Chemical genomics as a tool for environmental toxicity testing

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WORKSHOP
Methodologies for prioritizing hazardous chemicals in European waters: the state of play and the need for improvement
24–25 June 2014 - Cité Universitaire - Paris, France

Outline

- Introduction: The NTP road map vision
- Tox21 Program NIH Chemical Genomics Center- The qHTS paradigm
- Case study- Information gathered
  - Mitochondrial toxicity
- Benefits and Limitations

Challenges

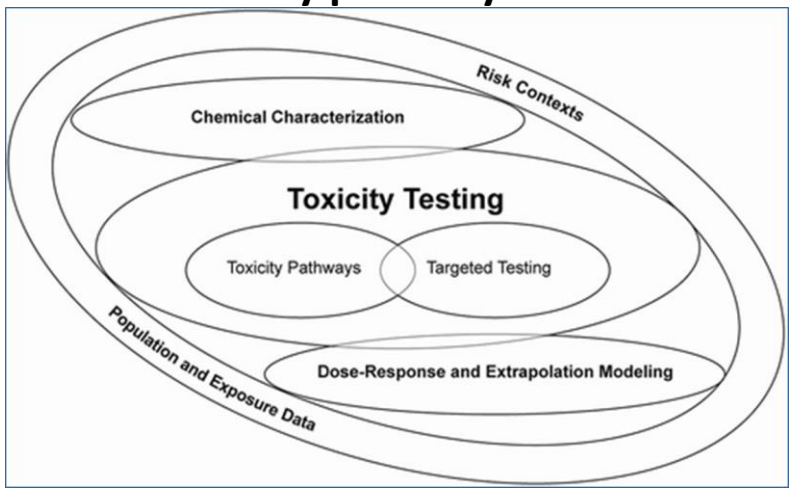
- Large numbers of substances that need to be tested
  - 80000 in USA with 2000 new chemical entities/year (Over 100000 in Europe)
  - Mixtures
  - Nanomaterials, biologicals
- Problems with traditional animal testing
  - Operational problems lead to high cost
  - Limitations interpreting the data
  - Societal concerns to reduce the use of animals

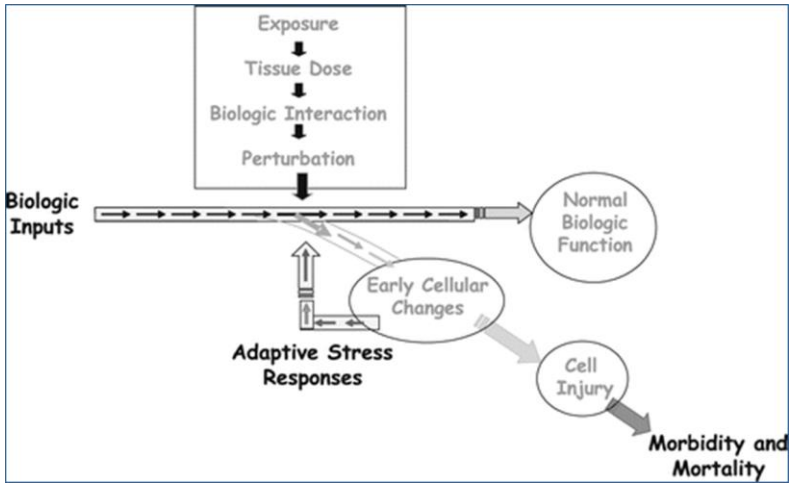
- ToxCast-EPA/Tox-21 Program-NTP NIEHS
  - to shift the assessment of chemical hazards from traditional experimental animal toxicology studies to target-specific, mechanism-based, biological observations largely obtained using in vitro assays
  - Incorporateing recent advances in molecular toxicology, computational sciences, and information technology to offer increased efficiency in tests design and costs

Tox-21 genesis

- **2004 NTP Roadmap**
  - Alternative assays for targeting the key pathways, molecular events, or processes incorporate them into a testing framework. NTP established a High Throughput Screening (HTS) program together with NCGC
- **2007 The National Academy of Sciences published its report "Toxicity Testing in the 21st Century: A Vision and Strategy“**
  - Perturbations of cellular responses in a suite of toxicity pathway assays using high throughput robotic assisted methodologies.
- **2008 Memorandum of understanding (MOU) NTP, EPA and NCGC**
- **2010, a new MOU was announced that was signed by the original three partners plus the U.S. Food and Drug Administration (FDA)**

