



**NTP**  
National Toxicology Program

# **NTP High Throughput Screening (HTS) Initiative**

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**National Institute of Environmental Health Sciences**

**SACATM**  
**June 12, 2007**



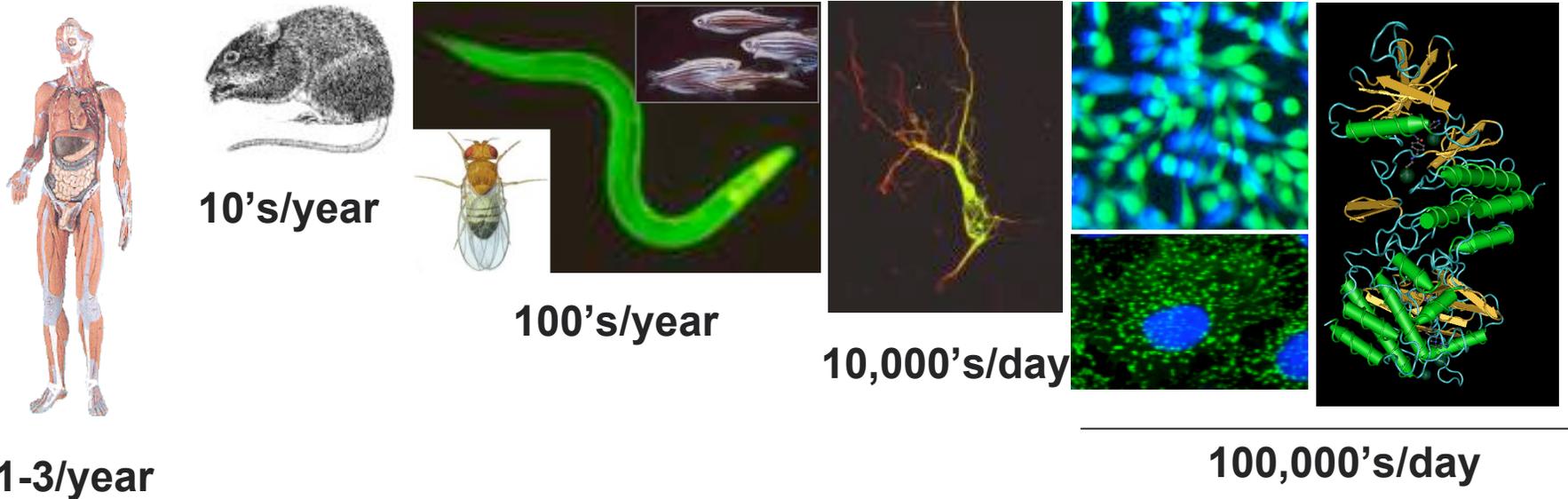


To meet the challenges of 21st century toxicology, the NTP Roadmap includes a major initiative to develop a high throughput screening (HTS) program with 3 main goals:

- Prioritize chemicals for further in-depth toxicological evaluation
- Identify mechanisms of action
- Develop predictive models for *in vivo* biological response



## What can be screened?





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# **NTP High Throughput Screening Assays Workshop**

December 14 - 15, 2005

Hyatt Regency Crystal City, Arlington, VA

Chair: Dr. Shuk-Mei Ho, University of Cincinnati

~70 participants from industry, academia, and government



## **Breakout Groups (BGs)**

### **1. Selection of Targets and Assays for High Throughput Screening**

**Co-chairs:** Dr. Kate Johnston (Cellumen Inc.), Dr. Tim Zacharewski (Michigan State Univ.)

### **2. Chemical Selection, Study Design, and Analytical Methods**

**Co-chairs:** Dr. Christopher Lipinski (Pfizer Global R&D), Dr. William Janzen (Amphora Discovery Corp.)

### **3. Data Storage, Analysis, and Interpretation**

**Co-Chairs:** Dr. Pauline Gee (CeMines Inc.), Dr. Alexander Tropsha (Univ. North Carolina at Chapel Hill)

### **4. Application of Data from HTS Assays in Regulatory Decision-making**

**Co-Chairs:** Dr. Jonathan Freedman (NIEHS), Dr. Hillary Carpenter (Minnesota Department of Health)



## **BG4 - Use of HTS Data in Regulatory Decision-Making**

### **Presently**

- Cannot be used for making regulatory decisions
- Could be used in priority setting for further evaluation

### **Requirements for use**

- Validation
- Uncertainty analysis
- Predictive ADME
- Chemicals must be tested in a large number of assays

### **Outreach**

- Workshops and training for regulatory scientists who will review and evaluate the data



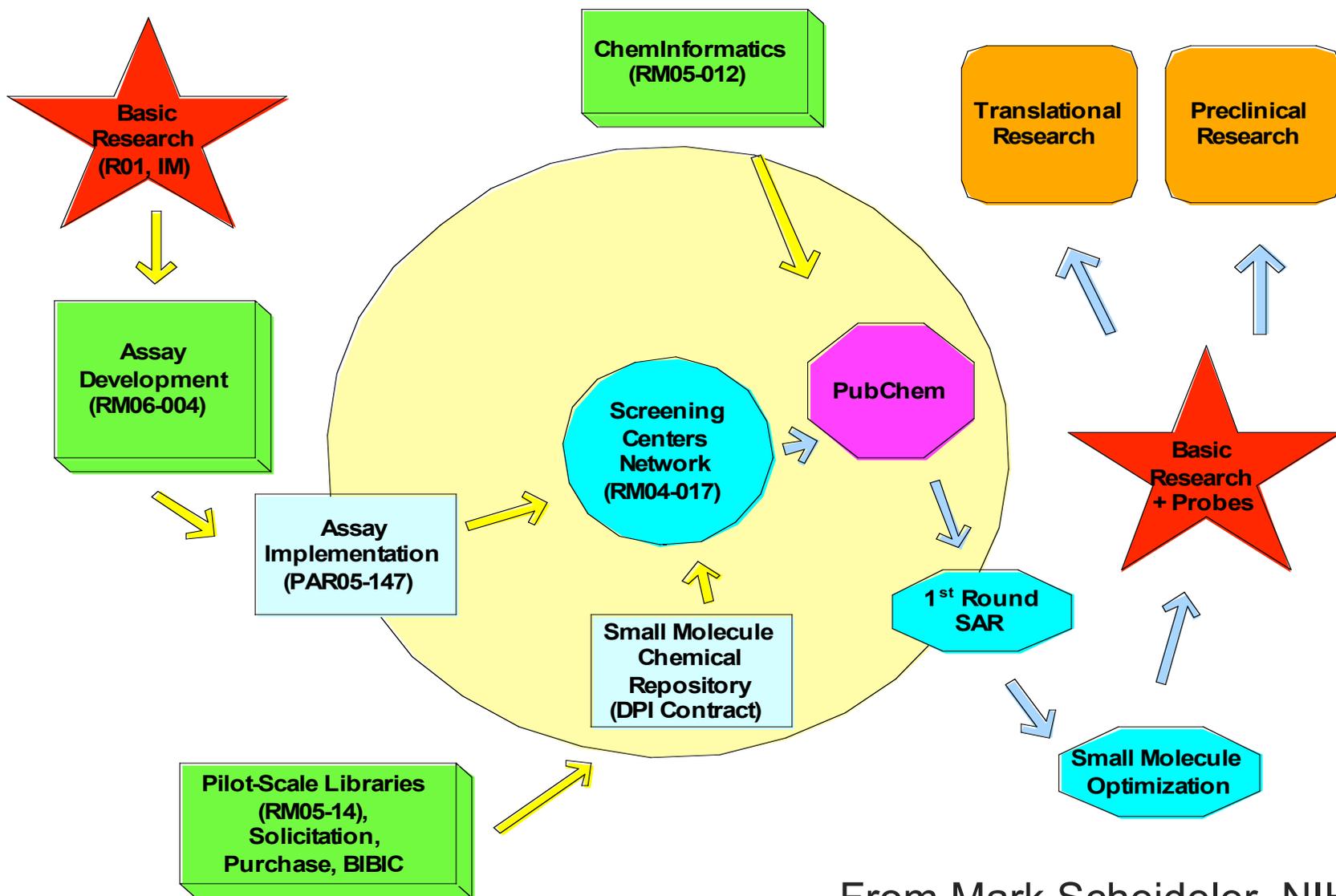
## **NIH Molecular Libraries Initiative**

