Generating GD211-Aligned Documentation for ToxCast to Support Assay Interpretation and Data Use

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ASCCT-ESTIV Award Winners Webinar Series

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Outline & Disclaimer

- ToxCast Overview
- Improved GD211 Assay Documentation

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Company or product names do not constitute endorsement by US EPA.

ToxCast Team

We are an interdisciplinary team (software developers, toxicologists, biologists, and statisticians) working alongside several internal/external collaborators (EPA labs, contract vendors, or other partners) to process data for a variety of stakeholder and user groups with multiple research needs.

Madison Feshuk

- *Lead, Pipelining and Curation*

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- *Lead, tcpl Development*

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- *Lead, tcplfit2 Development and Statistics*

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- *SME, DNT*

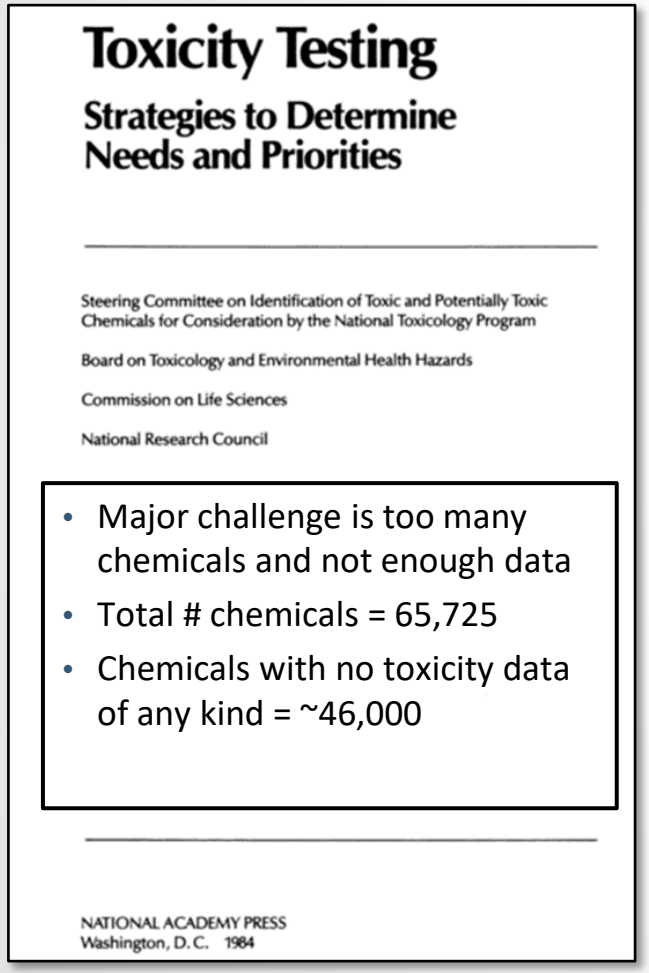
Katie Paul Friedman

- *SME, ToxCast Project Lead*

ToxCast Overview

Key Challenge:
Too many chemicals, not enough data or time

1984 NAS Report



Toxicity Testing

Strategies to Determine Needs and Priorities

Steering Committee on Identification of Toxic and Potentially Toxic Chemicals for Consideration by the National Toxicology Program

Board on Toxicology and Environmental Health Hazards

Commission on Life Sciences

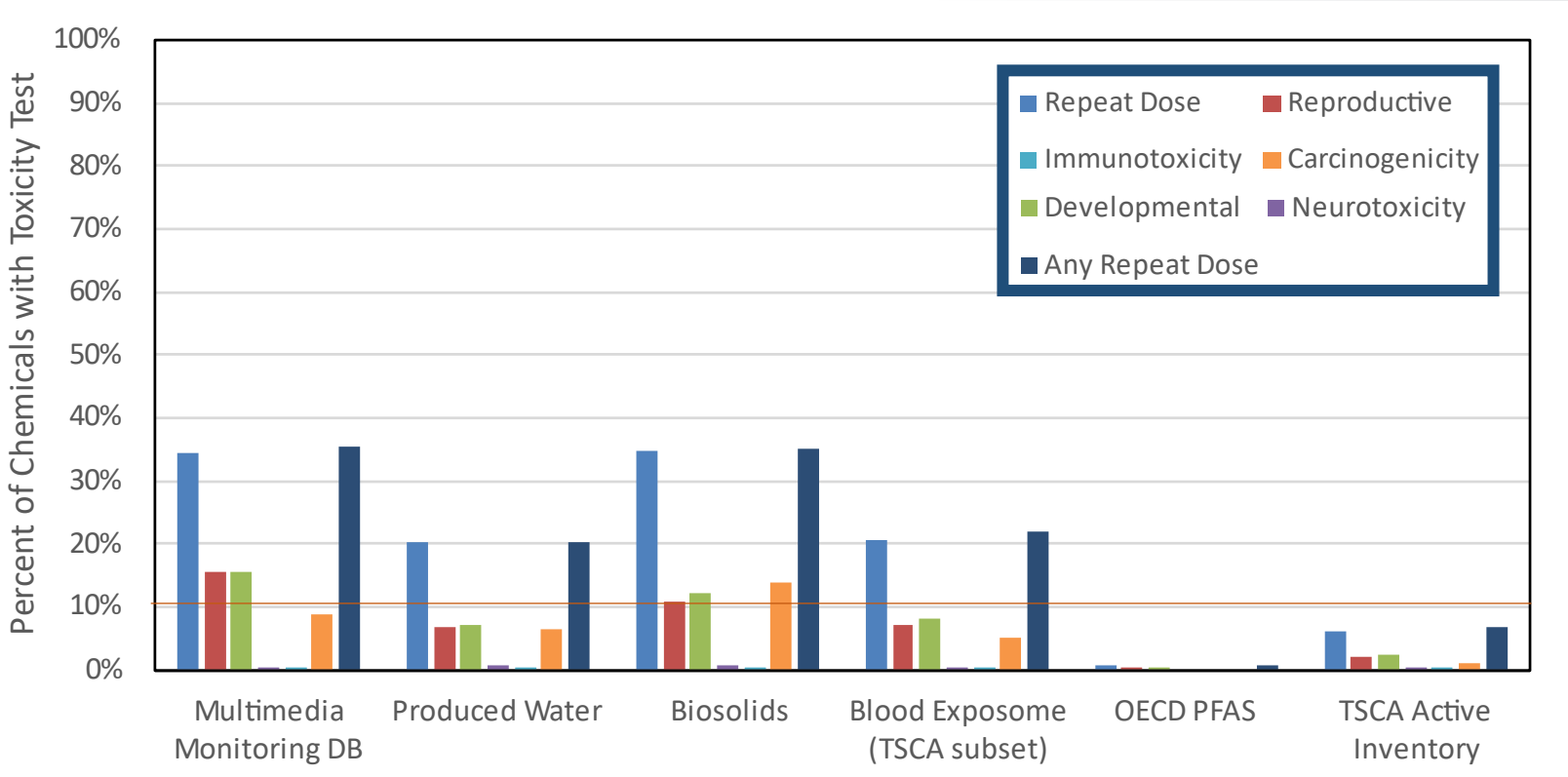
National Research Council

- Major challenge is too many chemicals and not enough data
- Total # chemicals = 65,725
- Chemicals with no toxicity data of any kind = ~46,000

NATIONAL ACADEMY PRESS
Washington, D.C. 1984

Toxicity Data and Human Health Assessments: 2022

2020 survey of 19 countries and regions: **350,000 chemicals and mixtures of chemicals** are registered in one or more inventories[1]

