ACEA_AR_agonist_AUC_viability

1. General Information

1.1 Assay Title: ACEA Biosciences xCELLigence Real-Time Cell Analysis on Androgen Receptor Agonism for Viability

1.2 Assay Summary: ACEA_AR_agonist is a cell-based, single-readout assay that uses 22Rv1, a human prostate cancer cell line, with measurements taken at 80 hours after chemical dosing in a 384-well plate, although TO5 and TO6 (mc0.srcf) used a 96-well plate. Differences in plate size can be ignored given data normalization. ACEA_AR_80hr is one of two assay component(s) measured or calculated from the ACEA_AR assay. It is designed to make measurements of real-time cell-growth kinetics, a form of growth reporter, as detected with electrical impedance signals by Real-Time Cell Electrode Sensor (RT-CES) technology. Data from the assay component ACEA_AR_AUC_viability was analyzed in the positive analysis fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of growth reporter, loss-of-signal activity can be used to understand changes in the viability. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.3 Date of Document Creation: September 05 2024

1.4 Authors and Contact Information:

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1.5 Assay Source: ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a realtime, label free, cell growth assay system called xCELLigence based on a microelectronic impedance readout.

1.6 Date of Assay Development: For date of assay development, see *Section 6: Bibliography*.

1.7 References: For complete list of references, see *Section 6: Bibliography*.

1.8 Proprietary Elements: Assay is non-proprietary; xCELLigence RTCA software and biosensor technology are available from ACEA Biosciences, Inc. and 22Rv1 cells are commercially available from American Type Culture Collection (ATCC HTB-133) with signed Material Transfer Agreement (MTA).

1.9 Assay Throughput: 384-well plate. The assay is conducted on 96-well plates with each plate containing positive controls for proliferation (testosterone) and cytotoxicity (MG132), negative controls (assay media, RPMI 1640), and two concentrations (0.5 percent and 0.125 percent) of DMSO solvent controls. Following a 24-hour incubation period, the cells are exposed to test chemicals for 80 hours and response is monitored no less than once per hour.

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 Toxicity Forecaster Program
tcpl: ToxCast Data Analysis Pipeline R Package
SSMD: Strictly Standardized Mean Difference

2. Test Method Description

2.1 Purpose: Electrical impedance is used to quantify changes to the growth of the cells where increase impedance is positively correlated with increased cell growth

The ACEA_AR assay exposed human prostate cell (22Rv1) cultures to the ToxCast library of diverse environmental chemicals using an eight-point, 1:4 dilution series concentration-response format (starting at a maximum final concentration of 100uM), using MG132 (cytotoxicity) and testosterone (proliferation) as positive controls and assay media and DMSO as a negative control and solvent control, respectively. All control chemicals were tested in quadruplicate on each plate. The ACEA_AR assay analyzed changes in cell adhesion and

morphology at the electrode: solution interface (located on the bottom of culture wells) using electronic microsensors. Changes in electrical impedance were monitored in real-time at the plate surface to investigate the potential activation of the androgen signaling pathway and subsequent increases in growth or changes in cell structure following 80-hour incubation with the test chemicals. The electrical signal produced by the experimental system can be used to detect changes in cell number, morphology and adhesion which occur in response to xenoestrogenic activation of AR-mediated pathways, and concentration-response curves were modeled for each chemical to determine half-maximal activity levels.

2.2 Scientific Principles: Endocrine disrupting chemicals (EDCs) interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Androgens, such as testosterone, are widely recognized for their importance in sexual development and differentiation but also play roles in metabolism, growth, development, and behavior and act as an intercellular signal (Bhasin et al., 2007; Monks & Holmes, 2018; Sumpter, 2005). Agonism of the androgen receptor is listed as a molecular initiating event in AOP #23, leading to reproductive dysfunction in fish (Villeneuve, 2021).

2.3 Experimental System: adherent 22Rv1 cell line used. 22Rv1 is a human prostate carcinoma epithelial cell line derived from a xenograft that was serially propagated in mice.

