High-throughput screening in the ToxCast Program and TSCA Data Needs

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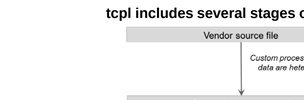
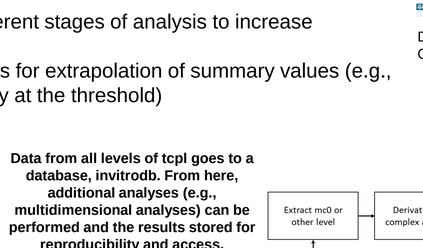
ToxCast bioactivity predictions (many assays)

Chemical space has increased in diversity and size over time

Set	Chemicals	Assays	Endpoints	Completion
ToxCast Phase I	293	~600	~700	2011
ToxCast Phase II	767	~600	~700	03/2013
ToxCast E1K	800	~50	~120	03/2013
ToxCast Phase III	~900	~300	~300	In progress
Tox21	~9000	~80	~150	In progress





Biological space: many sources, many targets

Biological responses

- Cell proliferation and death
- Cell differentiation
- Enzymatic activity
- Mitochondrial depolarization
- Protein destabilization
- Oxidative phosphorylation
- Reporter gene activation
- Gene expression
- Receptor binding
- Receptor transcriptional activation
- Steroid hormone synthesis
- Zebrafish behavior and development

Diverse assay designs, tissue sources, culture formats, and detection technologies

Information and predictions for specific targets/target families

Example: mitochondrial toxicity

26 assay endpoint IDs currently; more coming from NCCT

3 sources: Apredica, Tox21, NCCT (coming soon)	3 different technology categories: high content imaging, homogeneous fluorescence assay for membrane potential, and direct measure of oxygen consumption	Multiple biological processes: mitochondrial membrane depolarization, mitochondrial mass, mitochondrial function and oxygen consumption rate
Multiple cell types, inc.rat primary hepatocytes, and human liver and kidney immortalized cells		

Bioactive concentrations, without informing a mechanism

ToxCast Data Pipeline (tcpl)

Purpose: to perform initial concentration-response analysis in a reproducible and clear manner and store all of the information at each level of analysis in a uniform way to enable consistent access to data.

- Heterogeneous data from myriad platforms and sources can be analyzed to generate the same summary information
- Has created a vocabulary for referring to different stages of analysis to increase transparency and sharing
- Model fitting (Hill, model, and constant) allows for extrapolation of summary values (e.g., 50% activity concentration or AC50, or activity at the threshold)

tcpl includes several stages of analysis

Filer et al. 2017 PMID 27797781

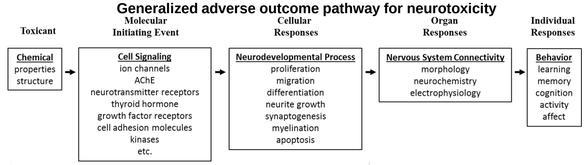
Data from all levels of tcpl goes to a database, invitrodb. From here, additional analyses (e.g., multidimensional analyses) can be performed and the results stored for reproducibility and access.

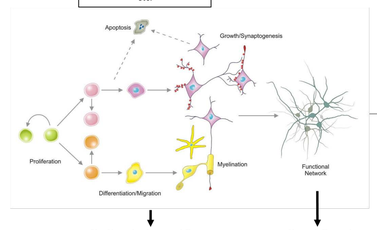
Screening approaches to characterizing potential neurotoxicity (filling gaps)

Purpose: Develop high-throughput methods that reliably and rapidly detect alterations in processes relevant to nervous system function & development.

- Thousands of chemicals which lack any information on neurotoxic potential
- Traditional neurotoxicity testing is resource intensive
- Need new approaches that efficiently identify and rank chemicals of concern

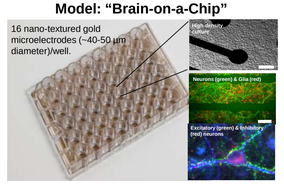
Generalized adverse outcome pathway for neurotoxicity

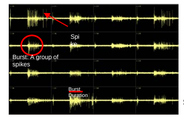




Functional Networks in Micro-Electrode Arrays (MEAs)