<http://nihroadmap.nih.gov/molecularlibraries/>

- HTS methods are being used to identify small molecules that can be optimized as chemical probes to study the functions of genes, cells, & biochemical pathways.
- In mid-2005, NTP became a formal participant in the MLI by establishing a collaboration with the NIH Chemical Genomics Center (NCGC).
- As a result, the NTP has the opportunity to link data generated from HTS assays for biological activity to toxicity data produced by the NTP's testing program.



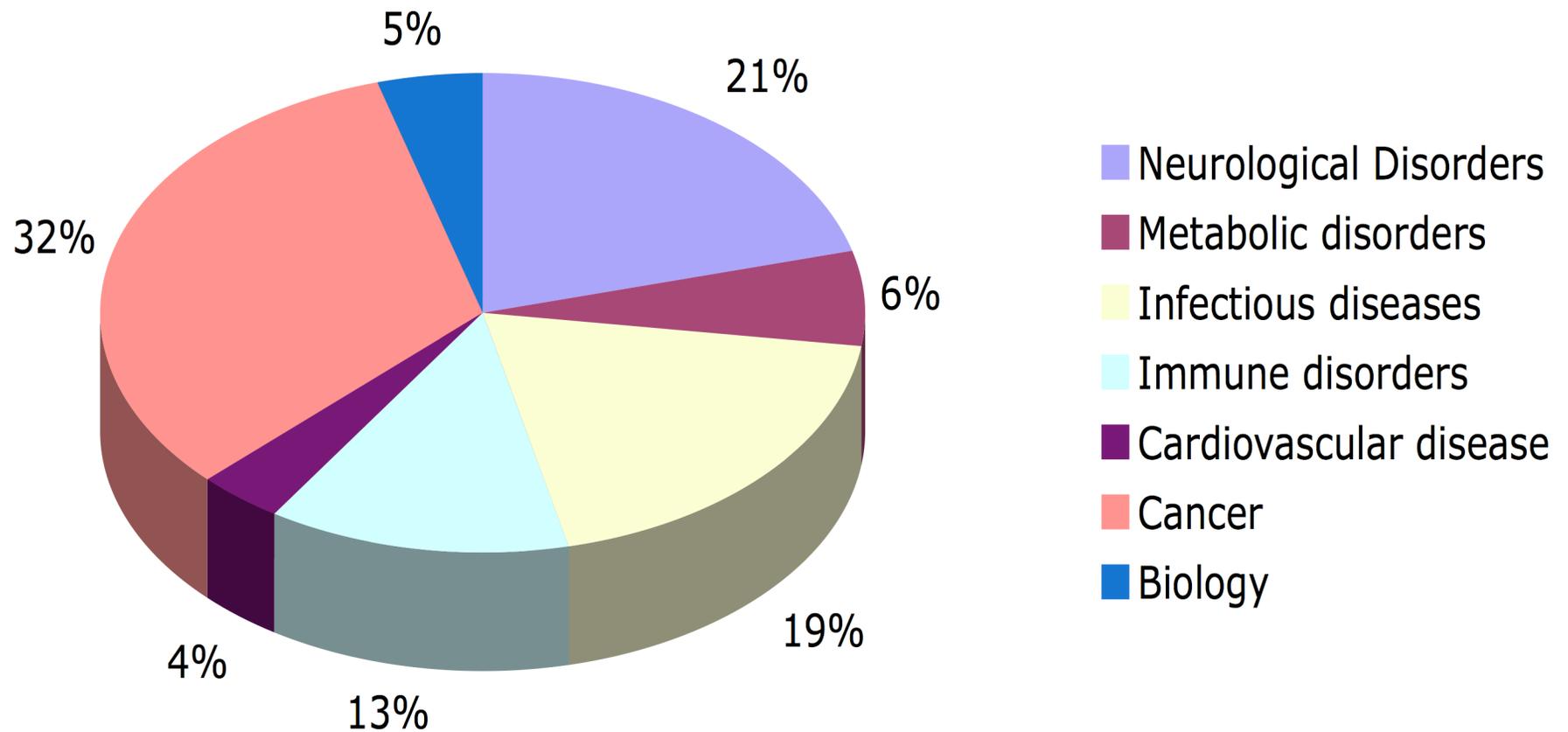
**Molecular Libraries Initiative (<http://nihroadmap.nih.gov/molecularlibraries/>)**