2.4 Metabolic Competence: The 22Rv1 cell line expresses androgen receptor (AR) and prostate-specific antigen (PSA), both of which are markers of prostate cancer. The presence of these markers in 22Rv1 cells confirms their origin from prostate cancer tissue and highlights their relevance in studying the disease. Importantly, the 22Rv1 cell line is unique in that it expresses both full-length and truncated forms of ARs. This mixed expression pattern is commonly observed in androgen deprivation resistant prostate cancers, making the 22Rv1 cell line a valuable model for studying the mechanisms underlying resistance to hormonal therapies. Morphologically, 22Rv1 cells exhibit epithelial characteristics and are cultured as adherent monolayers, providing a convenient system for in vitro experimentation.

2.5 Exposure Regime: The xCELLigence system Multi-E-Plate stations were used to measure the time-dependent response to chemicals. Each compound was tested in an eight-point, 1:4 serial dilution series starting at a maximum final concentration of 100 uM. A maximum starting concentration of 0.5% DMSO was present in the 100 uM chemical samples and was diluted along with the test article dilution series. The screen was performed in biological duplicate using two separate, 96-well, E-Plates 96 for each dilution series (n = 2). Positive controls (MG132 and testosterone) and a negative control (assay media) were tested in quadruplicate on each testing plate. Then, 0.5% and 0.125% DMSO were tested in duplicates in each plate to serve as solvent controls for the 2 highest concentrations of testing compounds: 100 uM and 25 uM. Reference compounds were tested with 8 concentrations with 1:5 serial dilutions. All screening was carried out by ACEA Biosciences, Inc. (San Diego, CA). 22Rv1 cells purchased from ATCC were maintained in media supplemented with 10% fetal bovine serum (FBS). Before screening, 22Rv1 cells were preconditioned in assay medium. Cells were then detached and seeded in E-Plates 96 in assay medium. After overnight monitoring of growth once every hour, compounds were added to T-47D cells and remained in the medium until the end of the experiment. Cellular responses were then recorded once every 5 min for the first 5 h, and once every hour for an additional 100 h.

ASSAY DESIGN SUMMARY	
Nominal number of tested concentrations: 7	Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.144 µM	Standard maximum concentration tested: 105 µM
Key positive control: MG 132	Neutral vehicle control: DMSO
Baseline median absolute deviation for the assay (bmad): 8.956	
Response cutoff threshold used to determine hit calls: 26.869	
Detection technology used: RT-CES (Label Free Technology)	

2.6 Response: Increased cell proliferation in response to xenoestrogenic interference with AR-mediated pathways as measured by monitoring electrical impedance at the cell-plate interface. One possible effect of endocrine disrupting chemicals is increased cell growth through perturbation of pathways linked to cell cycle regulation. Activation of the androgen receptor (AR) signaling pathway, for example, is one possible mechanism that underlies cell proliferation in hormonally sensitive tissues such as mammary and endometrial tissue. This assay was designed to identify those chemicals with the potential to affect cell growth by activating the androgen receptor-mediated cell proliferation pathway. The assay uses electronic microsensors located at the bottom of the cell culture well to detect changes in cell number, morphology, and adhesion through electrical impedance measurement at the electrode–solution interface following 80-hour incubation with test chemicals.

2.7 Quality and Acceptance Criteria: 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 Technical Limitations: 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 Related Assays: For related assays, consult the following assay lists or intended target families. This assay is present in the following assay lists:

Cytotoxicity Burst: Assays used to defne the cytotoxicity burst region

Additionally, this assay was annotated to the intended target family of cell cycle.

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 Responses captured in prediction model: See *Section 2.6* for additional information on responses measured.

3.2 Data Analysis: Data were collected from the xCELLigence system which converts raw impedance values into the Cell Index (CI) value; this is a measure of adhesion where CI = (impedance at time point n – impedance in the absence of cells)/nominal impedance value. These data were then converted to a Normalized Cell Index according to the equation NCI(Ti) = CI(Ti)/CI(Tk), where i = 1,2,3,….N where CI(Tk) is the last time point before chemical addition, CI(Ti) is the cell index at the i-th measured time point, and N is the total number of time points. Data were grouped by chemical and smoothed to combine replicates using a simple moving average (as the replicates were assessed in duplicate on separate plates so the time points were not identical). DMSO controls were considered as baseline for activity, and testosterone was used as a positive control and 100 percent activity for all the test chemicals on that plate. For cell loss, the NCI value at the time of compound administration was considered to represent complete (100%) viability. MG132 (2 uM), a proteasome inhibitor and known cytotoxic agent, was used as the positive control for cell loss and was tested in quadruplicate on each plate. The minimum average response on each plate was used as a positive control for cell loss for all the test chemicals on the corresponding plate. If a chemical sample was run on two different plates, then the minimum NCI values for MG132 were averaged. If an NCI value for MG132 fell below zero, the response was considered to be below the limit of detection and was replaced with the minimum value greater than zero across all plates. All smoothened NCI values were then converted to a percentage of positive control, which was considered to represent no (0%) viability. Concentration response curves were generated using smoothed NCI values and 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:

