Research activities for thyroid-related bioactivity screening in EPA-ORD

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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

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]

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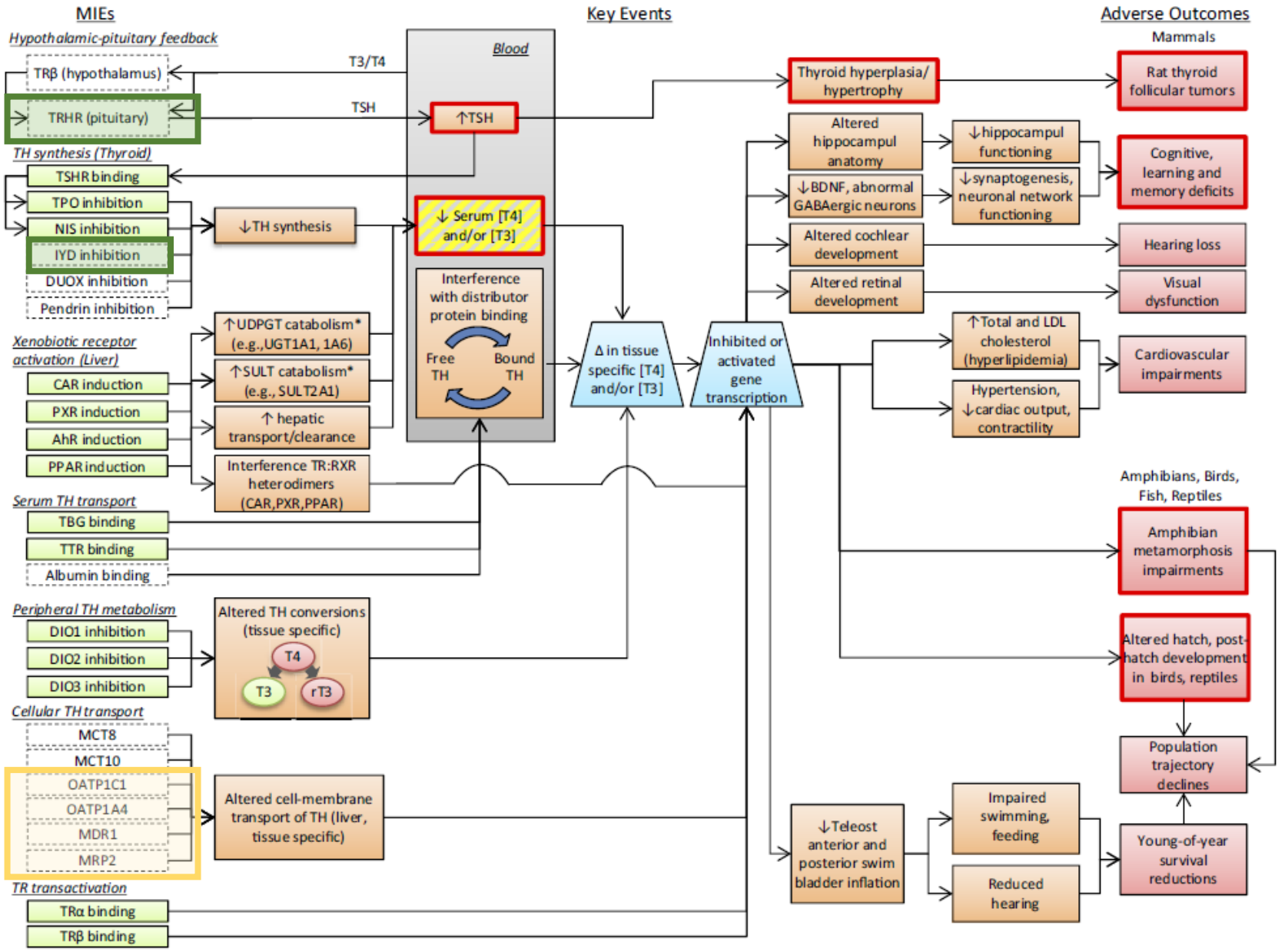
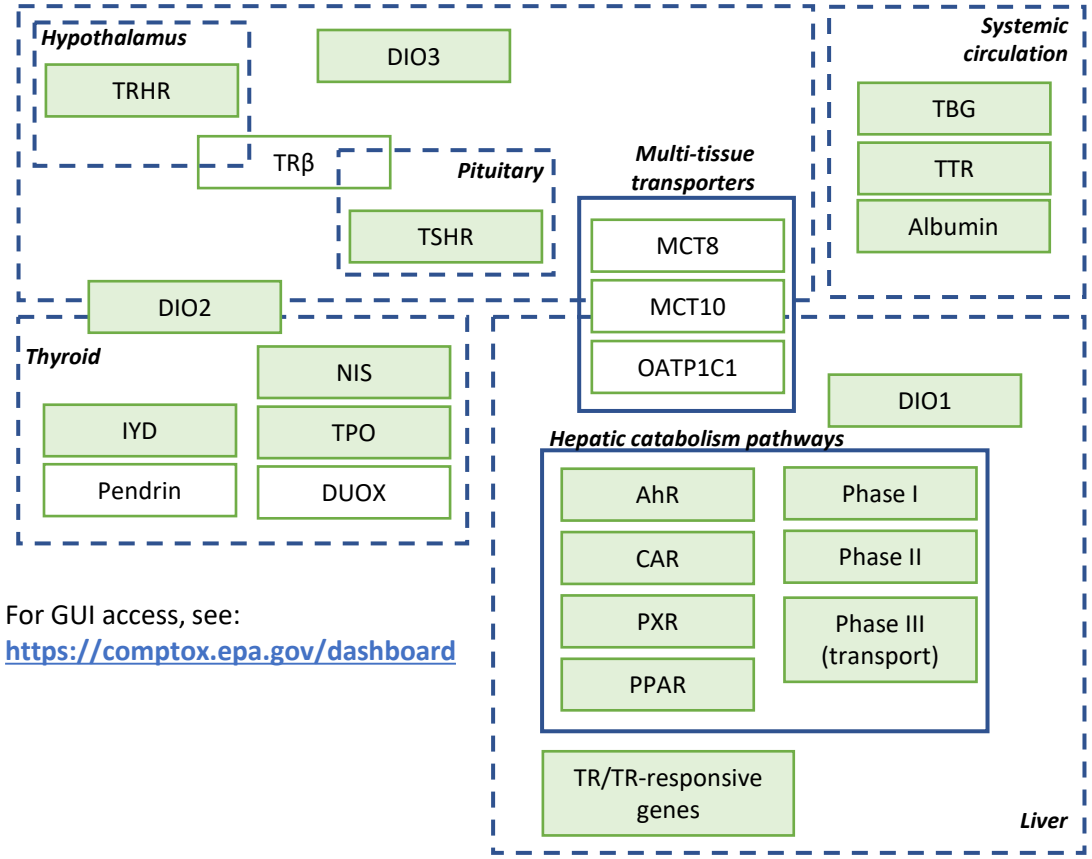


