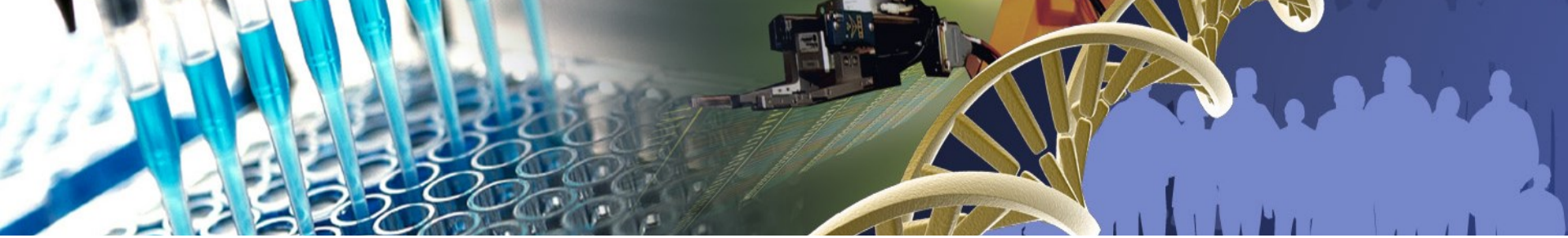


In Vitro and High Throughput Screening (HTS) Assays

Raymond Tice, Ph.D.
Special Volunteer @ NIEHS/NTP
(tice@niehs.nih.gov)

EFSA Expo
Shaping the Future of Food Safety, Together
Novel Chemical Hazard Characterization Approaches

Milano, Italy
October 16, 2015

Formation of the U.S. Tox21 Community

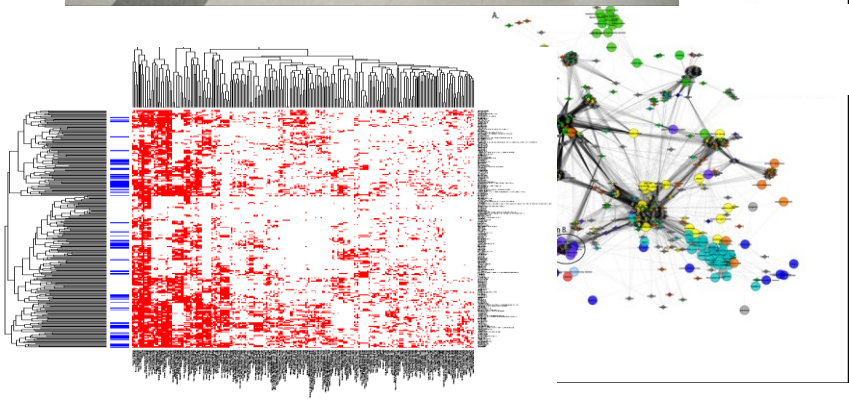
- 5-year Memorandum of Understanding (MoU) on "High-Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings" made public on Feb 14, 2008 signed by NHGRI (F.S. Collins), NIEHS/NTP (S.H. Wilson), and EPA (G.M. Gray).

- Revised 5-year MoU to add FDA made public on July 19, 2010 signed by NHGRI (E.D. Green), NIEHS/NTP (L.S. Birnbaum), EPA (P.T. Anastas), and FDA (J. Woodcock).

- New 5-year MOU signed September 2015 by NCATS (C.P. Austin), NIEHS/NTP (L.S. Birnbaum), EPA (L.G. Kadeli), and FDA (S.T. Mayne).

Goals of Tox21

- Identify patterns of compound-induced biological response in order to:
  - characterize toxicity/disease pathways
  - facilitate cross-species extrapolation
  - model low-dose extrapolation
- Prioritize compounds for more extensive toxicological evaluation
- Develop predictive models for biological response in humans



Tox21 Phase I – Proof of Principle (2005 – 2010)