From Mark Scheideler, NIH



## Categorization of 110 MLSCN assays, by disease

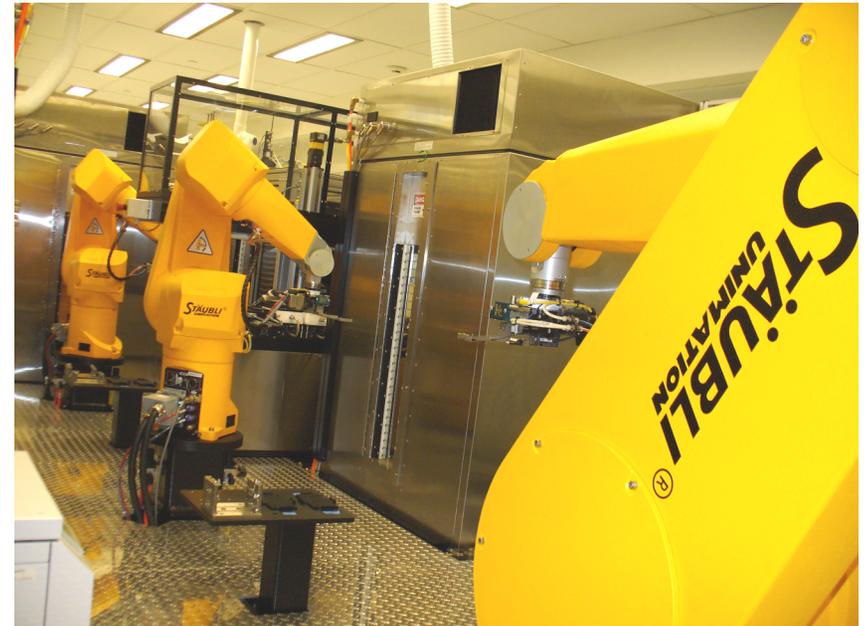




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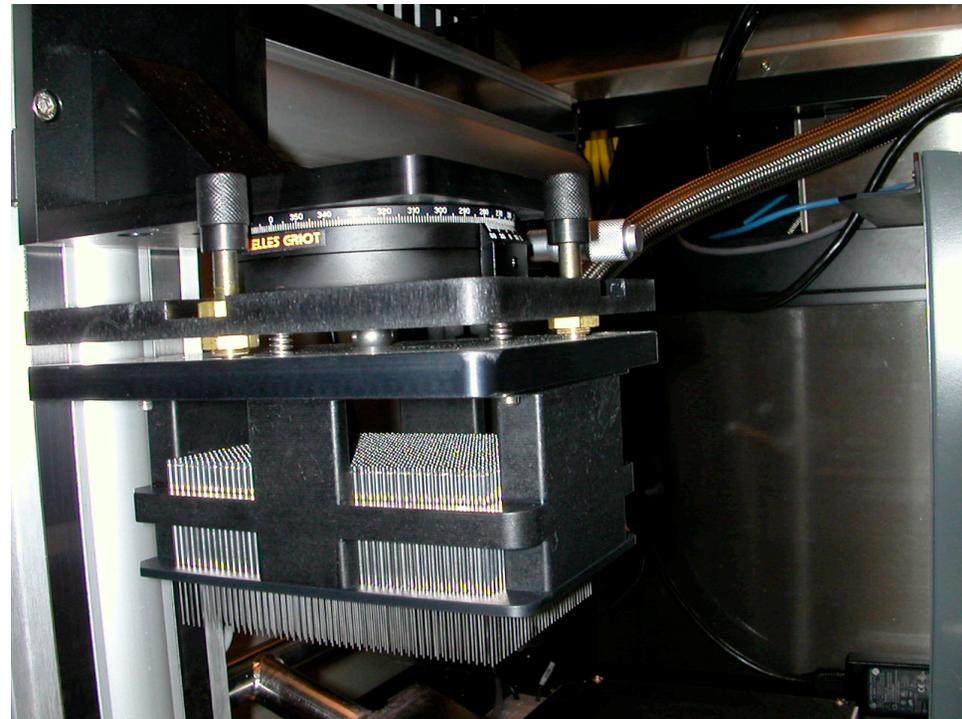
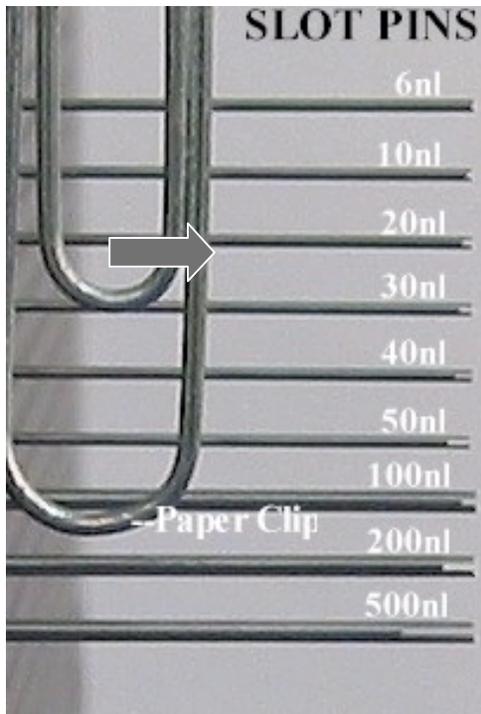


NIH CHEMICAL GENOMICS CENTER





## Pin-tool for compound delivery from inter-plate titrations series



- 1536 compound -to- 1536 assay plate transfer
- Volume range for Pin-based transfers: 10 nl to 0.5  $\mu$ L
- Transfer time  $\sim$  1-2 min per plate (includes wash cycle)
- No intermediate dilutions of compounds required