Research progress in EPA-ORD for thyroid tiered bioactivity screening

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

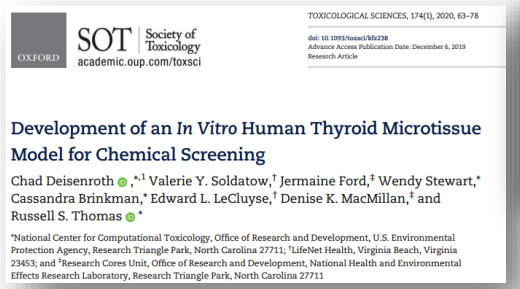


For GUI access, see:
https://comptox.epa.gov/dashboard

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.**

Tier 2-3 NAMs to confirm bioactivity and connect to KEs

Glandular TH synthesis


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

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

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

Chad Deisenroth, Valerie Y. Soldatow, Jermaine Ford, Wendy Stewart, Cassandra Brinkman, Edward L. LeCluyse, Denise K. MacMillan, and Russell S. Thomas

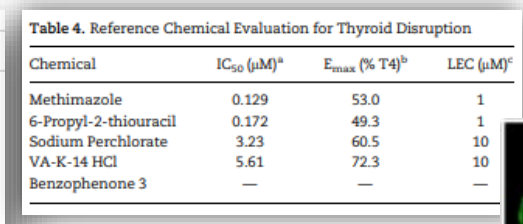
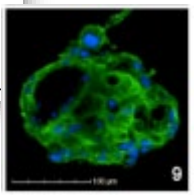


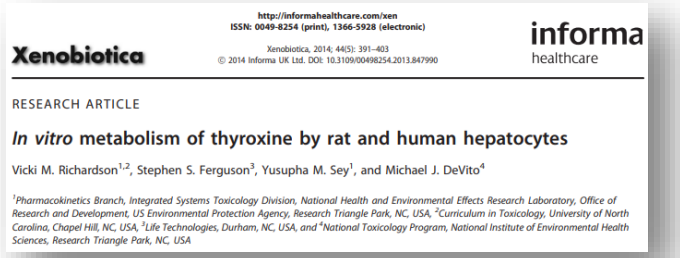
Table 4. Reference Chemical Evaluation for Thyroid Disruption

Chemical	IC_{50} (μM)[a]	E_{max} (% T4)[b]	LEC (μM)[c]
Methimazole	0.129	53.0	1
6-Propyl-2-thiouracil	0.172	49.3	1
Sodium Perchlorate	3.23	60.5	10
VA-K-14 HCl	5.61	72.3	10
Benzophenone 3	—	—	—



Human thyroid microtissue model could be used to confirm Tier 1 and 2 screening results that indicate possible effects on MIEs related to TH synthesis

Hepatic TH metabolism


http://informahealthcare.com/xen
ISSN: 0049-8254 (print), 1366-5928 (electronic)

Xenobiotica

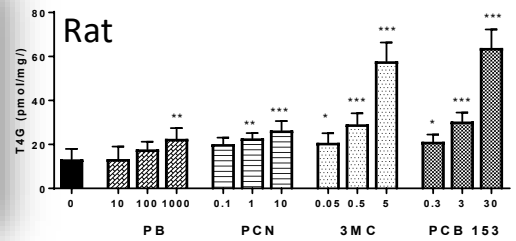
informa healthcare

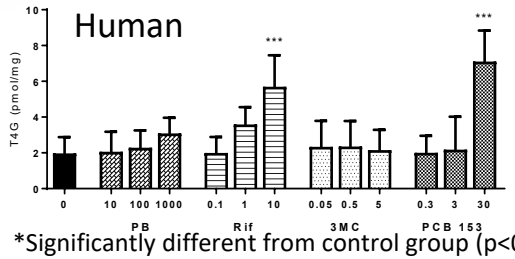
RESEARCH ARTICLE

In vitro metabolism of thyroxine by rat and human hepatocytes

Vicki M. Richardson, Stephen S. Ferguson, Yusupha M. Sey, and Michael J. DeVito

Richardson *et. al.*, (In preparation)





Rat and human hepatocyte models can be combined with analytical chemistry to detect THs and their metabolites to screen for chemical effects on TH status

*Significantly different from control group ($p<0.05$).

