ToxCast: A Program for Prioritizing Toxicity Testing of Environmental Chemicals

Keith A. Houck, David J. Dix, Matthew T. Martin, Ann M. Richard, Robert J. Kavlock

U.S EPA, ORD, National Center for Computational Toxicology

research & development

Abstract

Evaluating the potential of tens of thousands of chemicals for risk to human health and the environment is beyond the resource limits of the Environmental Protection Agency. The EPA's ToxCast program will explore alternative methods comprising computational chemistry, high-throughput screening and various toxicogenomics technologies to predict potential for toxicity and prioritize chemicals for detailed toxicity testing. The approach will center on bioactivity profiling of chemicals across a broad range of biochemical assays as well as cell-based assays with phenotypic, genomic, and metabolomic analyses. The proof-of-concept phase will use a set of chemicals with an existing, rich toxicological database including tumorigens, developmental and reproductive toxicants, neurotoxicants and immunotoxicants in order to provide an interpretive context for the ToxCast data. Combining the bioactivity profiling with physico-chemical parameters and predicted biological activities based on existing structure-activity models will yield a multidimensional dataset and informatics challenge requiring appropriate computational methods for integrating various chemical, biological and toxicological data into profiles and models predicting toxicity.

Research Goals

Build compound library consisting of chemicals with associated rich toxicological dataset for use in proof-of-concept phase

Acquire toxicological dataset on compound library to be used as reference data for various HTS and toxicogenomic technologies

Develop standardized vocabulary and relational database schema to manage reference toxicological data including study type, animal information, units, results, and endpoints

Assemble range of biochemical, cellular and toxicogenomic assays to provide broad sampling of biological target space

Test proof-of-concept chemicals is assay suite and build relational database of results

Develop computational tools and approaches to link reference toxicological data to biological activity and chemical information

Build predictive models that will prioritize chemicals with little or no toxicological data for potential for hazard

Environmental Chemical Domains with Potential ToxCast Applications

Antimicrobials 750
EDCs 1500
HPVs 3300
Pesticidal Inerts 3310
H2O CCLs 7324
MPVs 5400
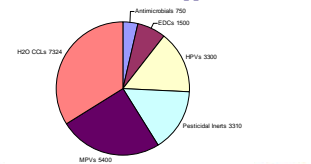
Compounds

### Criteria for Selection of Compound Screening Library:

1. Associated with rich toxicological history including endpoints for:
   - 90 day toxicity
   - Chronic toxicity
   - Multigenerational reproductive toxicity
   - Developmental toxicity
   - Developmental neurotoxicity
   - Immunotoxicity
2. Availability of analytical grade chemicals
3. Compatibility with HTS methods
4. Broad structural diversity with sufficient similarity clusters for correlation analysis

Decision: US Registered pesticide active ingredients (826)

NTP Testing Program

Select other chemicals with rich information base

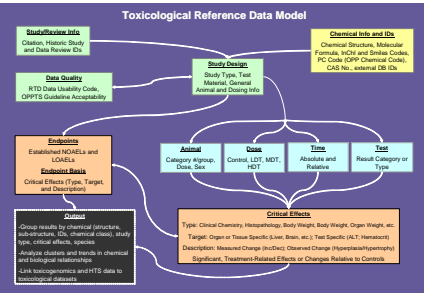### Library Parameters: ToxCast vs Pharma-Like Cmpds
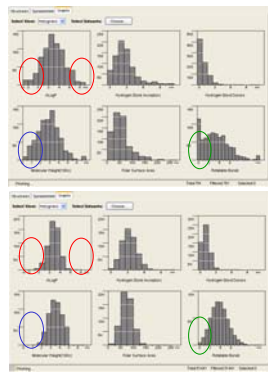
EPA PTC

NCGC MLI

EPA PTC compounds have wider ALogP range suggesting potential for solubility issues. Also many lower MW cmpds that may be manifested by less potency in *in vitro* assays.

Toxicology Reference

Targeted Toxicological Data Collection

Source:	EPA's Office of Pesticide Programs (OPP)
Format:	Data Evaluation Record (DER)
Chemical:	Conventional Pesticide Active Ingredients (~800)
Purity:	Technical Grade (>90%)
Dosing:	Primarily Orally Administered (based on availability and use pattern of pesticide)
Study Type:	Subchronic Toxicity (Rodents and Non-Rodents)
	Prenatal Developmental Toxicity
	Reproduction and Fertility Effects (2-generation)
	Chronic Toxicity (rat, mouse, and dog)
	Carcinogenicity (rat and mouse)
	Developmental Neurotoxicity
	Immunotoxicity

Data Collection Results
>4000 DER (2500 studies) for over 400 pesticides

Toxicological Reference Data Model

Study/Review Info: Citation, Historic Study and Data Review IDs

Chemical Info and IDs: Chemical Structure, Molecular Formula, InChI and Smiles Codes, PC Code (OPP Chemical Code), CAS No., external DB IDs