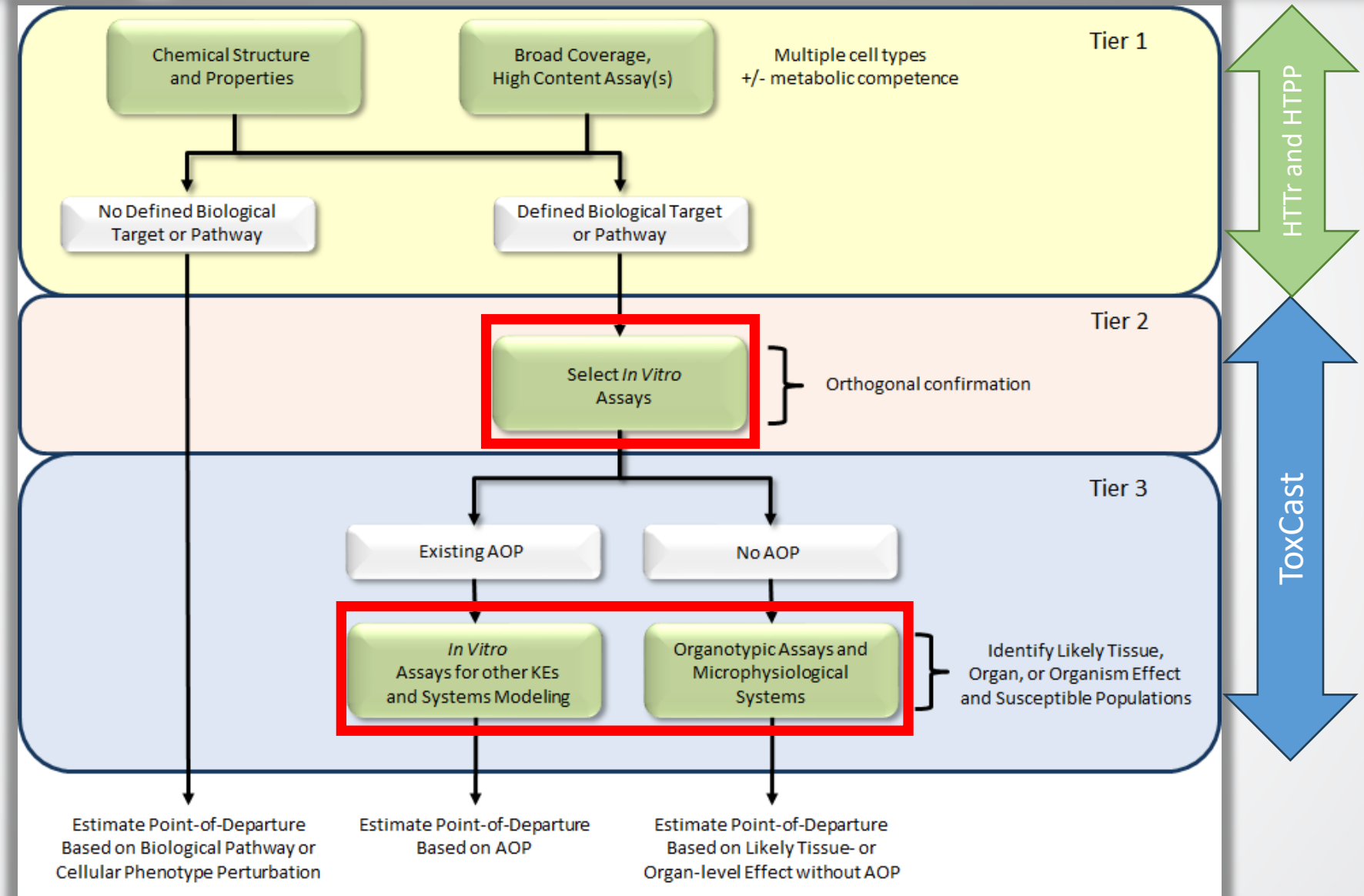


There is not enough time or money to generate traditional animal-based hazard data for all of these chemicals and their mixtures

Slide credit: Rusty Thomas

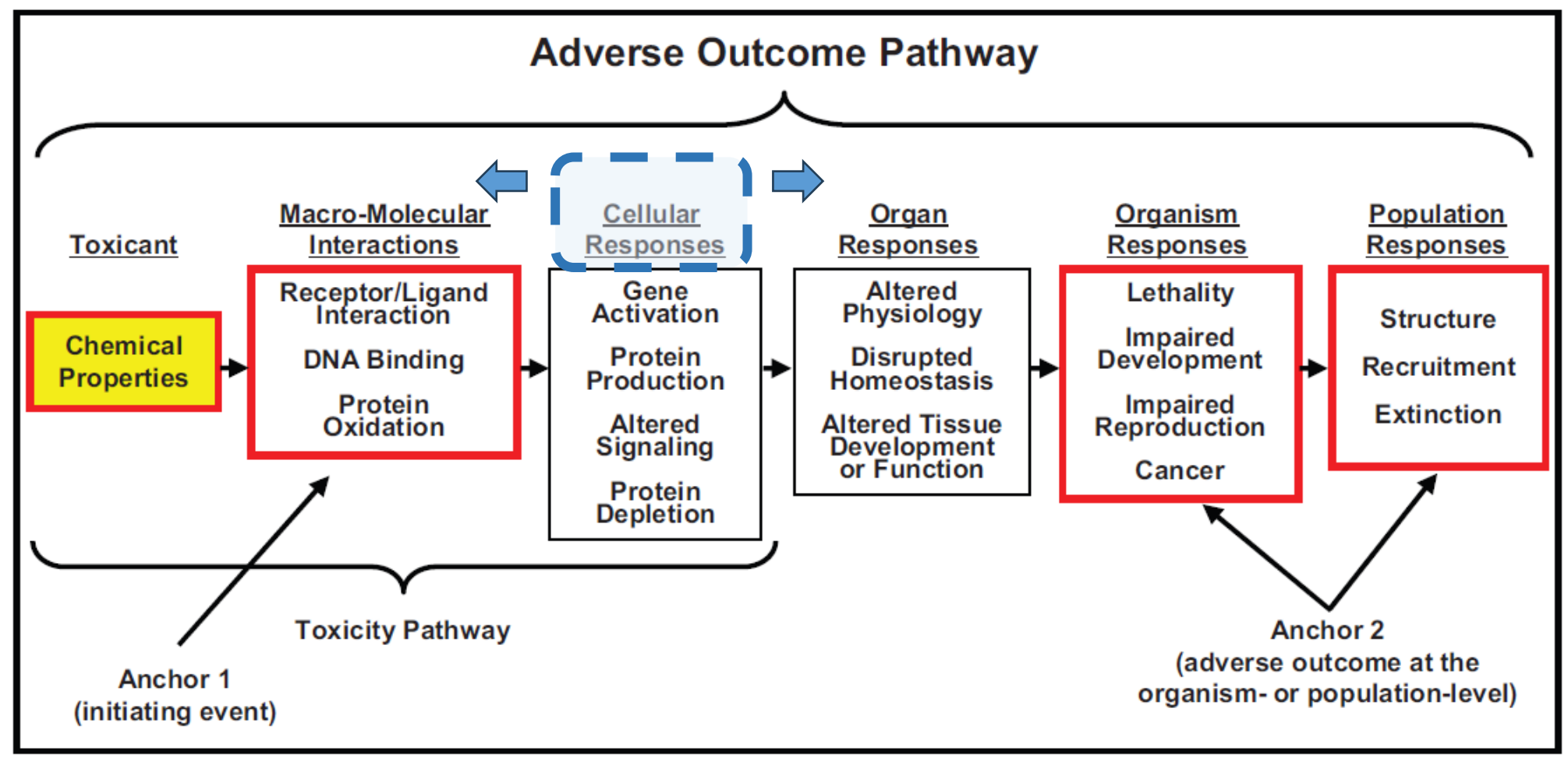
[1]Wang et al. *Environmental Science & Technology* 2020.

New Approach Methods (NAMs) can be targeted or provide more biological complexity



The NexGen Blueprint of CompTox at US EPA
Thomas et al. (2019) 10.1093/toxsci/kfz058

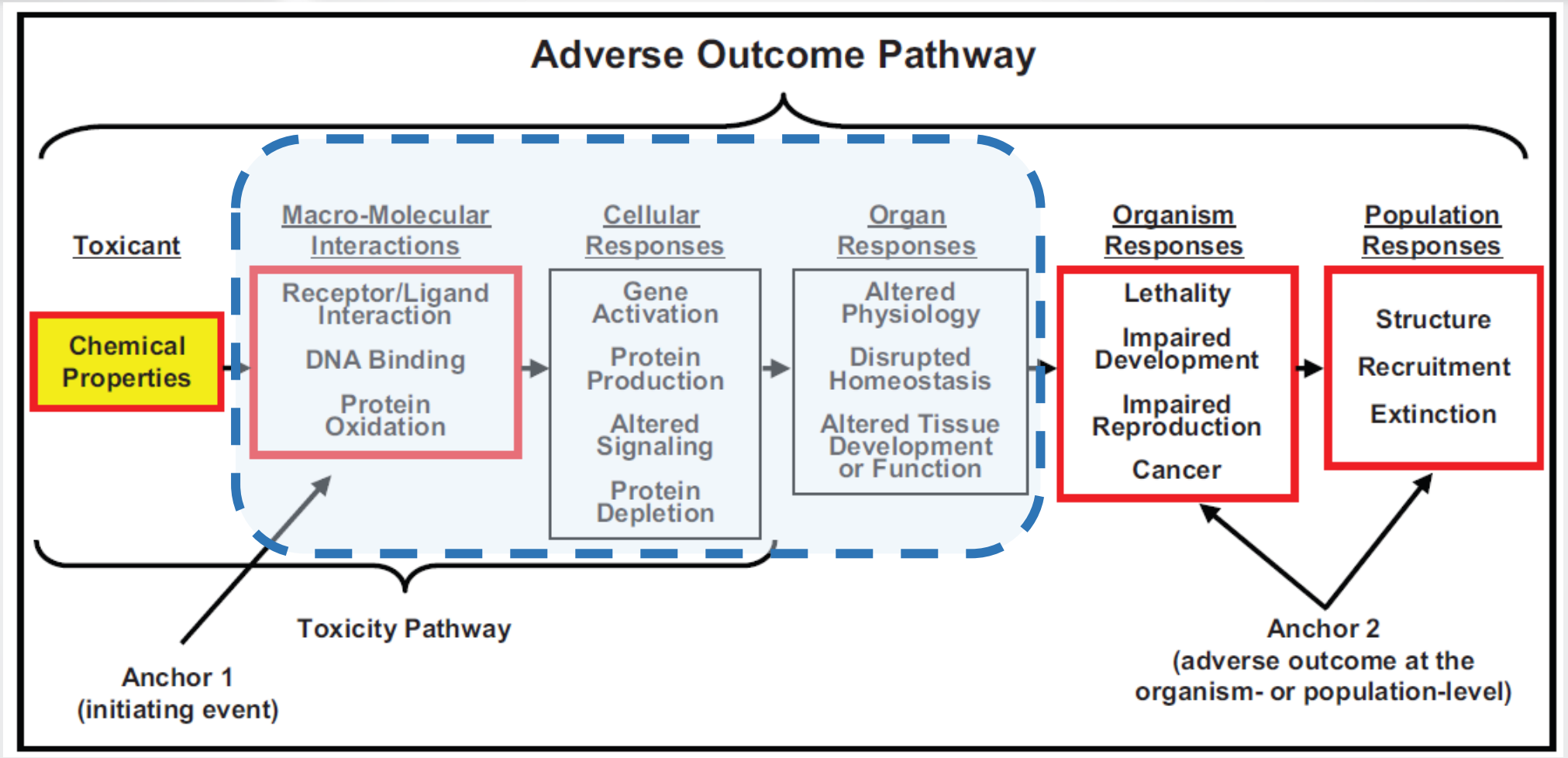
Broad profiling NAMs tend to probe cellular responses