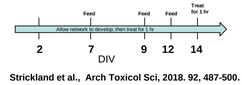
Model: "Brain-on-a-Chip"





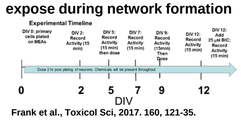
Data: Recordings enable quantification of complex aspects of network formation and function. These show a neurotoxicity profile

Acute Neurotoxicity Method: Plate, allow network formation, then expose

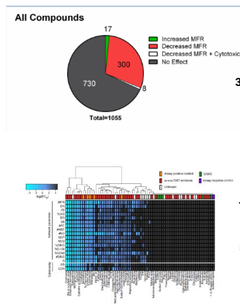


Strickland et al., Arch Toxicol Sci, 2018. 92, 487-500.

Developmental Method: Plate and expose during network formation



Frank et al., Toxicol Sci, 2017. 160, 121-35.

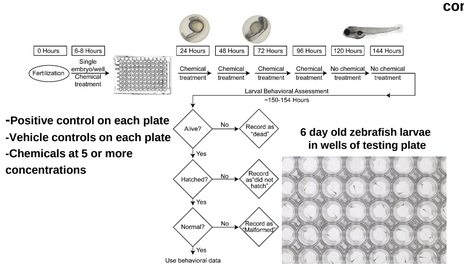


1055 ToxCast compounds screened. Approximately 30% were active.

70 compounds x 16 ontogeny parameters: separates active and inactive compounds

Development and Behavior in Zebrafish

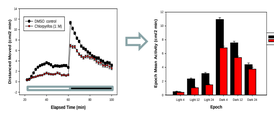
Model: Zebrafish in 96 well-plate



-Positive control on each plate
-Vehicle controls on each plate
-Chemicals at 5 or more concentrations

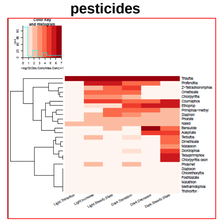
6 day old zebrafish larvae in wells of testing plate

Data: Zebrafish behavior under light and dark conditions provides an objective measure of activity



- Each 100 min of activity is condensed into 6 behavioral epochs (above)
- Identify concentrations that cause locomotor abnormalities using repeated measures analysis
- Developmental exposure may cause increases or decrease in activity in either the light or dark phases of testing.

Example: Organophosphorus pesticides



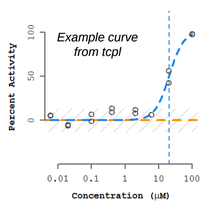
Profiles of locomotor perturbation patterns developed for each chemical class.

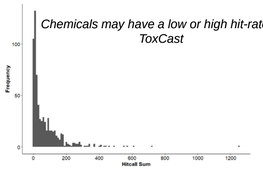
ToxCast data outputs for use

Dashboard: Comptox.epa.gov

Williams et al. 2017 PMID 29185060

From the curve fits, we have summary metrics such as AC50 and hit-call. These and other metrics can inform lots of questions.



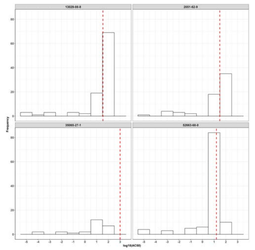


One chemical may have many positive "hitcalls" that we can add together to get a hitcall sum.

Here is an example of the number of hitcalls for nearly 500 chemicals in invitrodb.

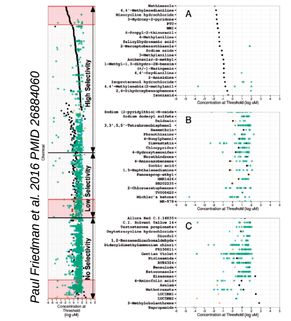
Chemicals with a very high hit rate may be cytotoxic, nonspecific, or cause assay interference.

In what concentration range is this chemical active? (related, what dose range would we extrapolate from in vitro to in vivo extrapolation?)



Example of the distribution of log10(AC50) values for 4 chemicals, with the cytotoxicity "burst" indicated by the red dashed line.

What is the selective or specific *in vitro* bioactivity, i.e., how far from the cytotoxicity "burst" is the AC50 of interest? (related, for some targets, can we prioritize positives?)



Example:using the burst to prioritize more selective positive hitcalls in the thyroperoxidase inhibition assay