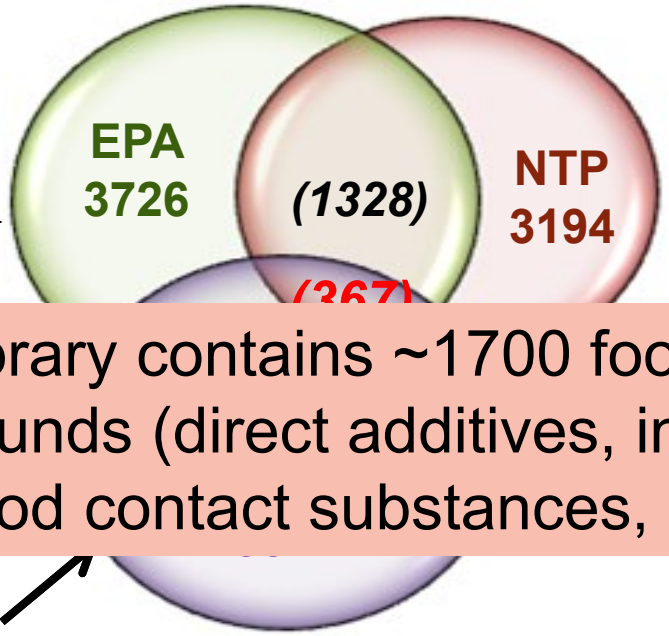
- EPA via ToxCast™ screened 320 compounds (309 unique, primarily pesticide actives and some endocrine active compounds) in ~550 assays.
  - Data made public via ACToR (Aggregated Computational Toxicology Resource; http://epa.gov/actor)
- NIH Chemical Genomics Center screened 1408 compounds (1353 unique) from NTP and 1462 compounds (1384 unique) from EPA in 140 qHTS assays representing 77 predominantly cell-based reporter gene endpoints.
  - Data made public via PubChem (http://pubchem.ncbi.nlm.nih.gov/) and CEBS (Chemical Effects in Biological Systems; http://www.niehs.nih.gov/research/resources/databases/cebs/)

Tox21 Phase II – Expanded Compound Screening (2011 – ?)

- EPA's ToxCast™: ~700 compounds in ~700 assays, ~1000 compounds in endocrine activity assays
- NCATS quantitative high throughput screening (qHTS):
  - 10K compound library screened 3 x at 15 concentrations
  - qHTS assays focused on:
    - nuclear receptor activation or inhibition
    - induction of cellular stress response pathways
    - characterizing human variability in response
- Data made public via:
  - EPA's CompTox website (http://www.epa.gov/comptox/)
  - NLM PubChem (http://pubchem.ncbi.nlm.nih.gov/)
  - CEBS (Chemical Effects in Biological Systems (http://www.niehs.nih.gov/research/resources/databases/cebs/)

Tox21 10K Compound Library – Version 1.0

ToxCast I and II compounds , Antimicrobial Registration Program, Endocrine Disruptor Program, Molecula Working FDA Drug Liver Injury Project, Failed drugs



NTP-studied compounds, NTP nominations and related compounds, NICEATM/ICCVAM compounds *vo* regulatory rnal ors (e.g., ing Institute, U.S. Army Public Health Command), formulated mixtures

The 10K library contains ~1700 food relevant compounds (direct additives, indirect additives/food contact substances, pesticides)

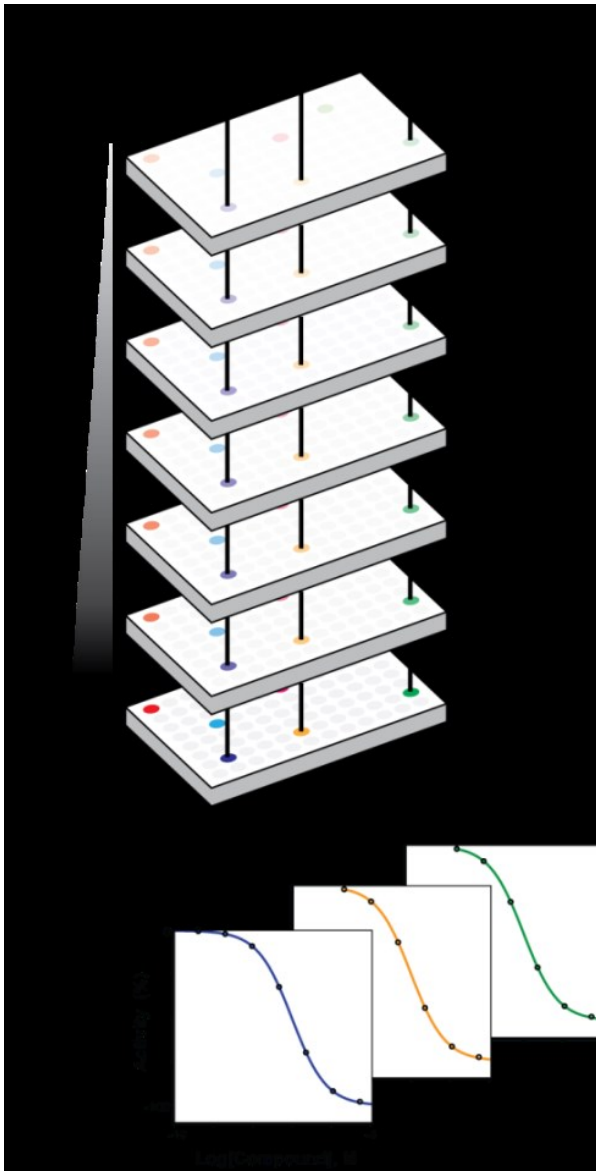
Unique	EPA	NTP	NCATS	Total	Total Unique	
GSIDs	3726	3194	3524	10444	8307	unique substances
Tox21 IDs	3729	3210	3733	10672	10496	unique solution IDs
wells	4224	3726	4224	12174	12174	total number of test cmpd wells

