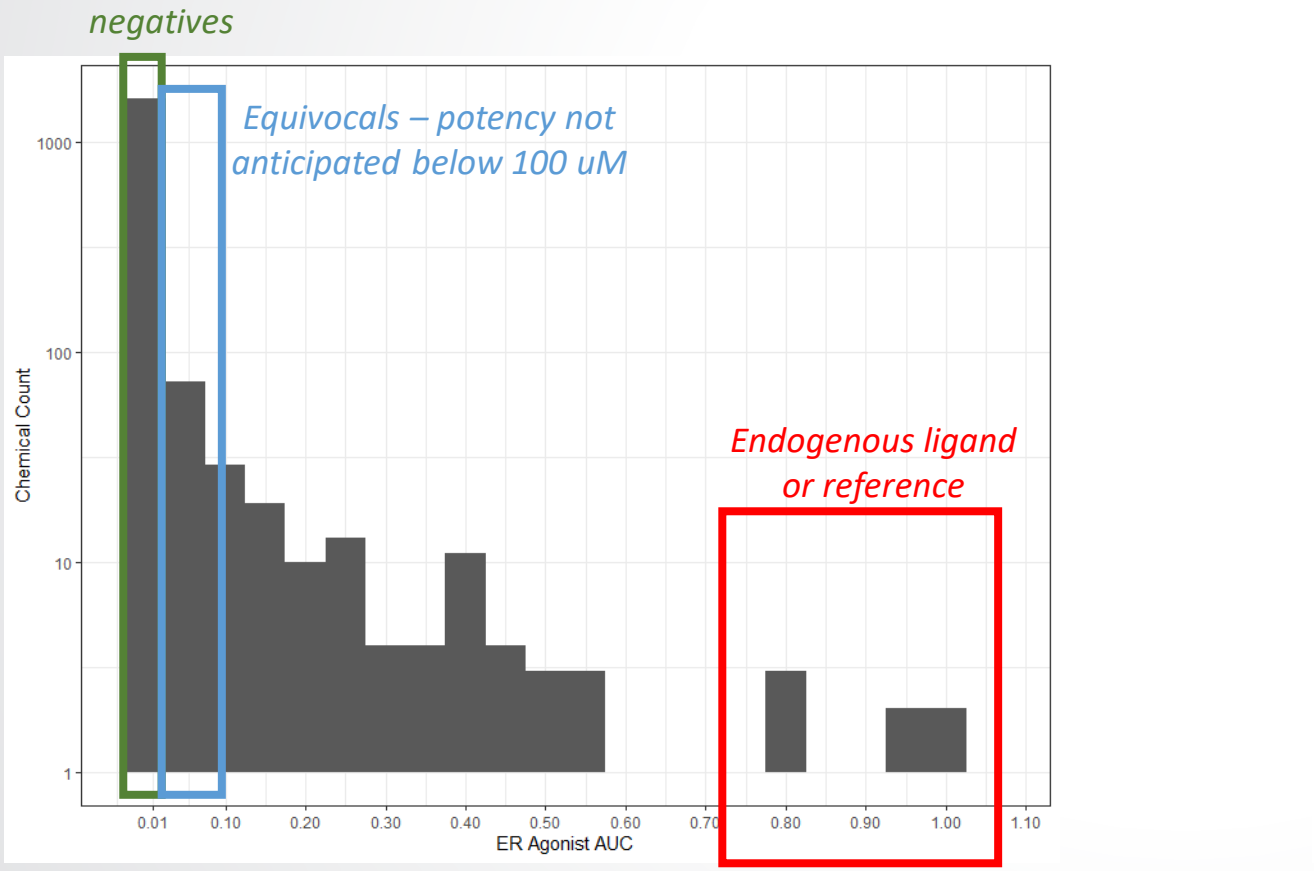
Where available, the Bioactivity > ToxCast Models provide the most reliable ER and AR predictions



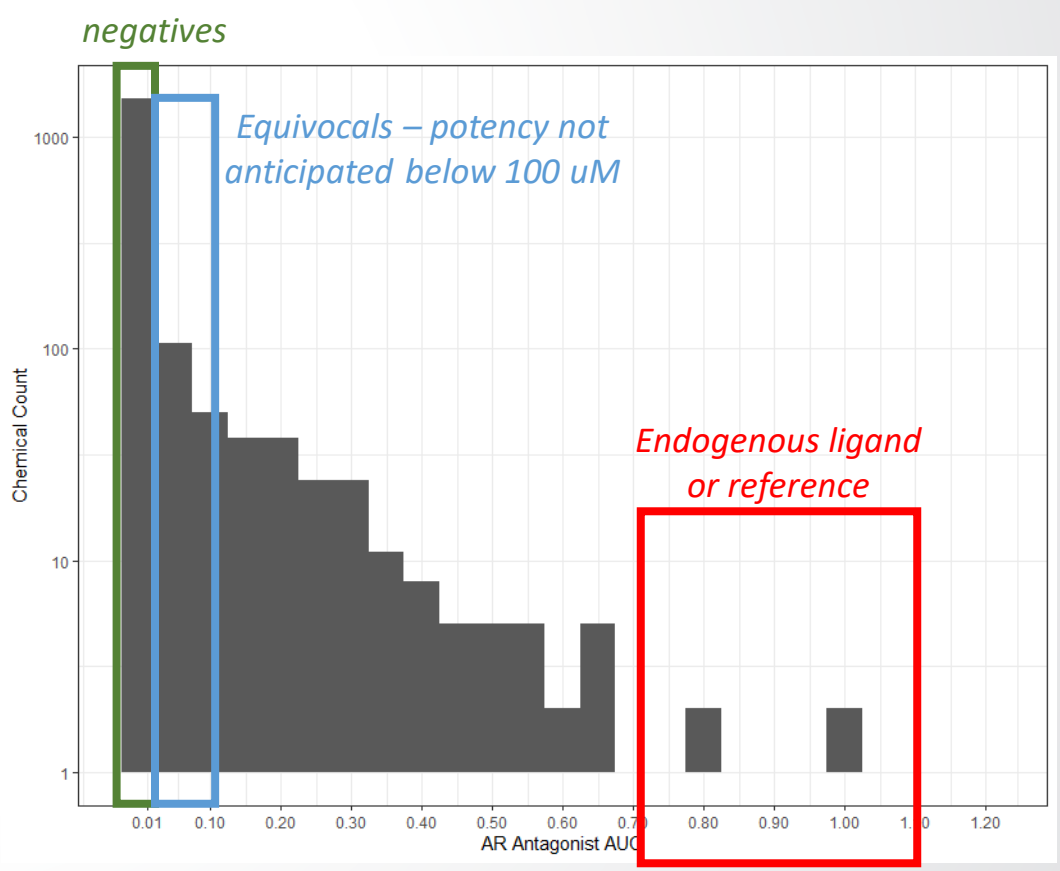
- 2 kinds of models are represented here: *in silico* consensus (Q)SARs and bioactivity-based ToxCast models
- For ToxCast models, >0.1 is positive; 0.001-0.1 is equivocal
- In the next slides, more background on each of these will be provided

Interpreting and using ToxCast pathway model scores: relative activity is important

Distribution of ToxCast ER Pathway Model Scores

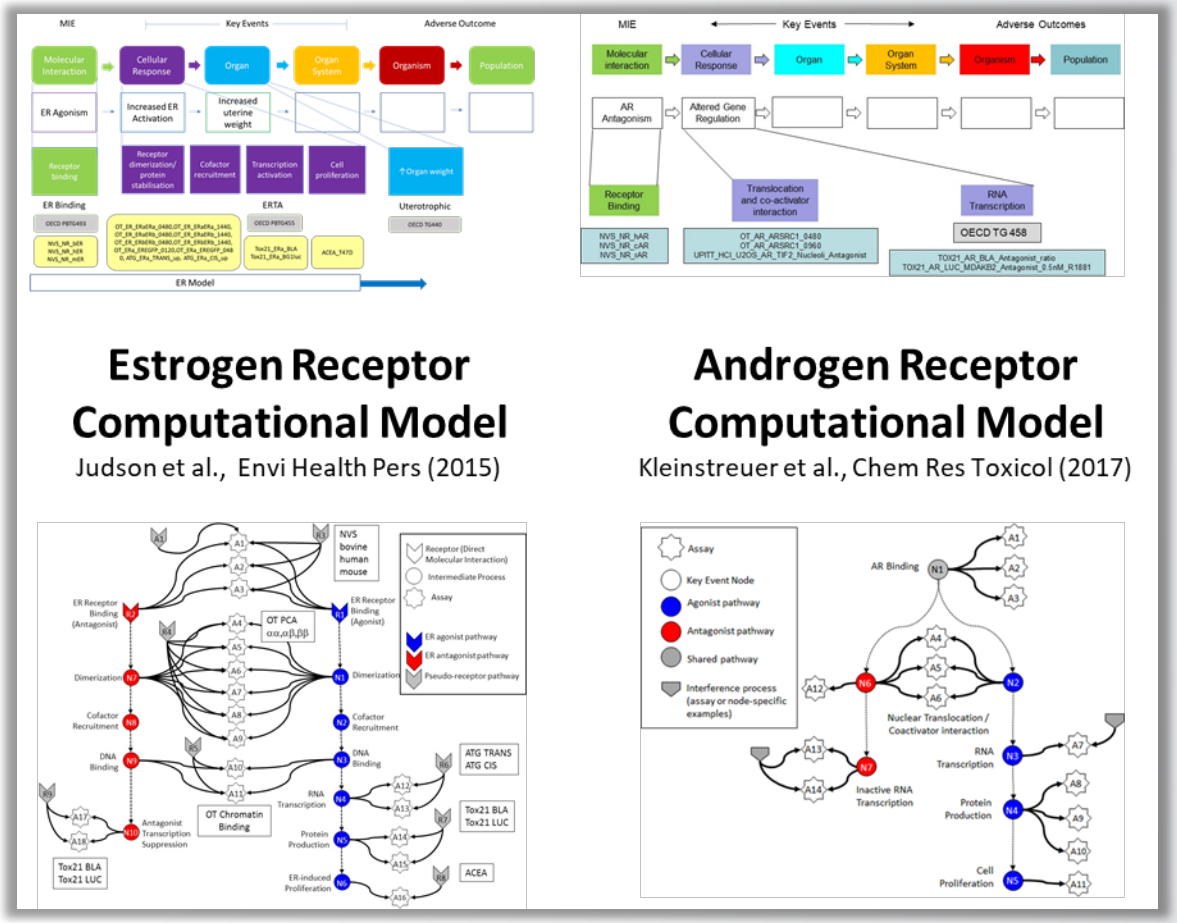


Distribution of ToxCast AR Pathway Model Scores

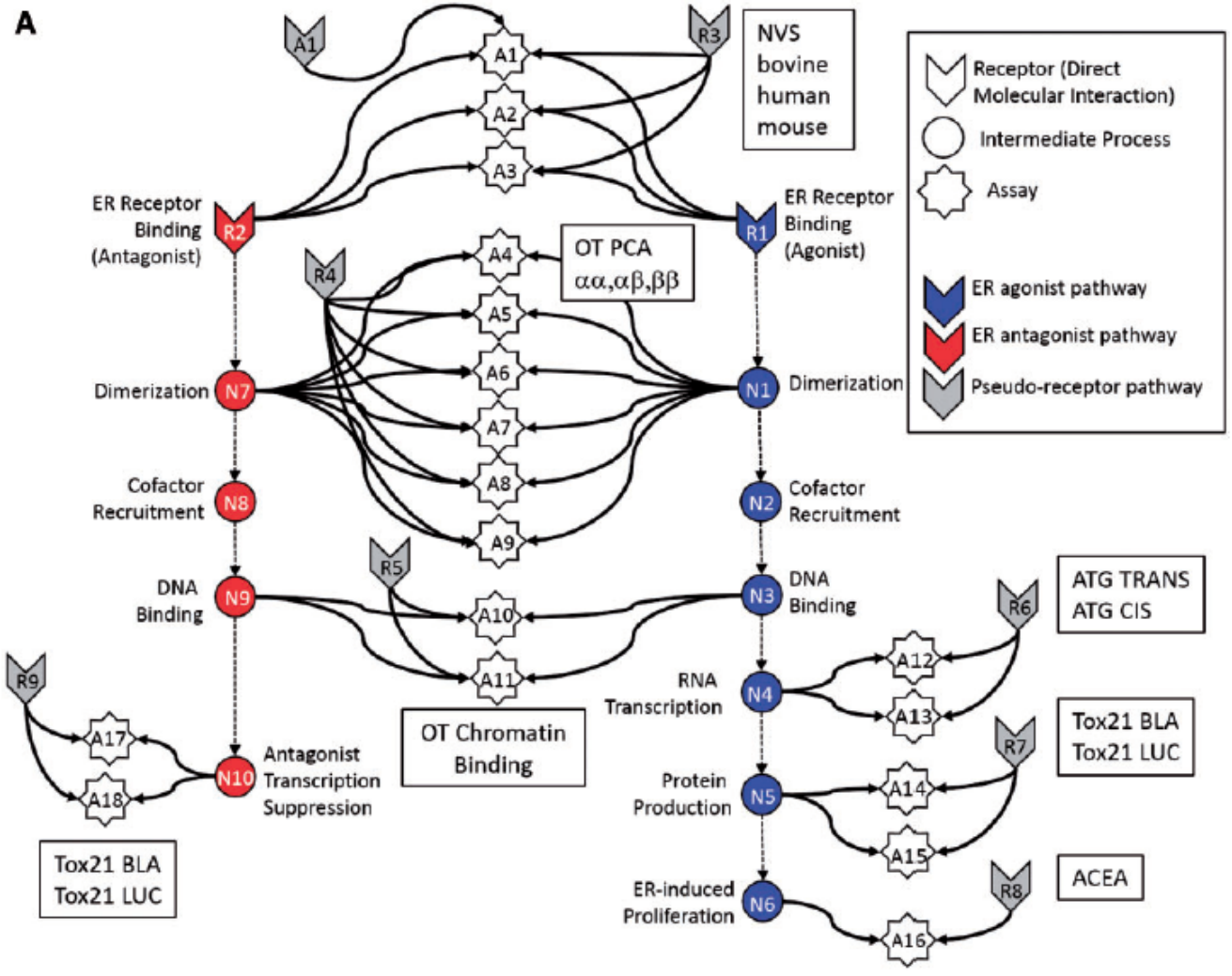


Systems biology modeling approach using *in vitro* ToxCast data

- Developed multiple high-throughput screening assays
  - Use multiple assays per pathway
    - Different technologies
    - Different points in pathway
  - No assay is perfect
    - Assay Interference
    - Noise
- Use a systems biology model to integrate assays
  - Model creates a composite dose-response curve for each chemical to summarize results from all assays



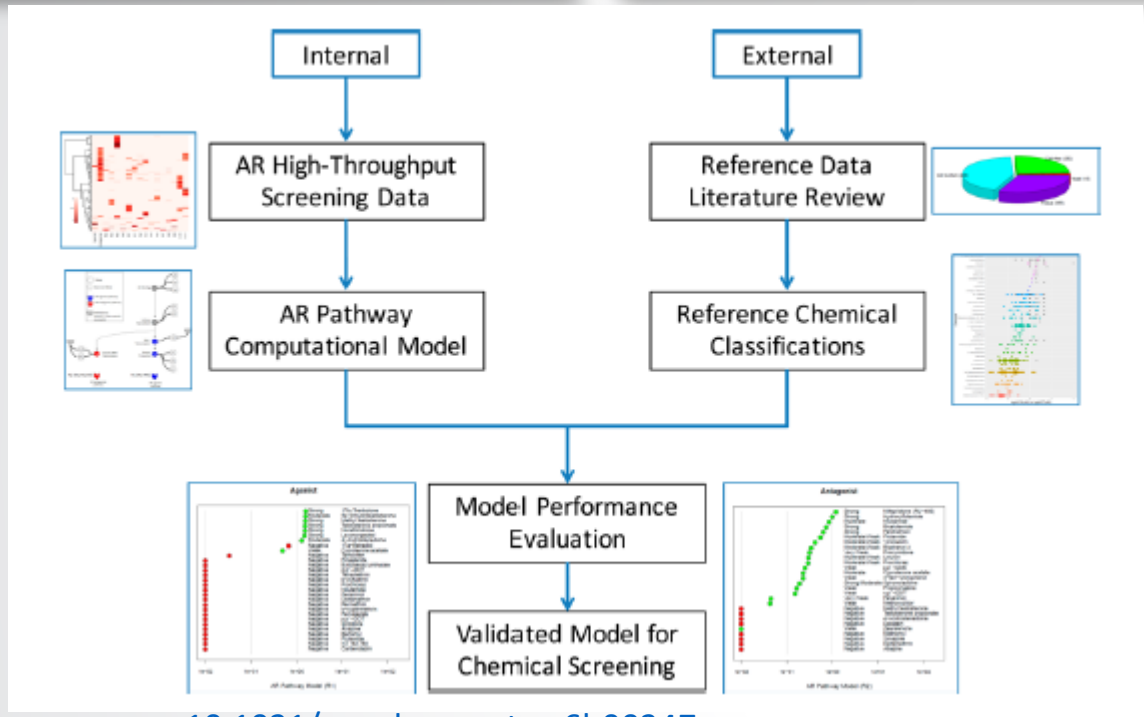
ToxCast ER model



https://doi.org/10.1093/toxsci/kfv168

- The current model in the CompTox Chemicals Dashboard is an update of the 2015 published model but still includes all 18 assays for agonist mode.
- This model has been accepted as an alternative for the ER binding, ER-TA, and Uterotrophic assays in the EDSP Tier 1 (https://www.federalregister.gov/documents/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice).
- A newer publication describes how only 4 assays that cover key “receptors” or events in the activation of ER can achieve similar performance as the full model (10.1016/j.yrtph.2017.09.022).

