CEETOX_H295R_11DCORT_noMTC

1. General Information

- 1.1 <u>Assay Title</u>: CeeTox H295R High-throughput Steroidogenesis Assay for 11-Deoxycortisol (11DCORT), maximum tolerated concentration (MTC) filtered results only
- 1.2 Assay Summary: CEETOX_H295R is a cell-based, multiplexed-readout assay that uses H295R, a human adrenal gland cell line, with measurements taken at 48 hours after chemical dosing in a 96-well plate. one of 12 assay component(s) measured or calculated from the CEETOX_H295R assay (11 hormones and 1 viability assay). It is designed to make measurements of hormone induction, a form of inducible reporter, as detected with absorbance signals by HPLC-MS-MS technology. The concentrations for these components have not been pre-filtered for the maximum tolerated concentration (MTC) based on the MTT assay (_noMTC). Data from the assay component CEETOX_H295R_11DCORT_noMTC was analyzed into 1 assay endpoint. This assay endpoint, CEETOX_H295R_11DCORT_noMTC, was analyzed with bidirectional fitting relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain or loss-of-signal activity using HPLC-MS-MS was used to understand synthesis of 11-Deoxycortisol in H295R cell line at 48hr of chemical exposure, without filtering concentrations for maximum tolerated concentration (MTC). To generalize the intended target to other relatable targets, this assay endpoint is annotated to the steroid hormone intended target family, where the subfamily is glucocorticoids.
- 1.3 Date of Document Creation: September 05 2024
- 1.4 Authors and Contact Information:

US Environmental Protection Agency (EPA) Center for Computational Toxicology and Exposure (CCTE) 109 T.W. Alexander Drive (Mail Code D143-02) Research Triangle Park, NC 27711

- 1.5 <u>Assay Source</u>: Ceetox, a part of Cyprotex, is a Contract Research Organization (CRO) that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.
- 1.6 Date of Assay Development: For date of assay development, see Section 6: Bibliography.
- 1.7 <u>References:</u> For complete list of references, see *Section 6: Bibliography*.
- 1.8 <u>Proprietary Elements</u>: This assay is not proprietary; it is a modification of the existing OECD H295R in vitro steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement
- 1.9 Assay Throughput: 96-well plate. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 C prior to HPLC-MS/MS quantification of steroid hormones.

1.10 Status: The assay is fully developed, and data are publicly available in ToxCast's invitroDB.

1.11 Abbreviations:

AIC: Akaike Information Criterion AOP: Adverse Outcome Pathway CV: Coefficient of Variation DMSO: Dimethyl Sulfoxide ToxCast: US EPA's <u>Toxicity Forecaster Program</u> tcpl: <u>ToxCast Data Analysis Pipeline R Package</u> SSMD: Strictly Standardized Mean Difference

2. Test Method Description

2.1 <u>Purpose:</u> steroid hormone biosynthesis

The CeeTox High-throughput Steroidogenesis assay was used to screen a large chemical library for changes in steroid hormone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells.

- 2.2 <u>Scientific Principles:</u> The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestogens, androgens, estrogens and glucocorticoids. To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis
- 2.3 <u>Experimental System</u>: adherent H295R cell line used. H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens
- 2.4 <u>Metabolic Competence:</u> H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals
- 2.5 <u>Exposure Regime:</u> Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines, with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 uL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following prestimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75uL each, and stored at -80 C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tertbutyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT. MTT procedures were as follows: after removal of test medium, 500 uL of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 C and 5% CO2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500uL anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard

Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10 μ M forskolin replicates to control for hormone stimulation, four 3 μ M prochloraz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100 μ M (where feasible within solubility limits of the specific test chemical) and targeting cell viability \geq 70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was \geq 70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

