

The Second Phase of ToxCast and Initial Applications to Chemical Prioritization

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research & development

Science Issues

Tens of thousands of chemicals and other contaminants exist in our environment, but only a fraction of these have been characterized for their potential hazard to humans.

ToxCast™ is closing this hazard data gap through high throughput screening (HTS) assays profiling activity in biological pathways relevant to systemic, carcinogenic, developmental and reproductive toxicity (Dix et al 2007).

ToxCast screening will provide hazard-based prioritizations ranking chemicals for targeted toxicity testing.

Research Goals

From Phase I of ToxCast, we recognized the need to refine and expand the 467 assay portfolio for broader coverage of biological pathways, and additional chemicals to probe more known modes of action.

Phase II will screen 700 additional chemicals in the expanded HTS assay portfolio of Phase I. High throughput genome-wide expression assays will again be considered, as will other 'omics technologies.

The 700 ToxCast Phase II chemicals will be a subset of the 10,000 chemicals being assembled for Tox21 HTS testing by NTP-NCGC-EPA.

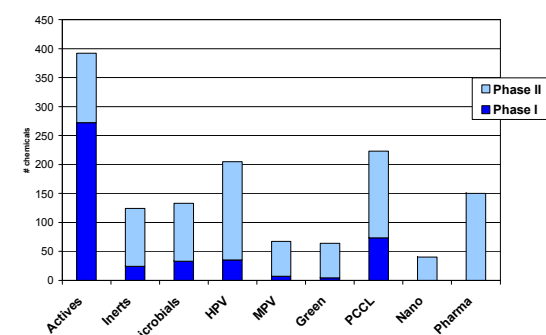
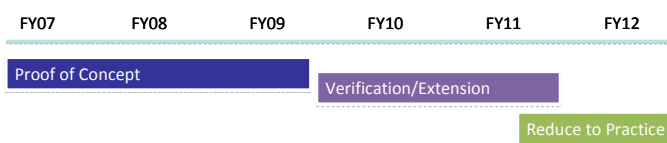
ToxCast HTS assay data will provide in vitro threshold concentrations for specific biological targets, genes, pathways and predictors.

High throughput pharmacokinetic modeling of HTS response will provide estimated equivalent doses at which similar activity is expected in vivo. These estimated equivalent doses will be used in combination with chemical structural descriptors, and existing in vivo toxicity data from ToxRefDB for building predictive models, supporting prioritization of chemicals for further toxicity testing.

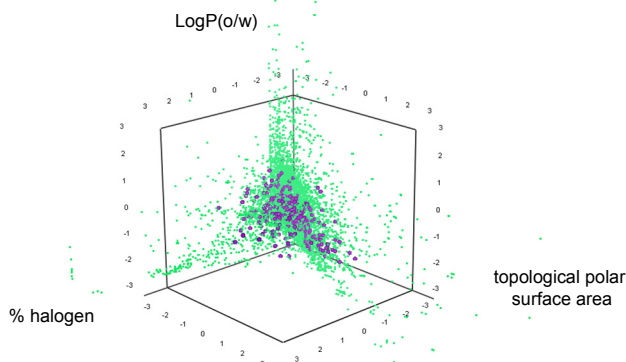
Chemicals

ToxCast Phases

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	320	Mostly Pesticide Actives	Signature Development	467	\$20k	FY09
II	700	Actives, Inerts, Antimicrobials, HPV, MPV, PCCL, Nano, Pharma	Validation	>400	\$20k	FY11
III	Thousands	Actives, Inerts, Antimicrobials, HPV, MPV, PCCL, Nano	Prediction and Prioritization	>300	\$15k	beginning FY12



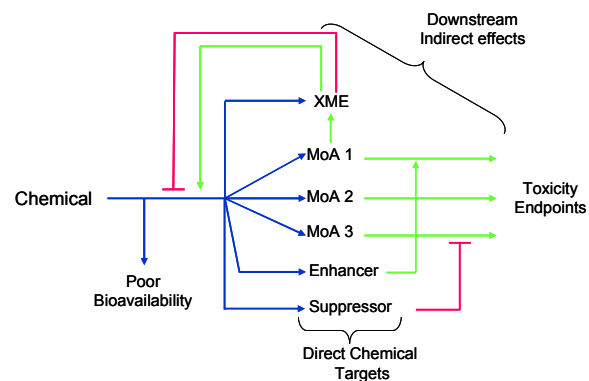
ToxCast Phase I and Phase II Chemicals: 272 of 309 Phase I chemicals were pesticide actives, whereas Phase II will be more diverse, even including pharmaceuticals and nanomaterials.



ToxCast Chemical Space: 309 Phase I chemicals (purple) plotted within a landscape of approximately 9,000 environmental chemicals from various lists from regulatory and research programs (green) (Judson et al 2009) against key physico-chemical properties. ToxCast Phase I and II chemicals are a subset drawn from the approximately 5,000 chemicals in the larger Tox21 set.

Methods/Approach

Assays



ToxCast Phase II Assays and MOA: the assays of Phase II will have to assess targets across many possible pathways and modes of action (MOA) for environmental chemicals.