ToxCast AR model



Chemical Research in Toxicology

Article

pubs.acs.org/crt

Development and Validation of a Computational Model for Androgen Receptor Activity

Nicole C. Kleinstreuer,*,† Patricia Ceger,‡ Eric D. Watt,§ Matthew Martin,§ Keith Houck,§ Patience Browne,∥ Russell S. Thomas,§ Warren M. Casey,† David J. Dix,⊥ David Allen,‡ Srilatha Sakamuru,# Menghang Xia,# Ruili Huang,# and Richard Judson§

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10.1021/acs.chemrestox.6b00347

- Reviewed by Scientific Advisory Panels in 2014 and 2017.
- The Dashboard provides values from the original model published in 2017; new full AR model presented in 2020 publication on minimal assay set (with more assays – now 14 considered).
- The use of the uncertainty bounds around both the ER and AR model scores can be helpful in understanding weak or borderline scores.

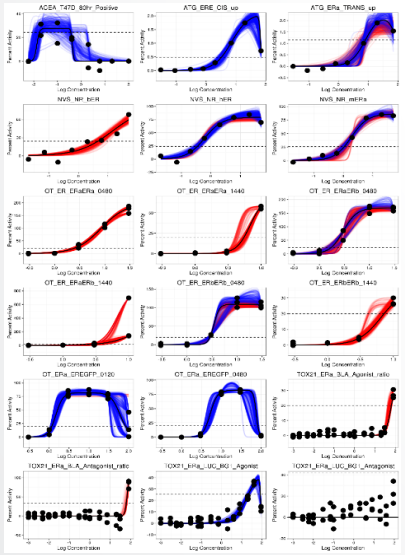
Uncertainty analysis for the ER and AR models

Major sources of uncertainty:

1. Qualitative: is an assay “hit” really due to ER/AR activity, or assay interference?
2. Quantitative: uncertainty around the true potency value (AC50)

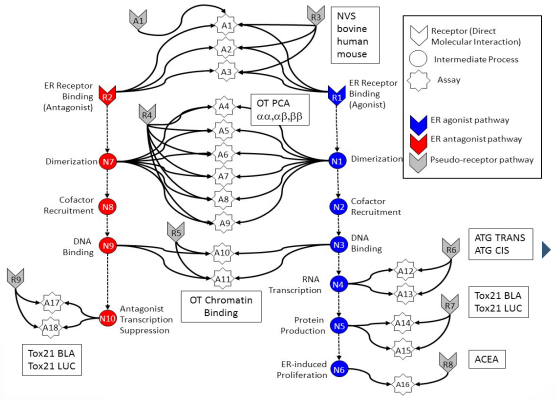
Both are now incorporated into the published ER and AR model results (not available on CCD currently)

Bootstrap Uncertainty in *In Vitro* Potency Values



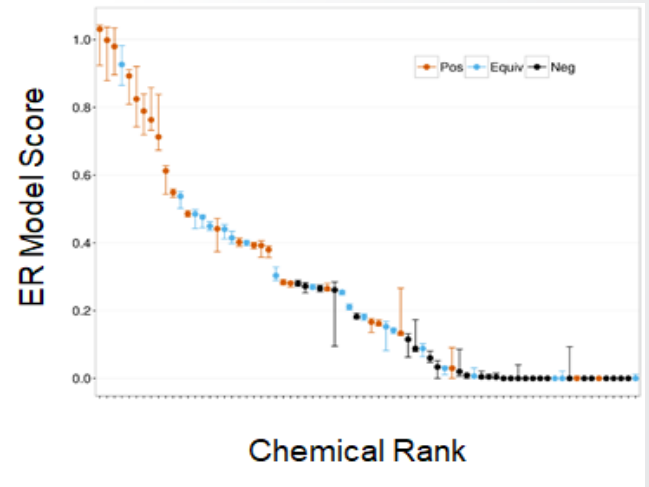
18 ER *In Vitro* Assays

Computational Modeling



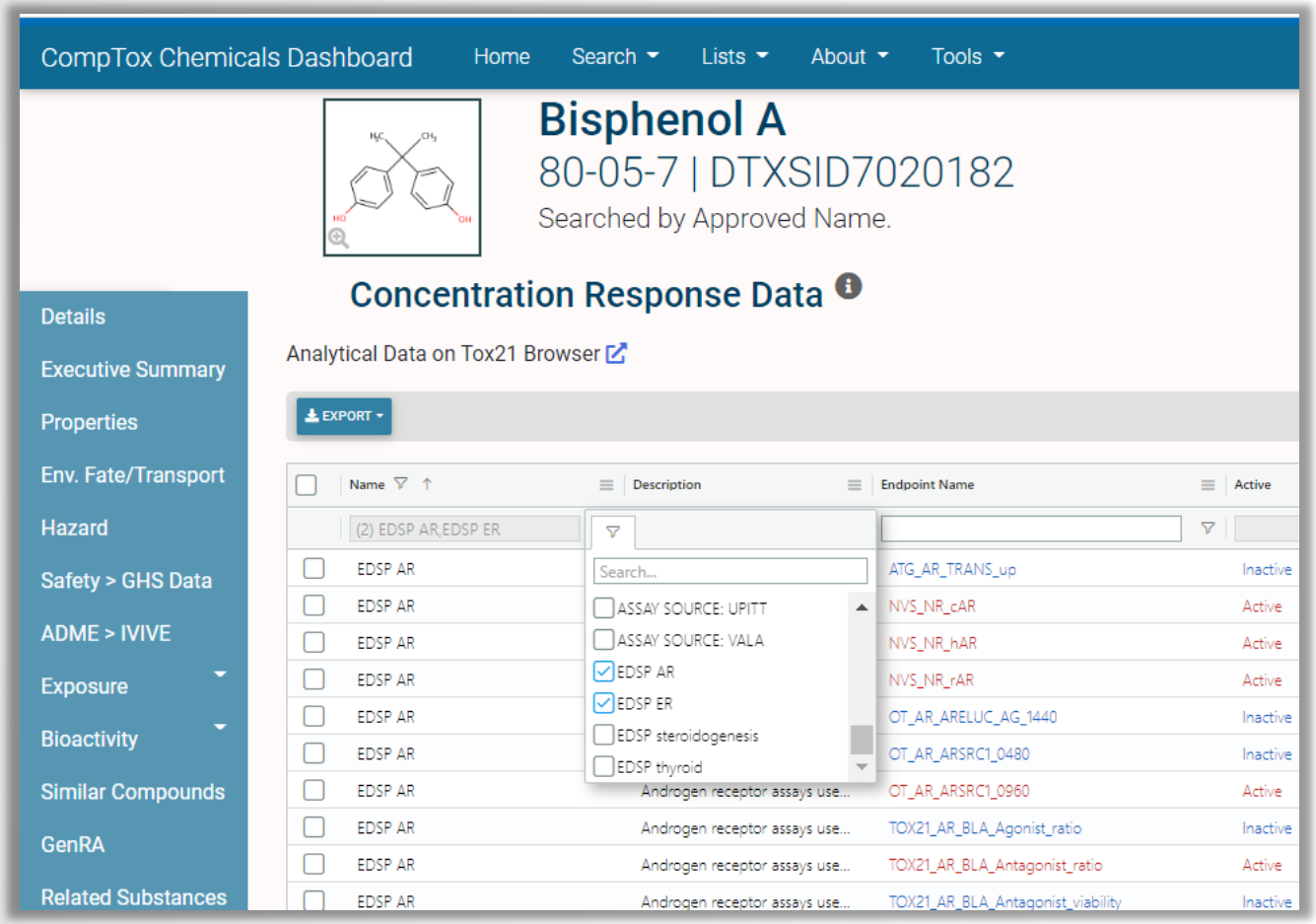
ER Pathway Model

Propagation of Uncertainty in Modeling Output



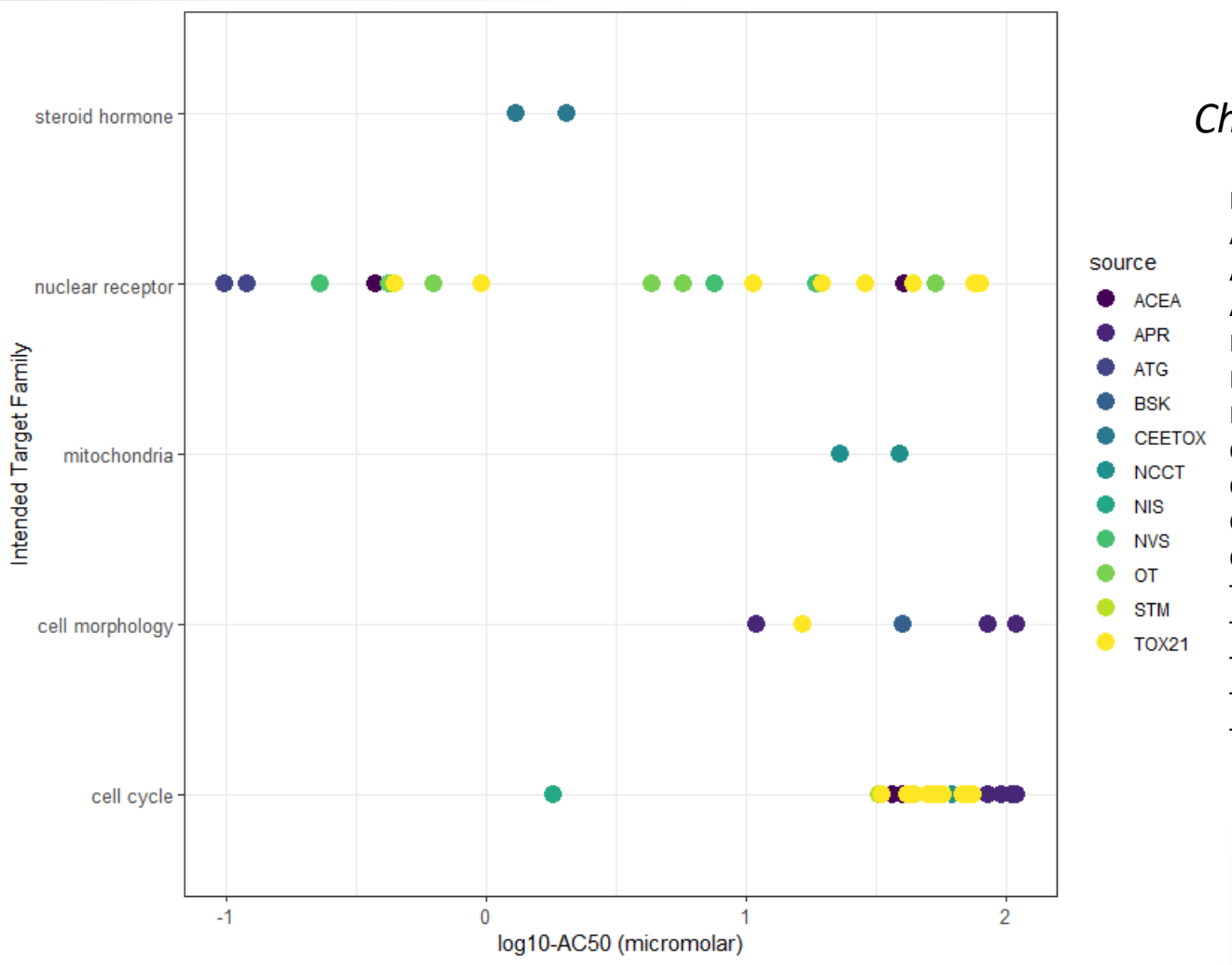
Watt and Judson, PLOS One 2018

Finding the assays that inform the ToxCast ER and AR Pathway models is a simple filtering step



- Bioactivity > ToxCast Conc. Response Data
- Filter for EDSP lists for ER to get the 18 ER assay endpoints and for AR to get the 11 AR assay endpoints

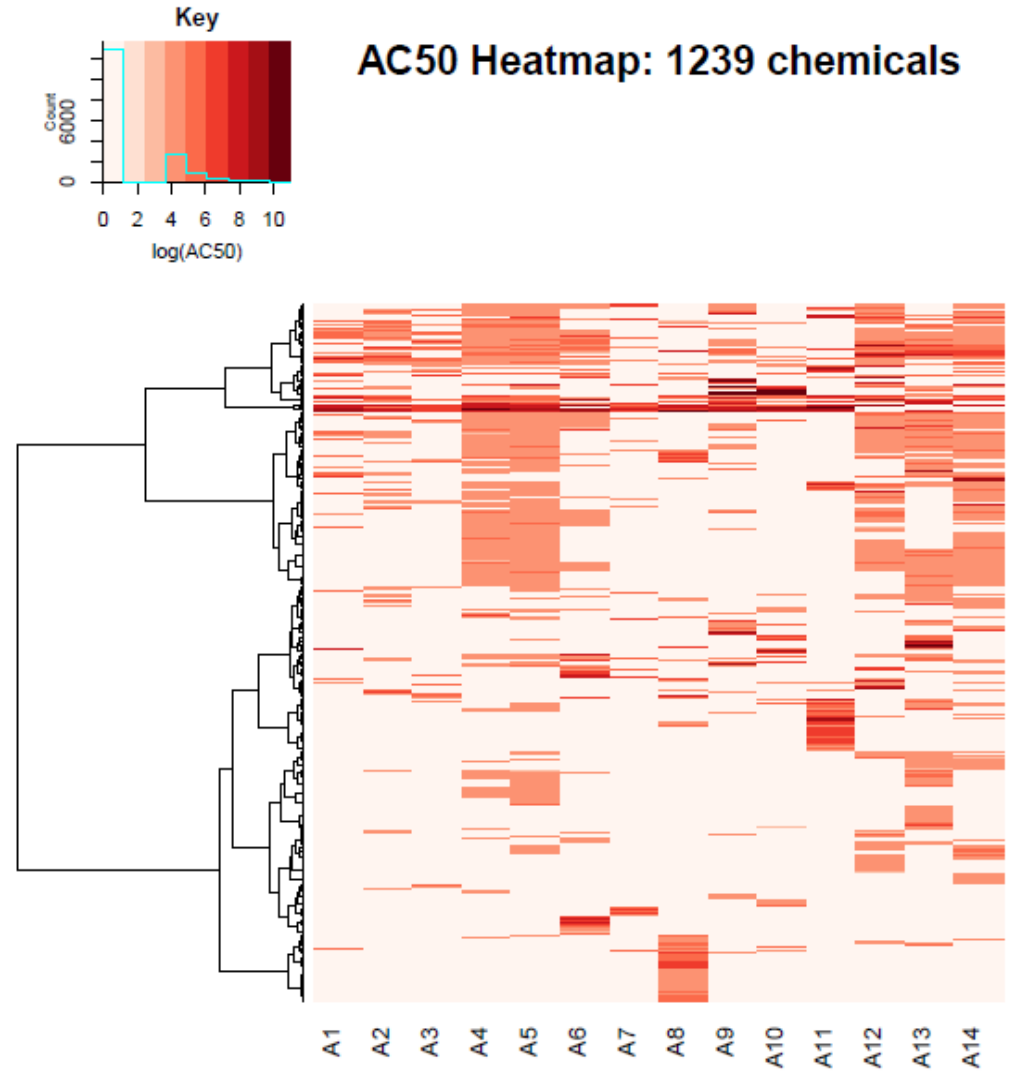
Export the data and dive deeper into the correspondence of the assays or comparison to other types of bioactivity



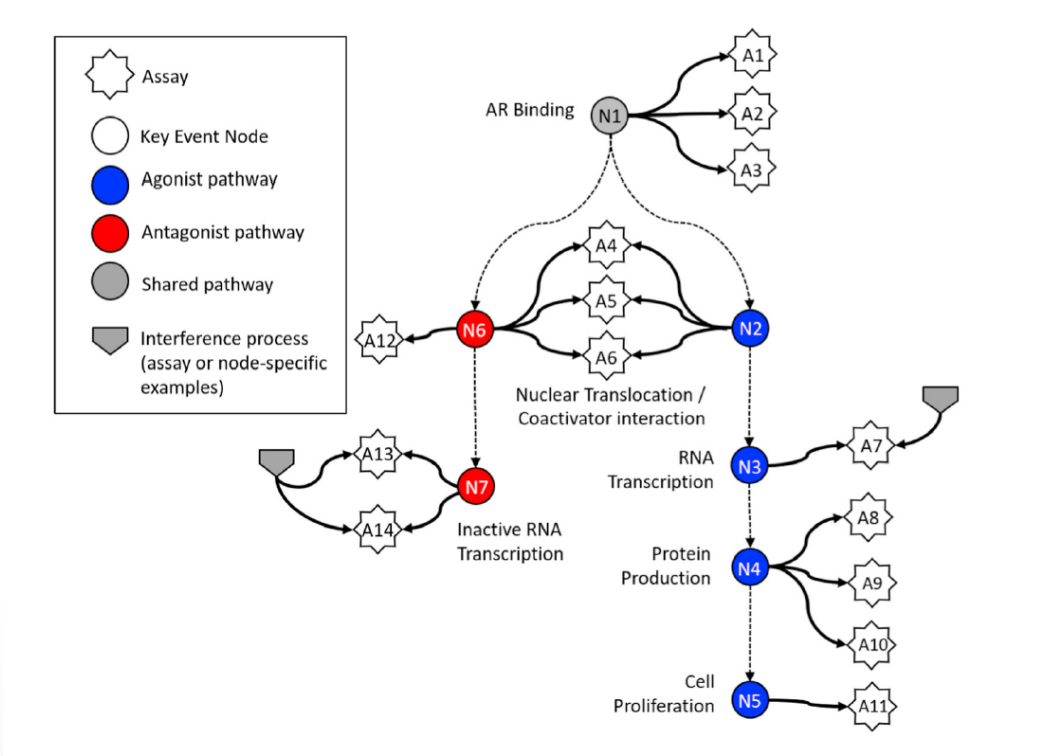
Downloaded ToxCast Summary from the CompTox Chemicals Dashboard, and filtered for one gene of interest