ASSAY DESIGN SUMMARY			
Nominal number of tested concentrations: 6	Target (nominal) number of replicates: 2		
Standard minimum concentration tested: 0.412 μM	Standard maximum concentration tested: 100 μM		
Key positive control: prochloraz; forskolin	Neutral vehicle control: DMSO		
Baseline median absolute deviation for the assay (bmad): 0.068			
Response cutoff threshold used to determine hit calls: 0.	409		
Detection technology used: HPLC-MS-MS (HPLC-MS-MS)			

- 2.6 <u>Response</u>: Decreased or increased production of 11-deoxycortisol following interference with steroidogenesis was quantified by HPLC-MS/MS.
- 2.7 <u>Quality and Acceptance Criteria:</u> Each assay may utilize different acceptance criteria and quality assurance methods as it pertains to the individual assay platform and implementation. Pre-processing transformations may indicate issues in plates or wells by setting well quality (wllq) values to 0. Analytical QC calls per sample and substance can also be considered to understand the applicability domain of the chemicals for screening.
- 2.8 <u>Technical Limitations:</u> ToxCast data can provide initial (screening) information about the capacity for a chemical to illicit a biological response; caution is advised with extrapolation of these results to organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems but can aid in the prioritization of compound selection for more resource intensive toxicity studies. See Section 4.4. for more information on the chemical applicability of the assay.
- 2.9 <u>Related Assays:</u> For related assays, consult the following assay lists or intended target families. This assay is present in the following assay lists:

Steroidogenesis Bioactivity: Assays related to steroidogenesis

Additionally, this assay was annotated to the intended target family of steroid hormone.

3. Data Interpretation

The ToxCast Data Analysis Pipeline (tcpl) R package includes processing functionality for two screening paradigms: (1) single-concentration ("SC") and (2) multiple-concentration ("MC") screening. SC screening consists of testing chemicals at one concentration, often for the purpose of identifying potentially active chemicals to test in the multiple-concentration format. MC screening consists of testing chemicals across a concentration range, such that the modeled activity can give an estimate of potency, efficacy, etc. MC data is the focus of this documentation, with SC data processing metrics to be incorporated in the future.

- 3.1 <u>Responses captured in prediction model</u>: See Section 2.6 for additional information on responses measured.
- 3.2 <u>Data Analysis:</u> The concentrations for these components were pre-filtered for the maximum tolerated concentration (MTC) based on the MTT assay (_noMTC). Data were log2-transformed and analyzed as a fold-

change compared to DMSO control wells as baseline response. All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals.

Prior to the data processing, all the data must go through pre-processing to transform the heterogeneous data into a uniform format before it can be loaded into a database. Level 0 pre-processing is done in R by vendor/dataset-specific scripts with all manual transformations to the data documented with justification. Common examples of manual transformations include fixing a sample ID typo or changing well quality value(wllq) to 0 after identifying problems such a plate row/column missing an assay reagent.

Once data is loaded into the database, tcpl utilizes generalized processing functions provided to process, normalize, model, qualify, and visualize the data. To promote reproducibility, all method assignments must occur through the database and should come from the available list of methods for each processing level. Assigned multiple concentration processing methods include:

Level 2: Component-specific corrections include:

1: none (Use corrected response value (cval) as is; cval = cval. No additional mc2 methods needed for component-specific corrections.)

Level 3: Endpoint-specific normalization include:

7: resp.log2 (Transform the response values to log-scale (base 2).), 9: resp.fc (Calculate the normalized response (resp) as the fold change, i.e. the ratio of the corrected (cval) and baseline (bval) values; resp = cval/bal.), 17: bval.apid.nwllslowconc.med (Calculate the baseline value (bval) as the plate-wise median, by assay plate ID (apid), of the corrected values (cval) of test compound wells (wllt = t) with a concentration index (cndx) of 1 or 2 or neutral control wells (wllt = n).)

