

Modeling Steroidogenesis Disruption Using High-Throughput *In Vitro* Screening Data

A.L. Karmaus^{1,2} and M.T. Martin¹

¹National Center for Computational Toxicology, US EPA, Research Triangle Park, NC; ²Oak Ridge Institute for Science Education Fellow

Matt Martin

martin.matt@epa.gov | 919-541-4104

Abstract

Environmental chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis) causing adverse reproductive and developmental effects. Historically, a lack of assays resulted in few chemicals having been evaluated for effects on steroidogenesis. The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes, yet the only enzyme in the pathway for which a high-throughput screening (HTS) assay has been developed is aromatase (CYP19A1), responsible for the aromatization of androgens to estrogens. Recently, the ToxCast HTS program adapted the OECD validated H295R steroidogenesis assay using human adrenocortical carcinoma cells into a model to quantitatively assess the concentration-dependent (0.003-100 μM) effects of chemicals on 10 steroid hormones including progestagens, androgens, estrogens and glucocorticoids. These results, in combination with two CYP19A1 inhibition assays, comprise a large dataset amenable to clustering approaches supporting the characterization of putative mechanisms of action (pMOA) for steroidogenesis disruption. In total, 514 chemicals were tested in all CYP19A1 and steroidogenesis assays. 216 chemicals were identified as CYP19A1 inhibitors in at least one CYP19A1 assay. 208 of these chemicals also altered hormone levels in the H295R assay, suggesting 96% sensitivity in the ability of hormone quantification to identify CYP19A1 inhibitors. Interestingly, only 18 of these chemicals were identified in both CYP19A1 assays, 17 were also identified in the steroidogenesis assay. Of these 17 chemicals, all elicited effects on estrogen levels and 13 had effects on androgen levels, as expected with CYP19A1 inhibition. The steroidogenesis assay data were also used to model CYP17A1 inhibition. We suggest that increases in progestagens and glucocorticoids with simultaneous decreases in the levels of androgens and estrogens comprise a profile indicative of CYP17A1 inhibition, which correctly identified known CYP17A1 inhibitors such as conazole fungicides including prochloraz. Cumulatively, our examples demonstrate that computational profiling of *in vitro* data provides a model that characterizes pMOAs for steroidogenesis disruption to support the identification of endocrine disruptive chemicals.

Introduction & Objectives

There are a broad spectrum of environmental chemicals that disrupt endocrine function resulting in adverse health effects. While endocrine disruption has been largely evaluated as chemical effects on hormone receptor signaling, disruption of hormone biosynthesis and metabolism (steroidogenesis) can also constitute endocrine disruption and lead to reproductive and developmental toxicity. High-throughput *in vitro* screening (HTS) assays have been developed to quantify chemical effects on the levels of steroid hormones, on aromatase enzyme inhibition, and on cholesterol transport essential for the delivery of substrate through to the inner mitochondrial membrane for steroidogenesis.

OBJECTIVES:

- ▶ Characterize the profile of effects across 10 steroid hormones quantified in the H295R high-throughput steroidogenesis assay
- ▶ Integrate HTS assay data for aromatase (CYP19A1) inhibition, translocator protein (TSPO) binding, and H295R hormone levels to model putative mechanisms of action for steroidogenesis disrupting chemicals

Table 1: ToxCast and Tox21 Assays Related to Steroidogenesis

Target	Assay Names	Study Design	Chemicals Tested	Chemicals with Conc.-Resp. Effect
TSPO Translocator Protein (aka. PBR)	NVS_MP_hPBR	Competitive chemical binding to the human translocator protein (TSPO) formerly known as PBR	2707	226
Steroid hormone levels	CEETOX_H295R	HPLC-MS/MS quantification of 10 steroid hormones in H295R cells	2060	336 effect on ≥1 hormone
Aromatase (CYP19A1)	NVS_ADME_hCYP19A1, Tox21_Aromatase_Inhibition	Two separate technologies that evaluate chemical effects on aromatase enzyme activity	1876 8306	72 1316 } 42 effect in both