NAME	GENE_SYMBOL	HIT_CALL	AC50
ACEA_ER_80hr	ESR1	ACTIVE	0.373
ATG_ERE_CIS_up	ESR1	ACTIVE	9.81E-02
ATG_ERa_TRANS_up	ESR1	ACTIVE	0.119
NVS_NR_bER	ESR1	ACTIVE	0.421
NVS_NR_hER	ESR1	ACTIVE	0.23
NVS_NR_mERa	Esr1	ACTIVE	0.257
OT_ER_ERaERa_0480	ESR1	ACTIVE	5.73
OT_ER_ERaERa_1440	ESR1	ACTIVE	4.31
OT_ERa_EREGFP_0120	ESR1	ACTIVE	0.424
OT_ERa_EREGFP_0480	ESR1	ACTIVE	0.631
TOX21_ERa_BLA_Agonist_ratio	ESR1	ACTIVE	0.962
TOX21_ERa_BLA_Antagonist_ratio	ESR1	ACTIVE	43.5
TOX21_ERa_LUC_VM7_Agonist	ESR1	ACTIVE	0.445
TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2	ESR1	ACTIVE	75.1
TOX21_ERa_LUC_VM7_Agonist_10nM_ICI182780	ESR1	ACTIVE	19.6

But, keep in mind no assay is perfect (ToxCast AR model, published in 2017 and refined in 2020)

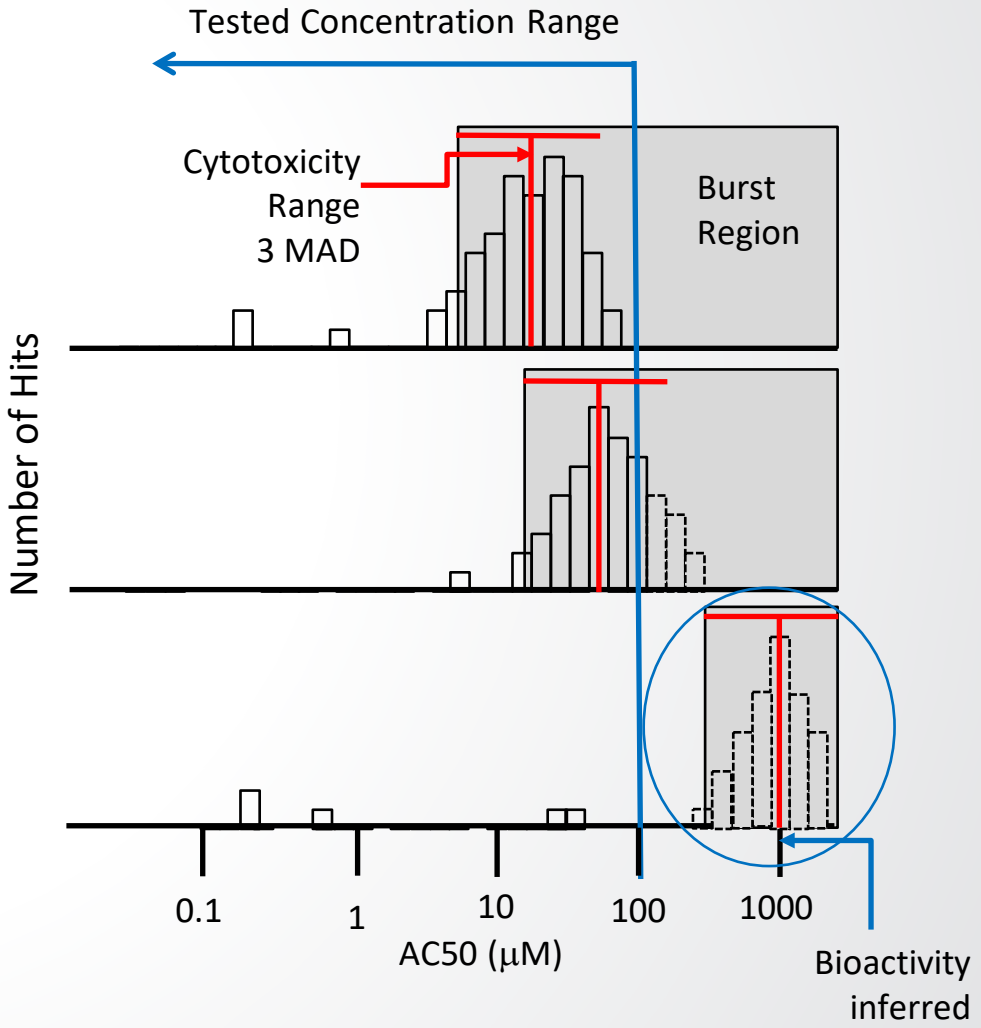


- Consider the subset of 1239 substances for which at least one AR assay endpoint in the set of 14 is positive.
- Not all assay endpoint positives are specific to the pathway (interference processes), and selectivity (distance from cytotoxicity) can be helpful in distinguishing AR antagonism from cytotoxicity (see Judson *et al.* 2016, 10.1093/toxsci/kfw092)



Cytotoxicity threshold or "burst" is incorporated into the ToxCast ER/AR models

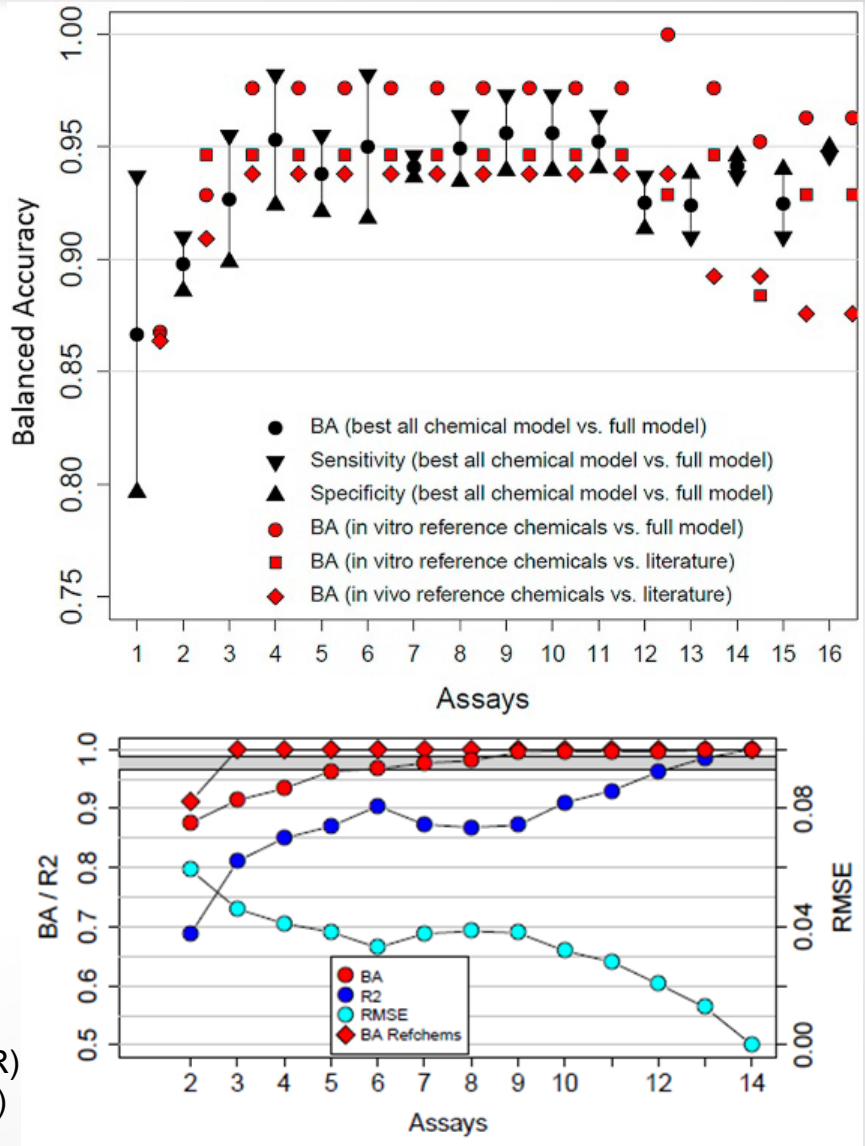
- Most chemicals display a "burst" of potentially non-selective bioactivity near the cytotoxicity concentration.
- This is often "false positive" activity
  - E.g. Activity in an ER assay in the "burst" region is likely due to cell stress and not true ER binding activity
- "Z-score" method can be used to filter out this false positive activity before drawing conclusions about ER, AR (or other specific target) activity



Judson et al. Tox.Sci. (2016)

Practically, how many assay endpoints are needed to maintain model performance?

- Original ER and AR models used many redundant assays to help understand the types of noise and assay interference occurring in *in vitro* assays
- "Subset models" were developed: Rebuild the original models using all subsets of assays (2, 3, 4, … n assays)
- Results show that subsets with fewer assays have acceptable performance against the full model, and the *in vitro* and *in vivo* reference chemicals.
- The acceptable subsets all have assays that:
  - probe diverse points in the pathway
  - use diverse assay reporting technologies
  - use diverse cell types
- ER Agonist: 4 or more assays
- AR Antagonist: 5 or more assays



Judson et al., Reg. Tox. Pharm. (2017) (ER)
Judson, et al. Reg. Tox. Pharm. (2020) AR)

Approach using *in silico* methods: CERAPP and COMPARA

- Large scale QSAR modeling projects to predict ER and AR activity
  - CERAPP - Collaborative Estrogen Receptor Activity Prediction Project
  - CoMPARA : Collaborative Modeling Project for Androgen Receptor Activity
- Use ER and AR Pathway model results to train QSAR models
- Use data from the open literature to evaluate
- Many expert groups from US, Europe, Japan and China submitted models, from which consensus models were derived
- Modes: Binding, Agonist, Antagonist
- Model types:
  - Qualitative (active, inactive),
  - Semi-quantitative (inactive, very weak, weak, moderate, strong)
- Results available through the CompTox Chemicals Dashboard as well as OPERA on GitHub (https://github.com/kmansouri/OPERA) and now OECD QSAR Toolbox (https://repository.qsartoolbox.org/Tools/Details/6703ab01-9529-4f86-814f-6efc49e1f59c)

CERAPP consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.93	0.58	0.85	0.94	0.67	0.18
Sp	0.97	0.92	0.98	0.94	0.94	0.90
BA	0.95	0.75	0.92	0.94	0.80	0.54

CoMPARA consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.99	0.69	0.95	0.74	1.00	0.61
Sp	0.91	0.87	0.98	0.97	0.95	0.87
BA	0.95	0.78	0.97	0.86	0.97	0.74

Forward Prediction Results

	CERAPP		CoMPARA	
	Active	Inactive	Active	Inactive
Binding	4001	28463	8202	40656
Agonist	2475	29989	1764	47094
Antagonist	2793	29671	9899	38959
Total	4001	28463	10623	47613

Mansouri et al., Environmental Health Perspectives (2016)
Mansouri et al., Environmental Health Perspectives (2020).

Conclusions for the ER and AR section

- Always use models over individual assays
- Model information for (Q)SARs and bioactivity-informed models are available for ER and AR activity

Overview of thyroid screening data in the CompTox Chemicals Dashboard

A thyroid adverse outcome pathway network as a guide

Public screening data is available for many MIEs in the AOP network.

- Green boxes indicate MIEs with HTS data in ToxCast or soon to be in ToxCast
- TRHR and IYD added since publication;
- Assays exist for TBG and TTR binding, but not in ToxCast (yet);
- Yellow box: Some indication of liver transporters from HepaRG data recently released (LTEA) and from primary hepatocyte data (CellzDirect).

Ongoing challenges

- Would be great to add high-throughput transcriptomics
- What about the need for redundancy/confirmation at assay targets?
- What about quantitative key event relationships?

Commentary

A Section 508-conformant HTML version of this article is available at https://doi.org/10.1289/EHP5297.

Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with *in Vitro* Testing and Adverse Outcome Pathway Approaches

Pamela D. Noyes,[1] Katie Paul Friedman,[2] Patience Browne,[3] Jonathan T. Haselman,[4] Mary E. Gilbert,[5] Michael W. Hornung,[4] Stan Barone Jr.,[6] Kevin M. Crofton,[2†] Susan C. Laws,[5] Tammy E. Stoker,[5] Steven O. Simmons,[2] Joseph E. Tietge,[4] and Sigmund J. Degitz[4]

[1]National Center for Environmental Assessment, Office of Research and Development (ORD), U.S. Environmental Protection Agency (EPA), Washington, DC, USA
[2]National Center for Computational Toxicology, ORD, U.S. EPA, Research Triangle Park, North Carolina, USA
[3]Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD), Paris, France
[4]Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory (NHEERL), ORD, U.S. EPA, Duluth, Minnesota, USA
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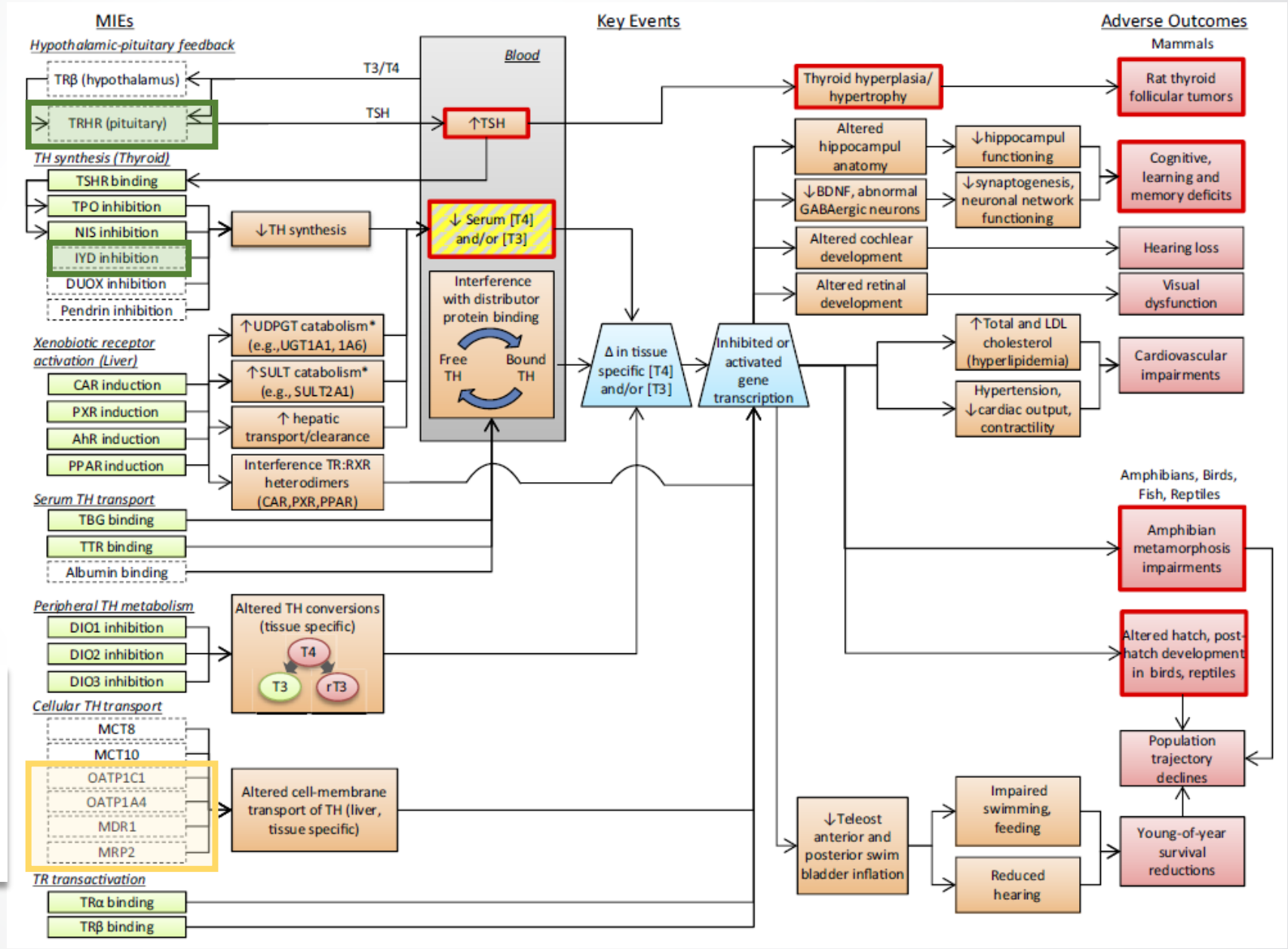
[6]Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. EPA, Washington, DC, USA