Ankley et al. (2010) 10.1002/etc.34

- Broad profiling NAMs in use interrogate gene expression and cell morphological responses
- These data may be used to infer upstream interactions or downstream organ responses

Heterogeneous targeted NAMs in ToxCast address a range of event types in the AOP framework

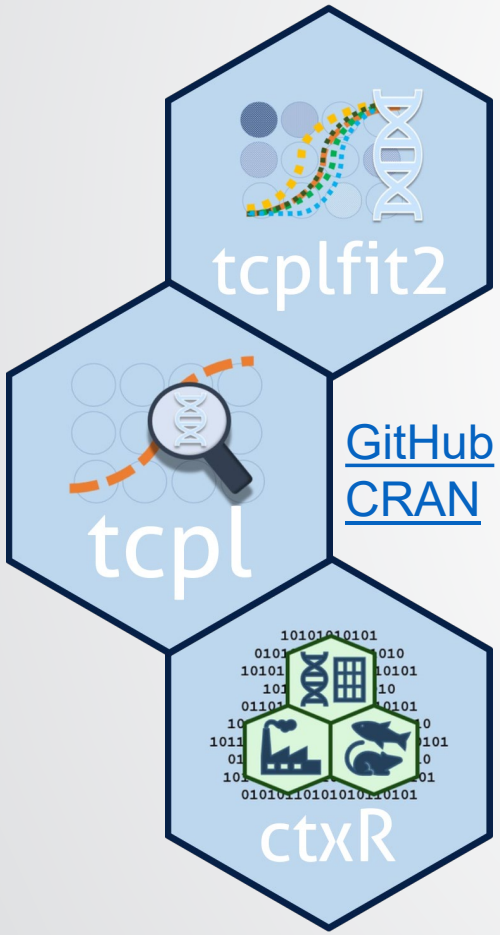


Ankley et al. (2010) 10.1002/etc.34

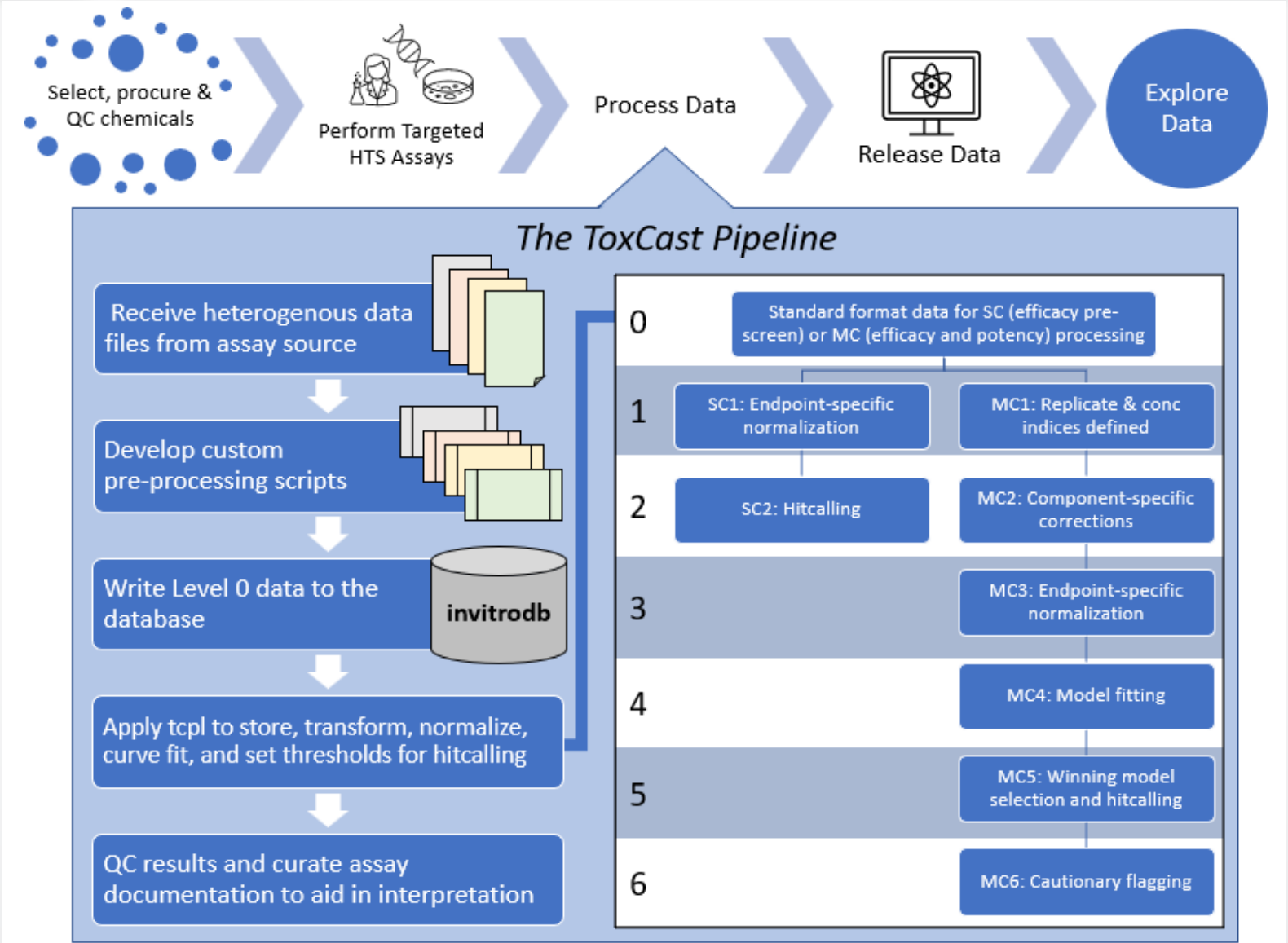
What is ToxCast?

Toxicity Forecaster (ToxCast) program encompasses pipelining software (*tcpl*, along with dependency *tcplfit2*), a database of NAM information (known as invitrodb), and efforts to curate and make these data informative.