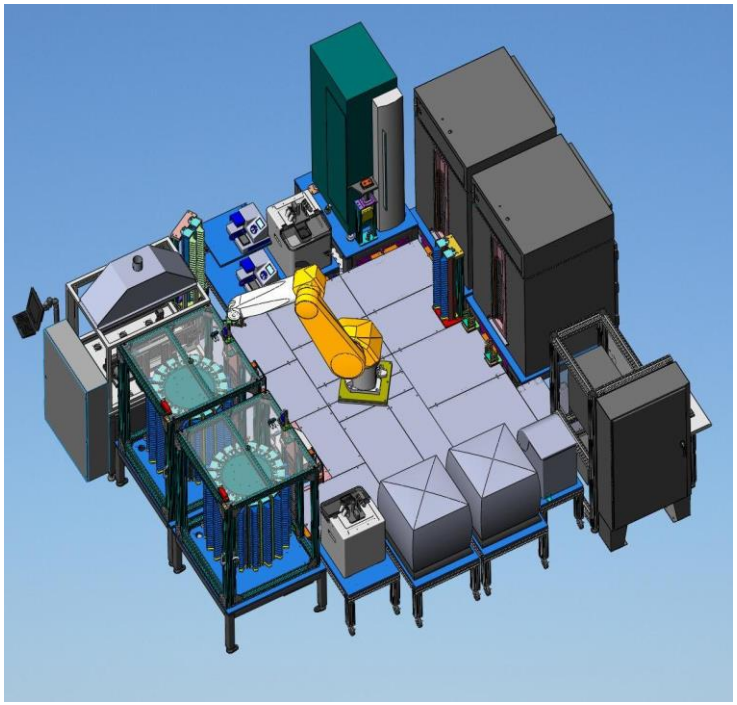
NSF Framework
Toxicity pathways



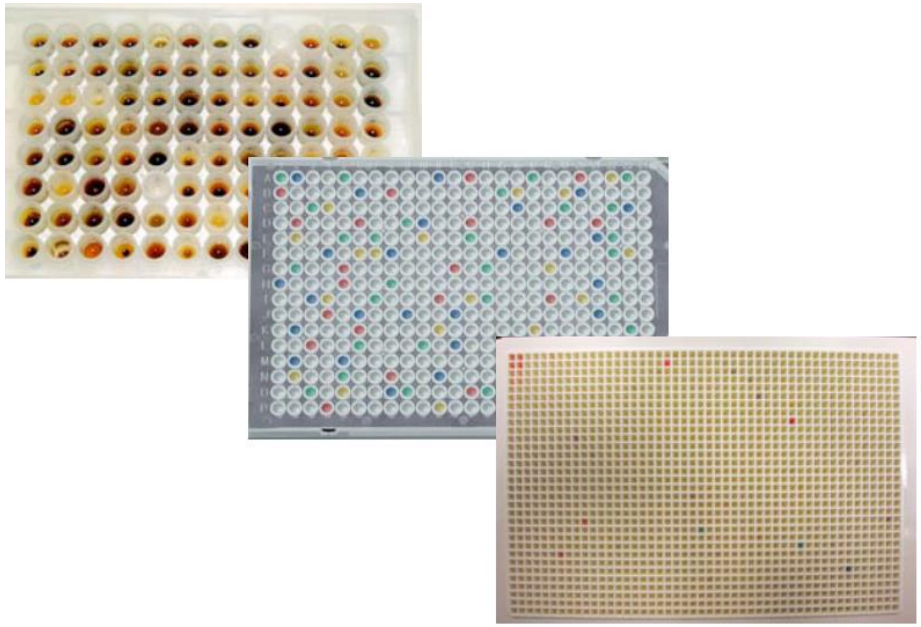


NIH Chemical Genomics Center

- **State-of-art HTS facility:**
  - Founded in 2004 National Human Genome Research Institute. Currently part of the DPI of the recently created NCATS.
  - Development of chemical probes for novel biology, and profiles of compound libraries, such as signatures of toxic substances. First center in NIH Molecular Library Screening Centers Network, now referred to as MLPCN
  - Collaborates with >200 investigators worldwide



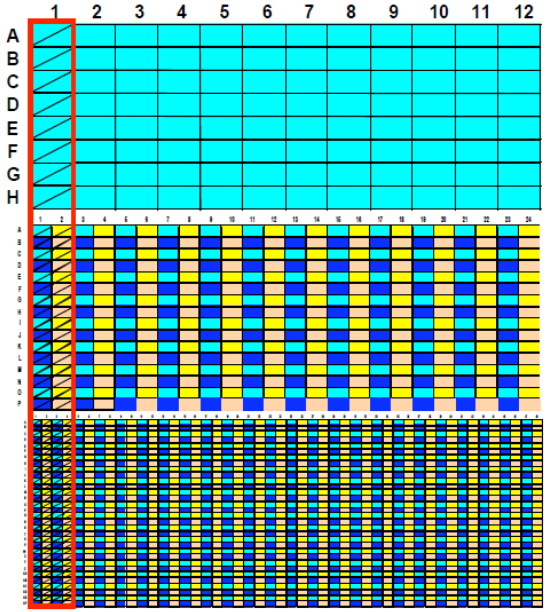
Screening Throughput



- 8 rows
- 12 columns (A-H)
- 88 test samples
- 8 controls (8.3%)

- 16 rows
- 32 columns (A-P)
- 352 test samples
- 32 controls (8.3%)

- 32 rows
- 48 columns (A-AF)
- 1,408 test samples
- 128 controls (8.3%)

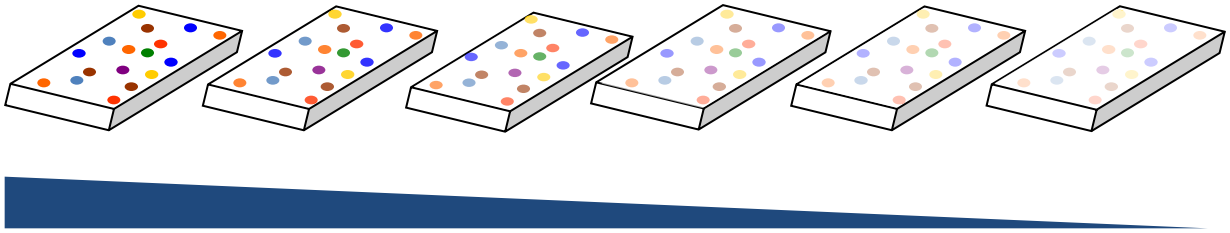


If @ 100 microtiter plates per day:

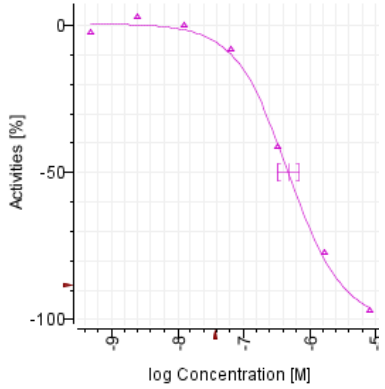
Plate format	samples§/day (wells/day)	Time to screen 1 MM samples
96-well	8,800 (9,600)	4 months
384-well	35,200 (38,400)	4 weeks
1,536-well	140,800 (153,600)	7 days

Quantitative High-Throughput Screening (qHTS)

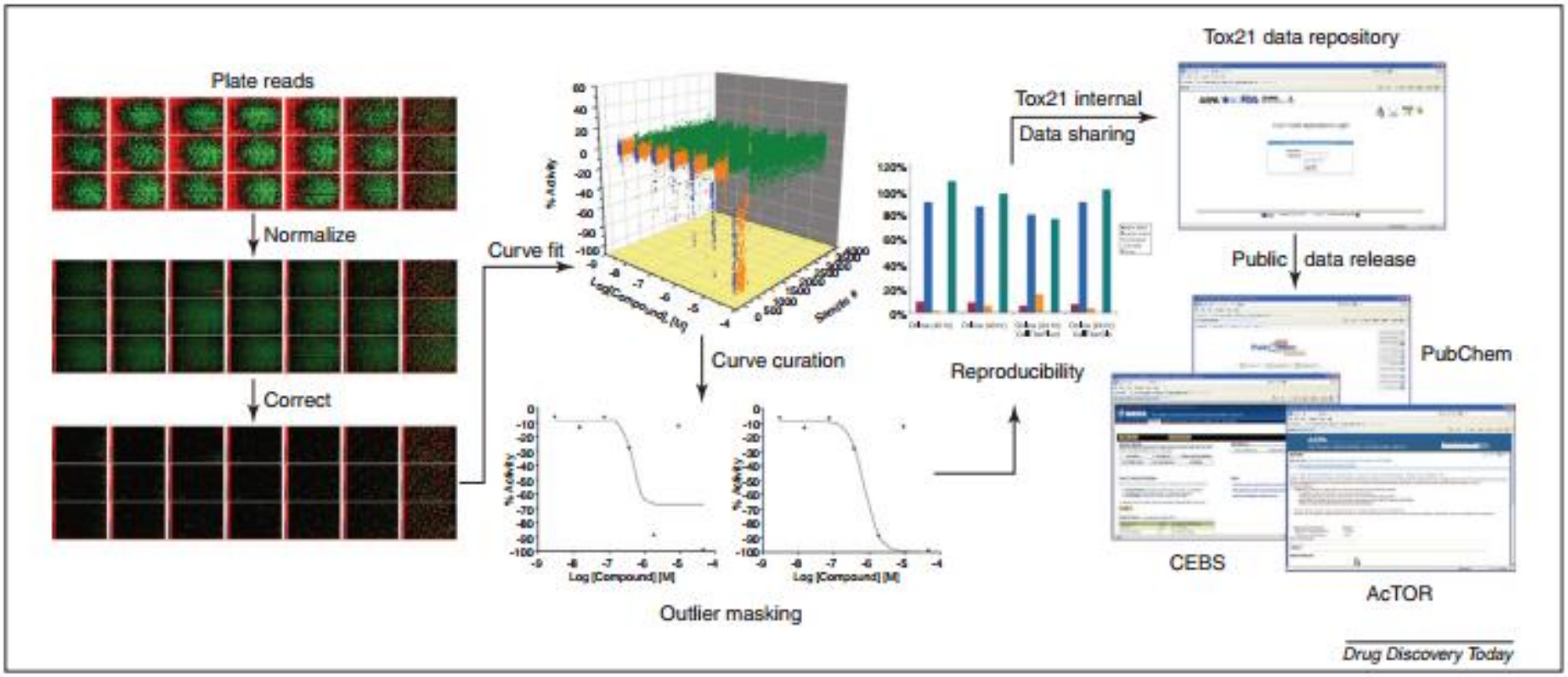
- Compounds are assayed at multiple concentrations
  - 7-15 concentrations
  - Concentration range 0.5 nM to 92 μM (over 6 orders of magnitude)
  - Concentration-response curve generated for each compound
- Assay volumes ~ 5 μL
- 1536-well plate format
- Informatics pipeline for data processing, curve fitting & classification, extraction of SAR