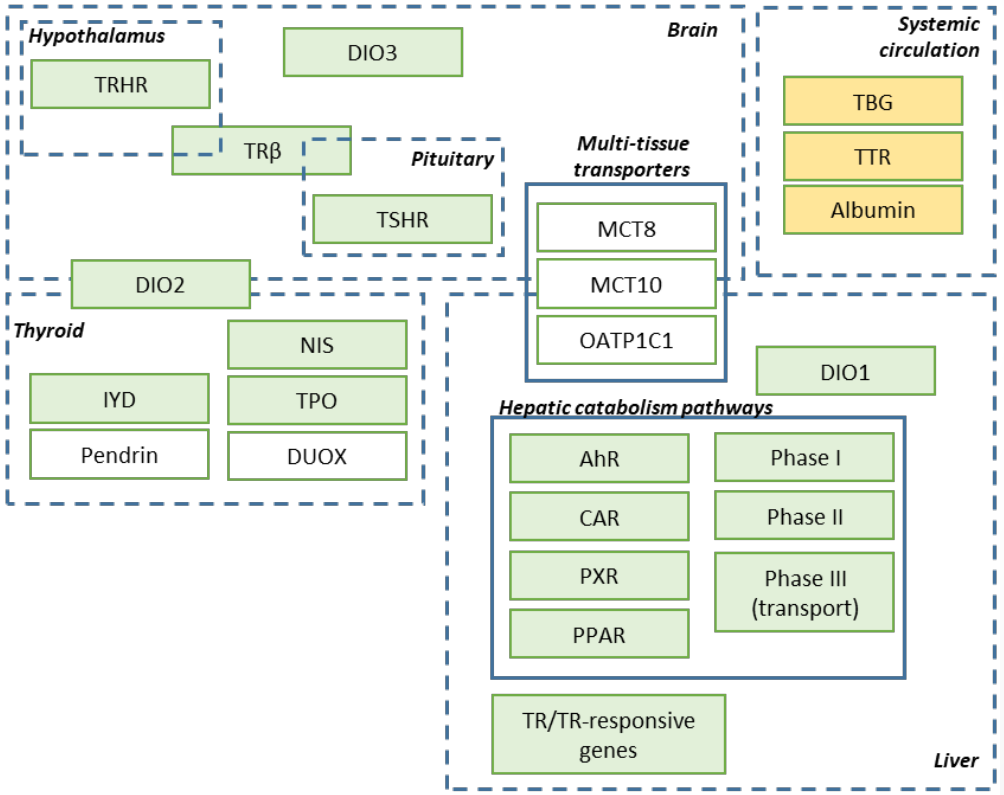
DOI: https://doi.org/10.1289/EHP5297



Thyroid-related screening can be imagined by groups of endpoints relevant to particular processes or tissues

Broad and Targeted (Tier 1-2) NAMs for bioactivity

Considering the MIEs by the tissues they may come from may steer us toward the Tier 2-3 NAM systems needed for confirmation of KEs and AOs

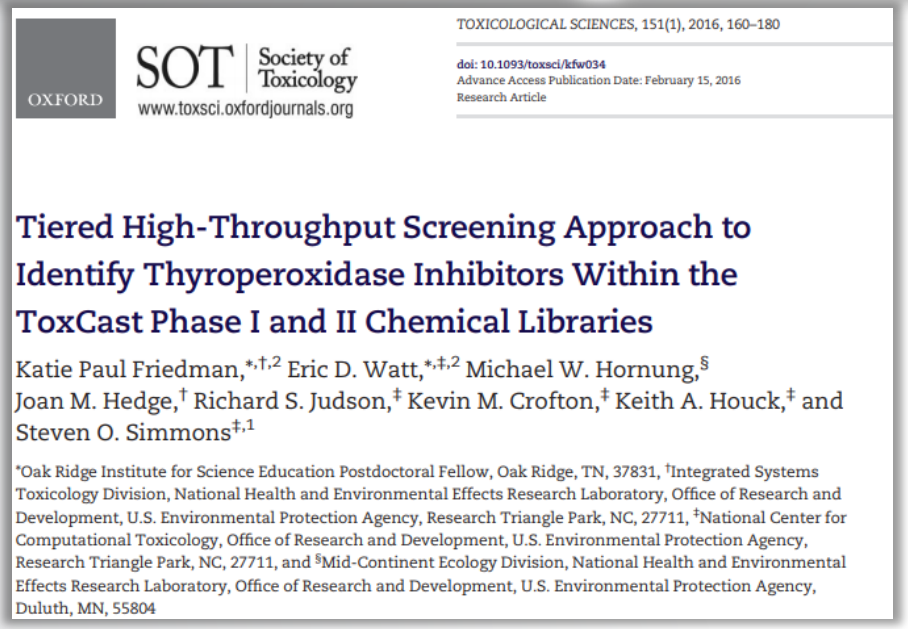


Green boxes = have some public screening methods and data in ToxCast or soon to be in ToxCast; clear boxes indicate not available in ToxCast. **Many scientists in EPA-ORD have contributed to a number of papers on these screening methods and results.**

Thyroid hormone synthesis (and peripheral metabolism)

aeid	Assay endpoint name (aenm)	Target grouping
1508	CCTE_Simmons_AUR_TPO_dn	TPO
1509	CCTE_Simmons_CellTiterGLO_HEK293T	TPO (parallel cytotoxicity)
1848	CCTE_Simmons_QuantiLum_inhib_2_dn	TPO (parallel nonspecific protein inhibition)
1824	CCTE_Simmons_GUA_TPO_dn	TPO
3090	CCTE_GLTED_hTPO_dn	TPO
2037	CPHEA_NIS_RAIU_inhibition	NIS
2110	NIS_HEK293T_CTG_Cytotoxicity	NIS (parallel cytotoxicity)
2309	CCTE_GLTED_hDIO1_dn	DIOs
2532	CCTE_GLTED_hDIO2_dn	DIOs
2533	CCTE_GLTED_hDIO3_dn	DIOs
3091	CCTE_GLTED_xDIO3_dn	DIOs
3032	CCTE_GLTED_hIYD_dn	IYDs
3092	CCTE_GLTED_xIYD_dn	IYDs

Assay principle of the current ToxCast Amplex UltraRed TPO (AUR-TPO) inhibition assay



OXFORD | SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 151(1), 2016, 160–180
doi: 10.1093/toxsci/kfw034
Advance Access Publication Date: February 15, 2016
Research Article

Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries

Katie Paul Friedman,*,†,2 Eric D. Watt,*,‡,2 Michael W. Hornung,§ Joan M. Hedge,† Richard S. Judson,‡ Kevin M. Crofton,‡ Keith A. Houck,‡ and Steven O. Simmons‡,1

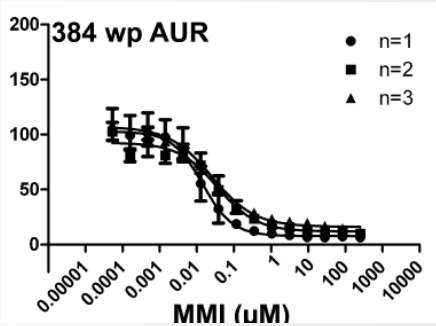
*Oak Ridge Institute for Science Education Postdoctoral Fellow, Oak Ridge, TN, 37831, †Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, ‡National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, and §Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN, 55804

Thyroid microsome (containing TPO)

Excess H_2O_2

Amplex UltraRed

Amplex UltroxRed (fluorescent)

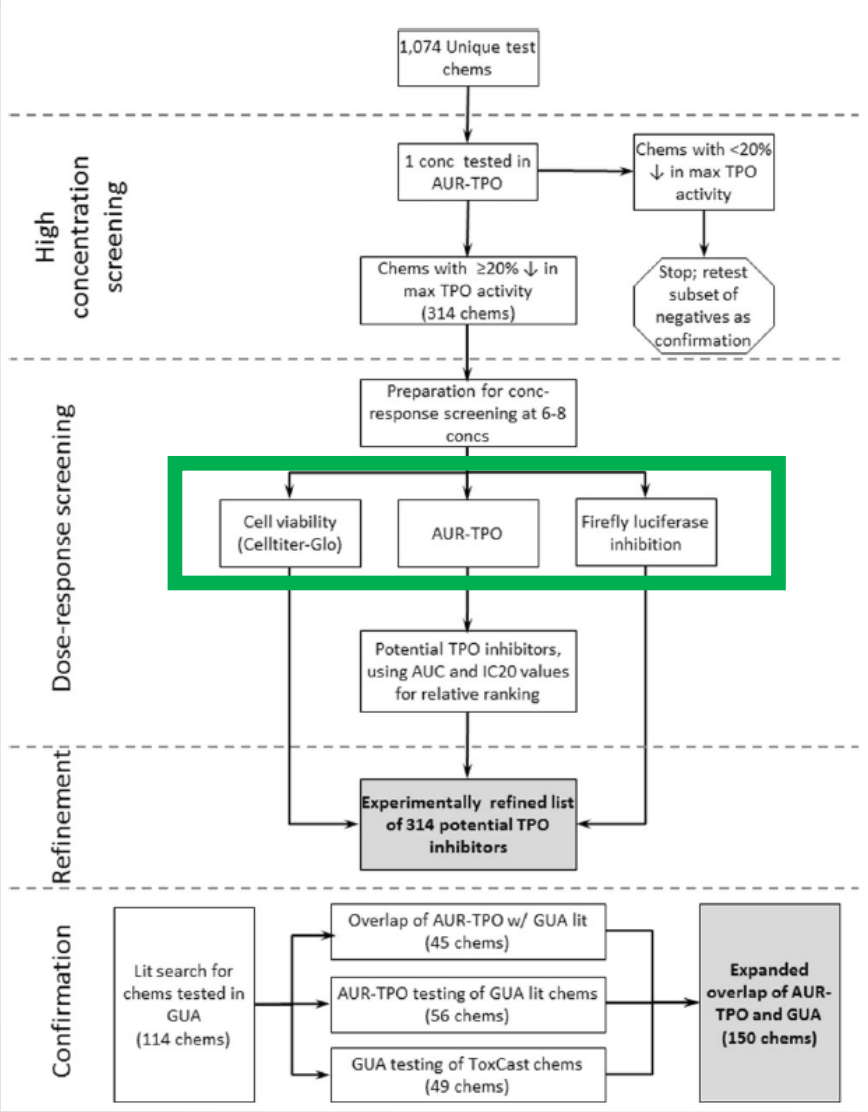


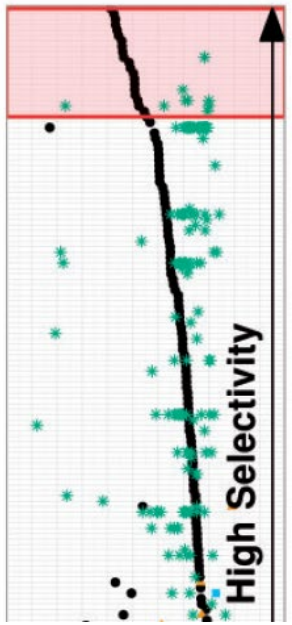
Paul Friedman K, Watt ED, Hornung MW, Hedge JM, Judson RS, Crofton KM, Houck KA, Simmons SO. (2016). Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors within the ToxCast Phase I and II Chemical Libraries. Toxicological Sciences. DOI: https://doi.org/10.1093/toxsci/kfw034

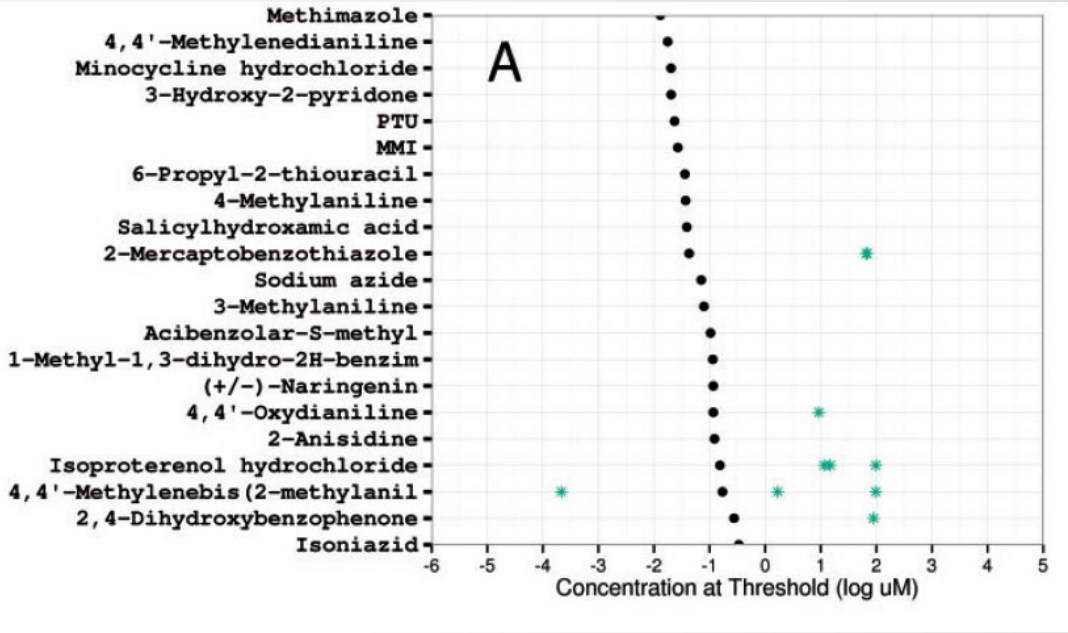
Paul KB, Hedge JM, Rotroff DM, Crofton KM, Hornung MH, Simmons SO. (2014). Development of a thyroperoxidase inhibition assay for medium through-put screening. Chemical Research in Toxicology. https://doi.org/10.1021/tx400310w

- Lead substance: methimazole (MMI)
- Other example positive reference chemicals: 6-propyl-2-thiouracil, dietary isoflavones, malachite green, ethylene bisthiocarbamates
- Also evaluated with a training set of reference chemicals
- Positive rate may approach 30% so context is important for filtering positives (consider sources of interference)
- Loss-of-signal assay

Context for interpretation

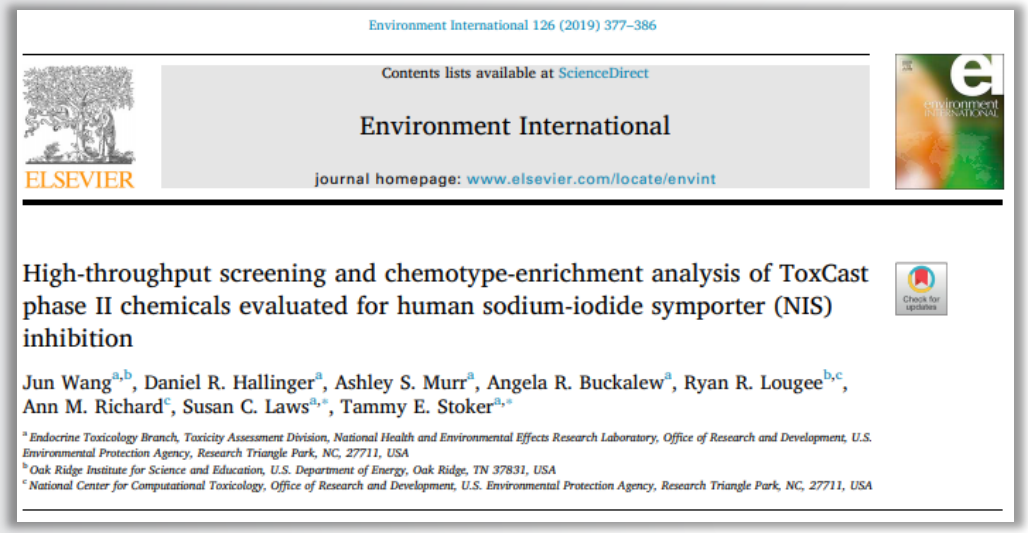






- Consider "selectivity": is the potency of TPO inhibition distinguishable from potency of nonspecific protein inhibition or cell viability (as an indicator of chemical reactivity/pertinent concentration range?
- This was a tiered screening – most of the chemicals screened in single concentration first.
- Consider the most potent and selective modes-of-action for these substances?