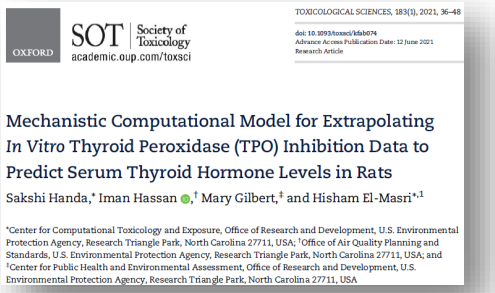
Research progress in EPA-ORD for thyroid tiered bioactivity screening

In silico NAMs for toxicokinetics, TH kinetics, and models to link screening to KEs and adverse outcomes

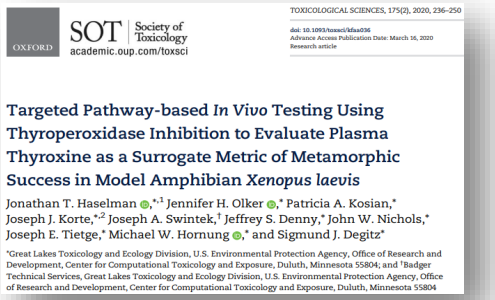
High-throughput toxicokinetic models

A maternal-fetal HTTK model, Kapraun, Wambaugh, et al. *in preparation; goal is to be able to understand how to prioritize chemicals based on exposure during critical windows of susceptibility for thyroid*

PBTK-TH kinetics



Connecting KEs to AOs



Prioritizing substances with exposures during critical windows of susceptibility

Understand if the bioactive concentration *in vitro* corresponds to internal exposures in humans

Connecting MIEs to critical key events in the AOP network (e.g., TPO inhibition to serum thyroid hormone levels)

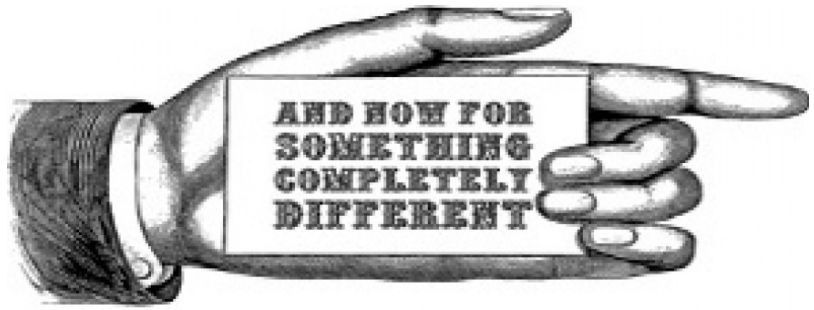
Connecting MIEs/KEs to adverse outcomes

Additional and ongoing research

	Examples
Orthogonal and confirmatory approaches	Cheminformatics, bioinformatics, etc. (e.g., ongoing work on TRHR using a cheminformatics approach) can be useful when a single screening assay is available
Combining all of the MIE data to prioritize for Tier 2 models	What is our confidence that changes at the MIE screening level translate to tissue level or serum TH outcomes?
Integration	How to use all screening data (ToxCast and international) for specific use cases?

Confirmatory screening in Tier 2-3 NAMs and these integrative models with available MIE screening data will be valuable for translating the data into information to evaluate the utility of screening data for use in weight-of-evidence, IATAs, DAs, etc.

And now for something completely different…in another session of the OECD EDTA meeting



A high-throughput H295R (HT-H295R) assay and model

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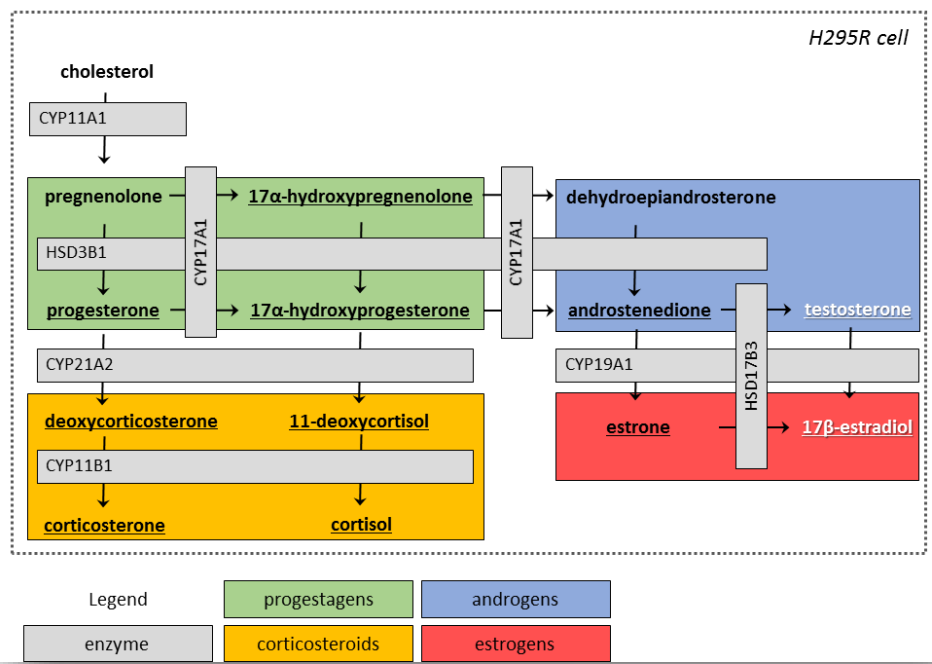
ToxCast HT-HT295R assay and model: evolution of a tool for potential regulatory applications

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)

QSAR approaches for HT-H295R bioactivity prediction
(Foster et al., in prep)