5: resp.pc (Calculate the normalized response (resp) as a percent of control, i.e. the ratio of the difference between the corrected (cval) and baseline (bval) values divided the difference between the positive control (pval) and baseline (bval) values multiplied by 100; resp = (cval-bval)/(pval-bval)*100.), 6: resp.multneg1 (Multiply the normalized response value (resp) by -1; -1*resp.), 15: pval.apid.medncbyconc.min (Calculate the positive control value (pval) as the plate-wise minimum, by assay plate ID (apid), of the medians of the corrected values (cval) for gain-of-signal single- or multiple-concentration negative control wells (wllt = m or o) by apid, well type, and concentration.), 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:

1: bmad3 (Add a cutoff value of 3 multiplied by the baseline median absolute deviation (bmad) as defined at Level 4.), 2: pc20 (Add a cutoff value of 20. Typically for percent of control data.), 27: ow_bidirectional_loss (Multiply winning model hitcall (hitc) by -1 for models fit in the positive analysis direction. Typically used for endpoints where only negative responses are biologically relevant.)

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*coff).), 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.), 19: viability.gnls (Flag series with an active hit call (hitc >= 0.9) if denoted as cell viability assay with winning model is gain-loss (gnls); if hitc >= 0.9, modl=="gnls" and cell_viability_assay == 1, then flag.), 20: no.med.gt.3bmad (Flag series where no median response values are greater than baseline as defined by 3 times the baseline median absolute deviation (bmad); nmed_gtbl_pos and nmed_gtbl_neg both = 0, where nmed_gtbl_pos/_neg is the number of medians greater than 3*bmad/less than -3*bmad.)

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: 1845	Number of chemicals tested: 1830

ACTIVITY HIT CALLS		
Active hit count: hitc≥0.9 490	Inactive hit count: 0≤hitc<0.9 1025	NA hit count: *hitc<0* *330*

WINING MODEL SELECTION	
Number of sample-assay endpoints with winning *hill* model:	104
gain-loss (gnls) model:	181
power(pow) model:	175
linear-polynomial (poly1) model:	535
quadratic-polynomial(poly2) model:	256
exponential-2 (exp2) model:	61
exponential-3 (exp3) model:	16
exponential-4 (exp4) model:	329
exponential-5 (exp5) model:	188

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 the selected winning model in long format. Activity 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 Software: 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 Robustness: 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*	173.792
Neutral control median absolute deviation, by plate: *nmad*	6.742
Coefficient of variation (CV%) in neutral control wells: *(nmad/nmed)*100*	4.03%
POSITIVE CONTROL (well type = "p")	
Positive control well median response value, by plate: *pmed*	223.133
Positive control well median absolute deviation, by plate: *pmad*	20.616
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($pmad^2$ + $nmad^2$)*	2.438
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*	73.087
Negative control well median absolute deviation value, by plate: *mmad*	2.686

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)*	-12.678
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 Reference Chemical Information: Reference chemical curation is ongoing, and this section will be updated as more information becomes available.

4.3 Performance Measures and Predictive Capacity: 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: 188.

4.4 Chemical Library Scope and Limitations: The ToxCast Chemical Library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. This chemical inventory incorporates toxicity data-rich 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 Context of Use: 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:** Judson R, Houck K, Paul Friedman K, Brown J, Browne P, Johnston PA, Close DA, Mansouri K, Kleinstreuer N. Selecting a minimal set of androgen receptor assays for screening chemicals. Regul Toxicol Pharmacol. 2020 Nov;117:104764. doi: 10.1016/j.yrtph.2020.104764. Epub 2020 Aug 14. PMID: 32798611; PMCID: PMC8356084.

7. **Supporting Information:**

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