Process Overview

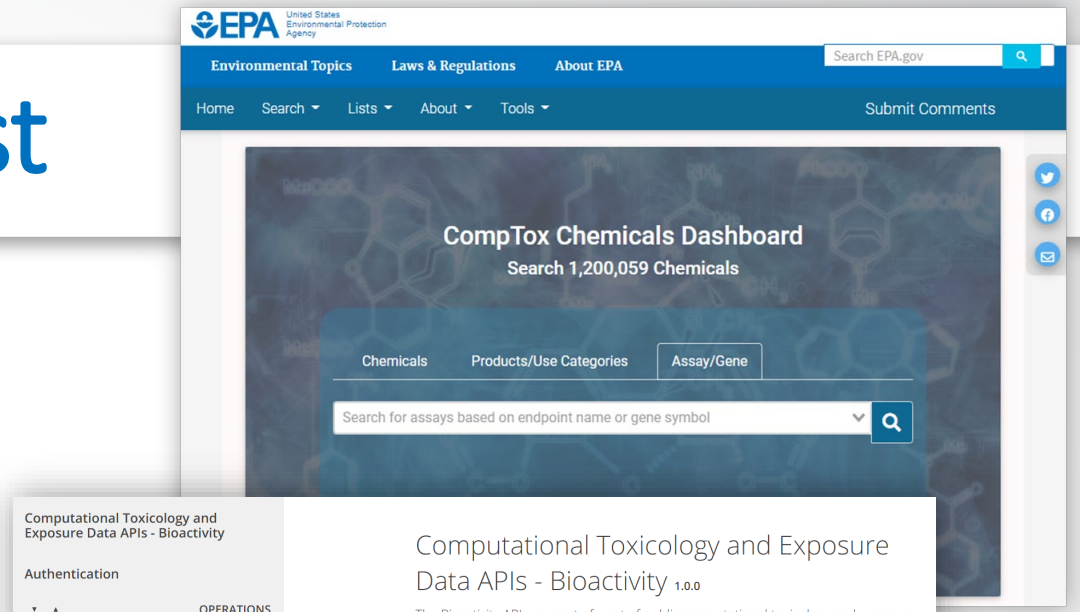


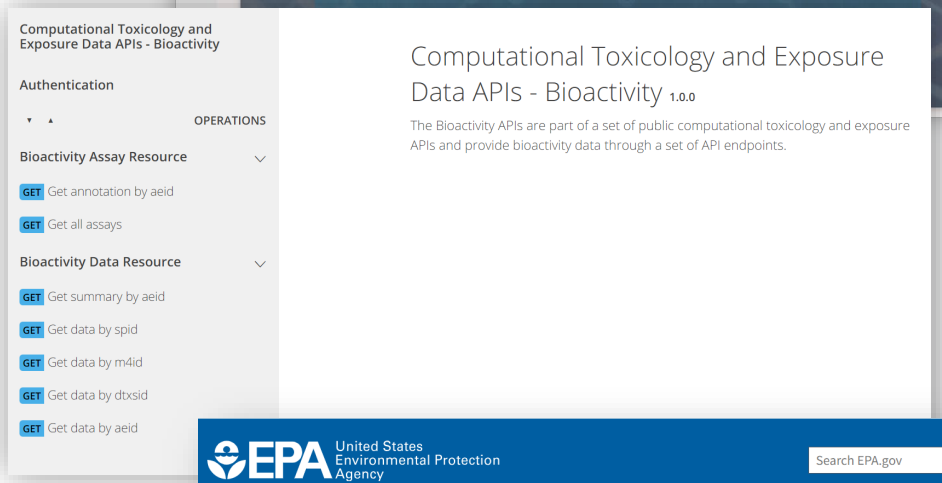
GitHub
CRAN

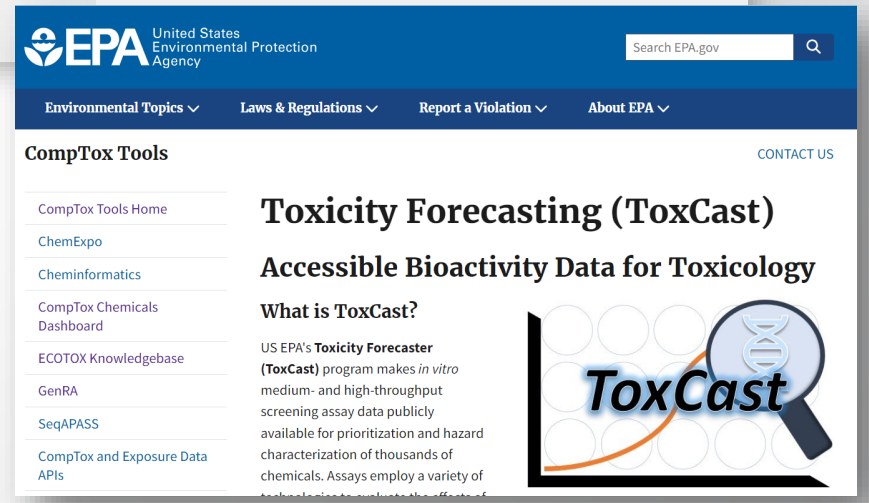


Exploring ToxCast

- ToxCast data is accessible via:
  - CompTox Chemicals Dashboard
  - Computational Toxicology and Exposure (CTX) Bioactivity API
  - Downloadable Data







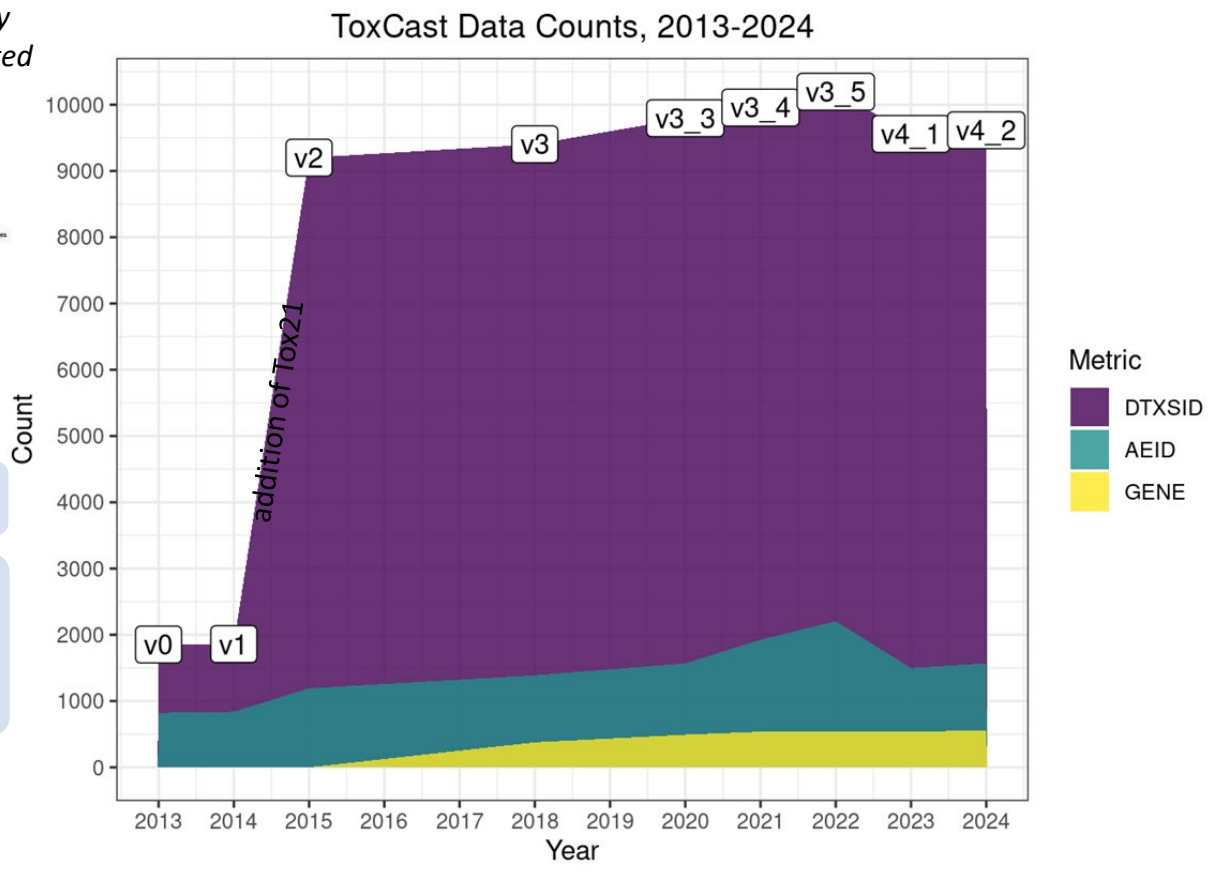
OECD GD211 Documentation Efforts

ToxCast Database Coverage

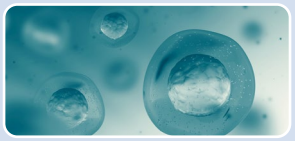
The **Toxicity Forecaster (ToxCast)** program curates and makes publicly available targeted bioactivity screening data. Latest database release (v4.2) includes:

- 26 Assay Sources
- 655 Unique Assays
- 1570 Unique Endpoints
- 9614 Chemicals

Including a TOX21 assay source for data generated by the TOX21 program



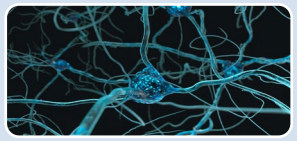
Diverse biology with ***over 500 mapped gene targets***, including:



Endocrine-Related: Estrogen Receptor, Androgen Receptor, Thyroid, Steroidogenesis

Cellular Signaling Pathways: Cytotoxicity, Proliferation, Stress, Mitochondrial Toxicity