Compound concentration



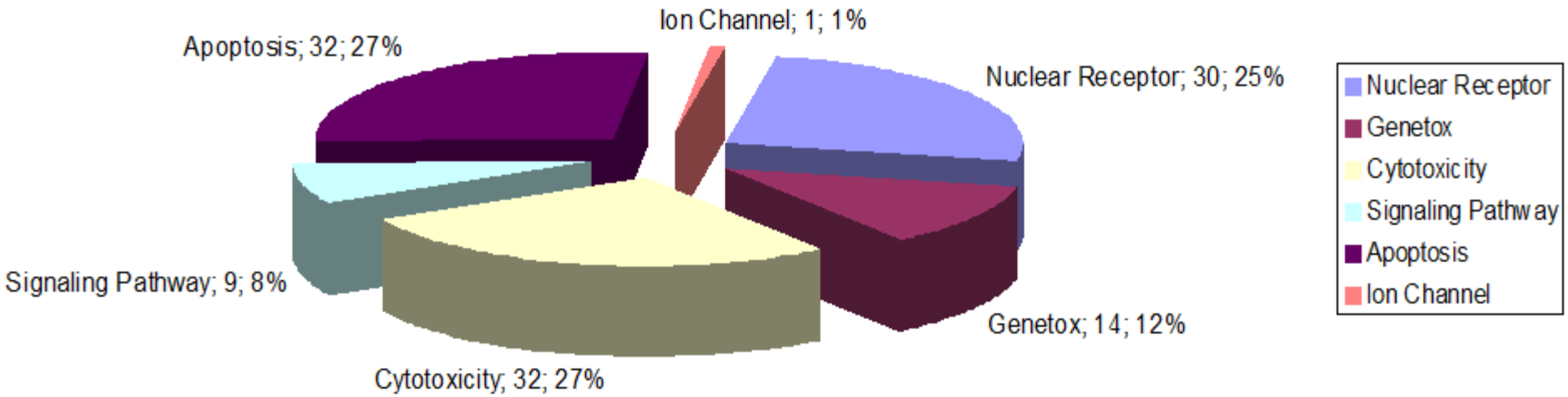
Quantitative High-Throughput Screening (qHTS)



Drug Discov Today. 2013 Aug;18(15-16):716-23.

