

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

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A wide variety of *in vitro* high-throughput screening (HTS) assay data is publicly available. These bioactivity data have the potential to facilitate the development of computational approaches for chemical assessments and provide mechanistic insight on chemical effects and hazard. However, linking HTS data to toxicologically-relevant mechanistic pathways or to regulatory endpoints remains a challenge and requires detailed information about assay technology and its related biological context. Here, we present the results of our efforts to use existing controlled bioassay ontologies to annotate over 2,000 assay endpoints from the US EPA's Toxicity Forecaster (ToxCast) program, including results from the Toxicology Testing in the 21st Century (Tox21) consortium. Use of existing ontologies facilitates stakeholder understanding, provides terminology that offers additional context, and informs upon the biological relevance of the many heterogeneous *in vitro* HTS assay readouts.

Assay annotations are leveraged to complete standardized data reporting templates, including the internationally recognized OECD guidance document (GD) 211 and OECD Harmonized Template (OHT) 201. 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. The template captures both assay technology information as well as mechanistic outcome results and interpretation (i.e., effects on molecular, subcellular, or tissue level relevant to hazard assessment) obtained from *in vitro*, *ex vivo*, or *in silico* methods.

Increased accessibility to annotated HTS data provides context that facilitates the identification of data gaps, mechanistic plausibility, and further investigation into regulatory-relevant endpoints such as endocrine disruption, carcinogenicity, developmental toxicity, and systemic effects. 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.

The OHT 201 output .i6z files will be made publicly available in the Integrated Chemical Environment (ICE, <https://ice.ntp.niehs.nih.gov/>) and IUCLID (<https://iuclid6.echa.europa.eu/>) with curation and molecular or cellular process annotations linking assay endpoints to regulatory endpoints of interest, thus allowing data to be easily searched, grouped, and visualized. Associated data visualizations assist users in reviewing a chemical's potential activity for selected regulatory endpoints. The GD 211 files that complement the OHT 201 data are available via the CompTox Chemicals Dashboard (<https://comptox.epa.gov/dashboard/>) along with complete concentration-response data and curated summary annotations from ToxCast. This presentation will walk through specific case studies that demonstrate how the OHT 201 and GD 211 templates can be used in tandem toward holistic HTS-assay based chemical analysis.

This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C. This abstract does not reflect official EPA policy.

Keywords: Systems and Integrative Toxicology; Computational Toxicology; Alternatives to Animal Testing