Protein Interactions: Receptors, Transporters, Ion Channels, Enzymes

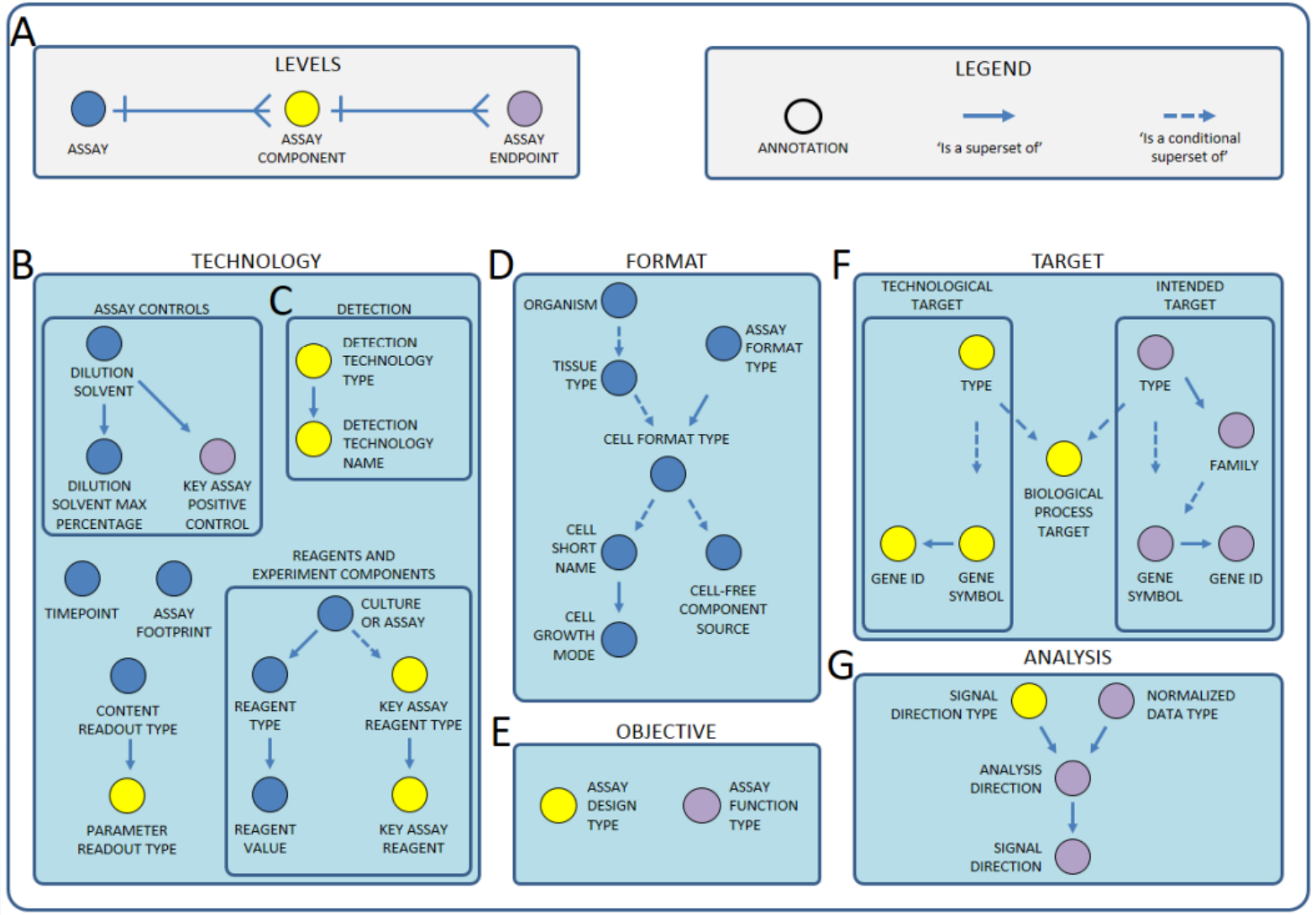


Complex Responses, e.g. Immune Response, Development, Neurotoxicity, etc.

As of October 2024, v4.1 data is available on CCD and CTX Bioactivity API. V4.2 is available to download.

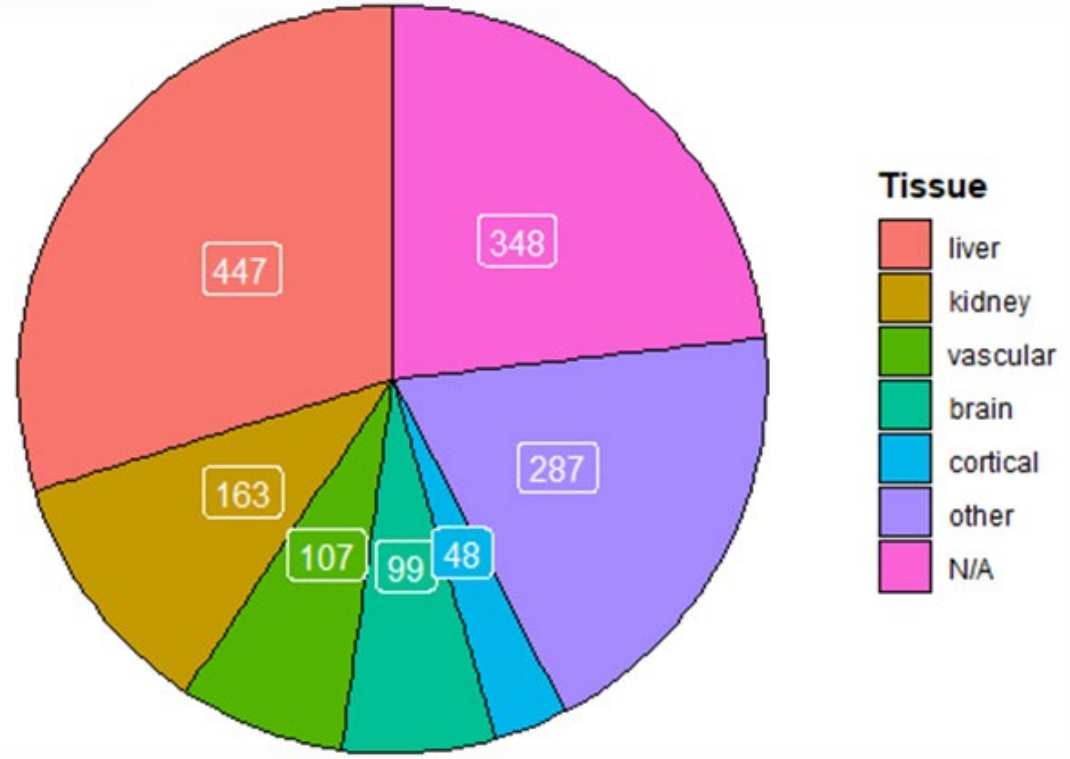
ToxCast assays have been annotated for many types of details

- Using the Bioassay Ontology (BAO) framework to capture four types of information, each annotation is assigned as a feature to an assay element level:
  - **Assay Source:** *Who* conducted the assay
  - **Assay:** *What* assay platform was used
  - **Assay Component:** "Raw" readout of *what* was measured
  - **Assay (Component) Endpoint:** *How* the measurement is interpreted (i.e. normalized component data)



Assay Element Annotations

- Most annotations employ controlled vocabulary within the database



Tissue *of origin across all assays*

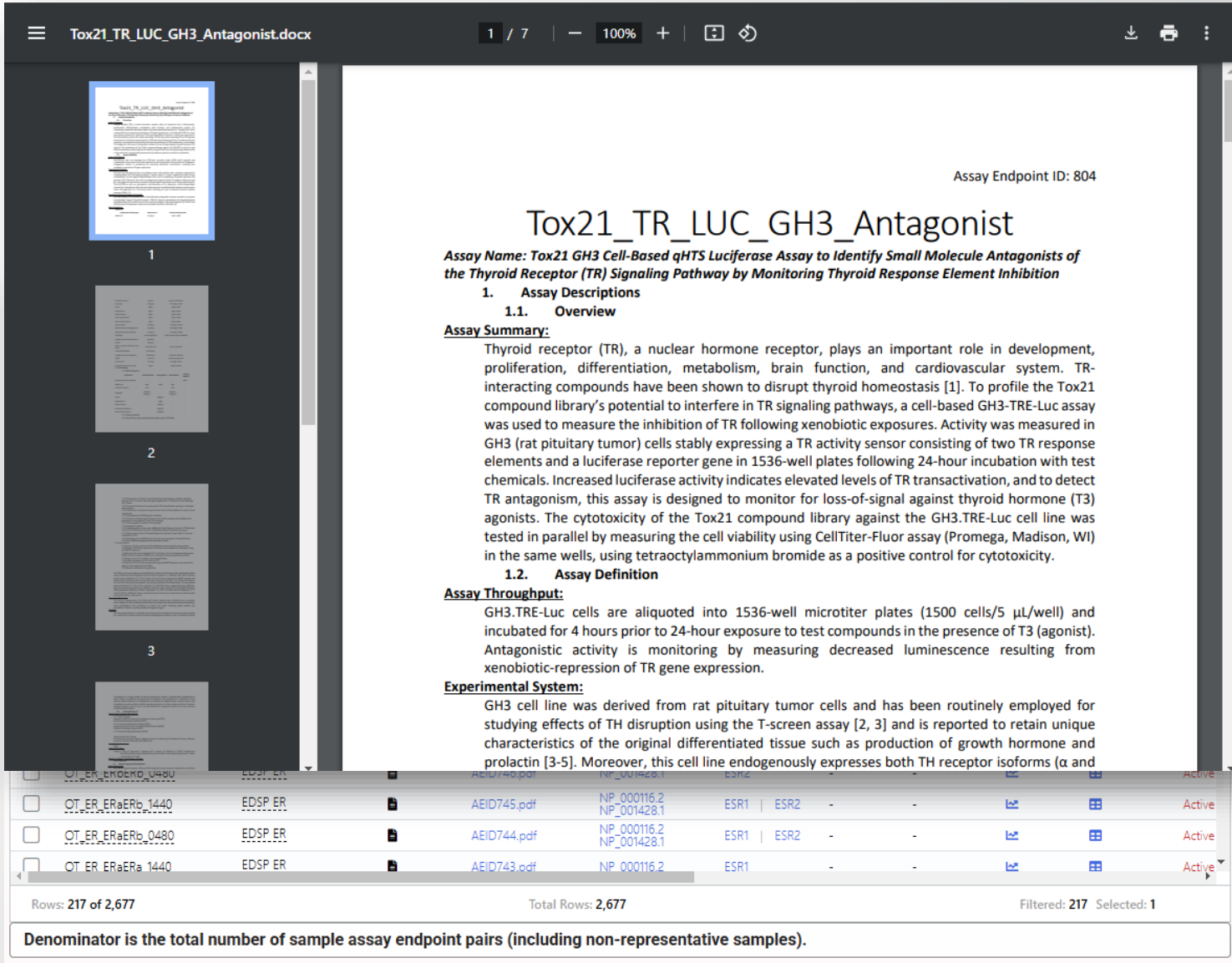
- Some annotations are hierarchical
  - e.g., general ‘intended_target_family’ and more specific ‘intended_target_family_sub’