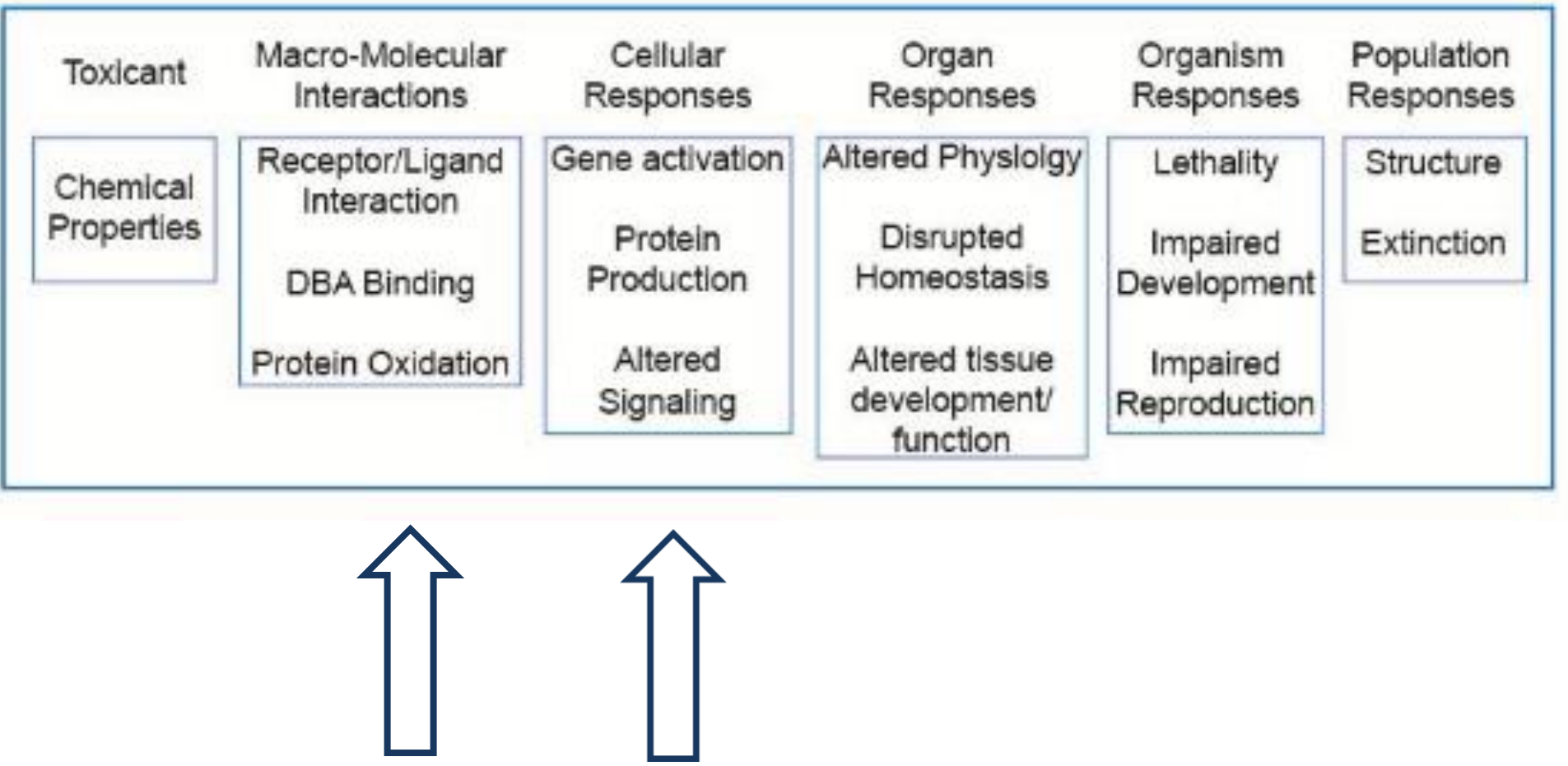
Tox21-Phase I Exploratory phase

- "proof of principle" libraries of 1,408 and 1,462 compounds, respectively, with each compound dissolved and stored in dimethyl sulfoxide (DMSO).
- Develop and optimize toxicological relevant assays

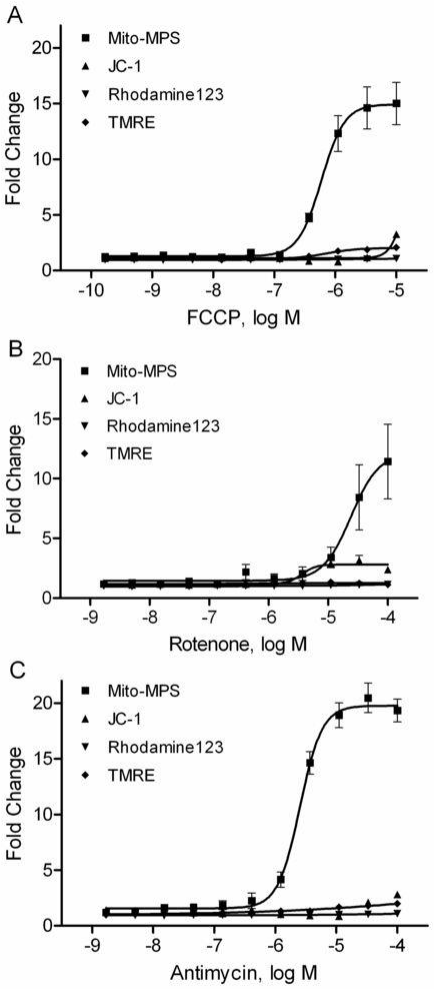


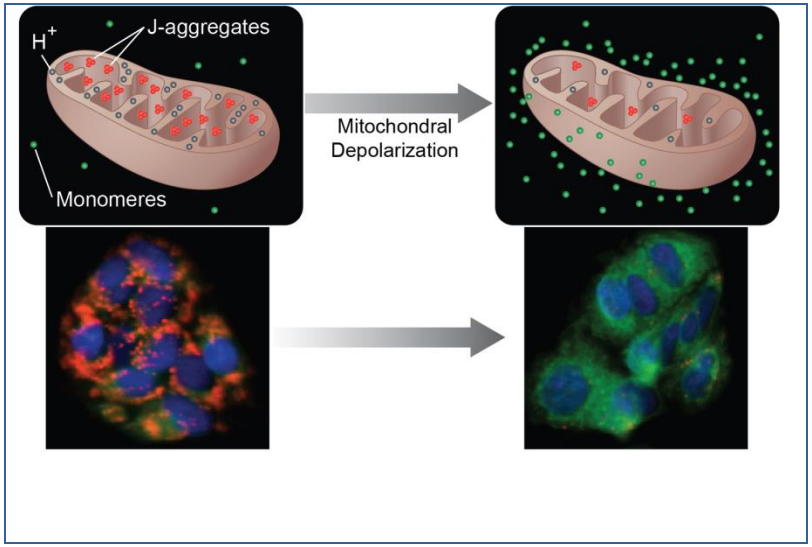
Environ Health Perspect 121:756-765 (2013)

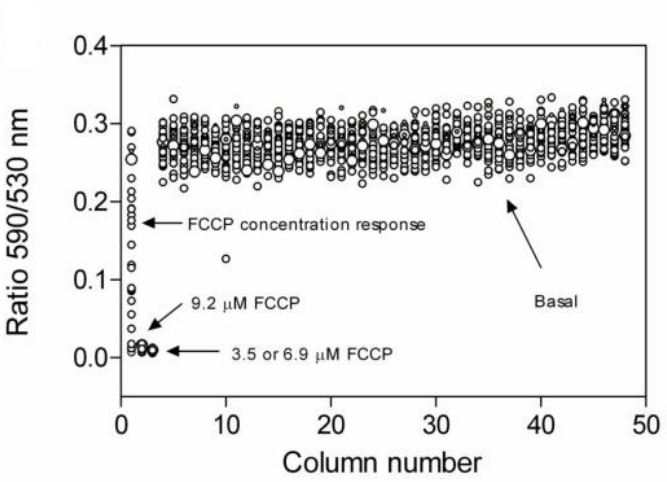
Adverse Outcome Pathways



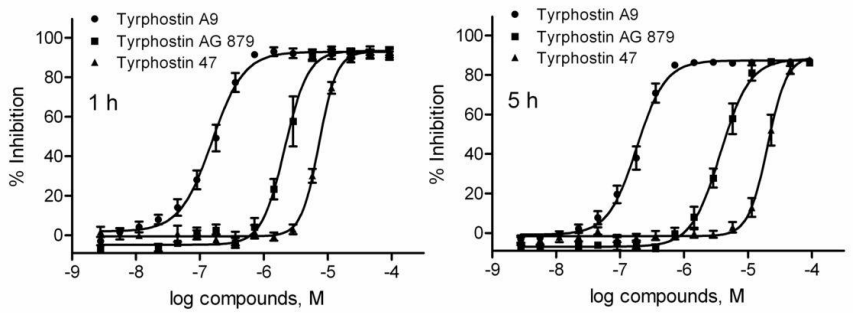
Mitochondrial membrane potential assay development