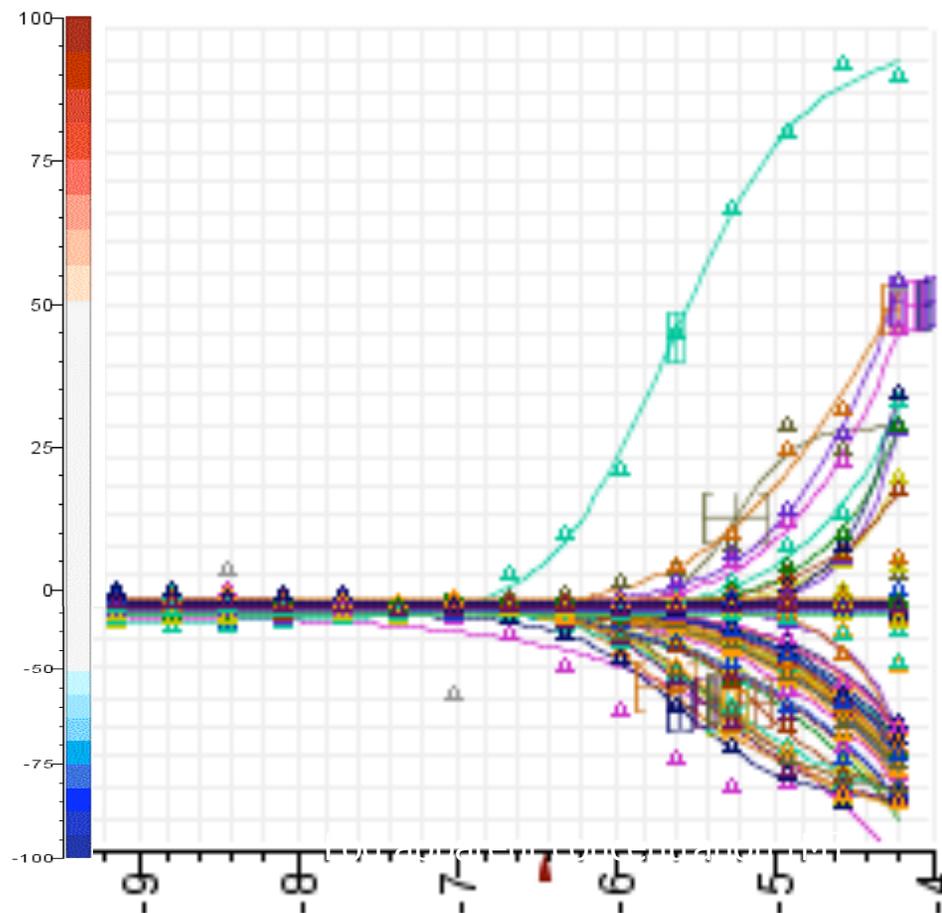


# NCGC data from a test primary screen: 1280 concentration-response curves

Positive  
modulation

Assay: *Pyruvate kinase*  
Library: LOPAC

Negative  
modulation



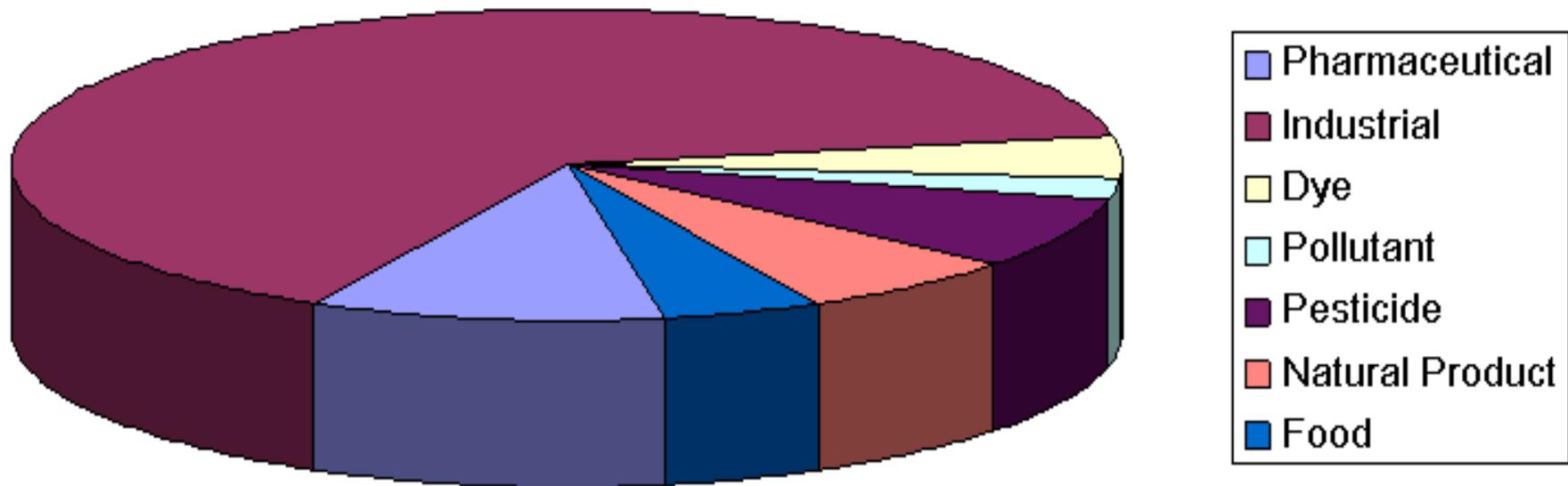


## The first NTP “1408” compound set

- All have been evaluated in one or more toxicological tests
  - 1353 unique compounds, 55 in duplicate to evaluate assay reproducibility
  - 1206 with NTP test data
  - 147 are ICCVAM reference substances recommended for the validation of alternative *in vitro* test methods (e.g., dermal corrosion, acute toxicity, endocrine activity).
- Selection was based on availability and solubility in DMSO at 10 mM, while avoiding excessive volatility and hazard.
- In addition to providing these compounds to the NCGC, we are providing the NTP compound library to the MLSCN repository so that other Centers, exploiting different HTS technologies, can have access to them.



## The NTP 1353 - Product Classes





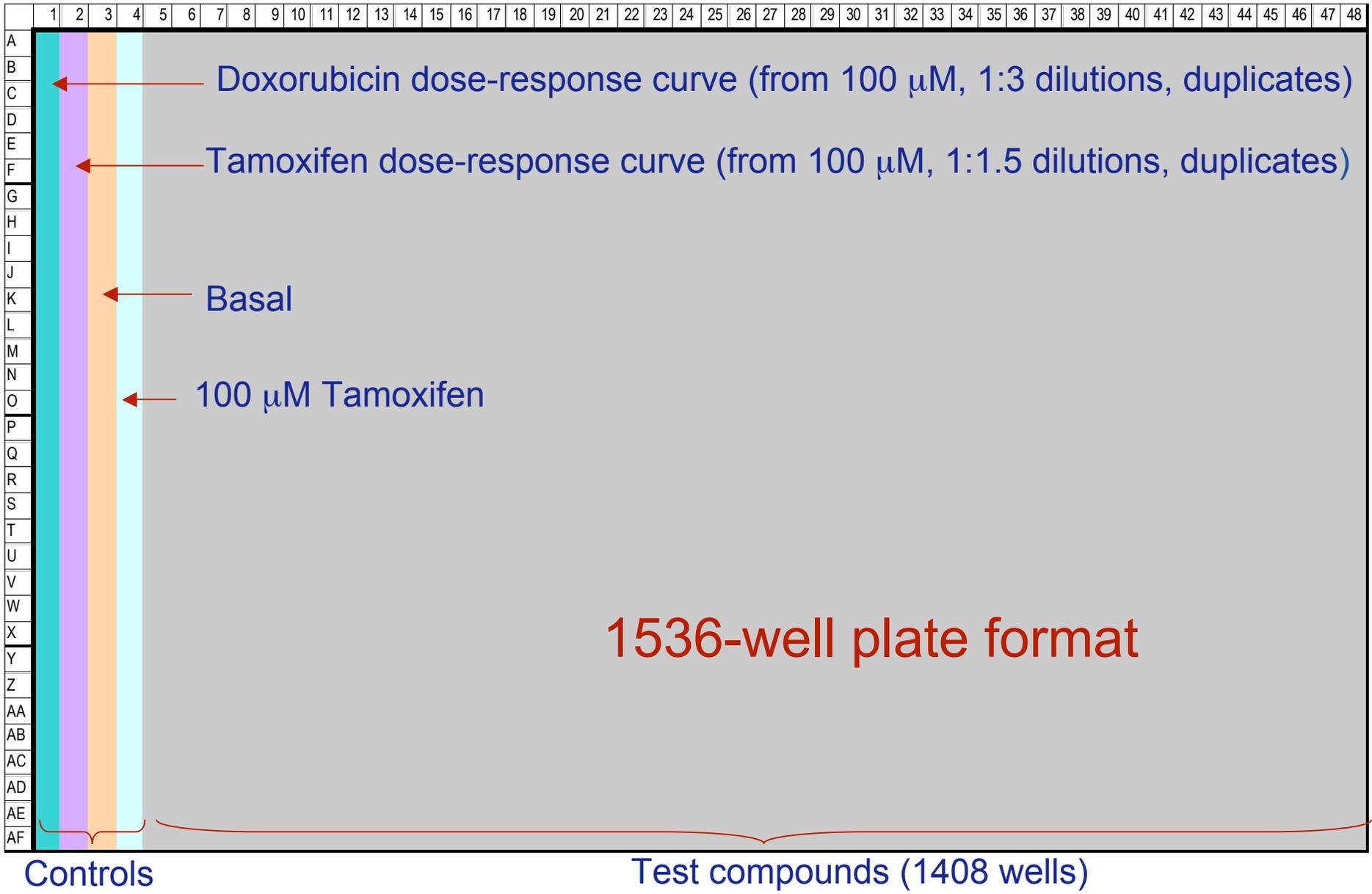
## HTS Assays Supplied to the NCGC

- **Cytotoxicity Assays** (selected because a measure of cytotoxicity is needed in virtually all cell-based HTS assays)
  - CellTiter-Glo® Luminescent Cell Viability Assay (measures ATP levels)
  - Cytotox-ONE™ Homogeneous Membrane Integrity Assay (measures release of lactate dehydrogenase from membrane-damaged cells)
- **Apoptosis Assays** (selected because a common pathway for many types of toxicity and diseases)
  - Caspase-Glo® 3/7 Assay
  - Caspase-Glo® 9 Assay
  - Caspase-Glo® 8 Assay
- **P-glycoprotein (Pgp-Glo™ Assay) ATPase Assay (aka MDR1 or ABCB1)** (involved in drug resistance)