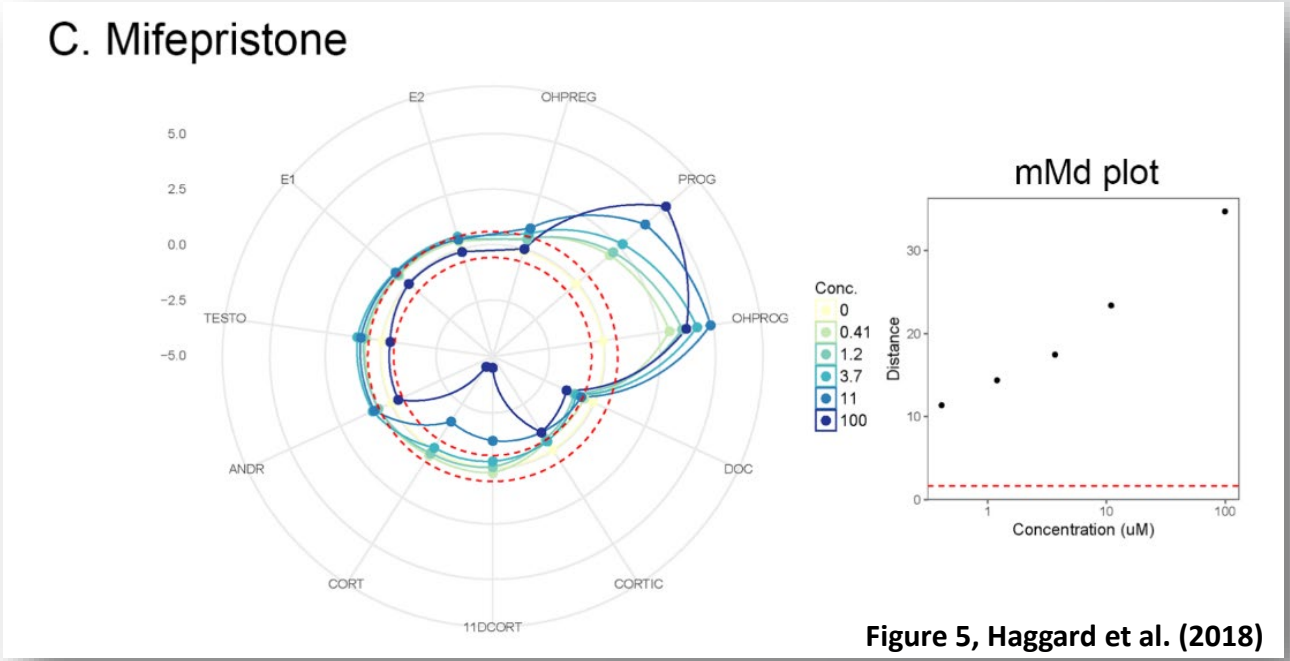


Figure 5, Haggard et al. (2018)

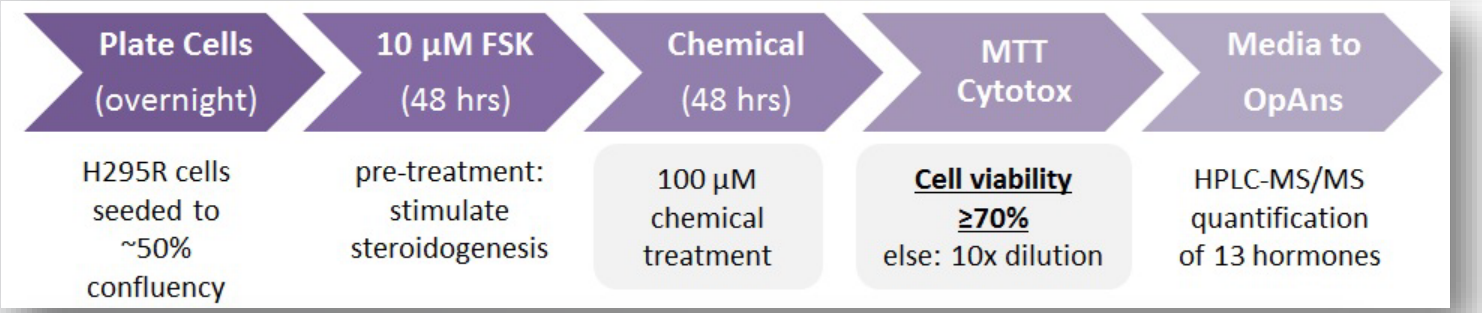
This HT-H295R assay implementation in ToxCast, and the model (using Mahalanobis distance), with comparison to OECD H295R assay validation study, were all presented to a FIFRA SAP in November 2017. https://www.regulations.gov/docket/EPA-HQ-OPP-2017-0214

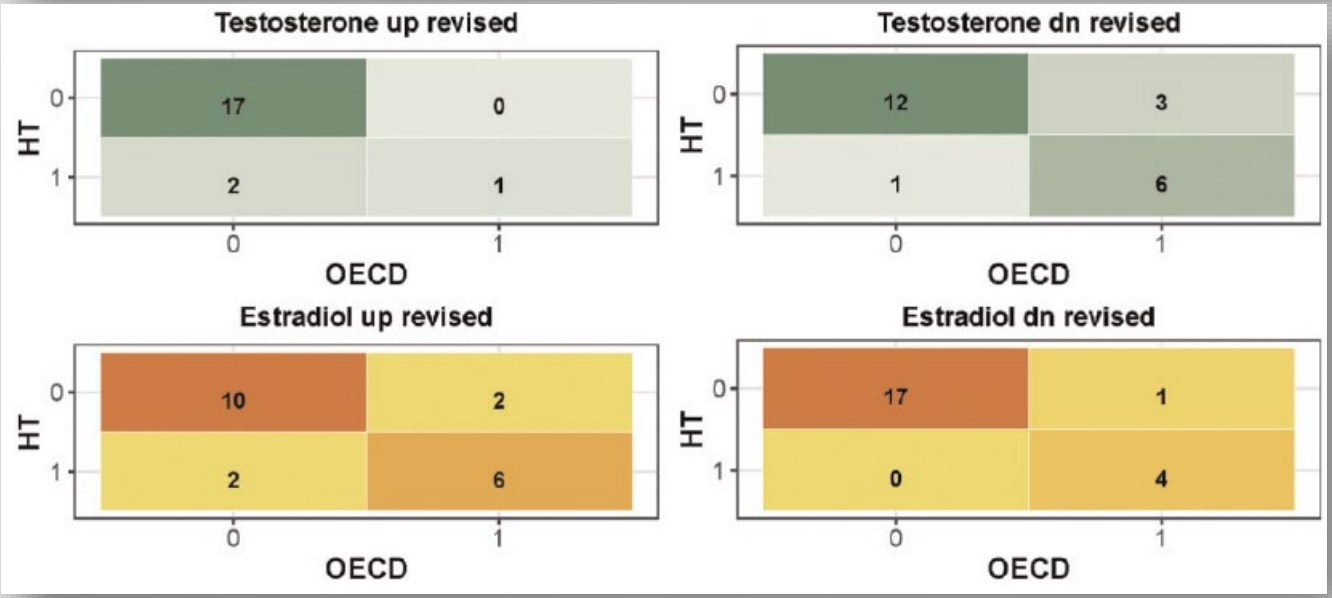
Research Status

Preparing a manuscript now on how to apply structure-activity relationships, including a machine learning approach and nearest neighbor approaches, to predict HT-H295R bioactivity for the rest of the EDSP Universe of chemicals