Assay principle of the ToxCast NIS inhibition assay



Environment International 126 (2019) 377–386

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/envint

High-throughput screening and chemotype-enrichment analysis of ToxCast phase II chemicals evaluated for human sodium-iodide symporter (NIS) inhibition

Jun Wang[a,b], Daniel R. Hallinger[a], Ashley S. Murr[a], Angela R. Buckalew[a], Ryan R. Lougee[b,c], Ann M. Richard[c], Susan C. Laws[a,*], Tammy E. Stoker[a,*]

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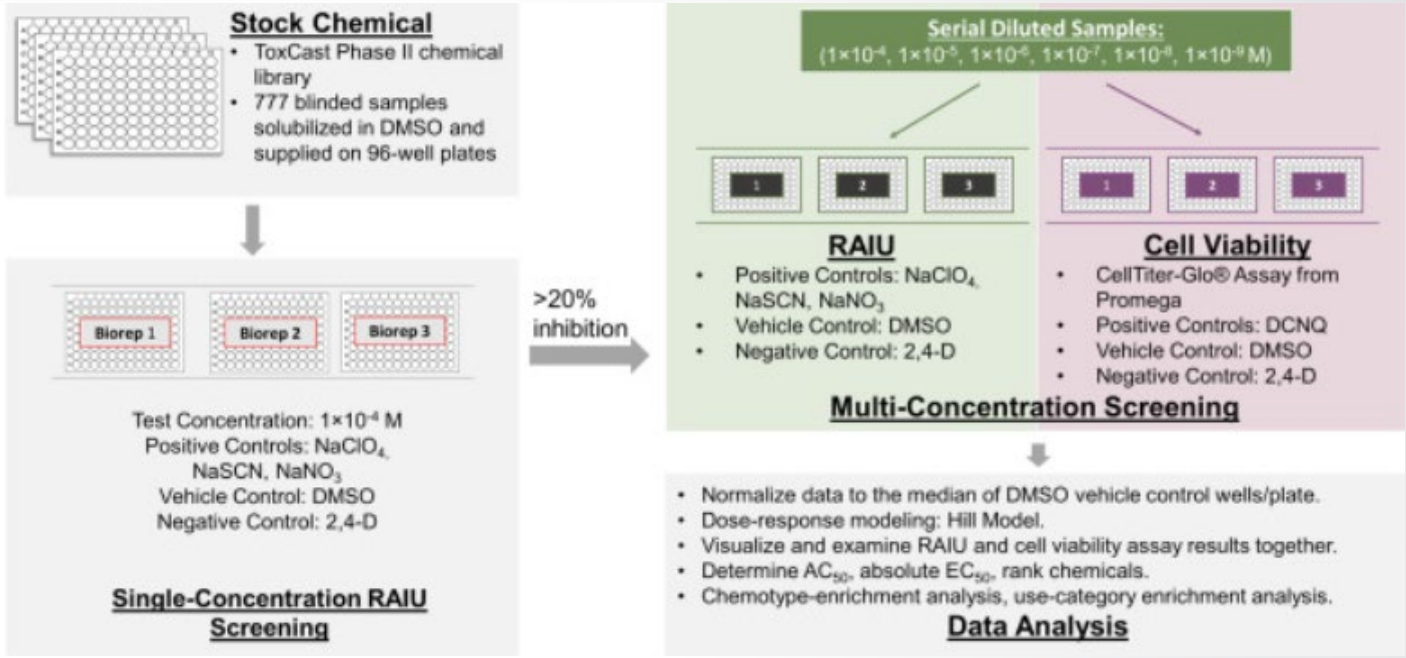
[b] Oak Ridge Institute for Science and Education, U.S. Department of Energy, Oak Ridge, TN 37831, USA
[c] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

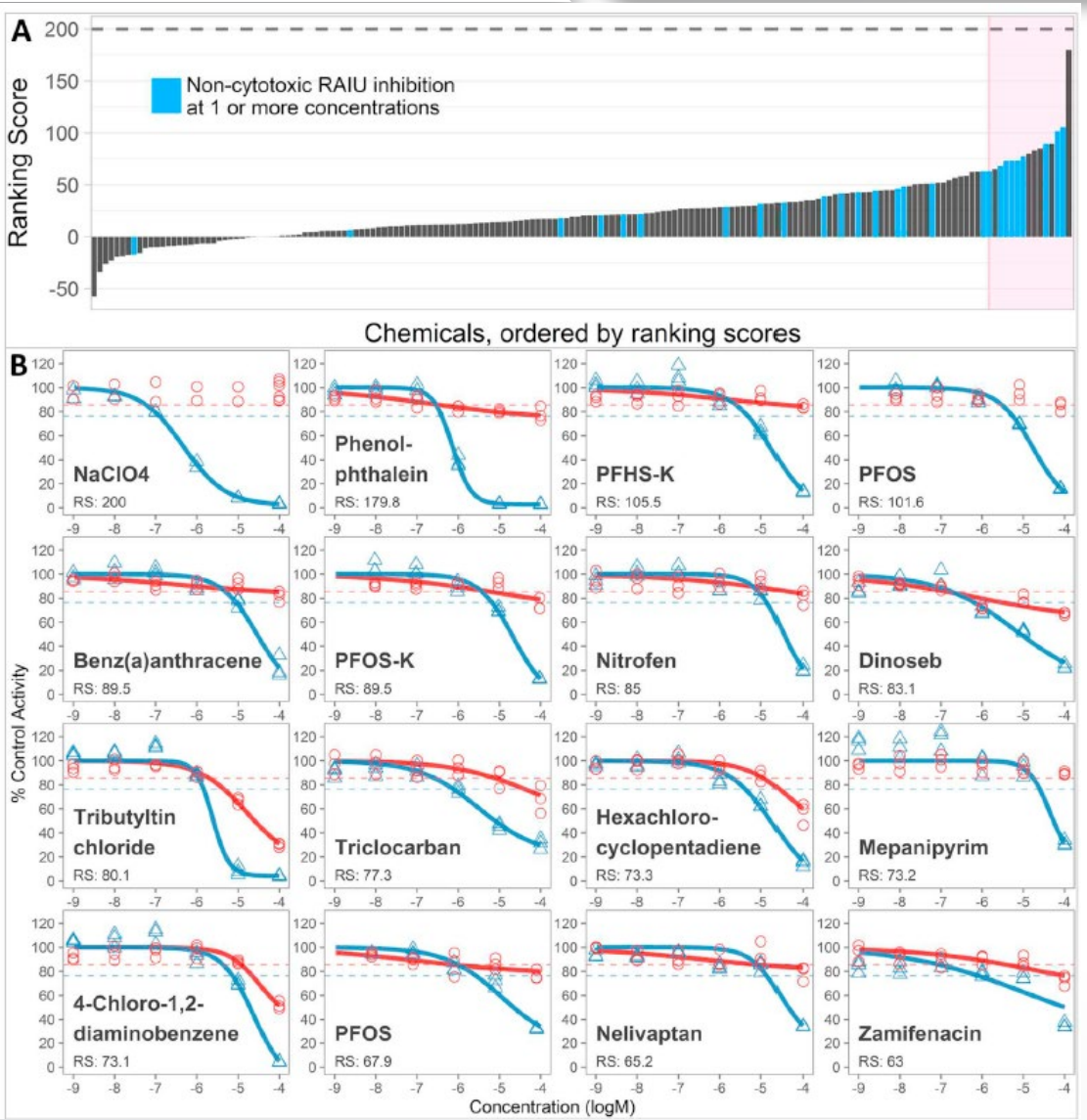


Wang J, Hallinger DR, Murr AS, Buckalew AR, Lougee RR, Richard AM, Laws SC, Stoker TE. (2019). High-throughput screening and chemotype-enrichment analysis of ToxCast phase II chemicals evaluated for human sodium-iodide symporter (NIS) inhibition. https://doi.org/10.1016/j.envint.2019.02.024

Wang J, Hallinger DR, Murr AS, Buckalew AR, Simmons SO, Laws SC, Stoker TE. (2018). High-throughput screening and quantitative chemical ranking for sodium-iodide symporter inhibitors in ToxCast Phase I chemical library. 10.1021/acs.est.7b06145

- Positive rate may approach 30-50% depending on the chemical library screened
- In screening ToxCast Phase 2, only 25 substances were considered selective

Context for interpretation



From Wang et al. 2019

- Tiered screening (single concentration screening followed by selected multi-concentration screening).
- Also a loss-of-signal assay with high hit-rate.
- Cytotoxicity may be a source of interference.
- Most potent and selective modes of action?

Lecat-Guillet N et al. 2008 identified organics that inhibited NIS beyond perchlorate and other monovalent anions

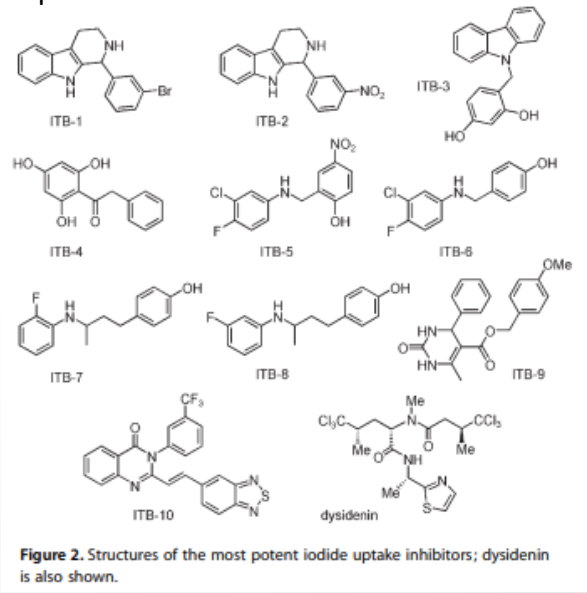


Figure 2. Structures of the most potent iodide uptake inhibitors; dysidenin is also shown.

Assay principle of the DIO inhibition assays

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Research Article

Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases

Jennifer H. Olker,[*,†,‡,§,1] Joseph J. Korte,[*,†,‡,§] Jeffrey S. Denny,[*,†,‡,§] Phillip C. Hartig,[*,†,‡,¶] Mary C. Cardon,[*,†,‡,¶] Carsten N. Knutsen,[||] Paige M. Kent,[|||] Jessica P. Christensen,[|||] Sigmund J. Degitz,[*,†,‡,§] and Michael W. Hornung[*,†,‡,§]

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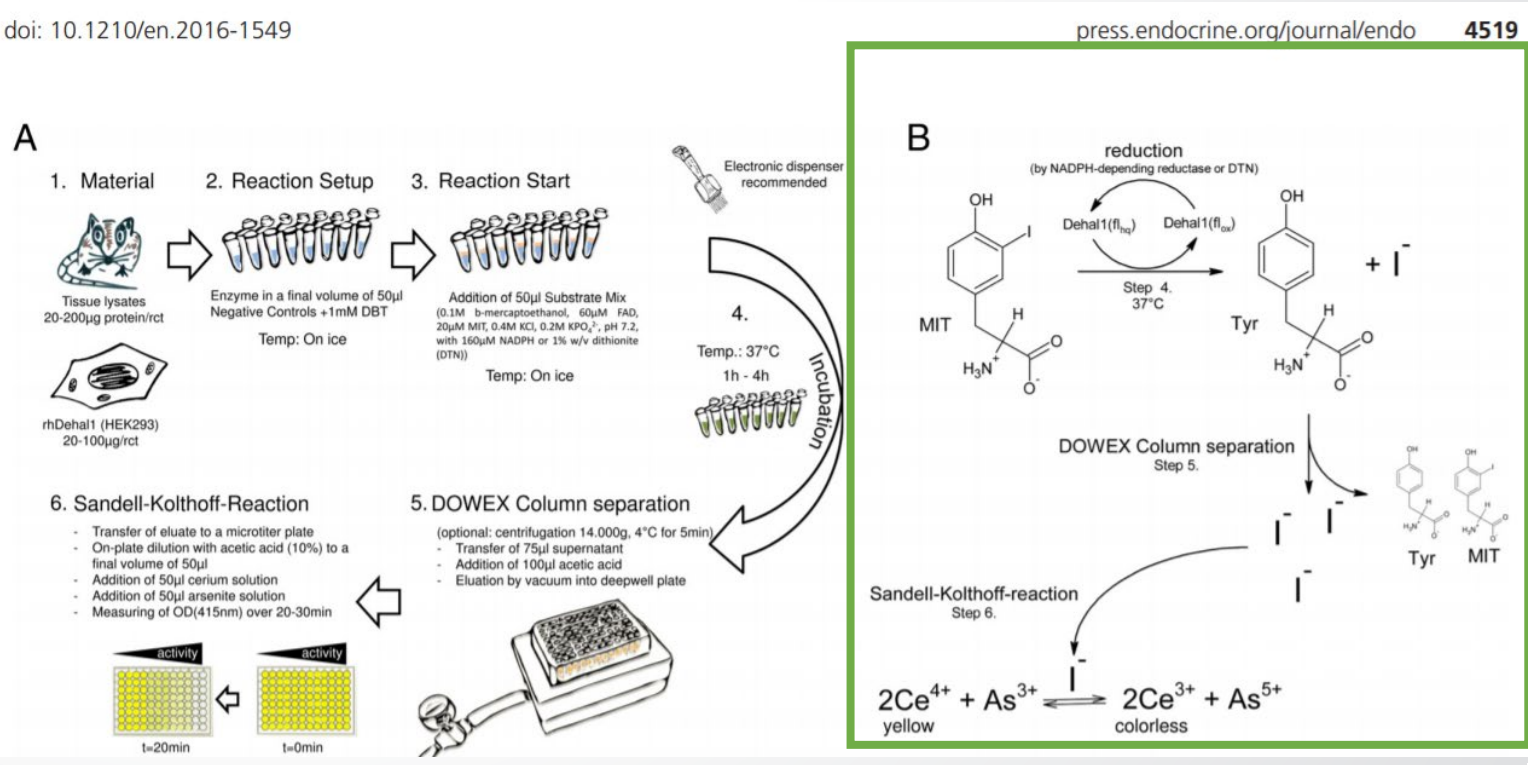
- HEK293 cell lysates overexpressing DIO1, DIO2, DIO3
- Method similar to Renko et al. 2016 (below) to detect excess iodide
- Examples: DIO1: genistein, PTU, iopanoic acid

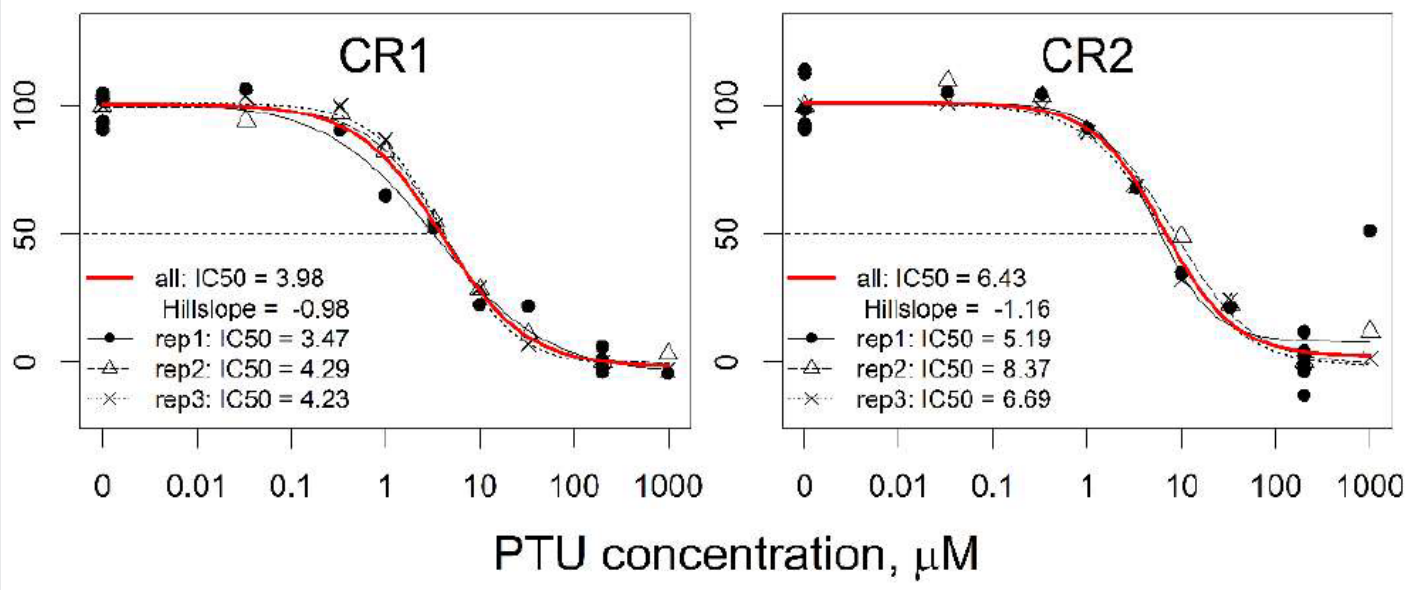
doi: 10.1210/en.2016-1549



Olker JH, Korte JJ, Denny JS, Hartig PC, Cardon MC, Knutsen CN, Kent PM, Christensen JP, Degitz SJ, Hornung MW. (2019). Screening the ToxCast Phase 1, Phase 2, and e1k Chemical libraries for Inhibitors of Iodothyronine Deiodinases doi: 10.1093/toxsci/kfy302

Hornung MW, Korte JJ, Olker JH, Denny JS, Knutsen C, Hartig PC, Cardon MC, Degitz SJ. (2018). Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity. 10.1093/toxsci/kfx279

Context for interpretation



Example highly reproducible PTU inhibition of DIO1 (from Hornung et al. 2018 Supp Figs)

- Hit rates are a bit lower than the TPO and NIS assays for 20% inhibition (~10-20%)
- Interference from surfactants or chemicals that disrupt membranes/nonspecific protein inhibition
- Iodine-containing substances are not amenable to the Sandell-Kolthoff chemistry
- Most potent and selective modes of action again might be considered

Indicators of hepatic catabolism

aeid	aenm
806	TOX21_AhR_LUC_Agonist
807	TOX21_AhR_LUC_Agonist_viability
116	ATG_CAR_TRANS_up
712	NVS_NR_hCAR_Agonist
713	NVS_NR_hCAR_Antagonist
1405	ATG_CAR_TRANS_dn
2047	TOX21_CAR_Agonist
2048	TOX21_CAR_Agonist_viabillity
2049	TOX21_CAR_Antagonist
2050	TOX21_CAR_Antagonist_viability
103	ATG_PXRE_CIS_up
135	ATG_PXR_TRANS_up
721	NVS_NR_hPXR
1474	ATG_PXRE_CIS_dn
1475	ATG_PXR_TRANS_dn
2362	TOX21_PXR_viability
2363	TOX21_PXR_Agonist

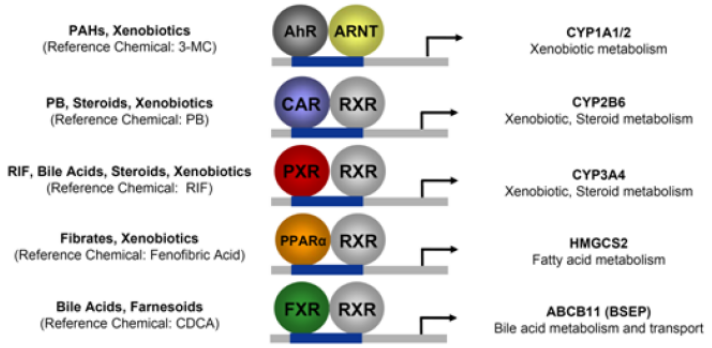
- ToxCast/Tox21 is so rich with assays to examine nuclear receptors and hepatic catabolism, but not all substances that activate these receptors and downstream metabolism cause thyroid effects *in vivo* (research/data gap).