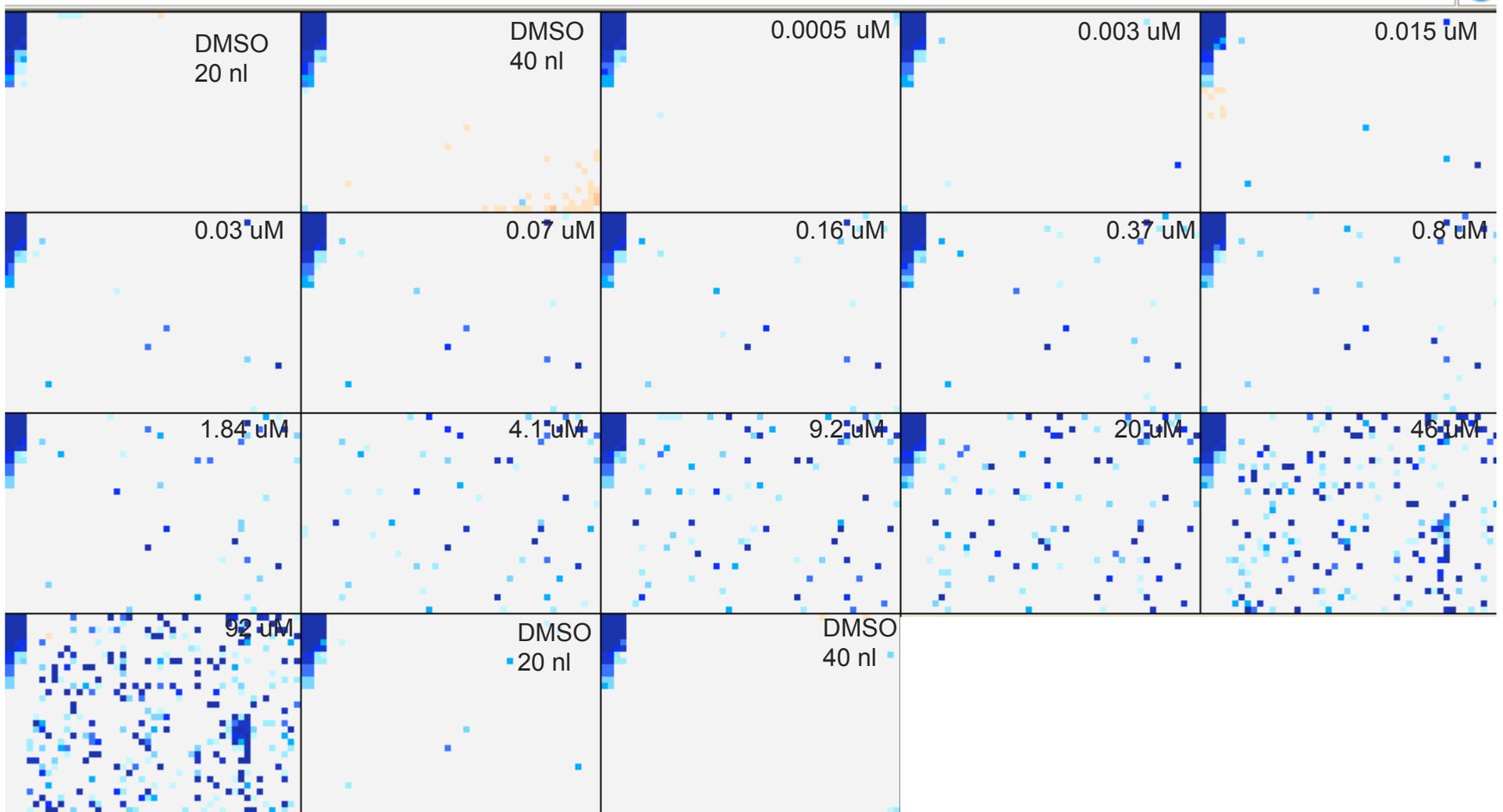
## NCGC HTS Assay Protocol for CellTiter-Glo Cell Viability Assay

1536 well plate format			
Sequence	Parameter	Value	Description
1	Reagent	5 $\mu$ l	1000 cells/well
2	Time	3-5 hr	37°C incubation
3	Compounds	23 nl	Compounds (0.59 nM - 92 $\mu$ M)
4	Time	40 hr	37°C incubation
5	Reagent	5 $\mu$ l	CellTiter Glo reagent
6	Time	20 - 30 min	Room Temperature
7	Detection	Luminescence	Viewlux plate reader





## NCGC qHTS results map for Jurkat cell screen



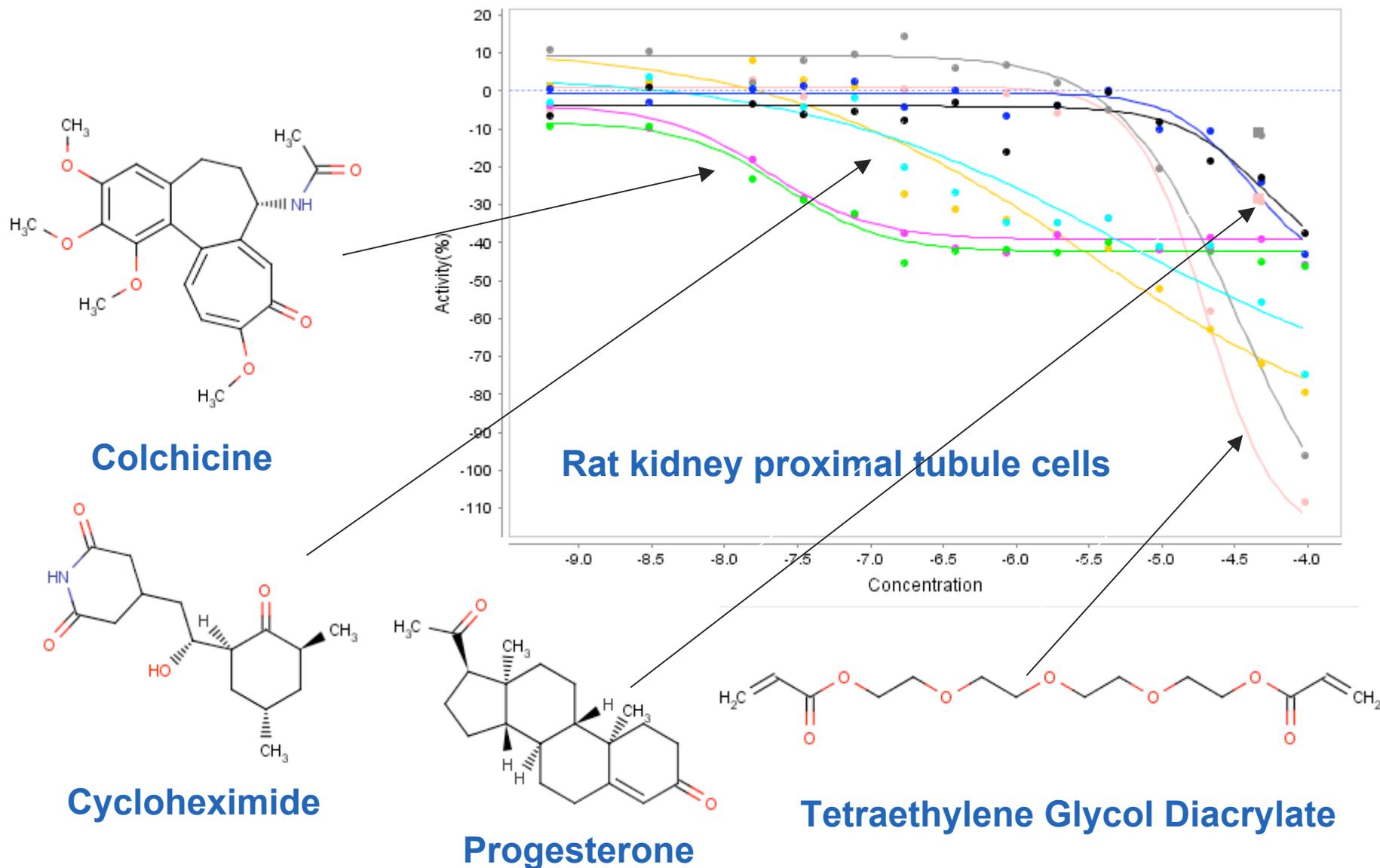


## NCGC: Human and Rodent Cell Types

Species	Cell names	Sources	
Human	Hek 293	Embryonic kidney cells	Transformed
Human	HepG2	Hepatocellular carcinoma	Transformed
Human	SH-SY5Y	Neuroblastoma	Transformed
Human	SK-N-SH	Neuroblastoma	Transformed
Human	Jurkat	T cell leukemia	Transformed
Human	BJ	Normal foreskin fibroblasts	Non-Transformed
Human	HUV-EC-C	Normal vascular endothelial cells	Non-Transformed
Human	MRC-5	Normal lung fibroblasts	Non-Transformed
Human	Mesangial cell	Normal cells from renal glomeruli	Non-Transformed
Rat	Proximal tubules	Normal cells from kidney	Primary
Rat	H-4-II-E	Hepatoma	Transformed
Mouse	N2a	Neuroblastoma	Transformed
Mouse	NIH 3T3	Fibroblasts from mouse embryo	Non-transformed