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

Haggard *et al.* (2018)

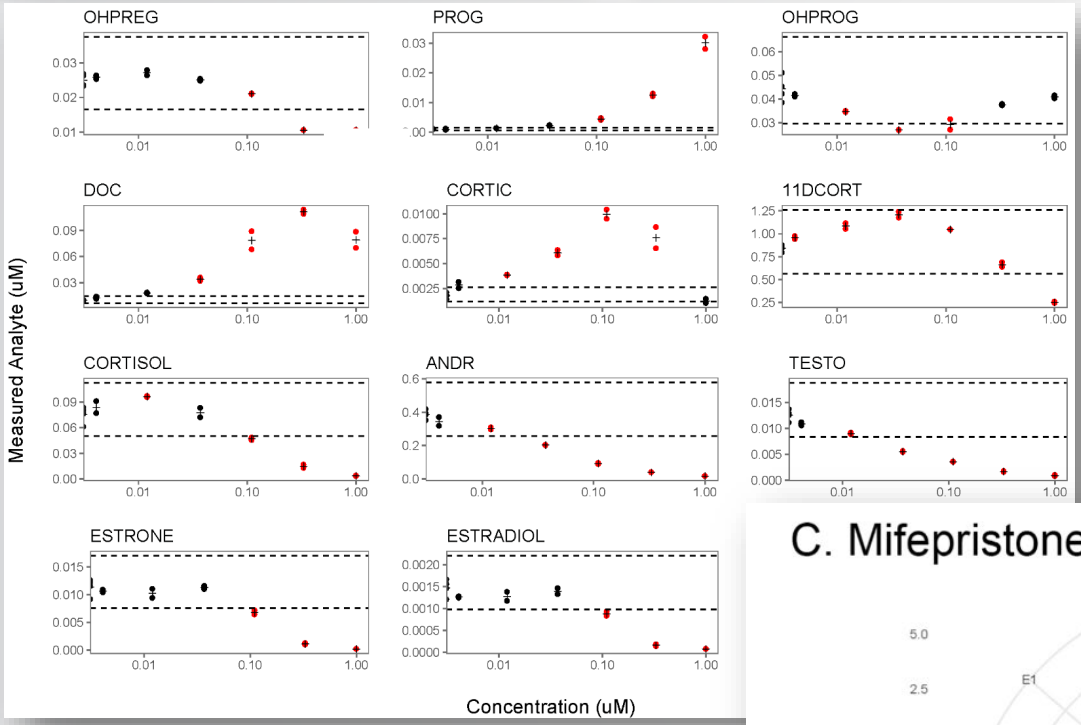
Revised Sensitivity	Revised Specificity	Revised Accuracy
1.00	0.89	0.90
0.67	0.92	0.82
0.75	0.83	0.80
0.80	1.00	0.95

OECD interlaboratory trial reproducibility

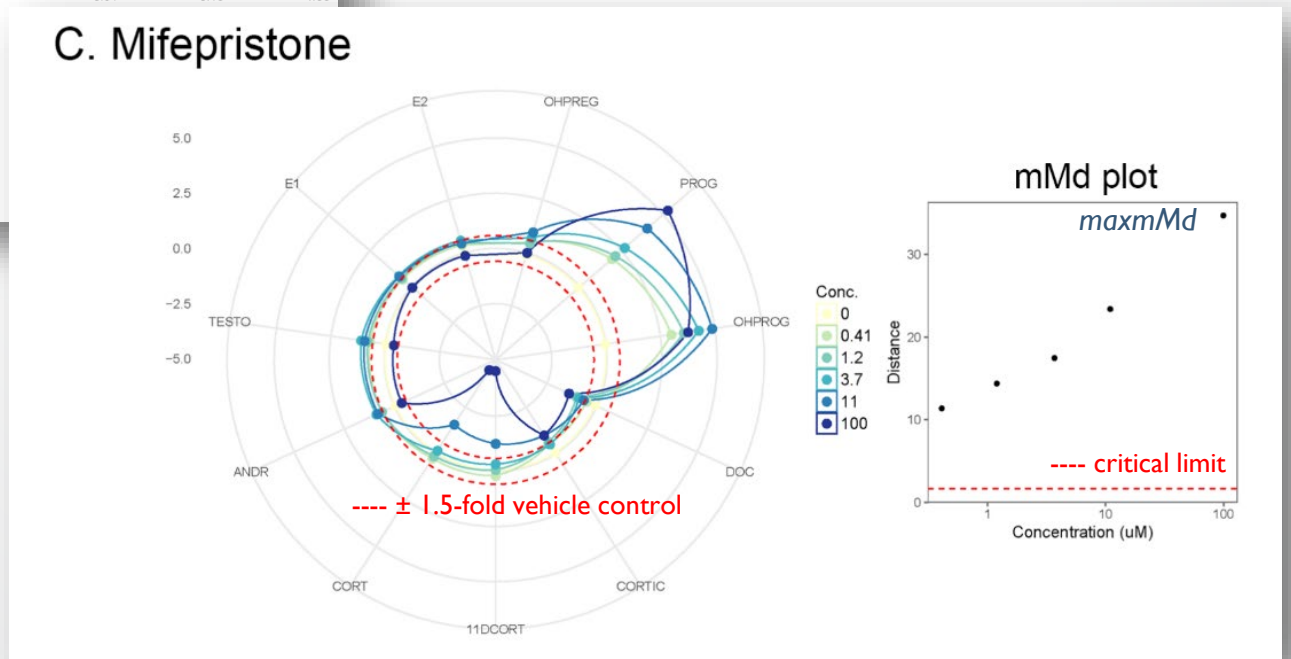
Chemical set	% concordance among labs	
	E2	T
12 core	0.95	0.88
16 supplemental	0.84	0.91
Total	0.89	0.90

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.

