National Institute of Environmental Health Sciences
Division of Translational Toxicology

Annotations for ToxCast and Tox21 High-Throughput Screening Assays: Facilitating Assay Interpretation and Data Use

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Background

- Building confidence in new approach methodologies (NAMs) for prioritization and hazard characterization requires accessible and easily interpretable bioactivity data.
- The U.S. Environmental Protection Agency (EPA) Toxicity Forecaster (ToxCast) program makes in vitro medium- and high-throughput screening (HTS) assay data publicly available for thousands of chemicals of interest. The assays included employ a variety of technologies to evaluate the effects of chemical exposure on diverse biological targets.
- To increase accessibility to annotated HTS data, the EPA and National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have annotated over 2,000 assay endpoints from the ToxCast program, including results from the Toxicology Testing in the 21st Century (Tox21) consortium.
- These HTS assay data were annotated using existing controlled bioassay ontologies to facilitate stakeholder understanding, provide terminology that offers additional context, and inform on the biological relevance of the many heterogeneous in vitro HTS assay readouts.

Key Goals for this Project:

1. **Identify fields from existing annotations for further reporting needs**
   Leveraging and expanding annotations for HTS data can provide context to facilitate the identification of data gaps, mechanistic plausibility, and further investigation into regulatory-relevant endpoints.
2. **Map existing annotations to standardized reporting templates**
   Existing assay annotations are mapped to complete standardized data reporting templates, including the internationally recognized OECD guidance document (GD) 211 and OECD Harmonized Template (OHT) 201.
3. **Ensure all data are publicly accessible and transparent**
   By offering users detailed assay descriptions using the GD 211 format and providing standardized OHT 201 formatted results for each chemical across all tested endpoints, this work renders these complex data streams more approachable and accessible, thereby increasing confidence for the adoption of HTS assay data in next generation chemical assessment.

Approaches to Annotation

Annotating Technological Assay Details

The ToxCast data pipeline, tcpl, is an open-source R package that stores, manages, curve-fits, and visualizes ToxCast data as well as populating the linked MySQL Database, InvitroDB. All ToxCast data is made accessible via the CompTox Chemicals Dashboard (comptox.epa.gov) under the Bioactivity section or download at: https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data. The ToxCast Summary page and well as examples of assay annotation fields describing assay platform and design are displayed below:

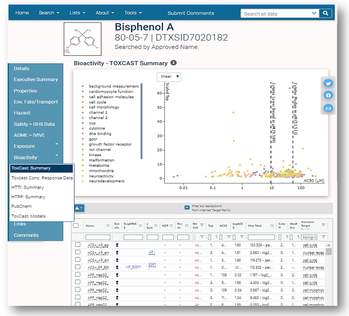


Table 1: Example Assay Annotation Fields

Normalized Data Type	Organism
Burst Assay	Tissue
Key Positive Control	Cell Format
Signal Direction	Cell Short Name
Intended Target Type	Cell Free Component Source
Parameter Readout Type	Cell Growth Mode
Assay Design Type	Assay Footprint
Biological Process Target	Assay Format Type
Detection Technology Type	Content Readout Type
Key Assay Reagent	Dilution Solvent
Technological Target Type	Dilution Solvent Percent Max
Timepoint Hr	Gene Symbol

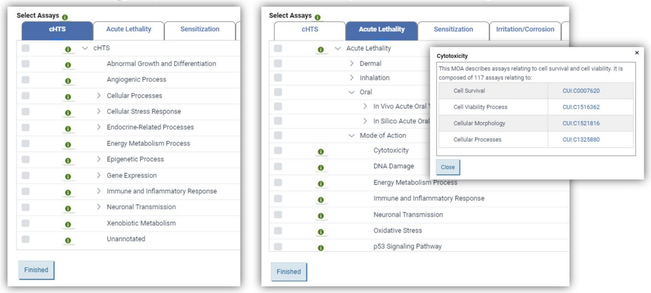
Understanding Biological Interpretation

NICEATM has developed the user-friendly and interactive Integrated Chemical Environment (ICE; ice.ntp.niehs.nih.gov). ICE provides data and computational tools to aid in finding, analyzing, and contextualizing NAMs. In the ICE Search tool, users can find curated HTS (cHTS) data via the Assay Selection feature where assays are grouped by controlled vocabulary terminology to facilitate retrieval of orthologous or complementary assays.