H295R Steroidogenesis Assay

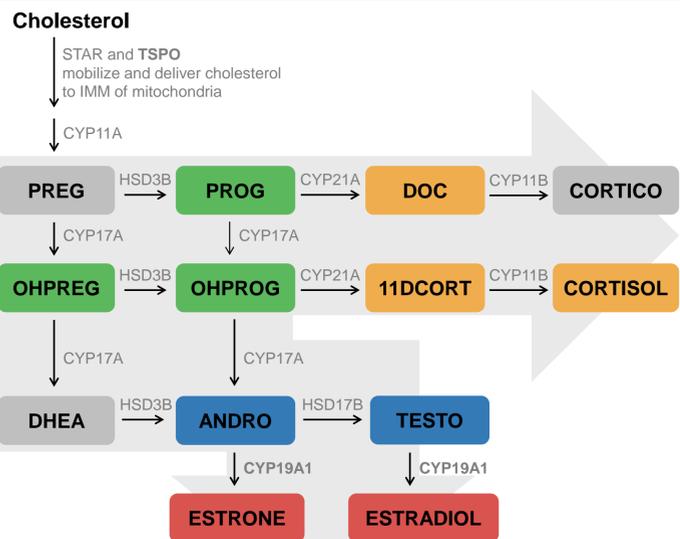


Figure 1. Steroidogenic pathway: H295R human adrenocortical carcinoma cells produce steroid hormones including progestagens (green), glucocorticoids (yellow), androgens (blue), and estrogens (red) in response stimulation by forskolin. Levels of PREG, CORTICO and DHEA were often below the limit of detection and omitted from subsequent analyses.

Profiles of Steroidogenesis Disruption vs. ToxCast

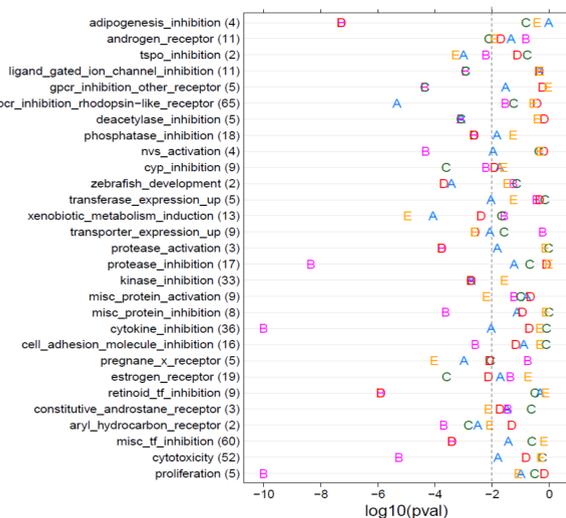
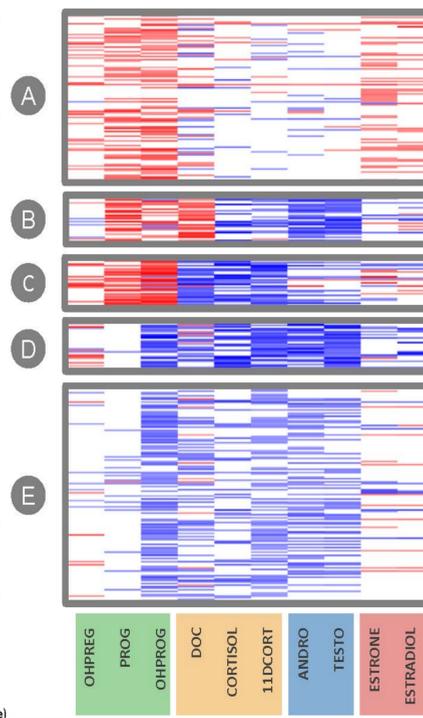


Figure 3. Profiles of steroidogenesis disruption compared to ToxCast assay endpoint groupings. The chemical clusters (A - E) from H295R steroidogenesis profiling were compared to the aggregate bioactivity across ToxCast assays. Assay endpoints were grouped based on intended target gene, target family, and signal direction. The aggregate bioactivity score per assay grouping was calculated by incorporating the hit call, AC50, and a measure of AC50 relative to chemical-specific cytotoxicity points. The chemicals in each chemical cluster were evaluated relative to chemicals not in the cluster across all assay groups using a t-test. The plot shows the log10-pvalue for each cluster in for each of the assay groups having at least one significant enrichment (p value ≤ 0.01).

Figure 2. Clustering of ToxCast Chemical-Mediated Effects on Steroidogenesis: The 336 chemicals that elicited concentration-dependent effects on hormone levels for at least one hormone were clustered using k-means to identify distinct profiles of steroidogenesis disruption. White indicates no data or no effect, concentration-dependent increase in hormone levels are indicated in red, concentration-dependent decrease in hormone concentration are shown in blue. Unique profiles that can be attributed to putative mechanisms of action (pMOA) include cluster D, which contains the control chemical prochloraz, known to inhibit CYP17A1.