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



- 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.



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

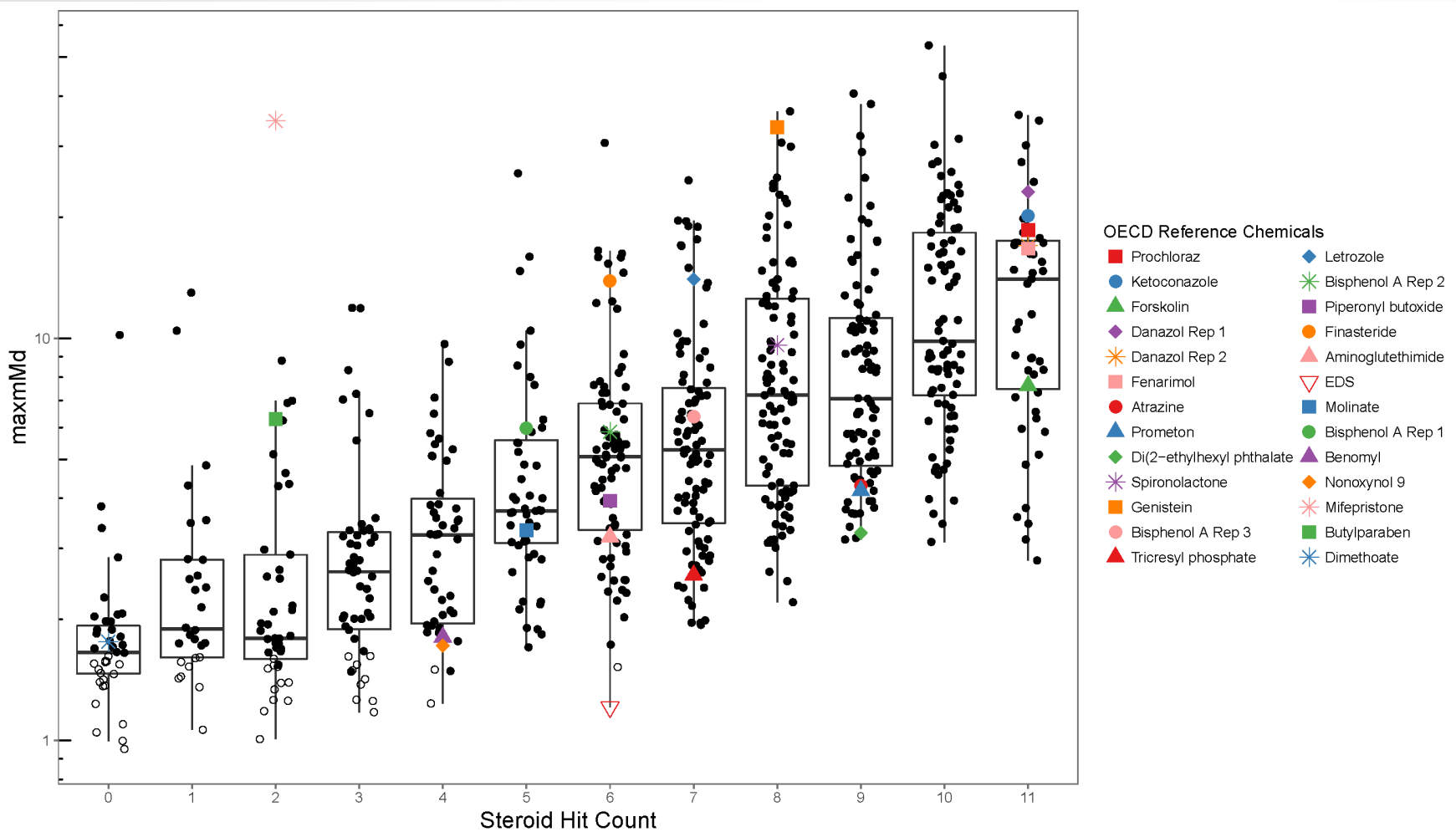
TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534
doi: 10.1093/toxsci/kfx274
Advance Access Publication Date: December 1, 2017
Research Article

High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis

Derik E. Haggard,*,† Agnes L. Karmaus,*,†,1 Matthew T. Martin,†,2 Richard S. Judson,† R. Woodrow Setzer,† and Katie Paul Friedman†,3

*Oak Ridge Institute for Science and Education Postdoctoral Fellow, Oak Ridge, TN. 37831; and †National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Durham, NC 27711