Intended_target_family *frequency across all endpoints*

Need for Standardized Documentation

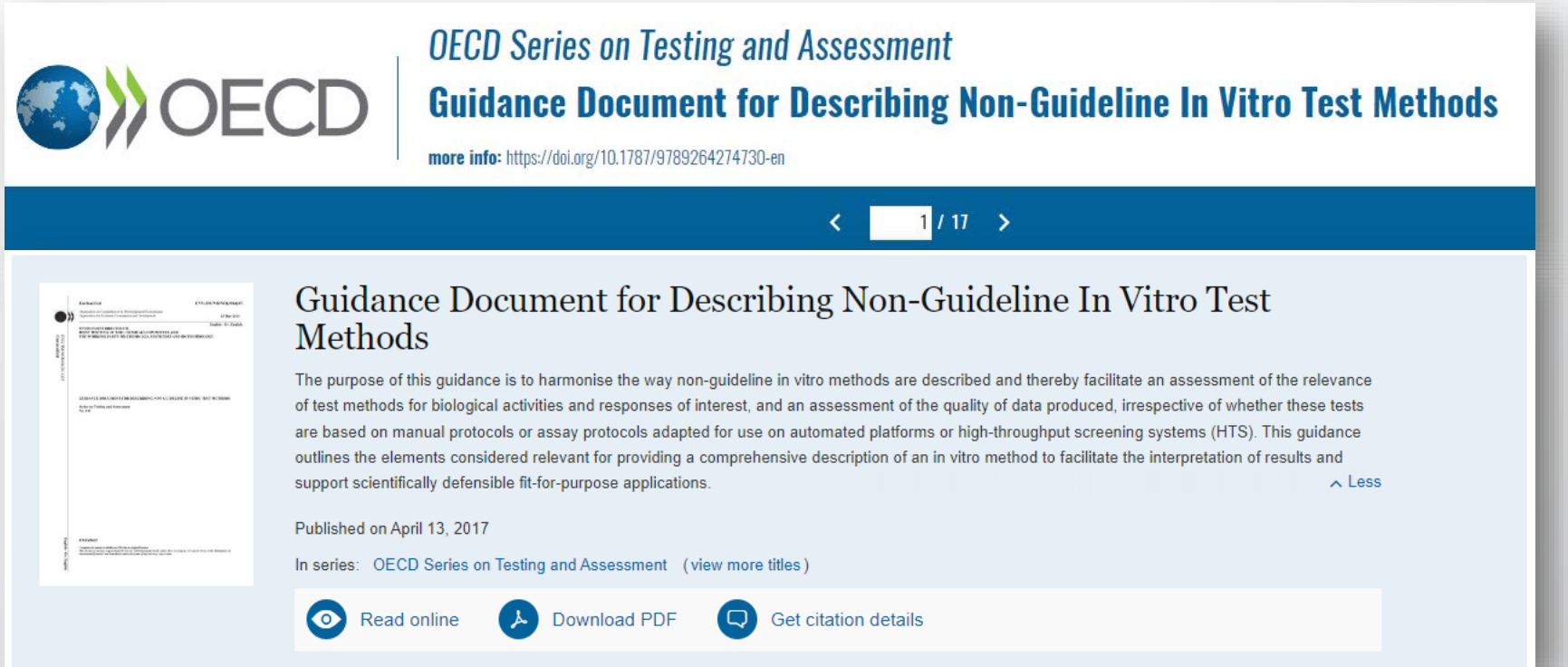
- Given ToxCast includes a heterogeneous set of assays across a diverse biological space, annotations help flexibly aggregate and differentiate processed data
- Assay documentation aligned with international standardization efforts can make data more useful and interpretable for use in decision-making.

The CCD bioactivity grid includes annotation columns for filtering as well as legacy GD211-aligned "Description" documents.



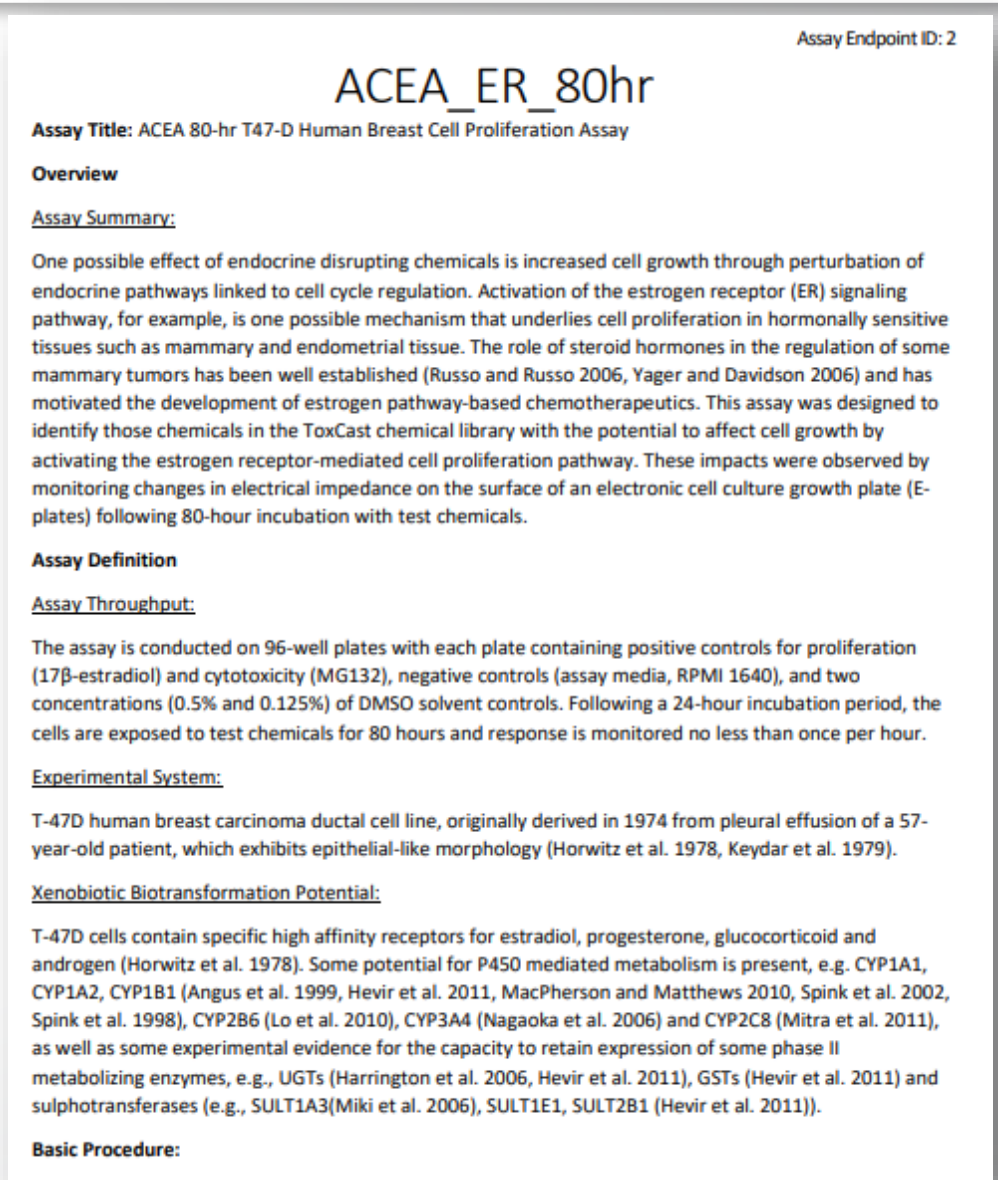
OECD Guidance Document 211 (GD211)

- A standard for comprehensive assay documentation describing non-guideline *in vitro* test methods and their interpretation
- Intended to harmonize non-guideline, *in vitro* method descriptions to allow assessment of the relevance of the test method for biological responses of interest and the quality of the data produced



Legacy Assay Description Documentation

- There have been past efforts to create a compiled report for ToxCast endpoints in a GD211-like format
  - Legacy documentation from February 2022 covered ~95 endocrine-related endpoints
    - *ACEA_ER_80hr example pictured*
  - *Note: a different set of documents from 2016 are available on CCD*
    - Method and assay objective descriptions may be valid, but performance metrics and assay quality statistics may be outdated
- **Manually maintaining documents is not sustainable or effective practice**



Assay Endpoint ID: 2

ACEA_ER_80hr

Assay Title: ACEA 80-hr T47-D Human Breast Cell Proliferation Assay

Overview

Assay Summary:

One possible effect of endocrine disrupting chemicals is increased cell growth through perturbation of endocrine pathways linked to cell cycle regulation. Activation of the estrogen receptor (ER) signaling pathway, for example, is one possible mechanism that underlies cell proliferation in hormonally sensitive tissues such as mammary and endometrial tissue. The role of steroid hormones in the regulation of some mammary tumors has been well established (Russo and Russo 2006, Yager and Davidson 2006) and has motivated the development of estrogen pathway-based chemotherapeutics. This assay was designed to identify those chemicals in the ToxCast chemical library with the potential to affect cell growth by activating the estrogen receptor-mediated cell proliferation pathway. These impacts were observed by monitoring changes in electrical impedance on the surface of an electronic cell culture growth plate (E-plates) following 80-hour incubation with test chemicals.

Assay Definition

Assay Throughput:

The assay is conducted on 96-well plates with each plate containing positive controls for proliferation (17β-estradiol) and cytotoxicity (MG132), negative controls (assay media, RPMI 1640), and two concentrations (0.5% and 0.125%) 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.

Experimental System:

T-47D human breast carcinoma ductal cell line, originally derived in 1974 from pleural effusion of a 57-year-old patient, which exhibits epithelial-like morphology (Horwitz et al. 1978, Keydar et al. 1979).