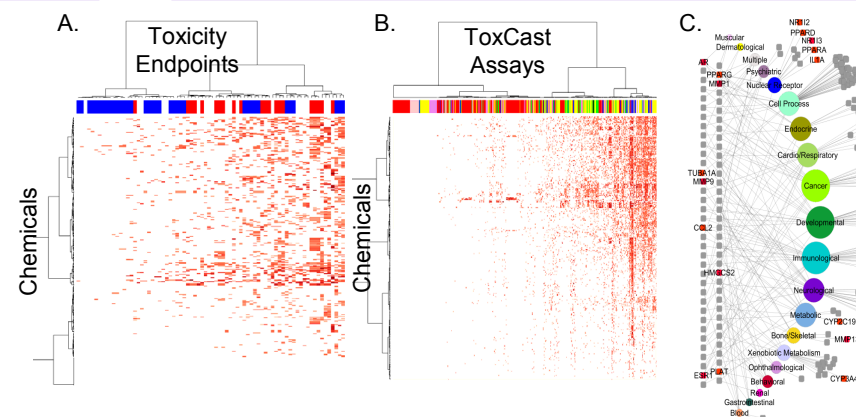


Human Toxicity Pathways in ToxCast Phase I: assays mapped to 231 unique human genes, 94 KEGG pathways, 99 Ingenuity pathways, and 1,126 GO ontologies. Assays, genes and pathways will be expanded upon in Phase II.

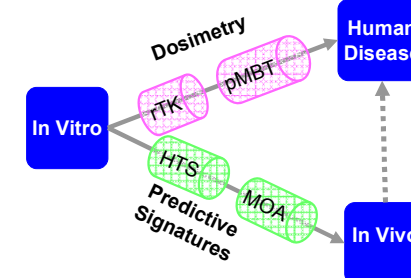
New Assay Sources for ToxCast Phase II:

- Possible new contracts for HTS genotoxicity, cell signaling pathways, cell differentiation, steroidogenesis, and xenobiotic metabolism
- ORD/NHEERL and Tox21 partners
- STAR grantees
- EU and OECD partners

Prioritization

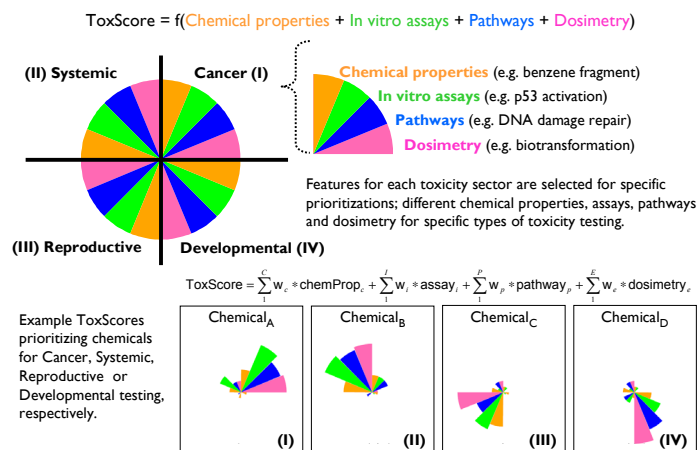


ToxCast Prioritization: initial prioritizations transition from ToxRefDB animal testing data (A) to ToxCast bioactivity profiles (B), but ultimately will be based on activity in human toxicity pathways and predicted causality of human disease (C).



ToxCast Predictions: HTS and pathway/mode-of-action (MOA) signatures will be refined in Phase II relative to human toxicity and disease through 'reverse toxicokinetics' (rTK), predicted metabolism and biotransformation (pMBT), and screening of known human toxicants (e.g., failed drug candidates).

ToxCast Hazard-Based Prioritization



Results/Conclusions

400 Phase II chemicals will be pesticidal actives, inerts or antimicrobials, or industrial chemicals rich in existing animal toxicity data and thus useful for verifying and expanding predictive toxicity signatures and pathways from Phase I screening.

150 Phase II chemicals will be pesticidal inerts, antimicrobials, industrial chemicals with limited toxicity data and in need of chemical categorization and prioritization.

Green chemicals, water contaminants, and nanomaterials are included in Phase II.

150 Phase II chemicals are failed pharmaceutical compounds with animal and human toxicity data for direct confirmation of human toxicity pathways and predictors.

ToxCast HTS assays include cell-free assays, cell-based assays in a variety of human and rodent primary cells and cell lines, and assays in zebrafish and other non-mammalian species.

Impact and Outcomes

Upon completion of Phase II, ToxCast will be prepared to conduct rapid, quantitative and high-quality hazard characterizations and subsequent prioritization of thousands of chemicals.

ToxCast will provide decision support software for chemical prioritizations (ToxMiner) with an integrated database and interface for data analysis, visualization and uncertainty assessment. ToxMiner will be user-friendly and freely available, facilitating widespread implementation.

ToxCast data will be publicly available through the ToxCast, ACToR, and DSSTox websites, and PubChem.

Future Directions

ToxCast has the capacity to screen and prioritize the thousands of untested environmental chemicals. Associated informatics tools will allow EPA to use this data to guide more intelligent, targeted testing.

References

- Dix et al (2007). The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol Sci* 95:5-12.
- Judson et al (2009). The toxicity data landscape for environmental chemicals. *Environ Health Perspect.* 117(5):685-95.