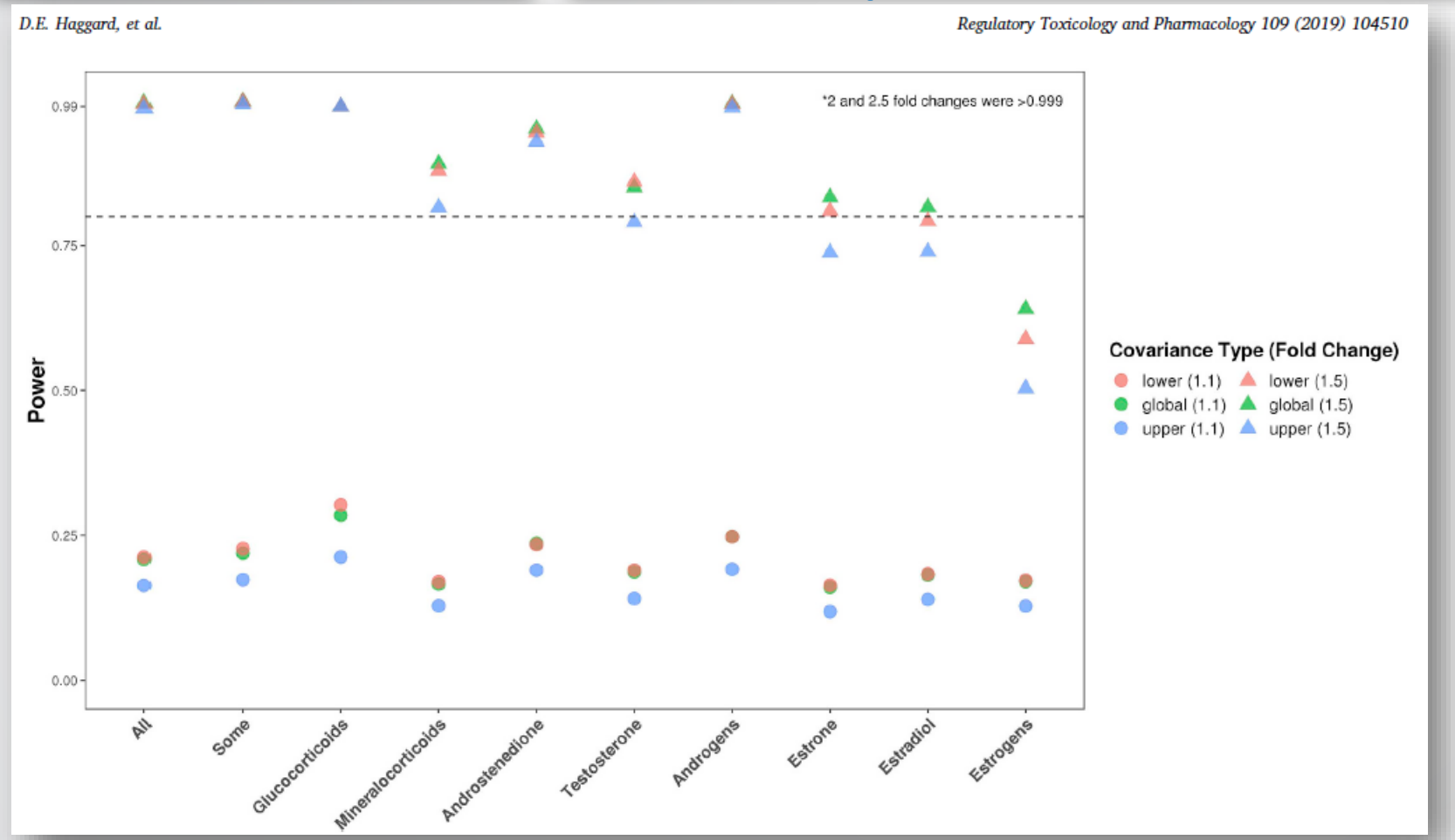
OECD interlaboratory validation reference chemicals typically affected 2+ hormones in the system



- 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) despite the count 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.

Haggard *et al.* (2018)

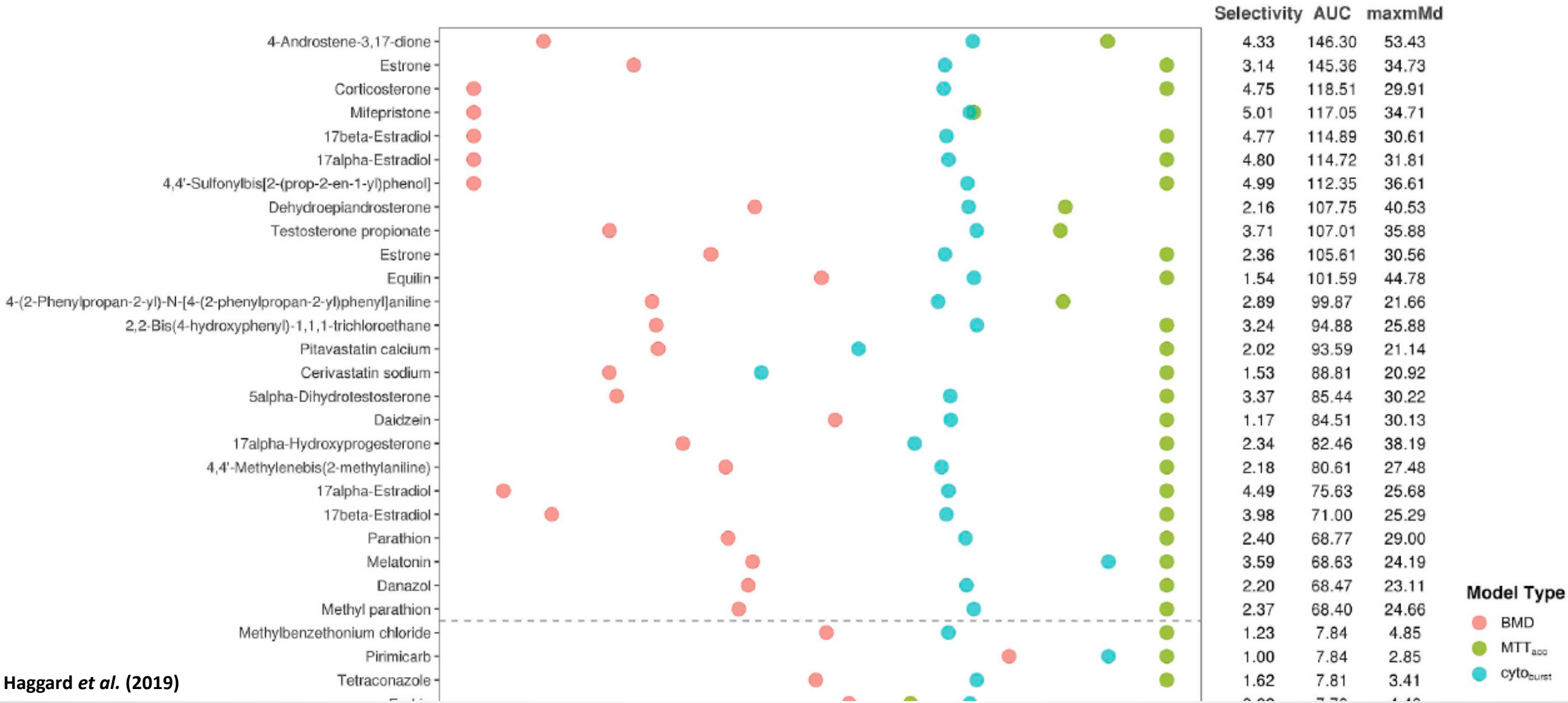
Data simulation aimed to address the stability of the statistical approach.