- The list of nuclear receptor related assays is still growing…too many to list…search by associated gene name

ToxCast liver-related models contain indicators of Phase I and II metabolism and transporters

CellzDirect (CLD): fewer genes, ToxCast Phase I only

- ToxCast 320 Chemical Library
- Fresh Primary Human Hepatocytes
- 2 human donors
- 6 Reference Chemicals (Rif, PB, 3-MC, Fenofibric Acid, CDCA, CITCO)
- 5 receptors targets (AhR, CAR, PXR, PPARα, FXR)
- 2 endogenous control gene targets (GAPDH, Actin)
- 14 relevant gene targets
- 3 Time Points (6,24,48 hours)
- 5 Concentrations (.004, .04,0.4, 4, 40 µM)



LifeTech Expression Analysis (LTEA): HepaRG cells, 1060 substances

- ToxCast Phase I and Phase II Chemical library
- 189 assay endpoints, including ~93 genes: biotransformation, transporters, cell cycle, disease state markers (inc microRNA), etc.

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www.nature.com/npjsba

ARTICLE OPEN

Check for updates

High-throughput toxicogenomic screening of chemicals in the environment using metabolically competent hepatic cell cultures

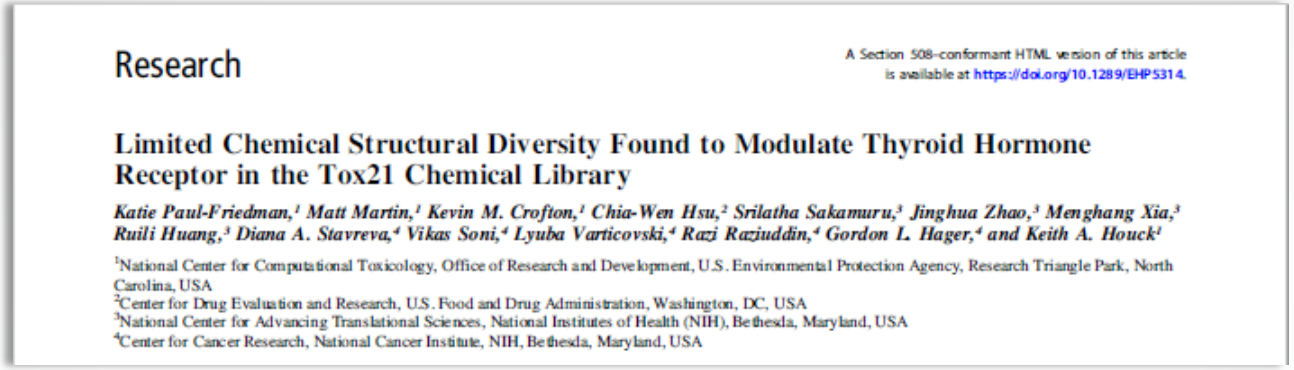
Jill A. Franzosa[1], Jessica A. Bonzo[2], John Jack[1], Nancy C. Baker[3], Parth Kothiya[1], Rafal P. Witek[2], Patrick Hurban[4], Stephen Siferd[4], Susan Hester[1], Imran Shah[1], Stephen S. Ferguson[5], Keith A. Houck[1] and John F. Wambaugh[1]✉

10.1038/s41540-020-00166-2

Thyroid hormone receptor assays

aeid	Assay endpoint name (aenm)	aeid	Assay endpoint name (aenm)
143	ATG_THRa1_TRANS_up	2226	TOX21_TR_LUC_GH3_Agonist_Followup
724	NVS_NR_hTRa_Antagonist	2226	TOX21_TR_LUC_GH3_Agonist_Followup
803	TOX21_TR_LUC_GH3_Agonist	2227	TOX21_TR_LUC_GH3_Antagonist_Followup
803	TOX21_TR_LUC_GH3_Agonist	2227	TOX21_TR_LUC_GH3_Antagonist_Followup
803	TOX21_TR_LUC_GH3_Agonist	2230	TOX21_TRA_COA_Agonist_Followup_ratio
804	TOX21_TR_LUC_GH3_Antagonist	2236	TOX21_TRB_BLA_Agonist_Followup_ratio
804	TOX21_TR_LUC_GH3_Antagonist	2237	TOX21_TRB_BLA_Agonist_Followup_viability
804	TOX21_TR_LUC_GH3_Antagonist	2240	TOX21_TRB_BLA_Antagonist_Followup_ratio
805	TOX21_TR_LUC_GH3_Antagonist_viability	2241	TOX21_TRB_BLA_Antagonist_Followup_viability
1094	LTEA_HepaRG_THRSP_dn	2244	TOX21_TRB_COA_Agonist_Followup_ratio
1095	LTEA_HepaRG_THRSP_up	2247	TOX21_TRB_COA_Antagonist_Followup_ratio
1369	ATG_THRb_TRANS2_up	2253	TOX21_TR_RXR_BLA_Agonist_Followup_ratio
1498	ATG_THRa1_TRANS_dn	2254	TOX21_TR_RXR_BLA_Agonist_Followup_viability
1499	ATG_THRb_TRANS2_dn	2689	ERF_NR_hTHRA_Agonist

Evaluating the hypothesis that the thyroid hormone receptor is less promiscuous than other steroid hormone receptors



Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5314.

Limited Chemical Structural Diversity Found to Modulate Thyroid Hormone Receptor in the Tox21 Chemical Library

Katie Paul-Friedman,[1] Matt Martin,[1] Kevin M. Crofton,[1] Chia-Wen Hsu,[2] Srilatha Sakamuru,[3] Jinghua Zhao,[3] Menghang Xia,[3] Ruili Huang,[3] Diana A. Stavreva,[4] Vikas Soni,[4] Lyuba Varticovski,[4] Razi Raziuddin,[4] Gordon L. Hager,[4] and Keith A. Houck[1]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA
[2]Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Washington, DC, USA
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[4]Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland, USA

- Hypothesis: TR modulators represent limited structural diversity.
  - X-ray crystallography of TR isoforms suggests the need for high homology to thyroid hormone.
  - Few known TRβ therapeutic selective agonists and antagonists and with limited diversity.
  - Some *in vitro* reports of TR modulation, possibly via interaction with recruitment of corepressors/coactivators to the receptor complex.
  - Examples in the literature: OH-PCBs, OH-PBDEs, BPA and TBBPA.

Integrating multiple assay endpoints: agonism and antagonism of thyroid hormone receptor (TR) occurs with a limited number of substances

We tested the hypothesis that TR has a more restrictive ligand-binding pocket than estrogen and androgen receptors using Tox21 screening and follow-up assays.

Table 1. Assay names (aenm) and assay end point identification (aeid) values used in the text and invitrodb database together with mode and purpose of assay.

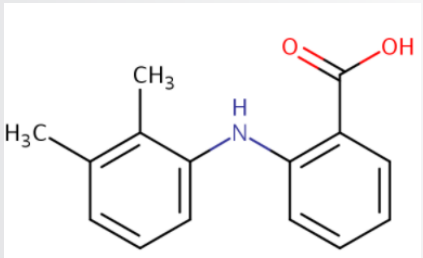
Assay short name	invitrodb: aenm	invitrodb: aeid	Cell line	Assay mode	Function
GH3-TRE-Ag	TOX21_TR_LUC_GH3_Agonist	803	GH3-TRE-Luc	Agonist	Primary qHTS
GH3-TRE-Antag	TOX21_TR_LUC_GH3_Antagonist	804	GH3-TRE-Luc	Antagonist	Primary qHTS
GH3-TRE-Via	TOX21_TR_LUC_GH3_Antagonist_viability	805	GH3-TRE-Luc	Viability	Cytotoxicity
GH3-TRE-Ag-Followup	TOX21_TR_LUC_GH3_Agonist_Followup	2226	GH3-TRE-Luc	Agonist	Confirmation
GH3-TRE-Antag-Followup	TOX21_TR_LUC_GH3_Antagonist_Followup	2227	GH3-TRE-Luc	Antagonist	Confirmation
TRb-bla	TOX21_TRB_BLA_Antagonist_Followup_ratio	2240	TRβ-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-bla-Ag	TOX21_TR_RXR_BLA_Agonist_Followup_ratio	2253	RXRα-UAS-bla HEK 293T	Agonist	Specificity
RXRa-bla-Antag	TOX21_TR_RXR_BLA_Antagonist_Followup_ratio	2257	RXRα-UAS-bla HEK 293T	Antagonist	Specificity
RXRa-Via	TOX21_TR_RXR_BLA_Antagonist_Followup_viability	2258	RXRα-UAS-bla HEK 293T	Viability	Cytotoxicity
TRa-coa	TOX21_TRA_COA_Agonist_Followup_ratio	2230	NA	Agonist	Orthogonal
TRb-coa	TOX21_TRB_BLA_Agonist_Followup_ratio	2236	NA	Agonist	Orthogonal
GFP-GR-TRb	NA	NA	GFP-GR-TRβ MCF7	Agonist and antagonist	Orthogonal

Note: Ag, agonist; Antag, antagonist; bla, beta-lactamase; coa, coactivator; GFP, green fluorescent protein;GH3, rat pituitary cell line; GR, glucocorticoid receptor; HEK 293T, human embryonic kidney cell line; LUC, luciferase; MCF7, human breast cancer cell line; NA, not applicable; qHTS, quantitative high-throughput screen; RXRa, retinoid X receptor alpha; TRa, thyroid hormone receptor alpha; TRb, thyroid hormone receptor beta; TRE, thyroid hormone receptor response element; UAS, upstream activating sequence; Via, viability.

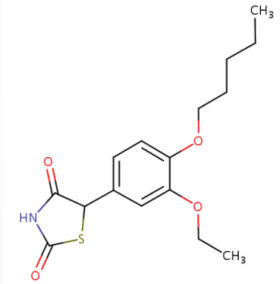
Agonism and antagonism of thyroid hormone receptor (TR) occurs with a limited number of substances

- 11 chemicals identified of 8,305 unique substances as putative direct TR ligands
  - 8 agonists
    - T3 analogs (see table to right)
  - Additional 9 chemicals, largely pharmaceuticals, that agonize RXR through TR:RXR heterodimer resulting in partial agonism in the transactivation assays (permissive heterodimer effect); no activity when RXR not present
  - 3 antagonists of higher confidence: pharmaceuticals, at concentrations exceeding therapeutic concentrations

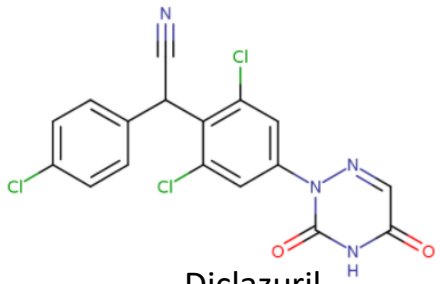
Chemical name
CP-634384
3,5,3′-Triiodothyronine
Levothyroxine
Tetrac
3,3′,5′-Triiodo-L-thyronine
Tiratricol
3,3′,5-Triiodo-L-thyronine sodium salt
Betamipron



Mefenamic acid
(NSAID, some evidence of plasma TH effects in rats)



Risarestat
(aldose reductase inhibitor for hypoglycemia assoc. with diabetes)



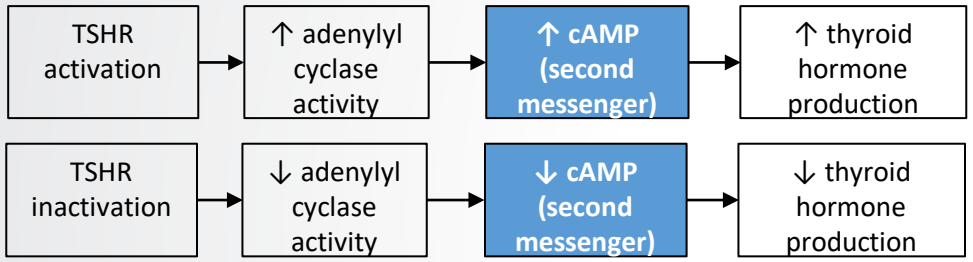
Diclazuril
(anticoccidal used in poultry)

Overall conclusion: work supports the hypothesis that TR is a very selective nuclear receptor.

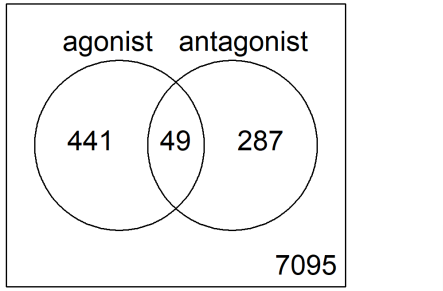
This work used a lot of expert judgment and substances with clear lead MOA were excluded from follow-up.

TOX21 TSHR assay principle

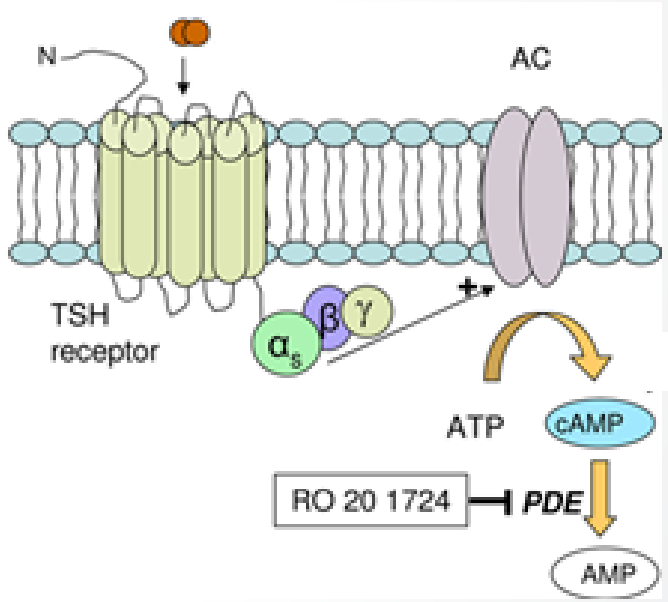
aeid	aenm
2040	TOX21_TSHR_HTRF_Agonist_ratio
2043	TOX21_TSHR_HTRF_Antagonist_ratio
2046	TOX21_TSHR_HTRF_wt_ratio



cAMP is the signal measured in this assay platform



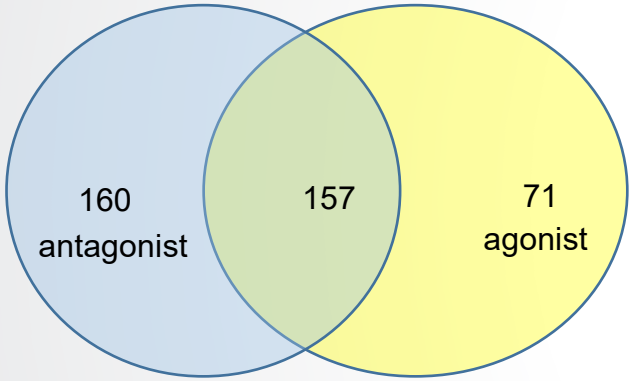
- TSHR is a GPCR with a few known agonists or antagonists.
- This assay measures agonism or antagonism for TSHR through the Gs-cAMP pathway.



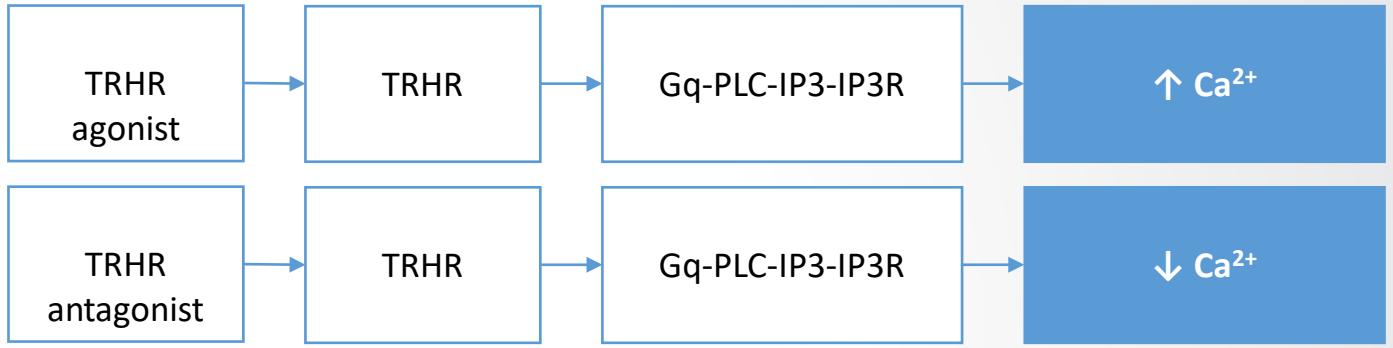
- Hits from the primary screen need to be confirmed or evaluated with orthogonal information.
- Assay interference may come from cytotoxicity, auto-fluorescent or blue dyes, agonists of other GPCRs may modulate cAMP, (e.g., B-adrenergic receptors) and other activators of adenylyl cyclase.