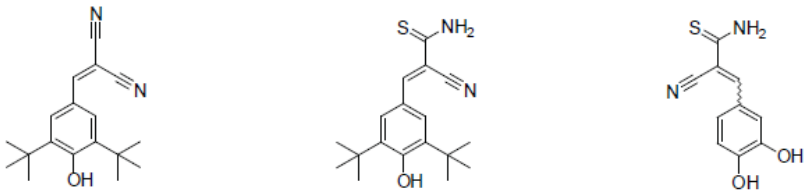






MMP Assay development cont

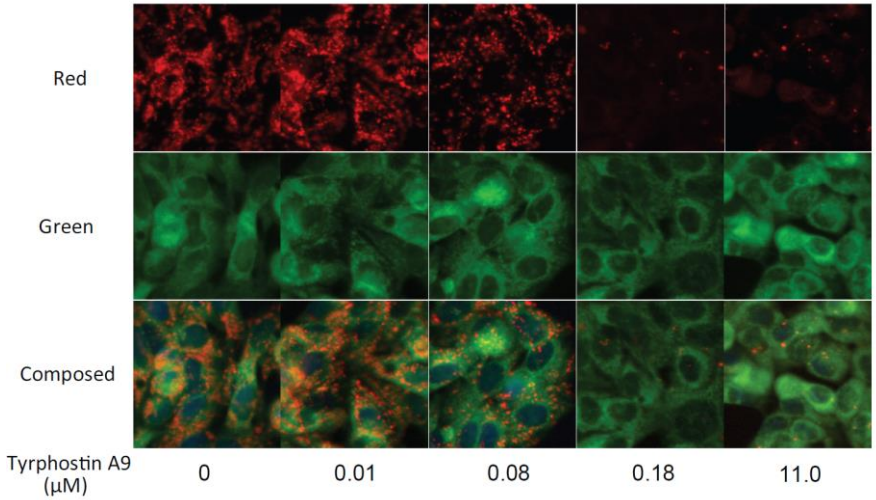




● Tyrphostin A9
IC_{50} = 0.15 μM, 1h
IC_{50} = 0.18 μM, 5h

■ Tyrphostin AG 879
IC_{50} = 2.13 μM, 1h
IC_{50} = 3.71 μM, 5h

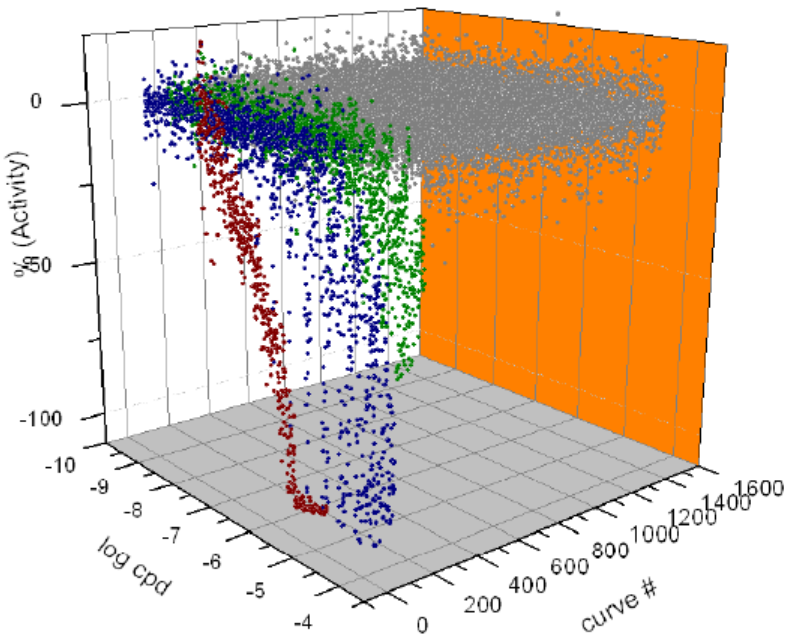
▲ Tyrphostin 47
IC_{50} = 7.29 μM, 1h
IC_{50} = 20.2 μM, 5h



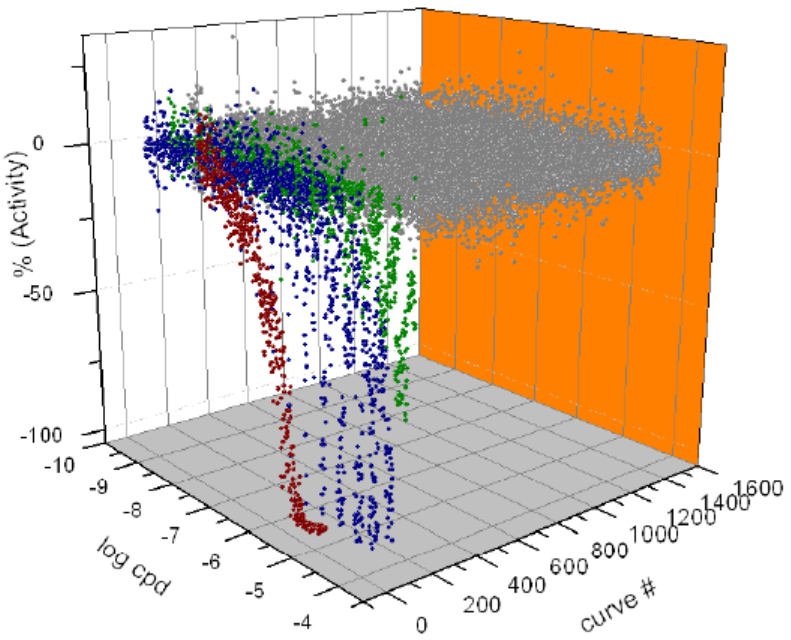
Physiol Genomics. 2012 May 1;44(9):495-503.