Haggard *et al.* (2019)

- Evaluated the robustness, reproducibility, and power of the HT-H295R statistical model per feedback received at Scientific Advisory Panel review.
- Considered a case study: does the HT-H295R assay and model detect aromatase inhibitors?
- Demonstrated the use of the HT-H295R statistical model in a selectivity-based prioritization exercise.

Parallel cytotoxicity (MTT assay) and cytotoxicity threshold estimates may help rank positives by selectivity



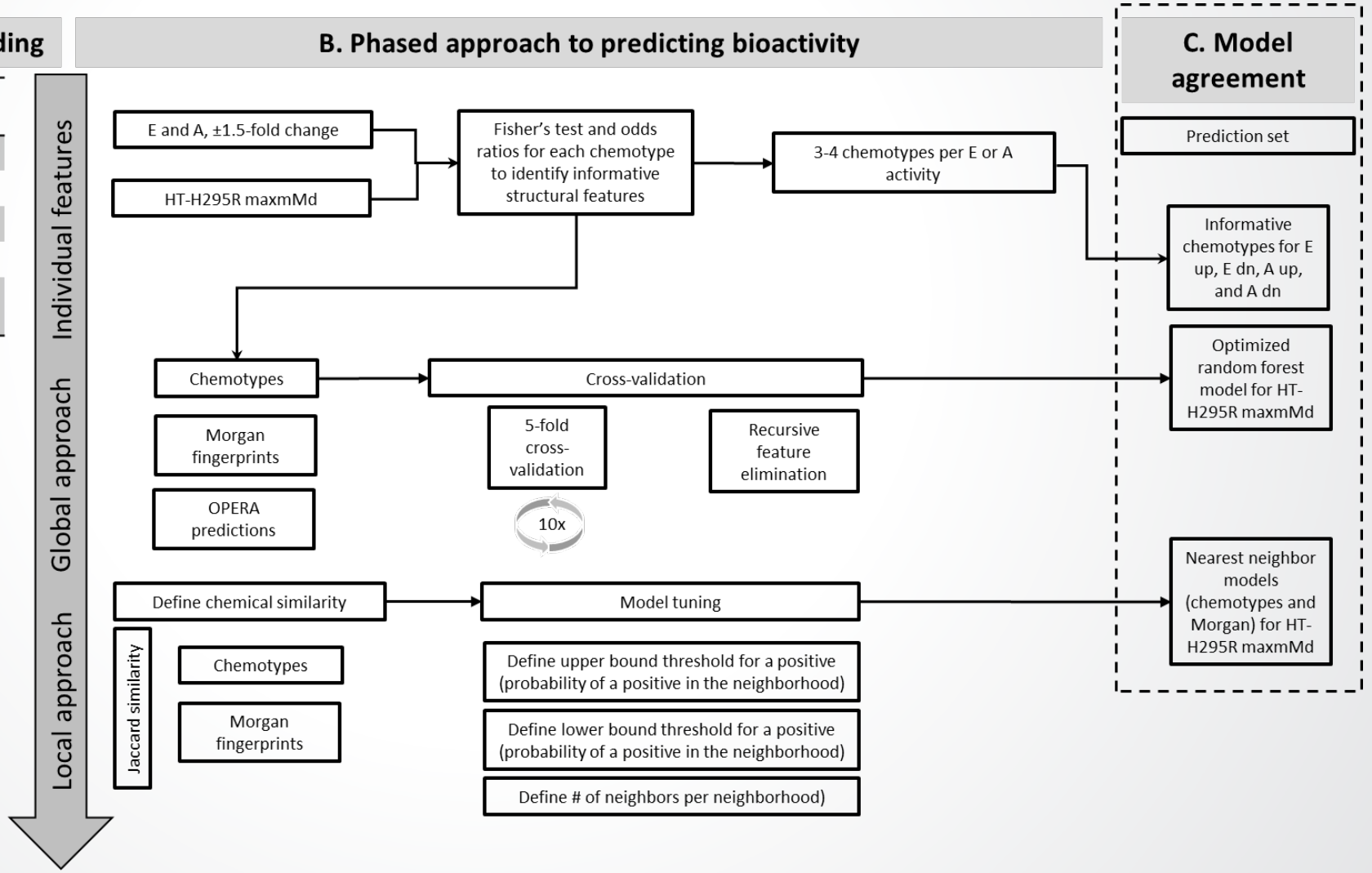
Haggard *et al.* (2019)

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

Foster *et al.* (in preparation) Evaluating structure-based activity in the high-throughput H295R assay for steroid biosynthesis.

A. Assay data for model building

Bioactivity Modeled	(+) chems	(-) chems
Estrogen up	52	590
Estrogen dn	40	602
Androgen up	4	638
Androgen dn	99	543
HT-H295R maxmMd	555	845



HT-H295R summary

- HT-H295R screening assay as an alternative for the OECD-validated, low throughput H295R assay performed well.
  - The ANOVA analysis and logic used herein 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 assays.
- Limitations: H295R assay results (LT or HT) are screening and on their own are unlikely to predict *in vivo* endocrine or reproductive outcomes **and** CyproTex is no longer providing this contract service as HT.

Thank you to EPA-ORD coauthors on these works

- Derik Haggard
- Woody Setzer
- Richard Judson
- Agnes Karmaus
- Matt Martin
- MJ Foster
- Grace Patlewicz
- Imran Shah