TOX21 TRHR assay principle

aeid	aenm
2364	TOX21_TRHR_HEK293_Agonist
2365	TOX21_TRHR_HEK293_Antagonist



388 Total Hits



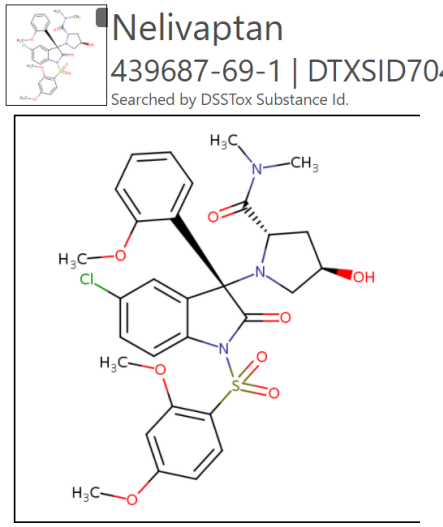
Calcium is detected using a fluorescence detection kit

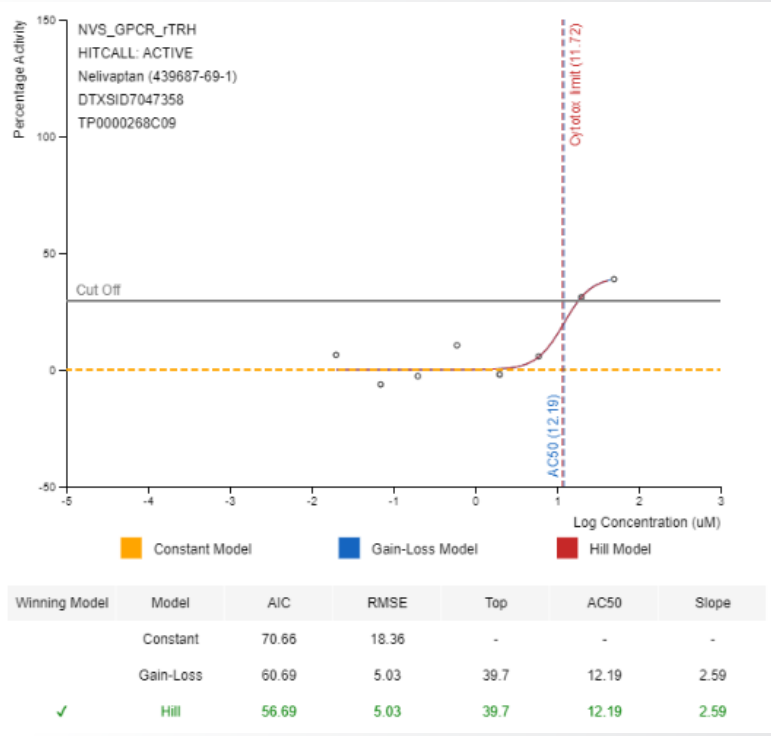
- Hits from the primary screen need to be confirmed or evaluated.
- Potential sources of interference: auto-fluorescence, nonspecific calcium interference, nonspecific GPCR activity, etc.
- Ongoing work to contextualize these results using molecular docking approaches.
- View these hits as putative until additional confirmation can be used.

Assay principle of the NVS TRHR assay

aeid	aenm
683	NVS_GPCR_rTRH

- Measures changes in scintillation (radioactivity) counts from [[3H]-(3-methylHis[2])-TRH] binding to rat TRHR.
- TRHR from rat forebrain membranes.
- 1000 substances screened in multi-concentration– limited overlap in the screen with the TOX21 TRHR screen, and nearly no overlap in hits.
- 35/1000 are hitcall=1; some clear interference from organometallic substances and detergents; borderline or noisy activity; possibly other GPCR modulators. Most of these hits seem easy to dismiss when inspecting the curves.





Winning Model	Model	AIC	RMSE	Top	AC50	Slope
	Constant	70.66	18.36	-	-	-
	Gain-Loss	60.69	5.03	39.7	12.19	2.59
✓	Hill	56.69	5.03	39.7	12.19	2.59

Nelivaptan is one of the only credible putative hits, but it has clear PXR activity at lower concentrations. This drug was developed for another GPCR, vasopressin receptor V1B in the anterior pituitary gland that works to release ACTH, prolactin, endorphins.

Conclusions for the thyroid bioactivity section

- Consider the specific molecular initiating event or group of molecular initiating events to locate the bioactivity data
- Additional effort is likely needed to identify the most relevant thyroid-related bioactivity, e.g. comparison to cytotoxicity, reactivity, or other bioactivities
- Redundant screening using confirmatory or orthogonal assay data is not available for all thyroid-relevant molecular-initiating events

Appendix for reference: progress on steroidogenesis

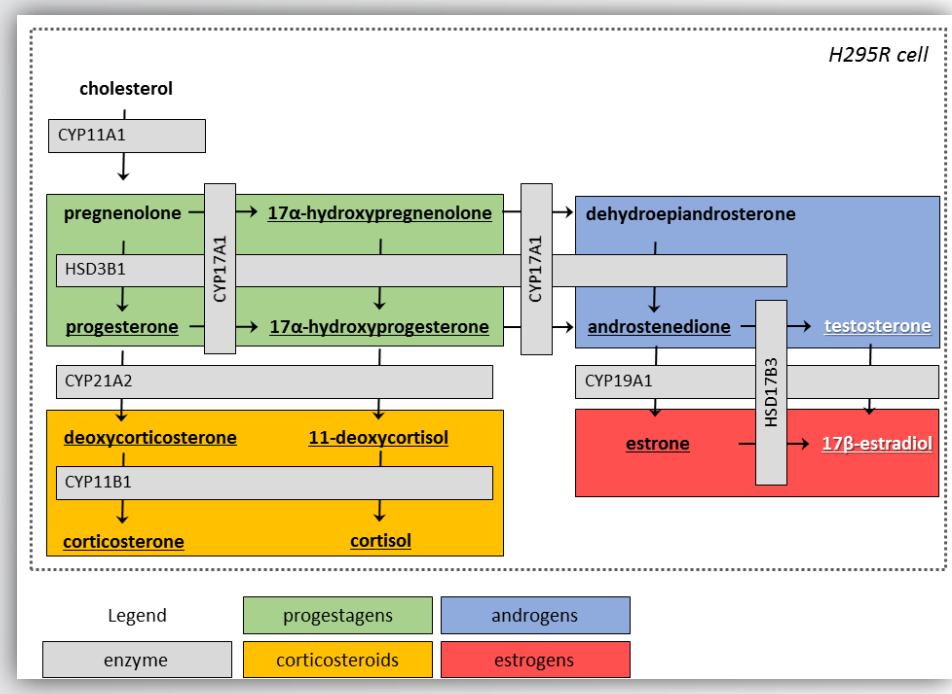
ToxCast HT-H295R assay, model, and structure-activity relationships: evolution of a tool for potential regulatory applications

Assay development
(Karmaus et al., 2016)

Model and comparison to OECD validation study results
(Haggard et al., 2018)

Further evaluation of the model and demonstration of prioritization
(Haggard et al., 2019)

(Q)SAR approaches for HT-H295R bioactivity prediction
(Foster et al., 2022)



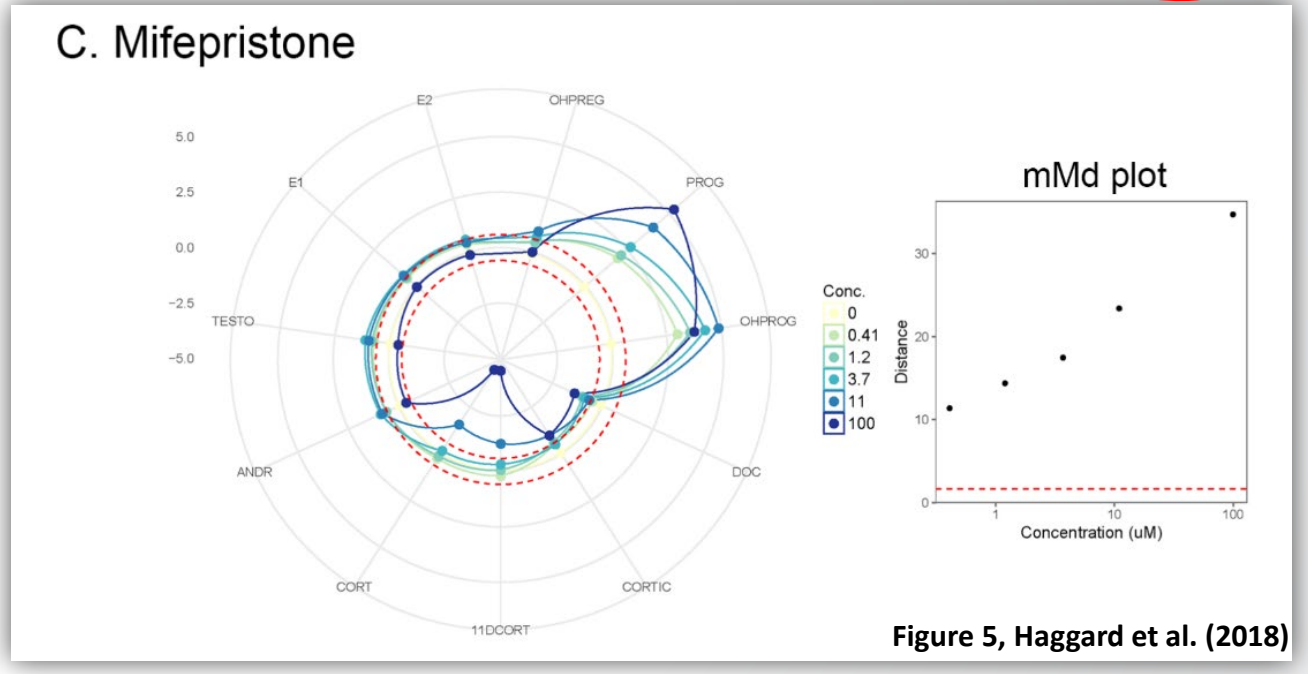


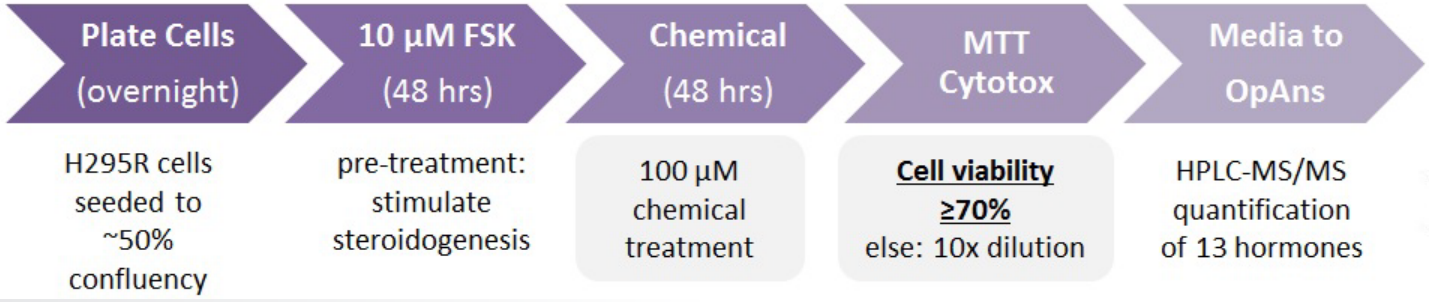
Figure 5, Haggard et al. (2018)

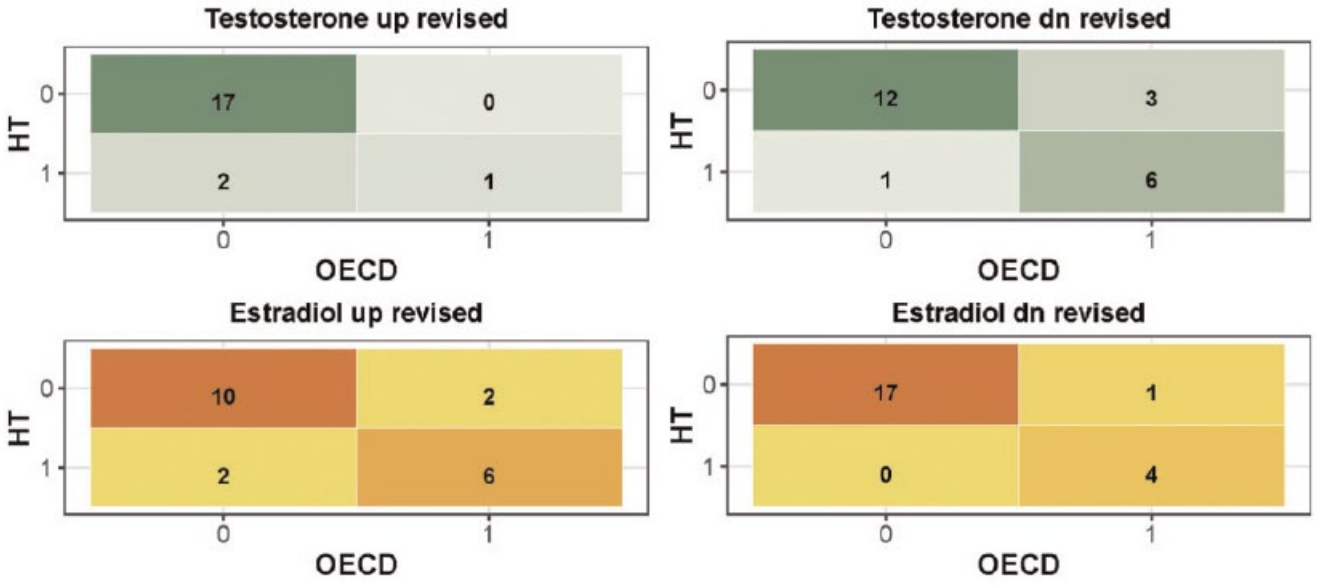
This HT-H295R assay implementation in ToxCast, and the model (using Mahalanobis distance), with comparison to OECD H295R assay validation study, were all presented to a FIFRA SAP in November 2017.
https://www.regulations.gov/docket/EPA-HQ-OPP-2017-0214

Latest research completed

Since there are > 6000 chemicals of interest for the EDSP that lack HT-H295R bioactivity results, this bioactivity can be predicted using a multi-strategy approach for structure-activity relationships, including preliminary structure alerts, machine learning, and nearest neighbor approaches (Foster *et al.*, 2022, Computational Toxicology).
ORD Lead: Katie Paul Friedman, ORD-CCTE

Comparison to the OECD interlaboratory validation exercise suggests that the HT-H295R assay performed well





Karmaus *et al.* (2016) and Haggard *et al.* (2018)

HT-H295R performance compared to OECD interlaboratory trial

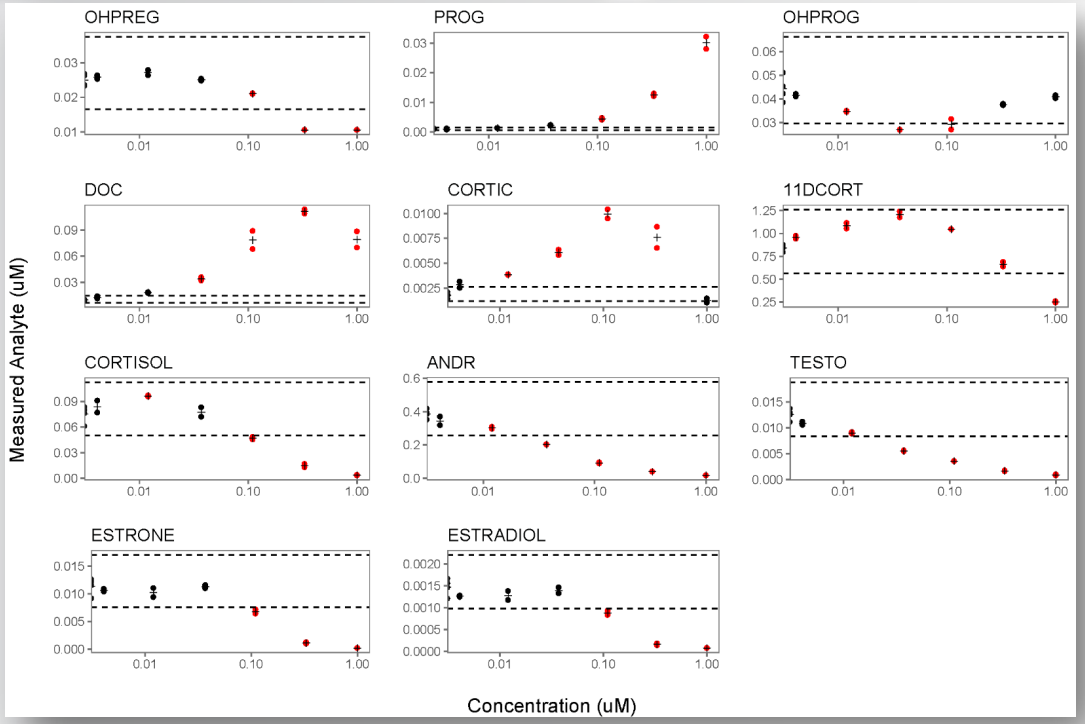
Effect	Revised Sensitivity	Revised Specificity	Revised Accuracy
Testosterone up	1.00	0.89	0.90
Testosterone dn	0.67	0.92	0.82
Estradiol up	0.75	0.83	0.80
Estradiol dn	0.80	1.00	0.95