Study Design: Study Type, Test Material, General Animal and Dosing Info

Data Quality: RTD Data Usability Code, OPPTS Guideline Acceptability

Endpoints: Established NOAELs and LOAELs

Endpoint Basis: Critical Effects (Type, Target, and Description)

Animal: Category w/group, Dose, Sex

Dose: Control, LDT, MDT, HDT

Time: Absolute and Relative

Test: Result Category or Type

Output:
- Group results by chemical (structure, sub-structure, IDs, chemical class), study type, critical effects, species
- Analyze clusters and trends in chemical and biological relationships
- Link toxicogenomics and HTS data to toxicological datasets

Critical Effects
Type: Clinical Chemistry, Histopathology, Body Weight, Organ Weight, etc.
Target: Organ or Tissue Specific (Liver, Brain, etc.); Test Specific (ALT, Hematocrit)
Description: Measured Change (Inc/Dec); Observed Change (Hyperplasia/Hypertrophy); Significant, Treatment-Related Effects or Changes Relative to Controls

Collaborations

NIEHS/NTP:	NIH/NCGC:	OECD
HTS initiative (R. Tice)	qHTS	Molecular Screening
C. elegans assays (J. Freedman)	(C. Austin, J. Inglese)	(EPA/OPPTS)

Screening

HTS in Drug R&D vs Environmental Toxicology

Descriptor	Pharma	Toxico
Chemical Space	Narrow	Broad
Chemical Numbers	10^4-10^5 ($10^{6?}$)	10^2-10^4
Intended MoA	Generally understood and narrow	May not exist
Target Potency	High	Generally low
Off Target Effects	Often understood	Poorly understood
Acceptance Rate	False negatives OK	False negative rate must be very low
Parent Activity	Design factor	Usually unknown

Assay Selection Considerations

- Capacity (hundreds to thousands of chemicals)
- Cover broad spectrum of gene/protein families
- Utilize genomic, proteomic and/or metabolomic tools applied to cellular or organismal assays
- Currently available
- Model organisms (non-mammalian)
- Linkage to known toxicological MOA
- Ability to test in concentration-response format
- Biotransformation capability
- Minimizing false negative rates

ToxCast Contract RFPs

- Chemical Procurement/Management
- Biochemical Assays
- Cellular Systems
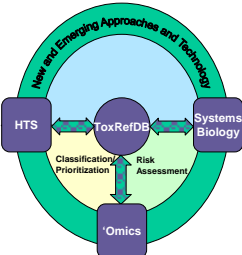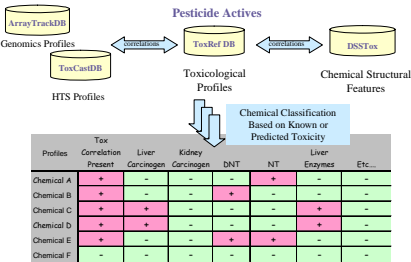- Genomic Analysis
- Model organisms
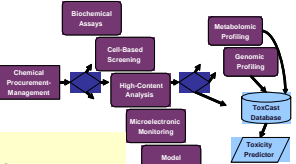
RFPs closing july-august 2006
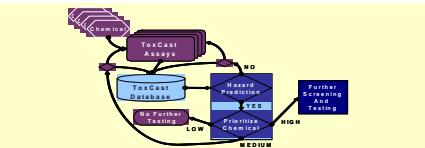
Awards target date october 2006

Results/Conclusions

ToxCast Chemical Classification of Environmental Chemicals

ArrayTrackDB — Genomics Profiles

ToxCastDB — HTS Profiles

TcxRef DB

DSSTox — Chemical Structural Features

Toxicological Profiles

Chemical Classification Based on Known or Predicted Toxicity

Profiles	Tox Correlation Present	Liver Carcinogen	Kidney Carcinogen	DNT	NT	Liver Enzymes	Etc...
Chemical A	+		-	-	-	-	-
Chemical B	+	-	-	-	-	-	-
Chemical C	+	+	-	-	-	+	-
Chemical D	+		-	+	-	+	-
Chemical E	+		+	+	-	+	-
Chemical F	-	-	-	-	-	-	-

New and Emerging Approaches and Technology

HTS — ToxRefDB — Systems Biology

Classification/Prioritization — Risk Assessment

'Omics

ToxCast: Potential Outcomes and Expansion Beyond Proof-of-Concept

- Availability of a science-based system to categorize chemicals of like properties and activities
  - Increasing confidence as database grows
  - Once operational, MOA leads for new chemicals
  - Provide EPA Program Offices with a relatively inexpensive predictive tool box that heretofore has been seriously lacking
- Improve the efficiency and effectiveness of the use of animals in hazard identification and risk assessment

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

COMPUTATIONAL TOXICOLOGY