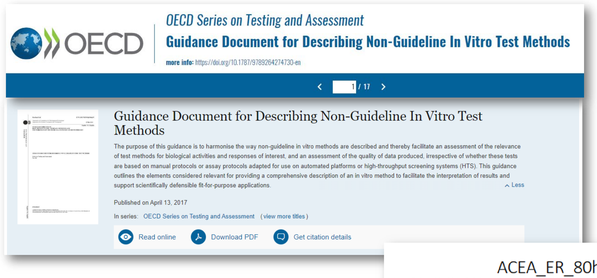
Mechanistic Target-Based
Based on biological process to

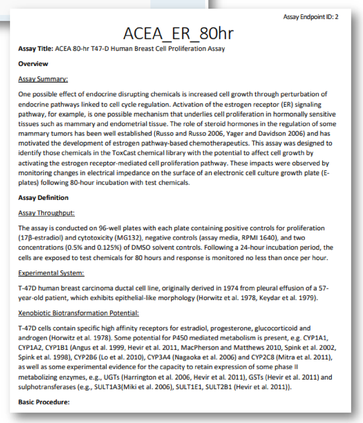
Mode of Action-Based Search
pathways relevant to toxicological

Integrated Chemical Environment



OECD Guideline Document 211 Generation





Annotations can be leveraged to populate fields for GD 211 and direct additional curation efforts

GD 211 serves as a standard for comprehensive assay documentation describing non-guideline *in vitro* test methods and their interpretation. The intent of GD 211 is 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.

ToxCast Assay Documentation details the experimental system, protocols, performance metrics, and assay quality statistics. Complete assay documentation is available for 95 endocrine-related assays, with additional assay curation ongoing. Major software and database enhancements to tcpl and invitroDB into an bidirectional curvefitting paradigm warrant a complete overhaul to existing assay description documentation.

OECD Harmonized Template 201 Generation

Annotations can be leveraged to populate fields in the OHT 201 form

The OHT 201 is a harmonized template for reporting chemical test result summaries for intermediate effects. An example for "Effect Identification" and "Process" sections is provided below:

OECD Template #201: Intermediate effects - mechanistic information *(Version [5.2]-[December 2022])*

Line no.	Field name	Field type Display type	Picklist Freetext template	Help text
16.	**Effect identification**	**Header 1**		Describe the mechanism that can be measured with the method by providing a 'Process', 'Object' and 'Action'. As a minimum, the 'Process' and 'Action' or the 'Object' and 'Action' must be identified. More than one combination can be provided (e.g. Cell Activation, CD54 molecule, increased & Cell Activation, CD86 molecule, increased). If both Process and Object are provided they have to be concordant with the chosen Action (e.g. both process and object are increased or decreased). See Yves et. al (2017) Please refer to the following P/O/A example from an existing OECD test guideline: TG442C, DPRA, kinetic DPRA, and ADRA: protein binding / - / increase
17.	Process	List sup. (picklist with remarks) Display: Basic	**Picklist values:** - apoptotic process - [GO:0008219] - biosynthetic process - [GO:0009058] - catalytic activity - [GO:0003824] - cell activation - [GO:0001775] - cell death - [GO:0008219] - cell differentiation - [GO:0030154] - cell migration - [GO:0016477] - cell proliferation - [GO:0008283]	Process represents the dynamics of the underlying biological system (e.g., receptor binding) (Ives et al, 2017). The Process is also used to annotate Key events in the Adverse Outcome Pathway Wiki (https://aopwiki.org/) as described in Ives et al, 2017, doi:10.1089/aivt.2017.0017). Select the process that best describes the mechanistic information observed or select 'other' to specify the Process and provide a term. Please consult the Ontology Lookup Service (OLS) which is available at https://www.ebi.ac.uk/ols/index to choose a Process term. If possible please select as Process one term belonging to the following ontology Gene Ontology (GO). For most terms there will be several options. It is therefore important to also copy the preferred ontology identifier into the remarks field. Cytotoxicity data should only be reported as a process (e.g. cell death) when it is the scope of the study to determine cytotoxicity. In cases where cytotoxicity is measured for supporting information e.g. for dose selection/elimination, it should not be considered as a process. Such data are reported as 'Other observations'.