Xenobiotic Biotransformation Potential:

T-47D cells contain specific high affinity receptors for estradiol, progesterone, glucocorticoid and androgen (Horwitz et al. 1978). Some potential for P450 mediated metabolism is present, e.g. CYP1A1, CYP1A2, CYP1B1 (Angus et al. 1999, Hevir et al. 2011, MacPherson and Matthews 2010, Spink et al. 2002, Spink et al. 1998), CYP2B6 (Lo et al. 2010), CYP3A4 (Nagaoka et al. 2006) and CYP2C8 (Mitra et al. 2011), as well as some experimental evidence for the capacity to retain expression of some phase II metabolizing enzymes, e.g., UGTs (Harrington et al. 2006, Hevir et al. 2011), GSTs (Hevir et al. 2011) and sulphotransferases (e.g., SULT1A3(Miki et al. 2006), SULT1E1, SULT2B1 (Hevir et al. 2011)).

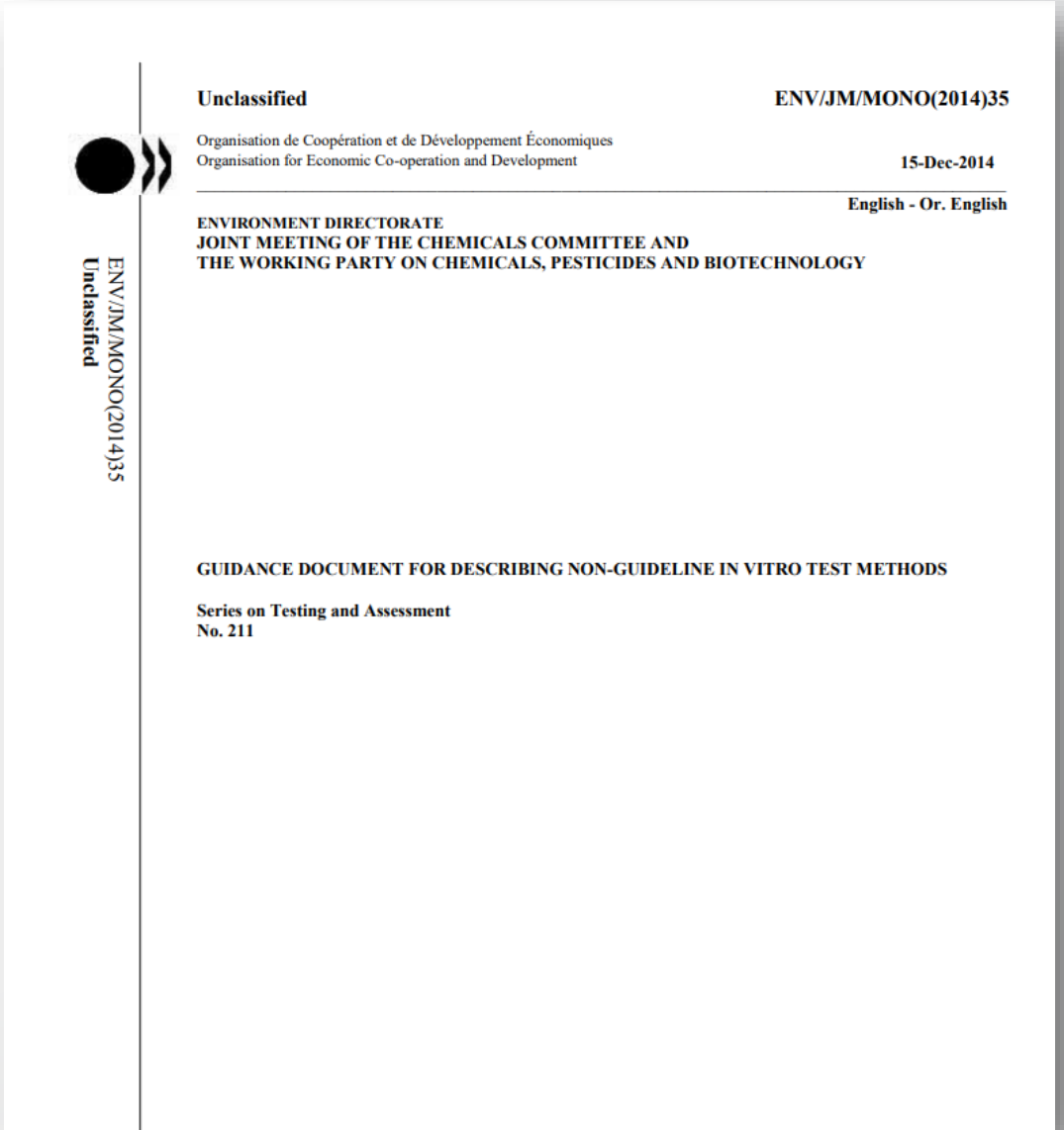
Basic Procedure:

Updated Assay Description Documentation

- Given major ToxCast software and database enhancements plus a desire to automate report generation, if possible, a complete overhaul to existing documentation process was undertaken
  - First, we reviewed existing database information to populate many GD211 stipulated fields
    - Annotations, auxiliary annotations, and past GD211 curations were leveraged
    - Different assay quality and endpoint metrics were derived using processed data
  - Missing fields were identified and selected for curation
    - Curation involves reviewing publications, SOPs, vendor statements of work, etc.
    - All information gets databased within the updated *assay_descriptions* table in invitrodb
  - A functionalized R script was developed to automate the generation of these new Word document reports

GD211 Sections

- Major sections include:
  1. General Information
  2. Test Method Description
  3. Data Interpretation
  4. Test Method Performance
  5. Potential Regulatory Applications
  6. Bibliography
  7. Supporting Information
- Each section includes subsections
  - These identified fields we're curating have no character limit for text whereas the annotations are often short in a standardized format or use a controlled term list


Unclassified ENV/JM/MONO(2014)35

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

15-Dec-2014

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2014)35
Unclassified

GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE IN VITRO TEST METHODS

Series on Testing and Assessment
No. 211

Test Method Description

field	description
assay_objectives	**2.1 Purpose of the test method:** *Inserted after assay_component_target_desc*; The claimed **purpose and rationale for intended use of the method** (e.g. alternative to an existing method, screening, provision of novel information in regulatory decision-making, mechanistic information, adjunct test, replacement, etc.) should be explicitly described and documented. The response measured in the assay should be put in the context of the biology/physiology leading to the in vivo response or effect. • If the biological activity or response refers to a key event or molecular initiating event (MIE), provide a short description indicating firstly what key event within an existing or developing AOP, or in relation to a mechanism or mode of action, the assay is aiming to characterize (i.e. which level of biological organization the assay may be attributed (e.g. sub-cellular, cellular, tissue, organ or individual), and secondly where the assay might fit in the context of an existing regulatory hazard (i.e. adverse outcome). • In the absence of any AOP, provide an indication of the plausible linkage between the mechanism(s) the assay is measuring and the resulting hazard endpoint.
scientific_principles	**2.2 Scientific principle of the method:** provide the scientific rationale, supported by bibliographic references to articles, for the development of the assay. A summary description of the scientific principle including the **biological/physiological basis and relevance** (e.g. modeling of a specific organ) and/or mechanistic basis (e.g. modeling a particular mechanism by biochemical parameters) should be described. If possible, indicate what the anchor point is within an AOP.
experimental_system	**2.3 Tissue, cells or extracts utilised in the assay and the species source:** indicate the experimental system for the activity or response being measured. Provide information on whether materials are readily available commercially or whether materials are developed in the laboratory (e.g. cell suspensions from tissue). Indicate source/manufacturer of biological material used. Indicated whether cryopreserved biological material can be used or only freshly prepared.
xenobiotic_biotransformation	**2.4 Metabolic competence of the test system:** describe and discuss the **extent to which the test system can be considered metabolically competent**, either by itself, or with the addition of an enzymatic fraction, if appropriate. Provide reference if available.
basic_procedures	**2.5 Description of the experimental system exposure regime:** provide a summary description of the essential information pertaining to the exposure regime (dosage and exposure time including observation frequency) of the test compounds to the experimental system including information on metabolic competence if appropriate; number of doses/concentrations tested or testing range, number of replicates, the use of control(s) and vehicle. Also, describe any specialized equipment needed to perform the assay and measure the response. Indicate whether there might be potential solubility issues with the test system, and solutions proposed to address the issue.
biological_responses	**2.6 Response and Response Measurement:** response here makes reference to **any biological effect, process or activity that can be measured**. Specify precisely and describe the response and its measurement.

Data Interpretation

field	description
Analytical description	3.2 Data analysis: Comment on the **response value in terms of a boundary or range to provide a context for interpretation**. E.g. putting into context what a negative value or >100% value might represent in a binding inhibition assay.

- Methods used in tcpl processing included as well as summary endpoint performance metrics

3. Data Interpretation

The tcpl package includes processing functionality for two screening paradigms: (1) single-concentration screening and (2) multiple-concentration screening. Single-concentration screening consists of testing chemicals at one concentration, often for the purpose of identifying potentially active chemicals to test in the multiple-concentration format. Multiple-concentration screening consists of testing chemicals across a concentration range, such that the modeled activity can give an estimate of potency, efficacy, etc.