Level 4: Baseline and required tcplFit2 parameters defined by:

1: bmad.aeid.lowconc.twells (Calculate the baseline median absolute value (bmad) as the median absolute deviation of normalized response values (rep) for test compound wells (wllt = t) with concentration index (cndx) equal to 1 or 2. Calculate one standard deviation of the normalized response for test compound wells (wllt = t) with a concentration index (cndx) of 1 or 2; onesd = sqrt(sum((resp - mean resp)^2)/sample size - 1). Onesd is used to establish BMR and therefore required for tcplfit2 processing.)

Level 5: Possible cutoff thresholds, where higher value for endpoint is selected, include:

6: bmad6 (Add a cutoff value of 6 multiplied by the baseline median absolute deviation (bmad). By default, bmad is calculated using test compound wells (wllt = t) for the endpoint.)

Level 6: Cautionary flagging include:

5: modl.directionality.fail (Flag series if model directionality is questionable, i.e. if the winning model direction was opposite, more responses (resp) would have exceeded the cutoff (coff). If loss was winning directionality (top < 0), flag if count(resp < -1° coff) < 2° count(resp > coff). If gain was winning directionality (top > 0), flag if count(resp > coff) < $2^{count}(resp < -1^{cont})$, 6: singlept.hit.high (Flag single-point hit that's only at the highest conc tested, where series is an active hit call (hitc >= 0.9) with the median response observed above baseline occurring only at the highest tested concentration tested.), 7: singlept.hit.mid (Flag single-point hit that's not at the highest conc tested, where series is an active hit call (hitc >= 0.9) with the median response observed above baseline occurring only at one concentration and not the highest concentration tested.), 8: multipoint.neg (Flag multi-point miss, where series is an inactive hit call (hitc < 0.9) with multiple median responses observed above baseline.), 9: bmd.high (Flag series if modeled benchmark dose (BMD) is greater than AC50 (concentration at 50 percent maximal response). This is indicates high variability in baseline response in excess of more than half of the maximal response.), 10: noise (Flag series as noisy if the quality of fit as calculated by the root mean square error (rmse) for the series is greater than the cutoff (coff); rmse > coff.), 11: border (Flag series if borderline activity is suspected based on modeled top parameter (top) relative to cutoff (coff); |top| <= 1.2(coff) or |top| >= 0.8(coff).), 13: low.nrep (Flag series if the average number of replicates per concentration is less than 2; nrep < 2.), 14: low.nconc (Flag series if 4 concentrations or less were tested; nconc <= 4.), 15: gnls.lowconc (Flag series where winning model is gain-loss (gnls) and the gain AC50 is less than the minimum tested concentration, and the loss AC50 is less than the mean tested concentration.), 17: efficacy.50 (Flag low efficacy hits if series has an active hit call (hitc >= 0.9) and efficacy values (e.g. top and maximum median response) less than 50 percent; intended for biochemical assays. If hitc >= 0.9 and coff >= 5, then flag when top < 50 or max_med < 50. If hitc >= 0.9 and coff < 5, then flag when top < log2(1.5) or max_med < log2(1.5).), 18: ac50.lowconc (Flag series with an active hit call (hitc >= 0.9) if AC50 (concentration at 50 percent maximal response) is less than the lowest concentration tested; if hitc >= 0.9 and ac50 < 10^logc_min, then flag.)

The following is an aggregate endpoint summary of the number of samples and chemicals tested, as well as active or inactive hit calls (hitc) and predicted winning models for all samples tested in this endpoint.