Table 2: OHT 201 "Effect Identification" source annotations

Invitrodb: Assay Component Endpoint Name
Invitrodb: Intended Target
Invitrodb:Technological Target Type
Invitrodb: Gene Symbol
Invitrodb: Signal Direction

Table 3: OHT 201 "Process" source annotations

Invitrodb: Assay Design Type
Invitrodb: Biological Process Target
ICE: Mode of Action
ICE: Mechanistic Target

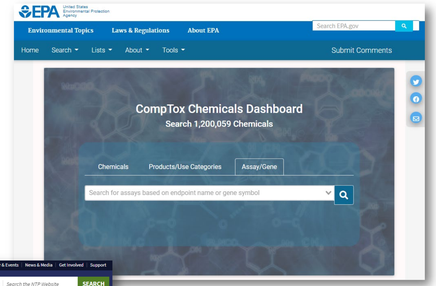
Accessing These Data

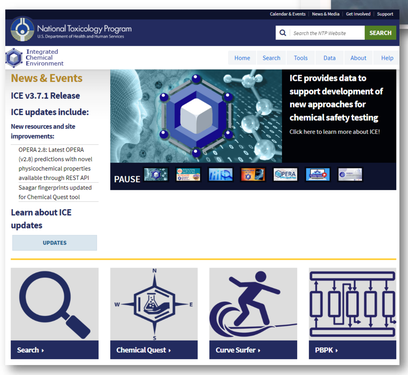
European Chemicals Agency (ECHA)'s IUCLID

IUCLID (iuclid6.echa.europa.eu) is a software developed explicitly for storing, maintaining, and exchanging data characterizing hazard properties of chemical substances. It was co-developed by ECHA and the OECD. Under REACH legislation, information submitted to ECHA has to be in IUCLID format. All OHT 201 forms will be completed, generated, and be retrievable using the IUCLID interface.

Comptox Chemicals Dashboard

Downloadable GD211 files are available from the data download page found on The CompTox Chemicals Dashboard website. All annotations and links to invitrodb download are also available. The Assay/Gene Search feature allows users to query and review HTS data, as well as download any retrieved results.





ToxCast Downloadable Data

In addition to access through the CompTox Chemicals Dashboard, the invitroDB database package, including the MySQL database, release note, summary files, assay description documentation, and concentration-response plots, are available for download. While developed primarily for ToxCast, the tcpl package is written to be generally applicable to the chemical-screening community. The tcpl R package can be installed from CRAN to interact with the database.

ICE

Search and download curated HTS data as well as visualize results with interactive plots. Ultimately the OHT 201 forms will be populated within the Curve Surfer tool to allow users to visualize results for chemicals tested in assays as well as download the OHT 201 summary forms.

Summary

Increased accessibility to annotated HTS data provides context that facilitates the identification of data gaps, mechanistic plausibility, and further investigation into regulatory-relevant endpoints

- Tox21 and ToxCast annotations were retrieved from invitroDB and ICE's cHTS data.
- Assay annotations are leveraged to work toward completing standardized data reporting templates OHT 201 and GD 211.
  - GD 211 serves as a standard for comprehensive assay documentation describing non-guideline in vitro test methods and their interpretation.
  - The OHT 201 is a harmonized template for reporting chemical test result summaries for intermediate effects.
- Resulting standardized forms will be available from IUCLID, CompTox Chemicals Dashboard, and ICE web tools.

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