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

3.2 Data Analysis: analytical_description

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. In the ToxCast program, level 0 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, the tcpl R package 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:
ZZZM2
Level 3: Endpoint-specific normalization include:
ZZZM3
Level 4: Baseline and required tcplFit2 parameters defined by:
ZZZM4
Level 5: Possible cutoff thresholds, where higher value for endpoint is selected, include:
ZZZM5
Level 6: Cautionary flagging include:
ZZZM6

The following describes an aggregate endpoint summary of number of chemicals tested, active or inactive hit calls (hitc), and predicted winning models.

Total number of samples tested: ZZZFA Number of chemicals tested: ZZZFN

Active hit count: hitc≥0.9	Inactive hit count: 0≤hitc<0.9	NA hit count: *hitc<0*
ZZZFB	ZZZFC	*ZZZFM*

Number of sample-assay endpoints with winning *hill* model:	ZZZFD
gain-loss (gnls) model:	ZZZFE
power(pow) model:	ZZZFF

Test Method Performance

- Robustness subsection:
  - Detailed assay quality statistics are derived from the raw concentration response data
    - z-prime
    - ssmd (strictly standardized mean difference)
    - Median and median absolute deviation are provided across all plate controls, where applicable
  - Plate-wise statistics commonly used in high throughput screening community

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*	ZZnmed_rplc
Neutral control median absolute deviation, by plate: *nmad*	aa_nmad
Coefficient of variation (CV) of neutral control wells: *(nmad/nmed)*	aa_cv
POSITIVE CONTROL (well type = "p")	
Positive control well median response value, by plate: *pmed*	aa_pmed
Positive control well median absolute deviation, by plate: *pmad*	aa_pmad
Z Prime Factor for median positive and neutral control across all plates: *1 - ((3 * (pmad + nmad)) / abs(pmed - nmed)*	aa_zprimp
Strictly standardized mean difference (SSMD) for positive compared to neutral control wells: *((pmed - nmed) / sqrt(pmad² + nmad²)*	aa_ssmdp
Positive control signal-to-noise: *((pmed-nmed)/nmad)*	aa_snp
Positive control signal-to-background: *(pmed/nmed)*	aa_sbp
NEGATIVE CONTROL (well type = "m")	
Negative control well median, by plate: *mmed*	aa_mmed
Negative control well median absolute deviation value, by plate: *mmad*	aa_mmad
Z Prime Factor for median negative and neutral control across all plates: *1 - ((3 * (mmad + nmad)) / absmpmed - nmed)*	aa_zprimm
Strictly standardized mean difference (SSMD) for negative compared to neutral control wells: *((mmed - nmed) / sqrt(mmad² + nmad²)*	aa_ssmdm
Signal-to-noise (median across all plates, using negative control wells): *((mmed-nmed)/nmad)*	aa_snm
Signal-to-background (median across all plates, using negative control wells): *(mmed/nmed)*	aa_sbm

Fall 2024 Release

- Curated information stored in the updated “assay_descriptions” table of invitrodb v4.2 and provided within a compiled report, to be available on the ToxCast Downloadable Data page.
- **809**/1570 endpoints will be included in new report. Descriptions will be available for the following endpoints:

Assay Sources		Biology

• ACEA
• ARUNA
• ATG
• BSK
• CLD
• CCTE_Shafer
• CCTE_Deisenroth
• CCTE_GLTED
• CCTE_Mundy
	• CCTE_Padilla
	• IUF
	• LTEA
	• OT
	• STM
	• TOX21
	• Tanguay
	• UKN
	• VALA
		• Androgen Receptor (AR)
		• Estrogen Receptor (ER)
		• Developmental Toxicity
		• Developmental Neurotoxicity
		  • DNT-IVB (Developmental Neurotoxicity In vitro battery)
		• Immunotoxicity
		• Steroidogenesis Bioactivity
		• Thyroid Bioactivity
		• Non-mammalian Vertebrate
		  • Zebrafish

2025 Priorities

- Curate missing info for the remaining publicly released ToxCast endpoints
  - Focus on TOX21, biochemical assays (Eurofins, Novascreen), and any remaining cytotoxicity burst endpoints
- Make template enhancements
  - Add AOPs and key event linkages if available
  - Leverage reference chemical lists from RefChemDB to derive predictive capacity metrics based on reference chemical lists
- Address any user feedback or recommendations
- Expand documentation practice to other tiers of screening

Summary

- These reports are a *work in progress*
- This documentation effort represents a novel, semi-automated and large-scale application of the GD211 template with all underlying information also databased.
- Complementary to any data generation and processing effort, assay documentation using internationally harmonized standards ensures data are transparent, accessible, and interoperable
  - Increasing confidence for the adoption of assay data in next generation chemical assessment.

Thanks for Listening

US EPA's Center for Computational Toxicology and Exposure
February 2024

- Please reach out with questions: Feshuk.madison@epa.gov
- Thank you to past contributors, collaborators, and our current ToxCast team
  - Ashley Ko
  - Manasvinee Mayil Vahanan
  - Kelly Carstens
  - Alison Harrill
  - Katie Paul Friedman

Extra Slides

Generating GD211-Aligned Documentation for ToxCast to Support Assay Interpretation and Data Use

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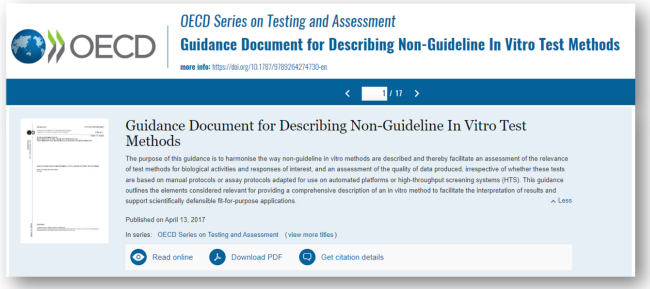
ToxCast Overview

The US EPA Toxicity Forecaster (ToxCast) program curates and makes publicly available a resource of targeted bioactivity assay data to inform chemical prioritization and hazard characterization. The ToxCast Data Analysis Pipeline R package, *tcpl*, is used to process, model, and visualize concentration-response screening data as well as populate a linked MySQL database, *invitrodb*. Given ToxCast includes heterogeneous assays across a diverse biological space, *invitrodb* contains manually curated and expert-reviewed assay annotation information, describing experimental and biological details that are essential for proper interpretation of screening results.

OECD GD211

The Organisation for Economic Co-operation and Development (OECD) guidance document 211 (GD211) suggests components of comprehensive assay documentation describing non-guideline *in vitro* test methods and their interpretation. The intent of the GD211 is to harmonize non-guideline, *in vitro* method descriptions to allow assessment of the relevance of the test method for biological response and data quality. Legacy GD211-like ToxCast documentation were manually maintained and covered less than 100 ToxCast assay endpoints.



ToxCast Assay Description Documentation

Given major ToxCast software and database enhancements plus a desire to expand assay coverage and automate report generation, a complete overhaul to the existing documentation process was undertaken.

- First, existing database information was reviewed to populate many GD211 stipulated fields.
  - Annotations, auxiliary annotations, and past GD211 curations were leveraged.
  - Different assay quality and endpoint metrics were derived using processed data.
- With missing fields identified, curation involved reviewing publications, standard operating procedures (SOPs), vendor statements of work, etc, to describe the assay design and protocols used. All information was databased within the updated *assay_descriptions* table in *invitrodb*.
  - Unlike *invitrodb* annotations which are often short in a standardized format or use a controlled term list, this curated text has no character limit, allowing for the highest level of detail.
  - The updated *assay_descriptions* table includes the following fields to fulfill GD211 specifications:
    1. assay_title
    2. assay_throughput
    3. assay_objectives
    4. scientific_principles
    5. analytical_description
    6. experimental_system
    7. xenobiotic_biotransformation
    8. basic_procedures
    9. biological_responses
    10. proprietary_elements
- Finally, a functionalized R script was developed to synthesize all information together and automate the generation of reports. Individual reports were then compiled.

These reports are a ***work in progress***. They will be iteratively updated as descriptions improve from user feedback and more information becomes available. For this iteration, assay description documents are available for **809** endpoints across the following assay sources and biological space:

Endpoint Report Coverage

Assay Sources		Biology
• ACEA	• CCTE_Padilla	• Androgen Receptor (AR)
• ARUNA	• IUF	• Estrogen Receptor (ER)
• ATG	• LTEA	• Developmental Toxicity
• BSK	• OT	• Developmental Neurotoxicity
• CLD	• STM	• DNT-IVB (Developmental Neurotoxicity *in vitro* battery)
• CCTE_Shafer	• TOX21	• Immunotoxicity
• CCTE_Deisenroth	• Tanguay	• Steroidogenesis Bioactivity
• CCTE_GLTED	• UKN	• Thyroid Bioactivity
• CCTE_Mundy	• VALA	• Non-mammalian Vertebrate
		• Zebrafish

A compiled report and assay description documents by endpoint were released in September 2024, accompanying the *invitrodb* v4.2 database release.

Visit the **Exploring ToxCast** webpage for more details:

Conclusions

Given limitations in traditional toxicology testing, new approach methods (NAMs) are needed to prioritize, evaluate and regulate the thousands of chemicals in commerce. Complementary to any data generation effort, assay documentation, along with the standardized and well-documented data processing procedures, ensure data are transparent and approachable, thereby increasing confidence for the adoption of assay data in next generation chemical assessment. This effort to develop thorough assay documentation aligned with the GD211 international standardization efforts can make ToxCast data more useful and interpretable for use in regulatory decision-making and research applications. *Disclaimer: This poster does not necessarily reflect U.S. EPA policy.*