SAMPLE AND CHEMICAL COVERAGE

Number of samples tested: 85

Number of chemicals tested: 84

ACTIVITY HIT CALLS				
Active hit count: hitc≥0.9 23	Inactive hit count: 0≤h 62	itc<0.9	NA hit count: <i>hitc<0</i> 0	
WINING MODEL SELECTION				
Number of sample-assay endpoints	with winning <i>hill</i> model:	6		
gain-loss	(gnls) model:	9		
power(po	<i>w)</i> model:	9		
linear-pol	ynomial (poly1) model:	19		
quadratic	<i>-polynomial(poly2)</i> model:	15		
exponent	<i>ial-2 (exp2)</i> model:	4		
exponent	<i>ial-3 (exp3)</i> model:	0		
exponent	<i>ial-4 (exp4)</i> model:	13		
exponent	<i>ial-5 (exp5)</i> model:	10		

For each concentration series, several point-of-departure (POD) estimates are calculated for the winning model. The major estimates include: (1) the activity concentration at the specified benchmark response (BMR) (bmd), (2) the activity concentration at 50% of the maximal response (ac50), (3) the activity concentration at the efficacy cutoff (acc), (4) the activity concentration at 10% of the maximal response, and (5) the concentration at 5% of the maximal response.

3.3 Prediction Model: All statistical analyses were conducted using R programming language, employing the tcpl package to generate model parameters and confidence intervals. Each chemical concentration response series is fit to ten predictive models, encoded by the dependency package tcplfit2. The models include the constant, Hill, gain-loss, two polynomials (i.e. linear and quadratic), power, and four exponential variants. The polynomials, power, and exponential models are all based on BMDExpress2. The winning model (modl) is selected based on the lowest AIC value and is used to determine the activity (or hit call) for the concentration series. If two models have equal AIC values, then the simpler model (i.e. model with fewer parameters) wins. In invitrodb, levels 4 and 5 capture model fit information. mc4 captures summary values calculated for each concentration series, whereas mc4_param stores the estimated model parameters for all models fit to data in long format. mc5 captures the winning model selected and the activity hit call, whereas mc5_param stores the estimated model parameters for each concentration-response series is determined by calculating a continuous hit-call for the winning model, which is the product of three proportional weights. The first weight reflects whether there is at least one median response outside the efficacy cutoff band. Second, the top (or maximal change in the predicted response) is larger than the cutoff.

The last weight reflects whether the AIC of the winning model is less than the constant model, i.e. the winning model is better fit than a flat line.

The continuous hit call value (hitc), fit category (fitc), and cautionary flags (mc6) can be used to understand the goodness-of-fit, enabling the user to decide the stringency with which to filter and interpret results. Hitc may be further binarized into active or inactive, depending on the level of stringency required by the user; herein, hitc greater than or equal to 0.90 is active, hitc between 0 and 0.90 is inactive, and hitc less than 0 is not applicable, but different thresholds may be used. Fitc was summarize curve behavior relative activity, efficacy, and potency comparisons between the AC50 and the concentration range screened. Cautionary flags on fitting were developed in previous versions of tcpl and have been stored at level 6. These flags are programmatically generated and indicate characteristics of a curve that need extra attention or potential anomalies in the curve or data. Users may review these filtered groupings to understand high-confidence curves.

3.4 <u>Software:</u> The ToxCast Data Analysis Pipeline (tcpl) is an R package that manages, curve-fits, plots, and stores ToxCast data to populate its linked MySQL database, invitrodb. Data for invitrodb v4.2 was processed using the tcpl v3.2. See *Section 7: Supporting Information* on the ToxCast program and tcpl R package.

4. Test Method Performance

4.1 <u>Robustness</u>: The following assay performance metrics surmise the robustness of the method i.e. the reliability of the experimental results and the prediction capability of the model used.