OECD interlaboratory trial reproducibility

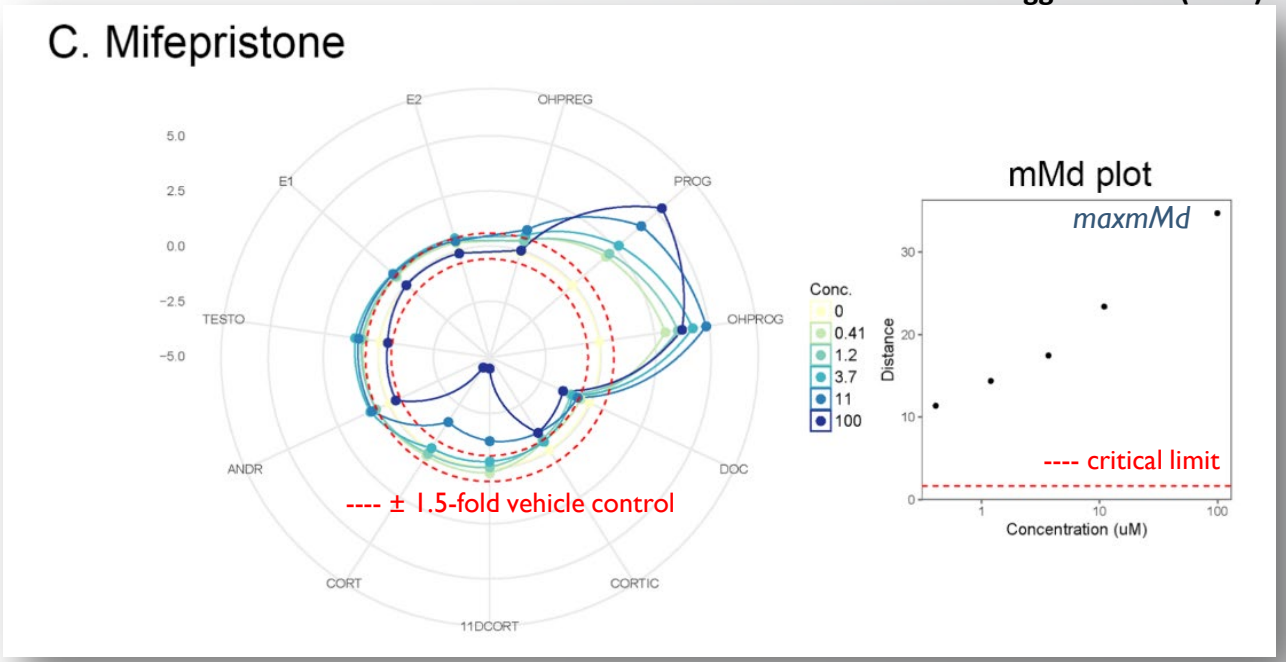
Chemical set	% concordance among labs	
	E2	T
12 core	0.95	0.88
16 supplemental	0.84	0.91
Total	0.89	0.90

Despite experimental differences to make the assay higher throughput, comparison of the HT-H295R E2 and T outcomes shows balanced accuracy similar to the maximum interlaboratory trial reproducibility for reference chemicals.

HT-H295R statistical model for prioritization: the maximum mean Mahalanobis distance (maxmMd)

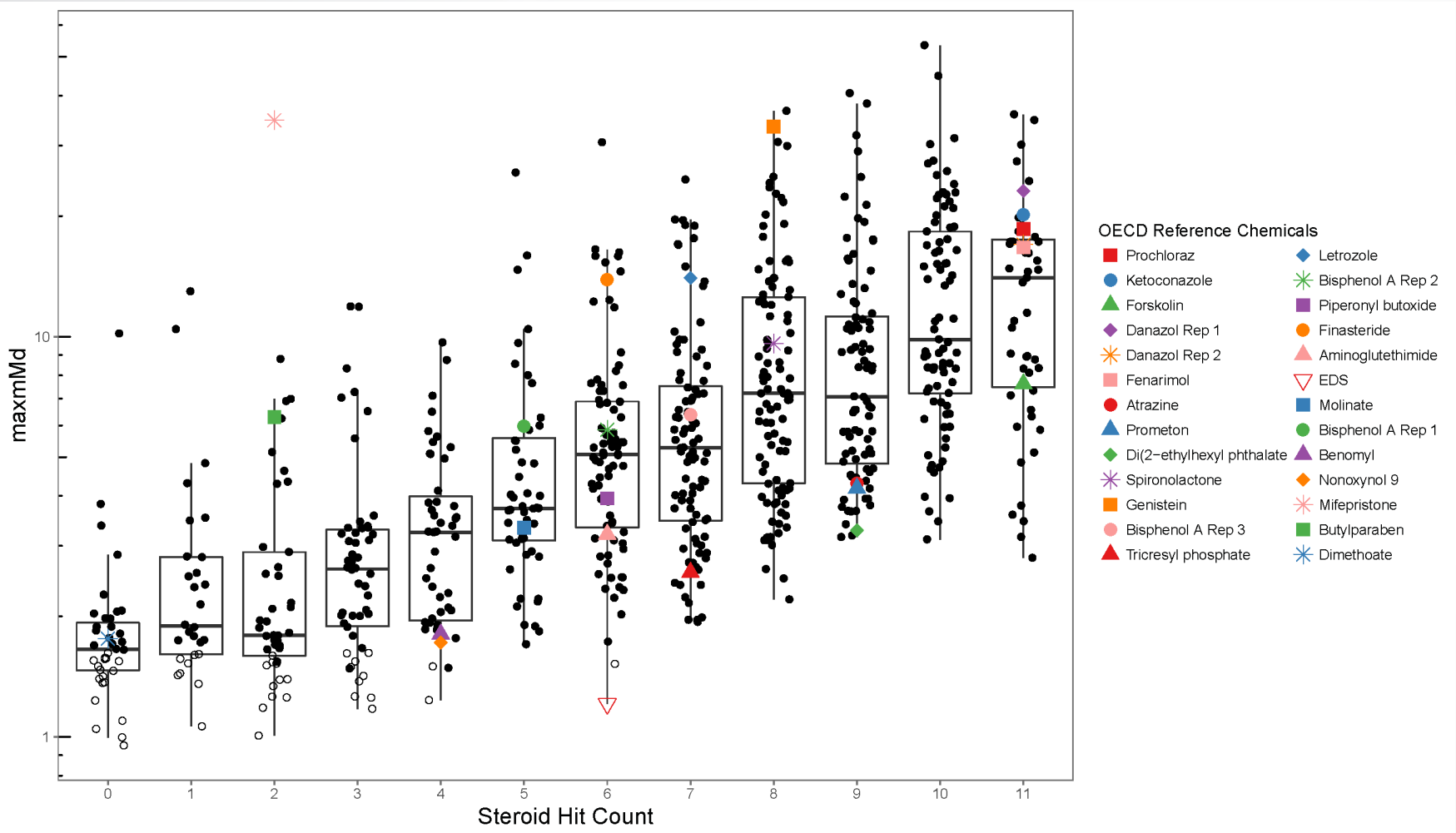


Haggard *et al.* (2018)



- Reduced an 11-dimensional question to a single dimension.
- Selection of the maxmMd appeared to provide a sensitive, reproducible, and quantitative approximation of the magnitude of effect on steroidogenesis.

Reference chemicals typically affected 2+ hormones in the HT-H295R assay, but had variable maxmMd by effect size

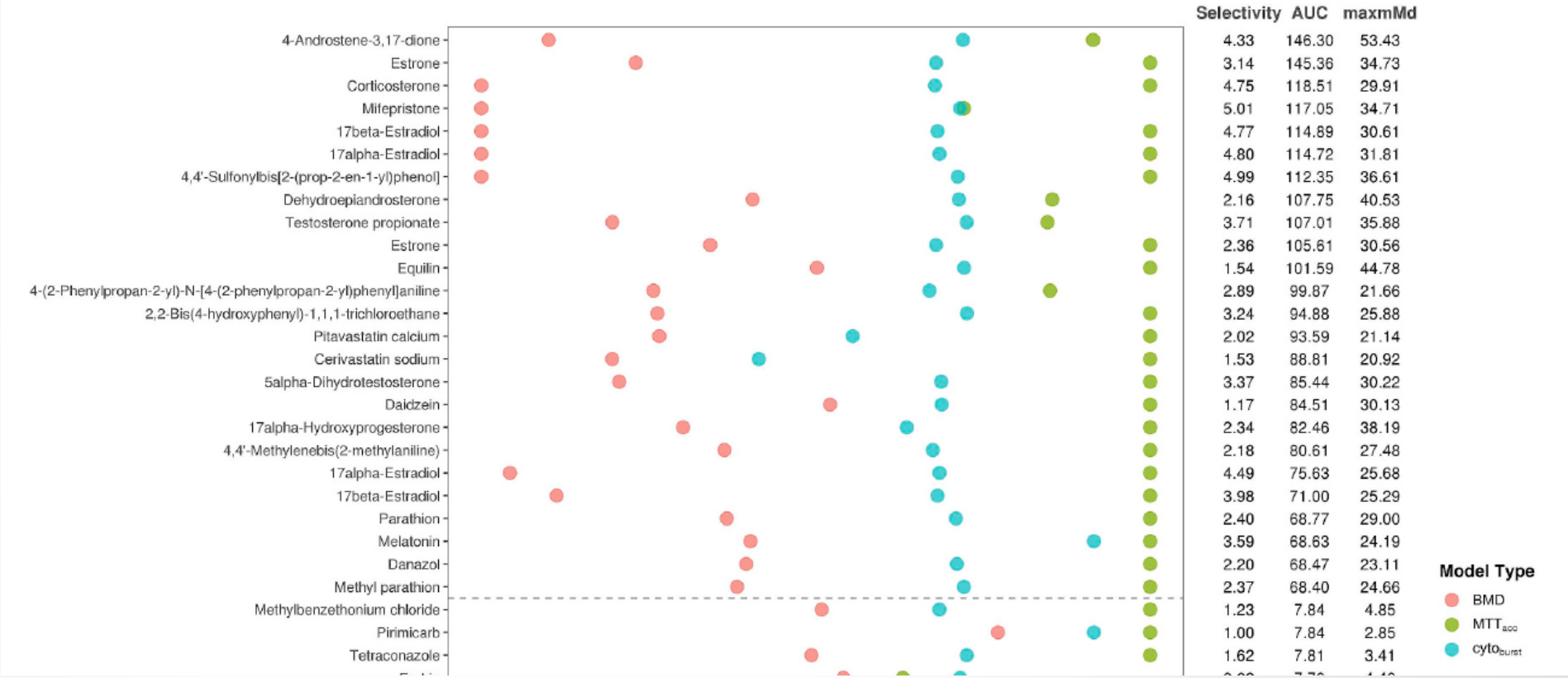


- Reinforced the idea that the H295R steroid biosynthesis is a dynamic and interdependent system.
- Illustrated that the maxmMd could distinguish chemicals with greater magnitude of effect (and potency), and that this value is distinct from the number of hormones affected.
- Presentation to a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel in Nov 2017 led to further investigation and demonstration of the approach (see Haggard *et al.* 2019).

Haggard *et al.* (2018)

Parallel cytotoxicity (MTT assay) and cytotoxicity threshold estimates may help prioritize chemicals with positive maxmMd by selectivity

- Top 25 most efficacious and most selective chemicals (above the dotted line) included many hormones, pharmaceuticals, and isoflavones.
- Cytotoxicity may provide context for relevant bioactive concentrations that perturb HT-H295R hormone synthesis.
- The maxmMd was a reasonable prioritization metric when combined with selectivity.



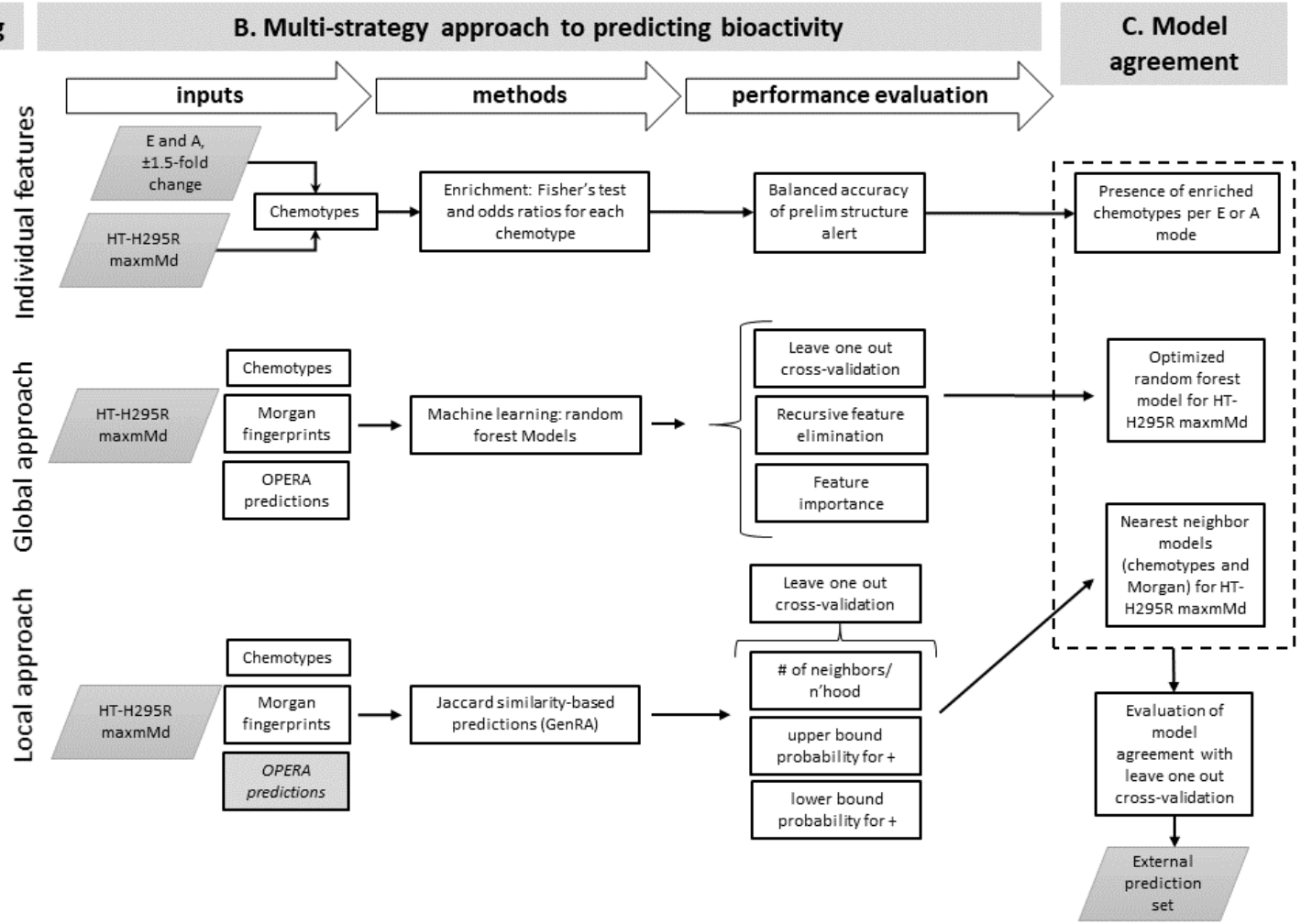
Haggard *et al.* (2019)

However, with only 654 chemicals with multi-concentration screening, and 2012 chemicals with single concentration screening, this approach would be insufficient to prioritize or inform the weight of evidence for all chemicals relevant to the EDSP.

How can we extend information from about ~2000 substances in the HT-H295R assay to larger chemical inventories of interest?

A. Assay data for model building

Bioactivity Modeled	(+) chems	(-) chems
Estrogen up	304	338
Estrogen dn	143	499
Androgen up	84	558
Androgen dn	267	375
HT-H295R maxmMd	555	845



- Individual features: whether a chemical shares structural features with chemicals that disrupt estrogen or androgen synthesis in HT-H295R
- Global approach: whether a chemical is predicted to perturb hormone biosynthesis in HT-H295R using a global random forest approach
- Local approach: whether a chemical shares structural features with chemicals that perturb HT-H295R bioactivity using a local nearest neighbor approach
- A heuristic model agreement score for HT-H295R bioactivity prediction was developed to easily communicate overall confidence in a chemical's positive or negative prediction for HT-H295R activity

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Summary of HT-H295R approaches

- HT-H295R screening assay as an alternative for the OECD-validated, low throughput H295R assay performed well.
  - The ANOVA analysis and logic used for the HT-H295R dataset to determine effects on the steroid biosynthesis pathway enabled a direct comparison of the OECD inter-laboratory validation data and the HT-H295R data.
- Novel integration of 11 steroid hormone analytes for pathway-level analysis using the HT-H295R assay data.
  - A mean Mahalanobis distance (mMd) was computed for each chemical concentration screened.
  - The mMd provided a set of unitless values from which the maximum mean Mahalanobis distance (maxmMd) could be calculated across the concentration range screened.
  - The maxmMd approach is reproducible in data simulations.
  - This maxmMd may be a useful prioritization metric.
- Structure-activity relationships may help identify chemicals of greatest interest for steroidogenesis screening in available high-throughput assay(s).
  - Extends the bioactivity screening information that was previously obtained for 2012 chemicals in the HT-H295R assay to address data gaps using an *in silico* method for thousands of substances of potential interest on the EDSPUOC list.
  - Assists with selection of chemicals for further evaluation of chemical effects on steroidogenesis or contribute to a weight-of-evidence approach for chemicals that have other sources of information regarding reproduction and hormone synthesis.

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