2255 replicate substances (GSIDs) across 3 inventories

Compound identity and structures available at http://www.epa.gov/ncct/dsstox/sdf_tox21s.html

Quantitative High Throughput Screening (qHTS)

- conducted at the National Center for Advancing Translational Science (NCATS
- homogeneous assays – add, mix, measure
- DMSO soluble compounds
- 536-well plate format on a robotics platform
- 15-point concentration-response curve
- 15 nM to 92 μM typical
- ~5 μL total assay volume/well
- ~1000-3000 cells/well
- **each assay run 3 times = ~36K conc. response curves**



Phase II qHTS Nuclear Receptor and Related Assays*

hAhR full length receptor in HepG2 cells (agonist completed but not antagonism)

hAR full length receptor in MDA kb2 cells; partial receptor in HEK293 cells

hCAR full length receptor in HepG2 cells

hERα full length receptor in BG1 cells; partial receptor in HEK293 cells

hFXR partial receptor in HEK293 cells

hGR full length receptor in HeLa cells

hPPARδ partial receptor in HEK293 cells

hPPARγ partial receptor in HEK293 cells

hRORγ partial receptor in CHO cells

hRXR partial receptor in HEK293 cells

rTRβ full length receptor in GH3 cells

hTSHR full length receptor in HEK293 cells

hVDR partial receptor in HEK293 cells

Inhibition of aromatase in MCF-7 cells

Retinol Signaling Pathway in C3H10T1/2 cells

NR assays conducted in agonist and antagonist modes

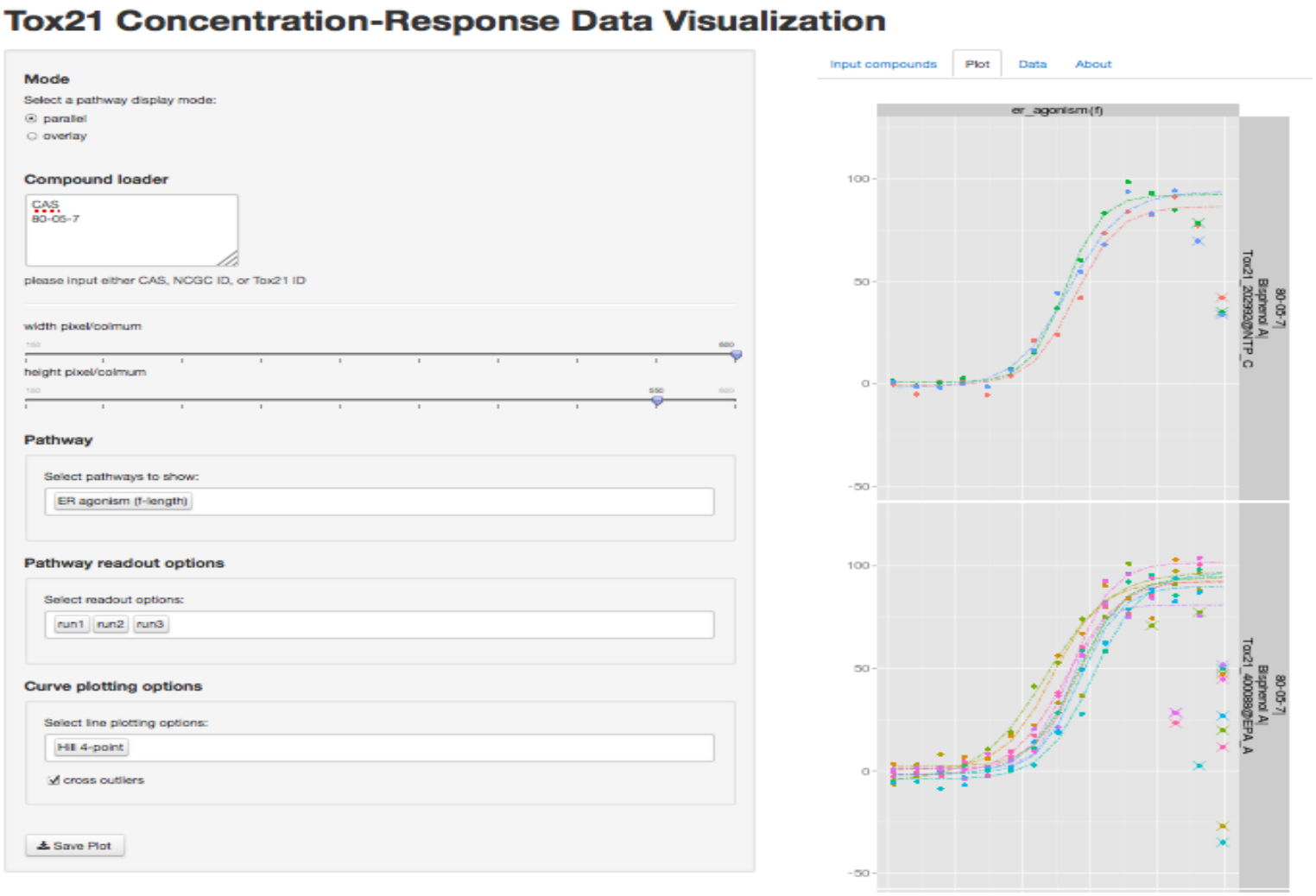
**Bolded text indicates completed assays*

