Adding Context to Tox21/ToxCast Data: Linking *In Vitro* Assays to Toxicity Outcomes

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BACKGROUND

- The ToxCast high-throughput screening program provides data for 1,387 assay component endpoints, with some endpoints having data for as many as 9,213 chemicals (invitrodb_v3, released by the US EPA/NCCT in Fall 2018). www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data
- Publicly downloadable ToxCast data include assay annotations, which provide detailed information about assay design, components, and biochemical targets to support biological interpretation.
- Predictive toxicology approaches can benefit greatly from mechanistic insight. To accomplish this, integrating *in vitro* assays to biological pathways and ultimately pathways to toxicity endpoints is needed.
- Modes of action (MOAs) for a number of toxicity endpoints have been identified by experts. While these are not as mechanistically detailed as adverse outcome pathways, these can be leveraged to help further contextualize and annotate ToxCast assays.
- By linking ToxCast assays to MOAs associated with toxicity outcomes, weight-of-evidence approaches and knowledge-driven modeling techniques can be applied with greater confidence.

ASSAY ANNOTATION

- Several informative entries are provided in the ToxCast annotation file that can be used for linking assays to MOAs attributed with toxicity outcomes:
  - Intended_target_family
  - Intended_target_family_sub
  - Intended_target_official_gene_symbol
  - Biological_process_target
- In addition to these data, expert knowledge was applied to refine assays mapped to MOA.

UTILITY OF ASSAY MAPPING

- Mapping assays to known MOAs contributing to apical toxicity outcomes creates assay groupings. These can be used in weight-of-evidence approaches or be leveraged for knowledge-driven modeling.
- While the ToxCast/Tox21 assays cover a broad spectrum of biological endpoints, coverage of certain MOAs is greater than others and there remain several MOAs lacking assay data. It is important to account for the uneven richness of data contributing to different MOAs when applying different analysis approaches.

Prioritization approach using ToxPi algorithm

- MOA-based groups of assays are used as the foundation for analyses utilizing the ToxPi approach, with each "slice" representing a specific MOA.

Knowledge-driven cluster-based modeling

- MOA-based groups of assay endpoints are being used to help "inform" (train) modeling efforts for making predictive models of toxicity outcomes. This uses chemical activity within a group of assays to characterize chemical-mediated effects rather than utilizing single assays as input (data not shown).

LINKING ASSAYS TO DEVELOPMENTAL TOXICITY

Table 1: Summary of Assay Counts Mapped to Various Modes of Developmental Toxicity

Mode of Action	# Tox21/ToxCast Assay Endpoints
Folate antagonism	1
Neural crest disruption *i.e., RAR, RXR, FGFR, endothelin, folate*	~20
Endocrine disruption *i.e., AR, ER, steroidogenesis, thyroid*	~50
Oxidative stress *i.e., NRF2, ER stress, p53, morphology*	~30
Vascular disruption	~80
GABA receptor	<10
5-HT receptors and transporters	<10
Other targets: *i.e., angiotensin II receptor, HDAC, COX1, NMDA, HMG-CoA synthase*	~10

The developmental toxicity MOAs in **Table 1** are from VanGelder *et al.* 2010. Other retrieved MOAs for which there are no mapped assays include genotoxicity.

The groups of assays, based on the MOAs in **Table 1**, were used as input to generate ToxPi charts (Marvel *et al.* 2018; toxpi.org). Input data are compiled by providing the potency (AC50) of any activity observed in the assays listed, per chemical. ToxPi charts help visualize the relative activity a chemical has for each MOA, providing an overall effect score to help rank chemicals for likelihood of bioactivity.

For example, **Figure 1A** (below) demonstrates that each slice of the ToxPi "pie" reflects a group of assays (i.e., an MOA). **Figures 1B-D** demonstrate three chemicals wherein different MOAs had various potency, illustrated by the relative sizes of the slices. Overall, the chemical in **Figure 1B** has the greatest weight of evidence for developmental toxicity, as many MOAs seem to have potent bioactivity.

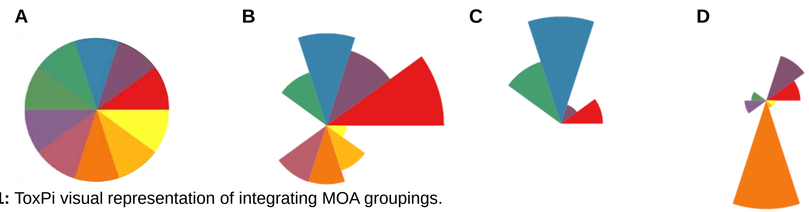

Figure 1: ToxPi visual representation of integrating MOA groupings.

LINKING ASSAYS TO ACUTE SYSTEMIC TOXICITY

Table 2: Summary of Assay Counts Mapped to Various Modes of Acute Systemic Toxicity

Mode of Action	Example Targets	# Tox21/ToxCast Assay Endpoints
Altered ion flow	*ligand-gated, K^+, CA^{2+}*	~20
Acetylcholinesterase inhibition	*AChE*	<5
Mitochondrial inhibition	*mito morphology*	~20
Damage to DNA and subcellular systems	*DNA morphology*	~10
Anticoagulation	*tissue factors*	<10
NMDA receptor antagonism	*NMDA receptor*	<5
Dopaminergic D2 receptor antagonism	*DRD2*	1
GABA receptor inhibition	*GABA receptors*	<10
Change in neurotransmitter function	*GPCRs, ion channels*	~60
Oxidative stress or ROS formation	*NRF2, MAO, redox*	~30
Cytotoxicity	*cell viability*	~40

Sources for acute systemic toxicity MOAs include Hamm *et al.* 2017 and NAS 2015. Other retrieved MOAs for which no assays were mapped in our approach include immune-mediated effects, increased permeability of cellular membranes, altered bioenergetics, Na/K ATPase inhibition, protein synthesis inhibition, Michael acceptor reactions, and GSH depletion.

SUMMARY & FUTURE DIRECTIONS

- MOAs for toxicity outcomes were identified from published literature.
- ToxCast assay endpoints were linked to MOAs for toxicity outcomes.
- The groups of assays mapped to MOAs can be utilized for various modeling and weight-of-evidence approaches.
- Limitations include the highly variable number of assays that can be linked to various MOAs; several MOAs had <5 assays and some are not represented by any assays at all from the current ToxCast/Tox21 inventory.
- Future directions include:
  - Incorporating assays from other sources (*e.g.,* PubChem) to expand biological coverage of assays used.
  - Identifying MOAs for other toxicity outcomes to broaden the scope of the assay groupings.
- Ultimately the assay groupings per MOA and the details regarding which MOAs can contribute to specific toxicity outcomes will be publicly available through the Integrated Chemical Environment (ICE) tool.

Visit ICE

https://ice.ntp.niehs.nih.gov/

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