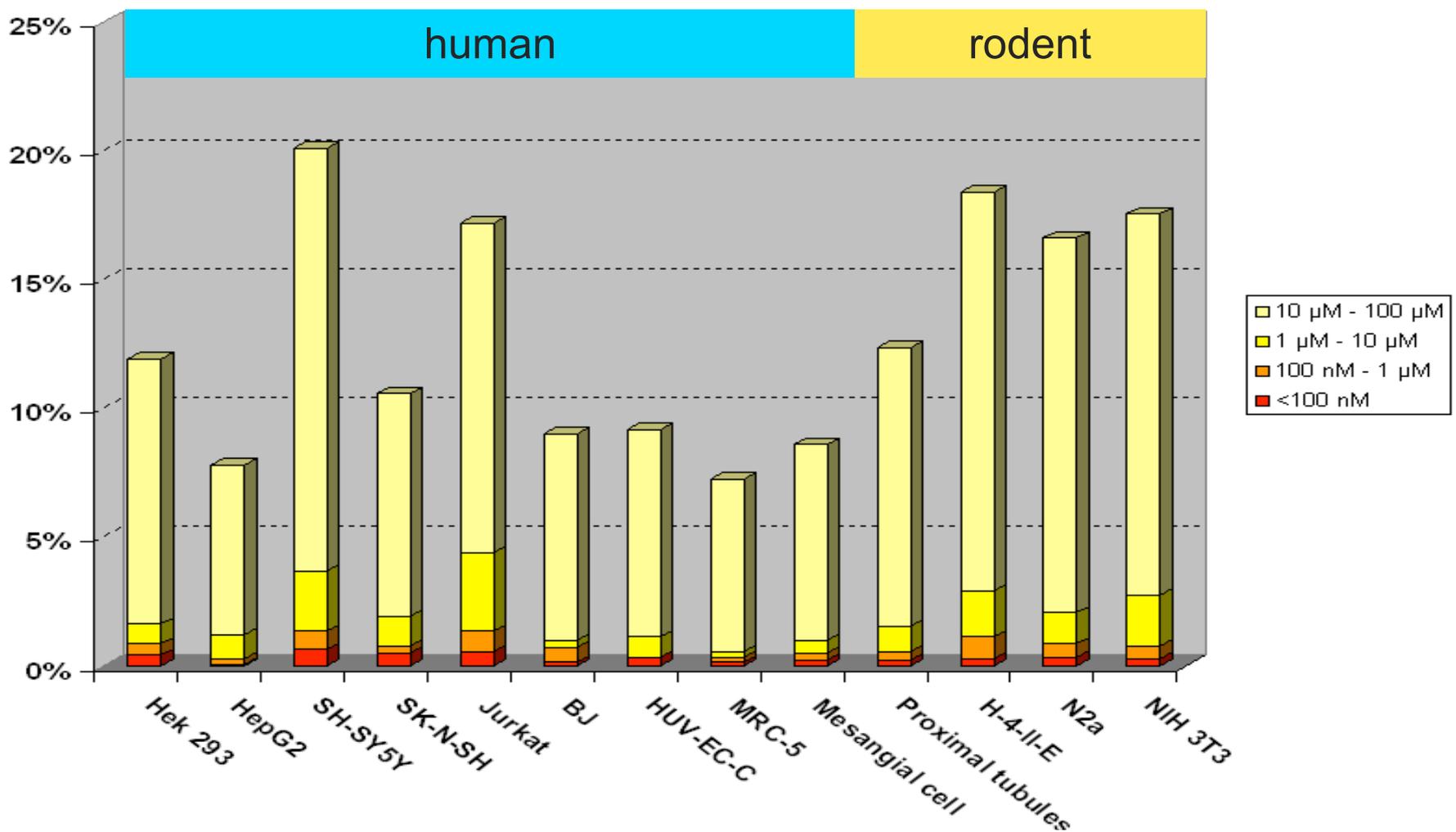


## Cytotoxicity Concentration Response Curves of Duplicate Compounds





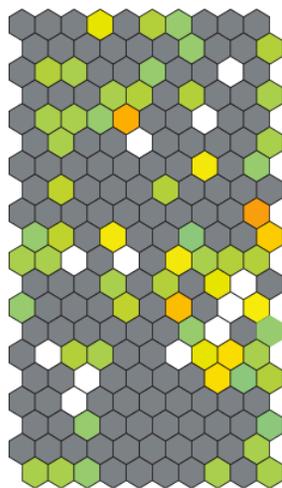
## Cytotoxicity potency distribution of the NTP 1408 compounds in 13 cell types