NEUTRAL CONTROL (well type = "n")				
Neutral control well median response value, by plate: nmed	375.998			
Neutral control median absolute deviation, by plate: nmad	8.199			
Coefficient of variation (CV%) in neutral control wells: (nmad/nmed)*100	2.19%			
POSITIVE CONTROL (well type = "p")				
Positive control well median response value, by plate: pmed	862.42			
Positive control well median absolute deviation, by plate: pmad	28.307			
Z Prime Factor for median positive and neutral control across all plates: (1 - ((3 * (pmad + nmad)) / abs(pmed - nmed))	NA			
Strictly standardized mean difference (SSMD) for positive compared to neutral control wells: ((pmed - nmed) / sqrt(pmad ² + nmad ²)	17.304			
Positive control signal-to-noise: ((pmed-nmed)/nmad)	NA			
Positive control signal-to-background: (pmed/nmed)	NA			
NEGATIVE CONTROL (well type = "m")				
Negative control well median, by plate: mmed	26.77			
Negative control well median absolute deviation value, by plate: mmad	1.205			
Z Prime Factor for median negative and neutral control across all plates: (1 - ((3 * (mmad + nmad)) / abs(mmed - nmed))	NA			
Strictly standardized mean difference (SSMD) for negative compared to neutral control wells: ((mmed - nmed) / sqrt(mmad ² + nmad ²)	-35.091			
Signal-to-noise (median across all plates, using negative control wells): ((mmed-nmed)/nmad)	NA			
Signal-to-background (median across all plates, using negative control wells): (mmed/nmed)	NA			

- 4.2 <u>Reference Chemical Information:</u> Reference chemical curation is ongoing, and this section will be updated as more information becomes available.
- 4.3 <u>Performance Measures and Predictive Capacity:</u> The performance and predictivity for a given assay may be evaluated with a variety of performance statistics but is dependent upon available data. Predictive capacity (i.e. false negative, false positive rates) will be assessed when reference chemical information is available. Ideally, assays will have sufficient data on reference chemicals (i.e. positive and negative controls) to enable estimation of accuracy statistics, such as sensitivity and specificity.

ToxCast targets may align to a range of event types in the Adverse Outcome Pathway (AOP) framework, however each assay technology may have specific limitations, which may require user discretion for more complex interpretations of the data.

The median root mean squared error (RMSE) across all winning models for active hits was calculated as: 10.

4.4 <u>Chemical Library Scope and Limitations:</u> The <u>ToxCast Chemical Library</u> was designed to capture a large spectrum of structurally and physicochemically diverse compounds. This chemical inventory incorporates toxicity datarich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also consider practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Under these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary *in vivo* and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. Analytical QC calls per sample and substance should be considered to understand the applicability domain of the chemicals for screening.

5. Potential Regulatory Applications

- 5.1 <u>Context of Use:</u> Examples of end use scenarios could include, but are not limited to:
 - Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across,
 - *Priority Setting:* The assay might help prioritize substances within an inventory for more detailed evaluation,
 - Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency; or
 - Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.
- 6. Bibliography: Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. High-Throughput Screening of Chemical Effects on Steroidogenesis Using H295R Human Adrenocortical Carcinoma Cells. Toxicol Sci. 2016 Apr;150(2):323-32. doi: 10.1093/toxsci/kfw002. Epub 2016 Jan 18. PMID: 26781511; PMCID: PMC4809454., Haggard DE, Karmaus AL, Martin MT, Judson RS, Setzer RW, Paul Friedman K. High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis. Toxicol Sci. 2018 Apr 1;162(2):509-534. doi: 10.1093/toxsci/kfx274. Erratum in: Toxicol Sci. 2018 Aug 1;164(2):646. PMID: 29216406., Haggard DE, Setzer RW, Judson RS, Paul Friedman K. Development of a prioritization method for chemical-mediated effects on steroidogenesis using an integrated statistical analysis of high-throughput H295R data. Regul Toxicol Pharmacol. 2019 Dec;109:104510. doi: 10.1016/j.yrtph.2019.104510. Epub 2019 Oct 29. PMID: 31676319.

7. Supporting Information:

More information on the ToxCast program can be found at: <u>https://www.epa.gov/chemical-research/toxicity-forecasting</u>. The most recent version of downloadable data can be found at: <u>https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data</u>. The ToxCast Data Analysis Pipeline (tcpl) R package is available on <u>CRAN</u> or <u>GitHub</u>. Check out tcpl's vignette for comprehensive documentation describing ToxCast data processing, retrieval, and interpretation.