Overlap of H295R with TSPO or Aromatase Assays

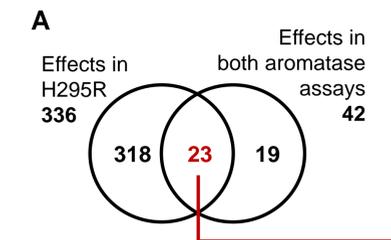


Figure 4. Identifying chemicals with concentration-dependent effects on both aromatase inhibition assays and H295R steroidogenesis: A. 23 samples, representing 18 unique chemicals, had effects on aromatase and hormone levels. B. The profile of hormone effects, with increase (red) or decrease (blue) in hormone levels used for hierarchical clustering, AC50 values in μM overlaid in white text.

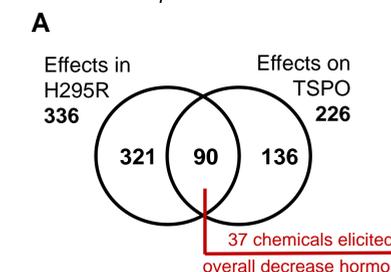
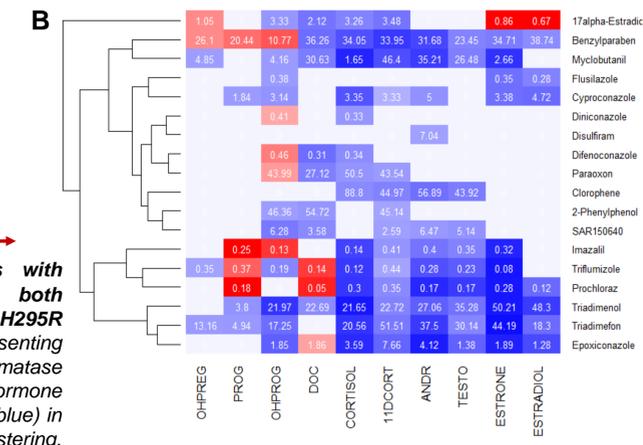
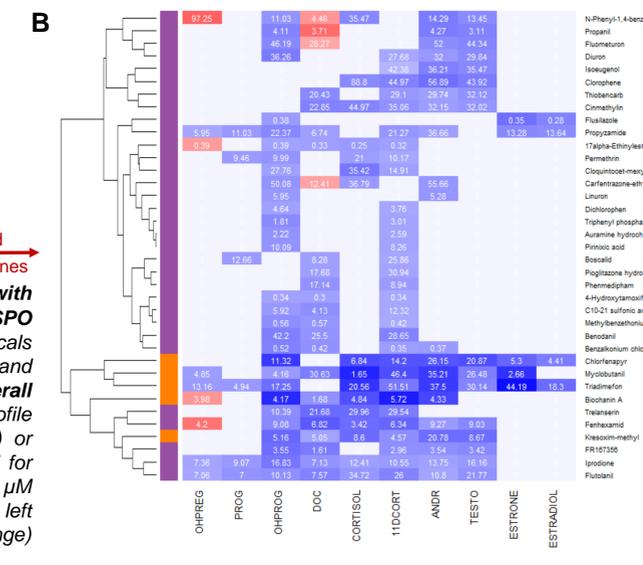


Figure 5. Identifying chemicals with concentration-dependent effects on TSPO and steroidogenesis: A. Of 90 chemicals having effects on both steroidogenesis and TSPO, 37 had profiles of overall decreased hormone levels. B. The profile of hormone effects, with increase (red) or decrease (blue) in hormone levels used for hierarchical clustering, AC50 values in μM overlaid in white text. Bar on the left identifies chemicals from cluster D (orange) or E (purple).



Summary and Future Directions

- ▶ Profiling chemical-mediated effects across the H295R assay by efficacy of effects on hormone levels using k-means clustering identified five distinct pMOA groups (Karmaus et al., 2016 Toxicol Sci.) and were associated to specific assay groupings informing pMOAs.
- ▶ Adding TSPO and aromatase HTS data more accurately defined pMOAs for subsets of chemicals that alter cholesterol transport and/or inhibit aromatase, respectively.

- ▶ Future efforts to evaluate chemical-mediated steroidogenesis disruption using high-throughput screening data will incorporate complementary assay data (e.g., CYPs and nuclear receptor assays) in order to improve performance of predictive modeling efforts.