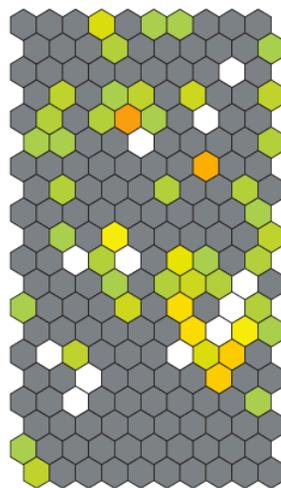


# Structure-Toxicity Relationships Across Assays

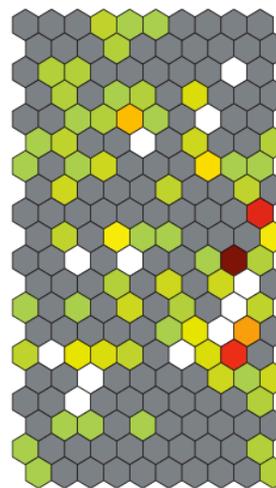
HEK293



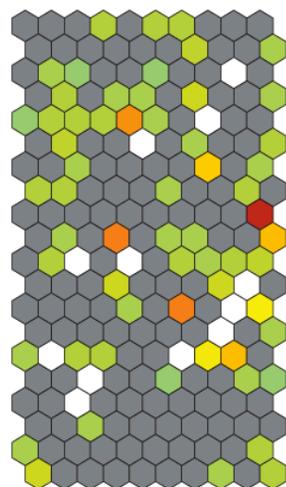
Mesangial



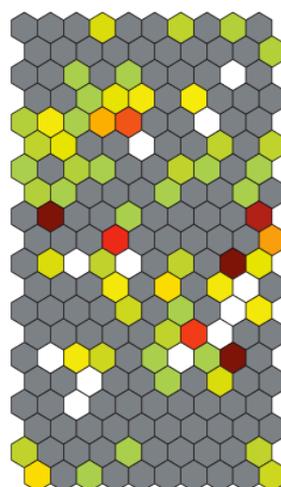
Rat Renal Proximal Tubule



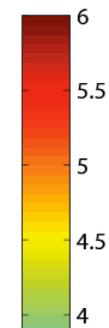
SK-N-SH



SH-SY5Y



Potent



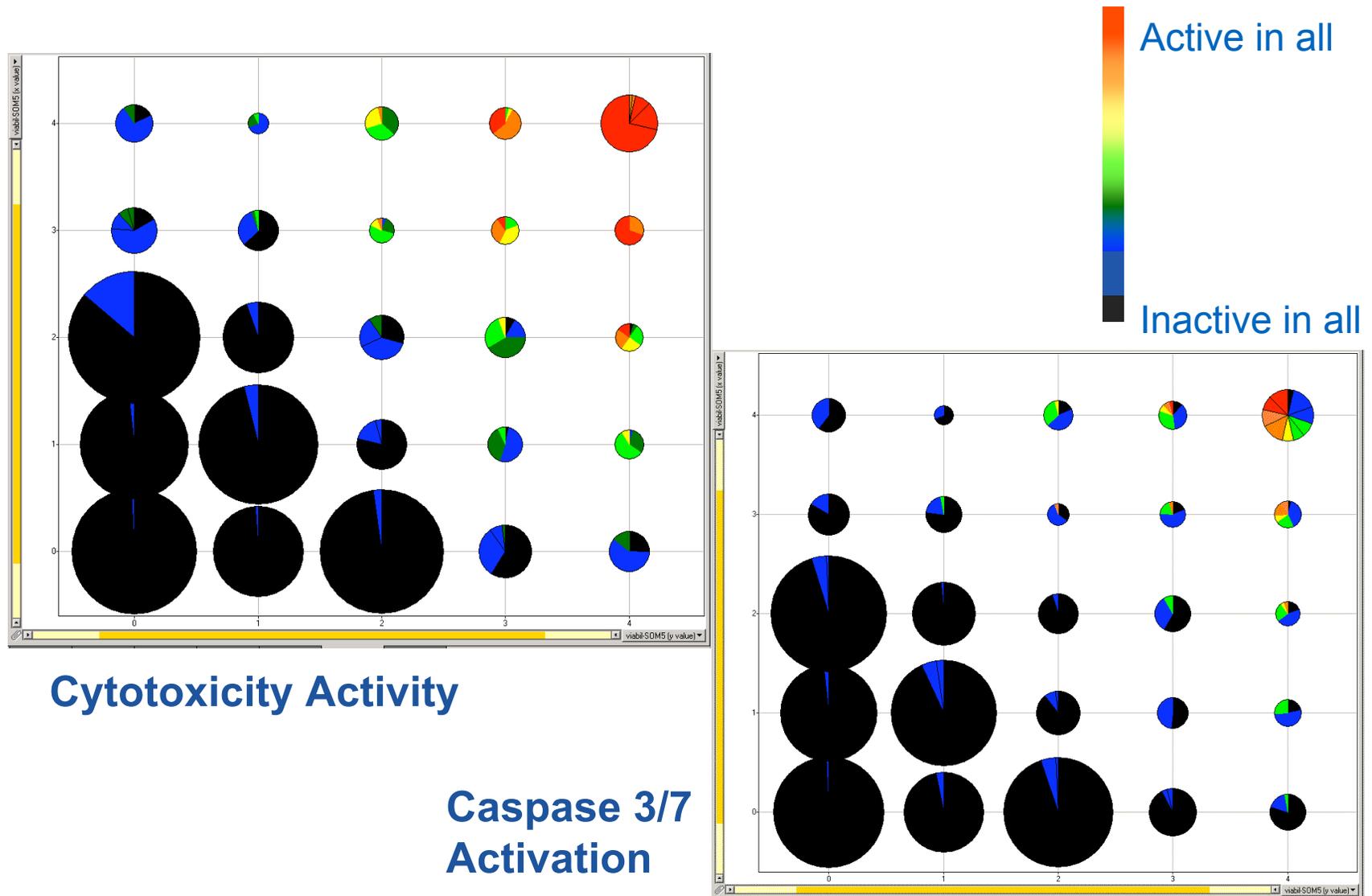
Inactive

Empty Cluster

1353 compounds are clustered based on chemical signatures/fingerprints

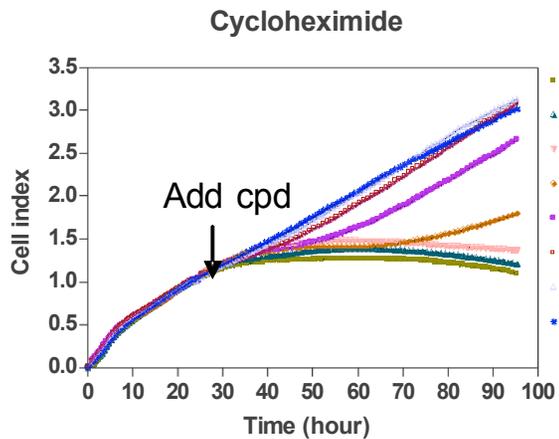


# Cytotoxicity SOMs and Caspase 3/7 activation activity

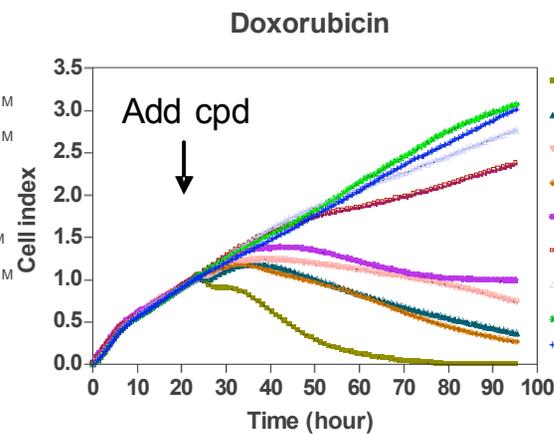




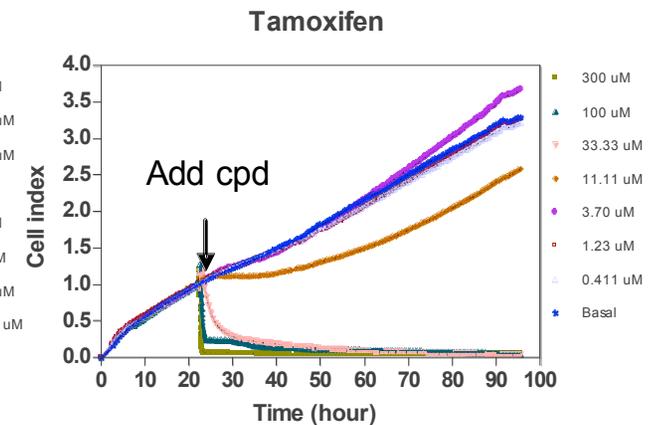
# Toxicant compound signatures determined by RT-CES system in HepG2 cells