MMP- Phase I

One hour



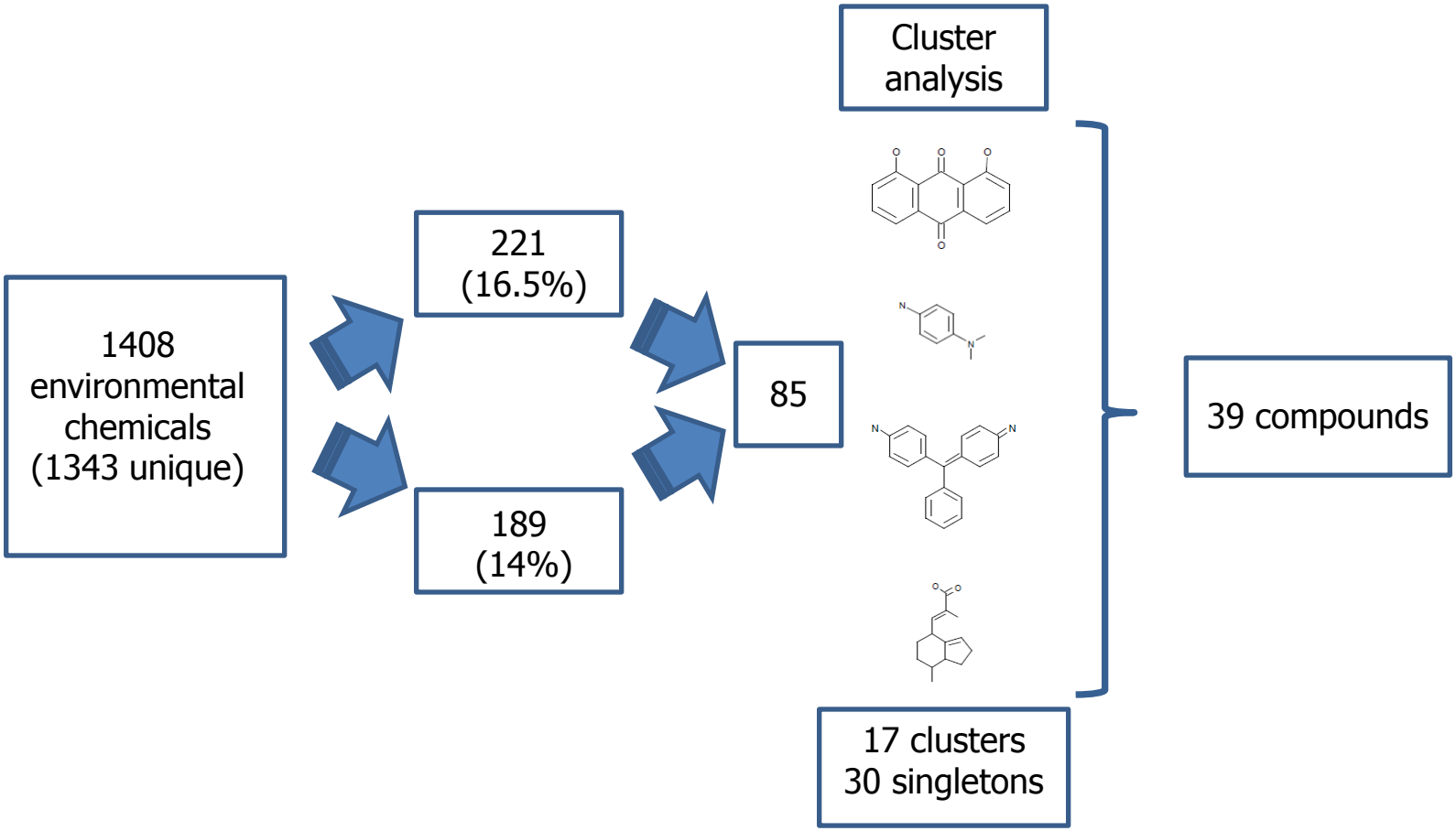
Five hours



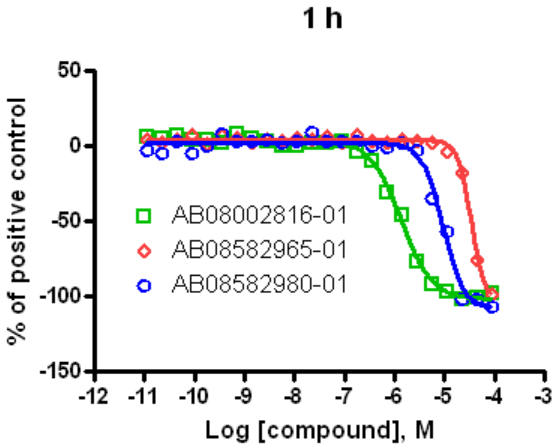
Positive Control
Actives
Weak actives; Inconclusive
Negatives

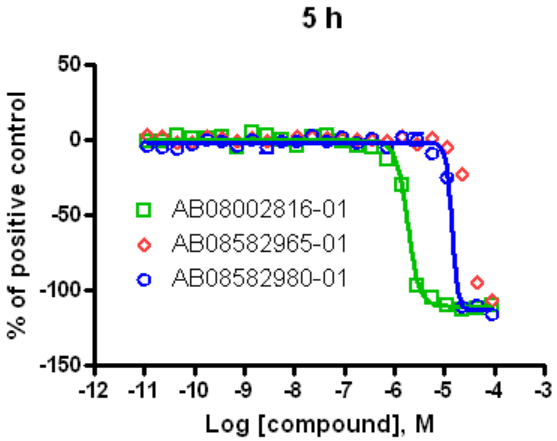
Chem Res Toxicol. 2013 Sep 16;26(9):1323-32.