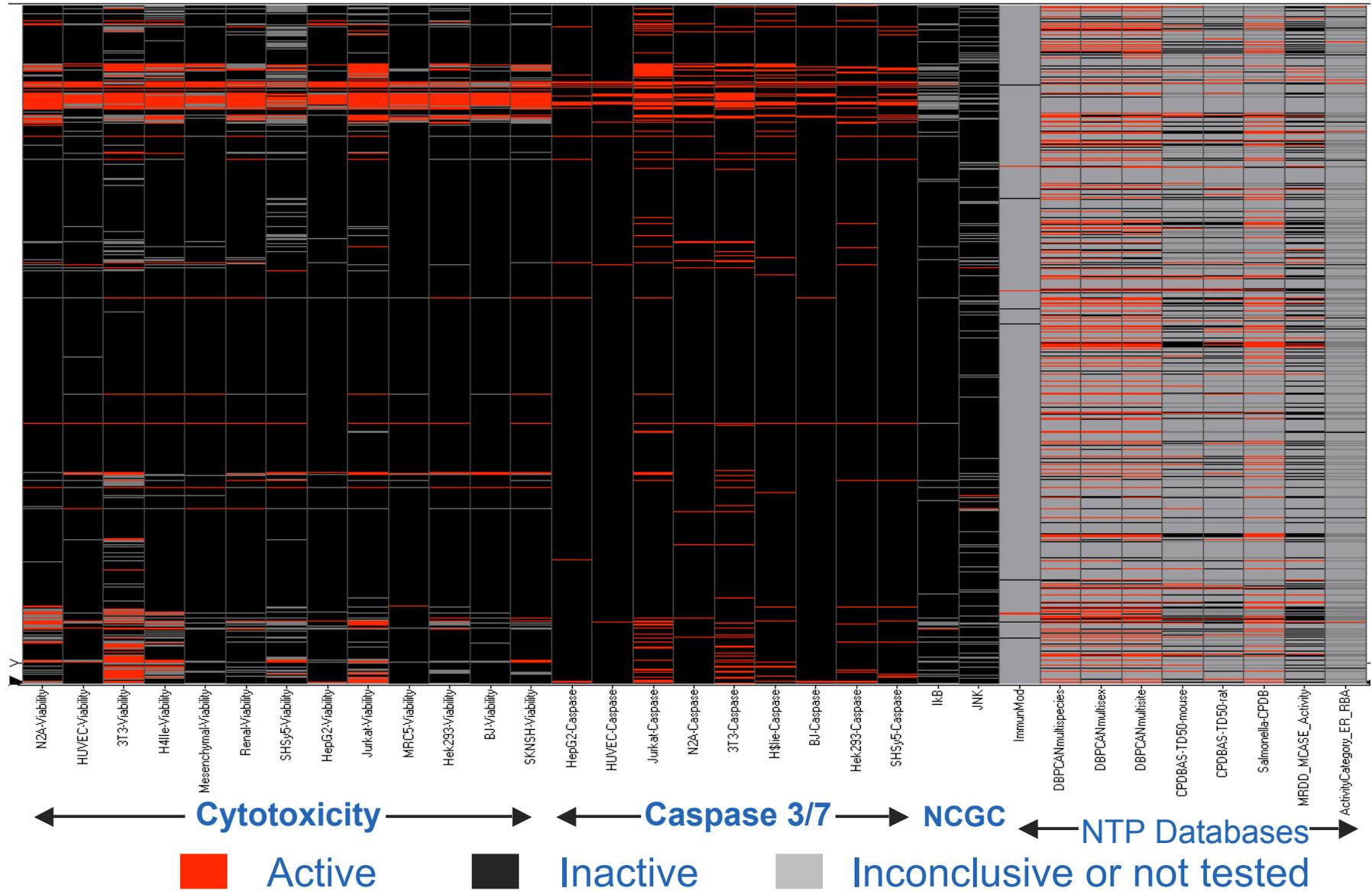
Protein synthesis inhibition



DNA damage



Apoptosis, Ion channel, Kinases





Address: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=427>

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## BioAssay Summary

**AID: 427** [?](#)

**Name:** Cell Viability - Hek293

**Data Source:** NCGC

**Test Results:** [Show](#) [Select](#) [Plot](#)

[Links](#) [Description](#) [Protocol](#) [Comment](#) [Definitions](#)

**Links:** [?](#)

**Compounds:** [?](#) All: 1335 Active: 74 Inactive: 1180 Inconclusive: 86

**Substances:** [?](#) All: 1408 Active: 80 Inactive: 1241 Inconclusive: 87

**Taxonomy:** 1 Link [?](#)

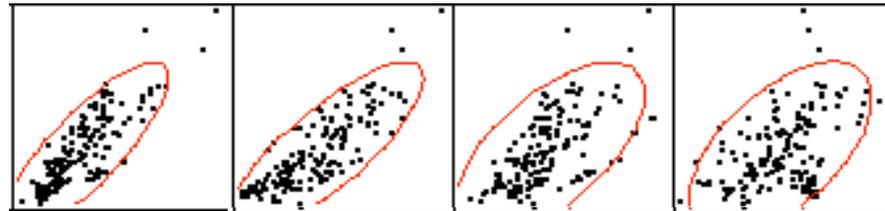
**Description:** [?](#)

NIH Chemical Genomics Center [NCGC]  
NIH Molecular Libraries Screening Centers Network [MLSCN]  
National Institutes of Environmental Health Sciences [NIEHS]  
National Toxicology Program [NTP]

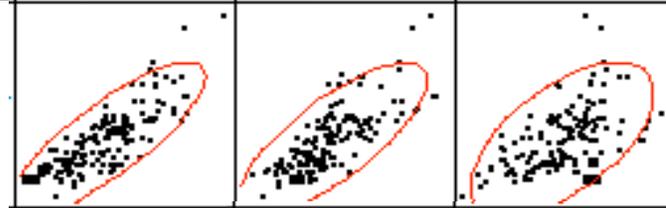


## Understanding toxicity from biological and chemical fingerprints (Chihae Yang, Leadscope)

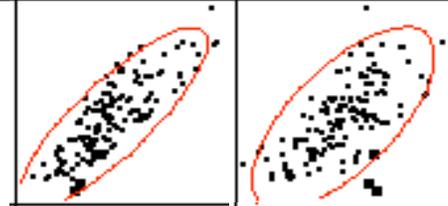
BJ viability



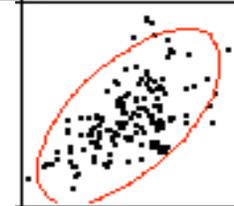
HEPG2 viability



Jurkat viability



N2A viability



BJ	0.58
HEPG2	0.60
Jurkat	0.68
N2A viability	0.63

**Viability and acute toxicity**

Rodent LD50



## Current Activities (1)

- In the next set of 1408 compounds for the NCGC, focus on:
  - compounds of specific interest for immune modulation and cancer
  - structurally-related compounds that have a range of activities
  - compounds that require metabolic activation and their metabolites
- In terms of assays, focus on those
  - that are representative of key steps in pathways important to cancer and immune modulation,
  - which are available through the 8 EPA ToxCast contracts (438 cell or biochemical endpoints evaluated), through the MLSCN, and potentially through other organizations
- In terms of cell types, expand the use of primary cells.



## Current Activities (2)

- Evaluate the differential responses among cell types to see if some are more informative than others (e.g., does using a primary kidney cell better identify kidney tumorigens?).
- Evaluate the relationship between HTS and mid-throughput screening assay data (*C. elegans*, zebrafish) and *in vivo* adverse health responses (e.g., acute toxicity, immunotoxicity, cancer, etc.)
- Incorporate various measures of chemical space (log p, molecular weight, number of rotatable bonds, number of hydrogen acceptors and donors) into the analysis.
- Establish an external advisory group.



## **NTP/NIEHS**

- John Bucher, Allen Dearry, Jennifer Fostel, Chris Portier, Fred Parham, Cynthia Smith, Kristine Witt

## **NCGC**

- Chris Austin, Ruilli Huang, Jim Inglese, Noel Southall, Menghang Xia

## **EPA**

- David Dix, Keith Houck, Bob Kavlock, Ann Richard



## **SACATM Discussion Questions**

**(Lead Discussants: Drs. DeGeorge, Becker, McClellen, Qu)**

- 1.** Do you have general comments on the NTP HTS Initiative in terms of its purpose, the approach being used, and/or the selection of assays, cell types, or compounds to test?
- 2.** Do you have specific comments on the approaches being used to identify a battery of HTS assays for predicting *in vivo* responses (e.g., immunotoxicity, carcinogenicity) for the ultimate goals of (1) identifying mechanisms of action and (2) prioritizing substances for further in-depth toxicological evaluation?