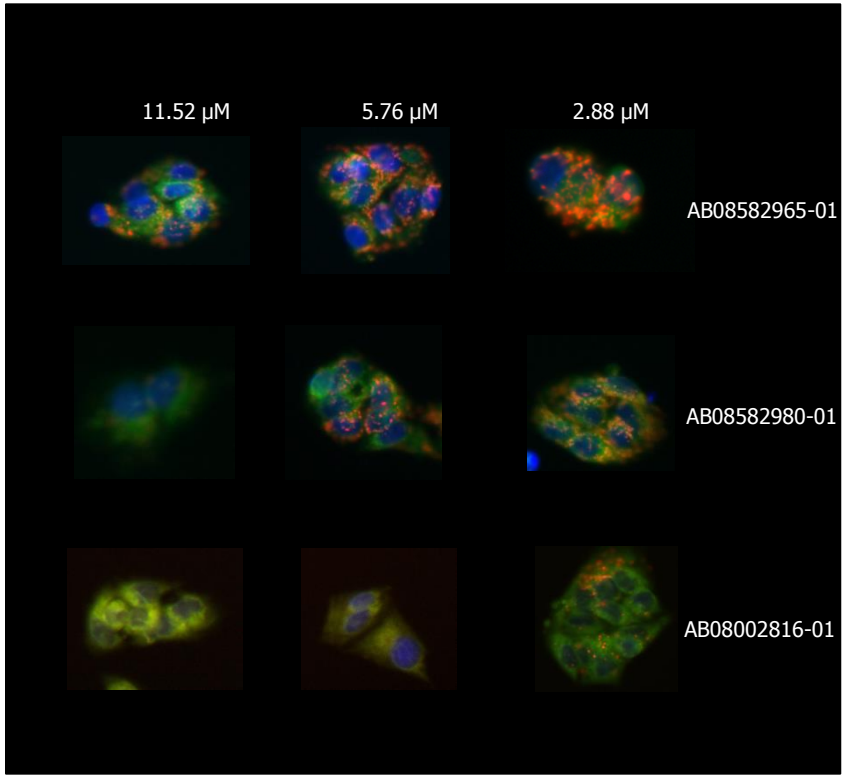
Screen data analysis

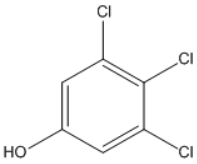


Confirmatory assays

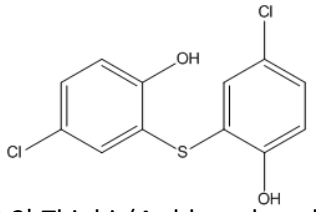






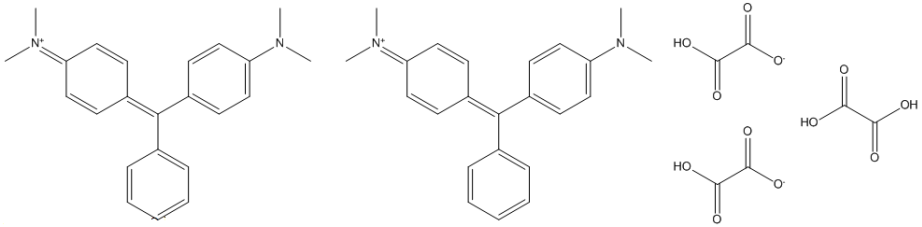


3,4,5-Trichlorophenol

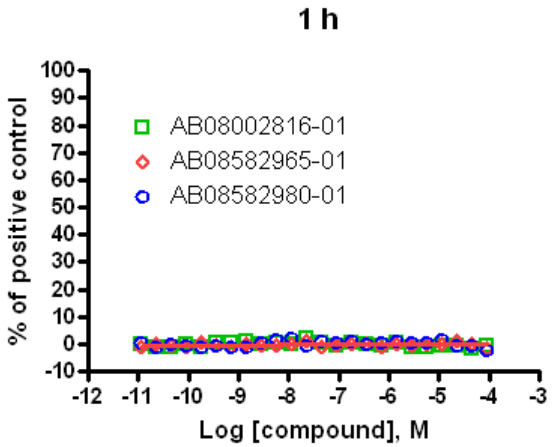


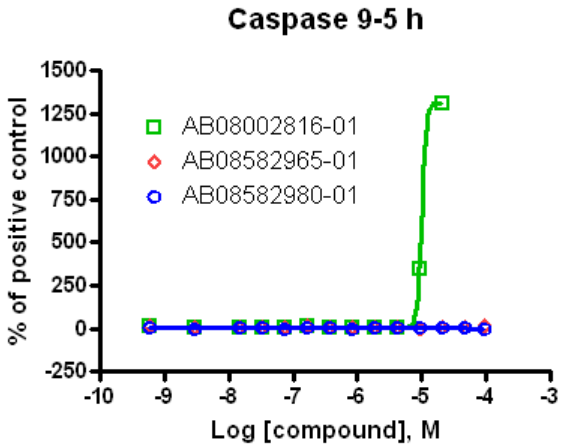
2,2'-Thiobis(4-chlorophenol)

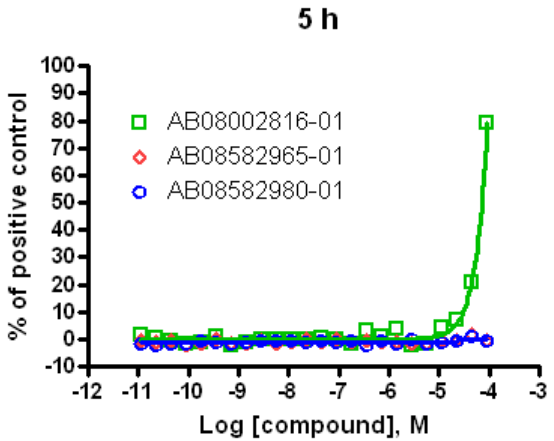
Malachite Green Oxalate

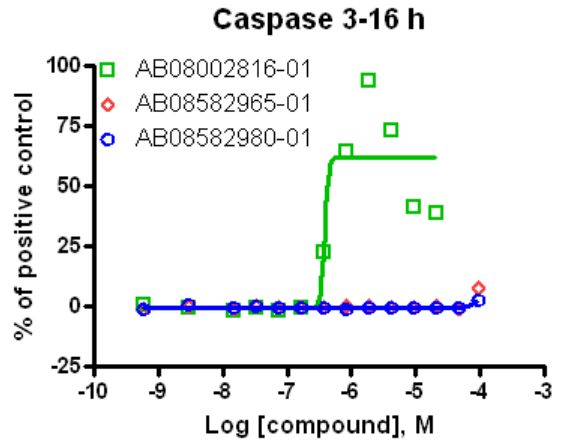


Mechanistic assays

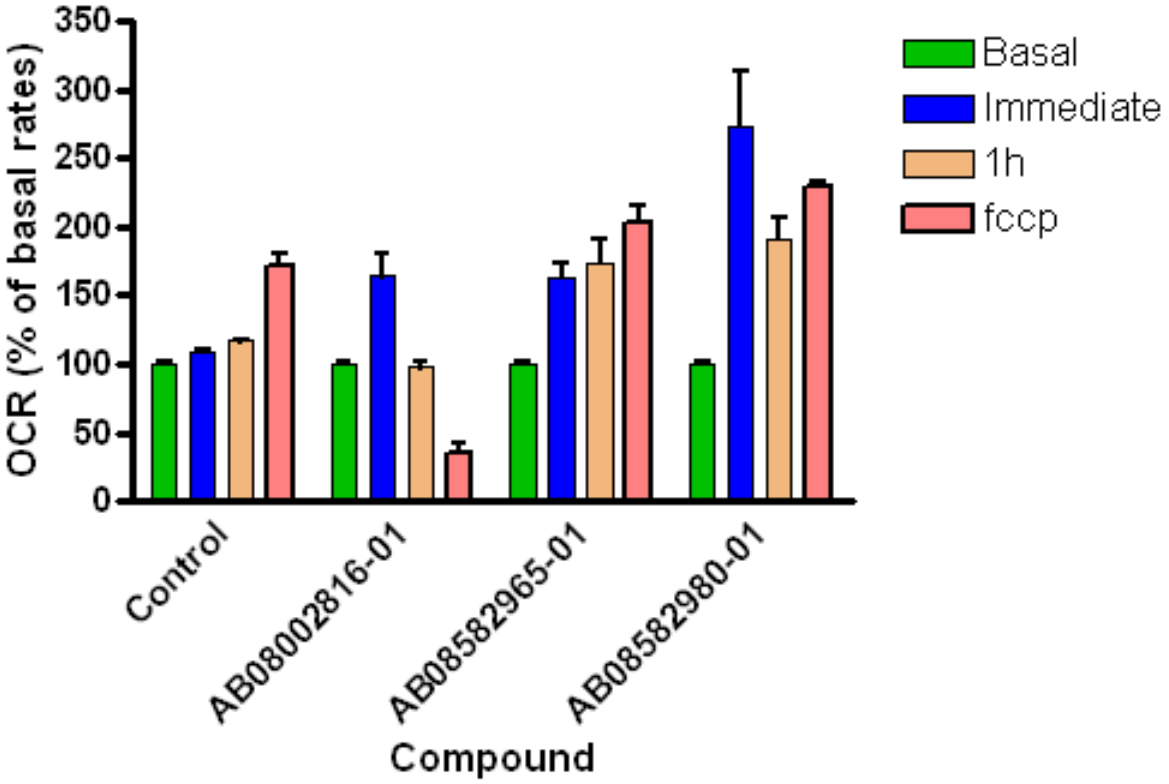




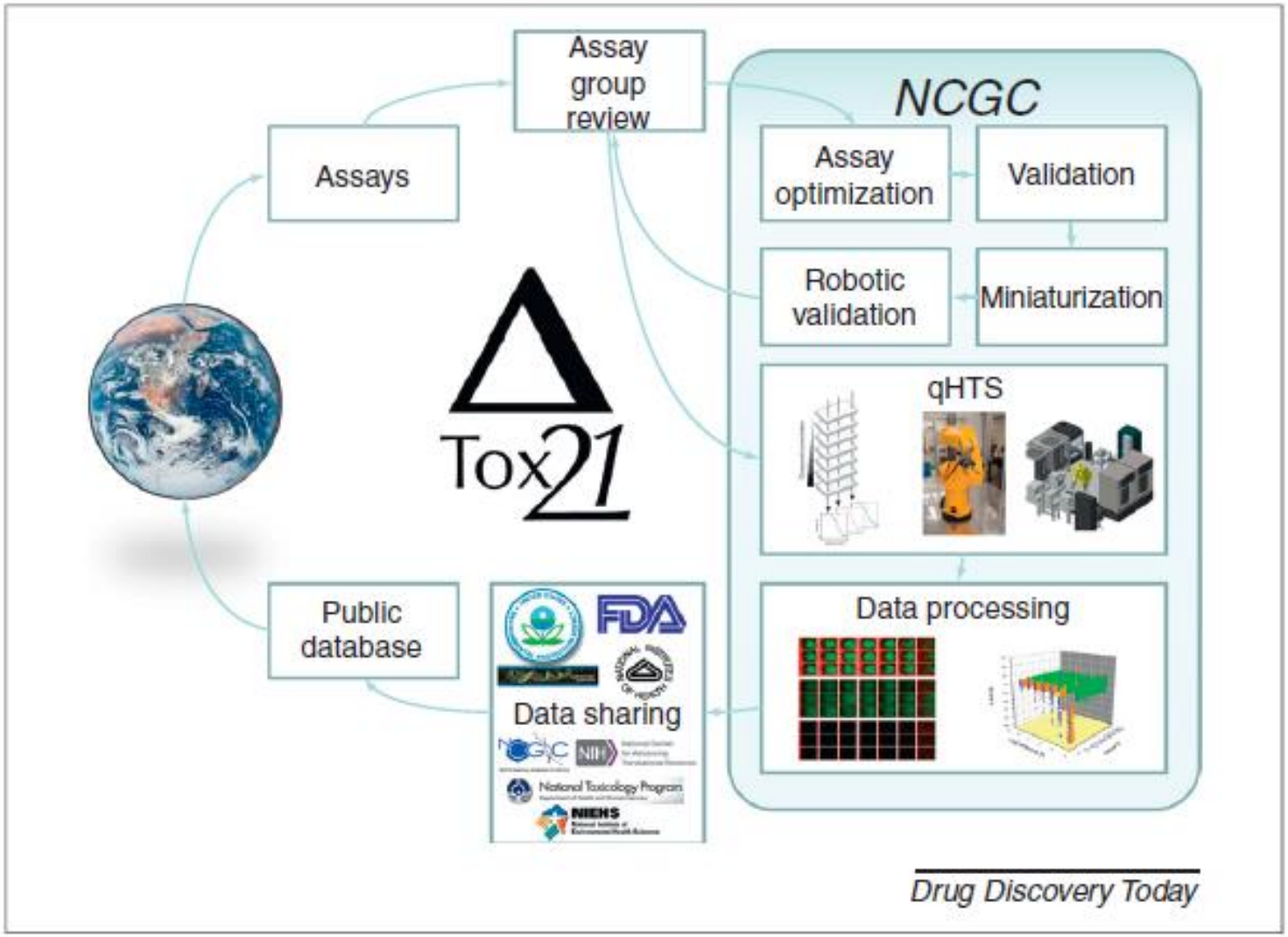




Mechanistic assays(Cont)



Tox21- Phase II Production phase phase



Drug Discov Today. 2013 Aug;18(15-16):716-23.

Benefits and Limitations

Objectives:

- Research, develop, validate, and translate innovative compound testing methods to characterize toxicity pathways.
- Identify compounds, assays, informatic analyses, and targeted testing needed to support development of the new methods.
- Identify patterns of compound-induced biological response in order to characterize toxicity pathways, facilitate cross-species extrapolation, and model low-dose extrapolation.
- Prioritize compounds for more extensive toxicological evaluation.
- Develop predictive models for biological response in humans.
- Make all data publicly available.

Benefits and Limitations

- Coverage of chemicals of interest is incomplete (i.e., volatiles).
- Lack of understanding how compounds interact (complex mixtures).

- Currently, Interactions between cells, tissues, organs and populations are poorly captured.
- Xenobiotic metabolism is lacking in many in vitro assays.
- Assessing the effects of chronic exposure conditions in vitro is not possible.
- Identifying when a perturbation to a gene or pathway would lead to an adverse effect in animals or humans remains a challenge.
- **Perfect** assays do not exist.

- Free concentration of a compound in vitro is unknown.
- Extrapolating from in vitro concentration to in vivo dose or blood levels is not straightforward.

Acknowledgments

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Kristine Witt

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Elizabeth Wagner
Yukako Komaki
Justin Pals

University of South Carolina
Craig Beeson

Gordon Research Conference on Water Disinfection Byproducts:

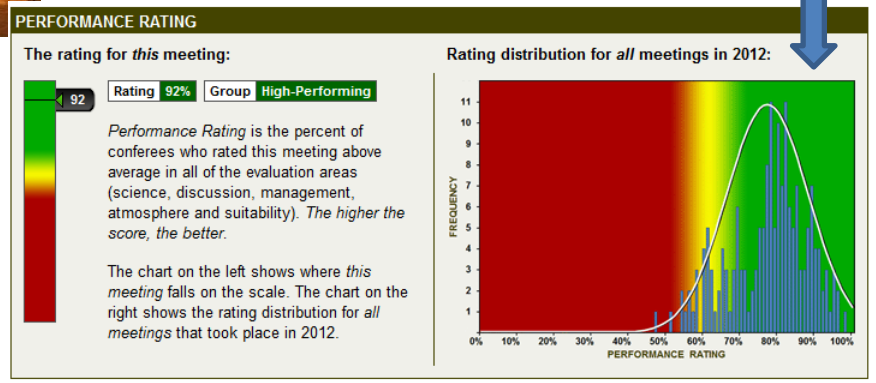
Charting the horizons of interdisciplinary research in water disinfection, byproducts, water reuse and public health

August 9-14, 2015

Mount Holyoke College, South Hadley, MA

August 8-9, 2015 Gordon Research Seminar organized for graduate students and postdocs. GRS Chair, Dr. Matias Attene-Ramos

Performance rating of 92% for the 2012 GRC on DBPs



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Stanford University

Keep these dates open and participate in your GRC!