Phase II qHTS Stress Response Pathway Assays*

Endoplasmic Reticulum stress	**EndoR (lipid damage) in HeLa cells**
Genotoxic stress	**p53 activation in HCT-116 colon cancer cells**
	ATAD5 levels in HEK293 cells (ATPase family AAA domain-containing protein 5 – a DNA damage response element)
	DT40 (DNA-repair mutant isogenic chicken cell clones) (Rev3 (-/-), rad54/ku70 (-/-), wild type)
	pH2AX induction in CHO cells
Heat shock	**HSE in HeLa or HepG2 cells**
Hypoxia	HRE (HIF-1α) in ME-180 cervical carcinoma cells
Inflammation	**NFκB in ME-180 cells**
Oxidative stress	**ARE/Nrf2 in HepG2**
Multiple stresses, cell death, specific toxicities	**AP-1 activation in ME-180 or HepG2 cells**
	Caspase 3/7 activation
	LDH release, ATP levels to assess cytotoxicity
	mitochondrial membrane potential in HepG2 cells
	Cell death/viability kinetic studies in 2 cell types

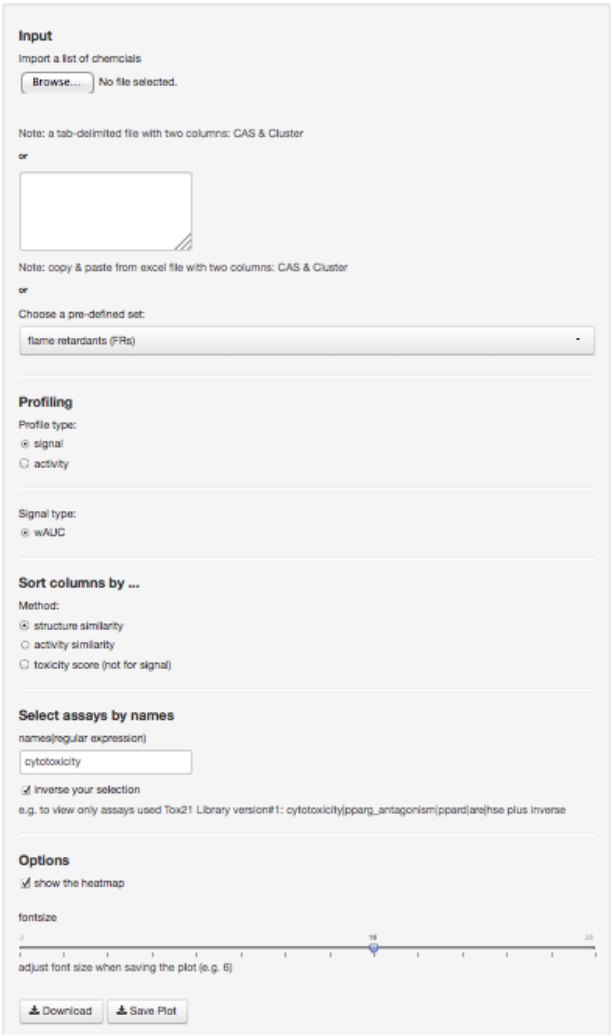
**Bolded text indicates completed assays*

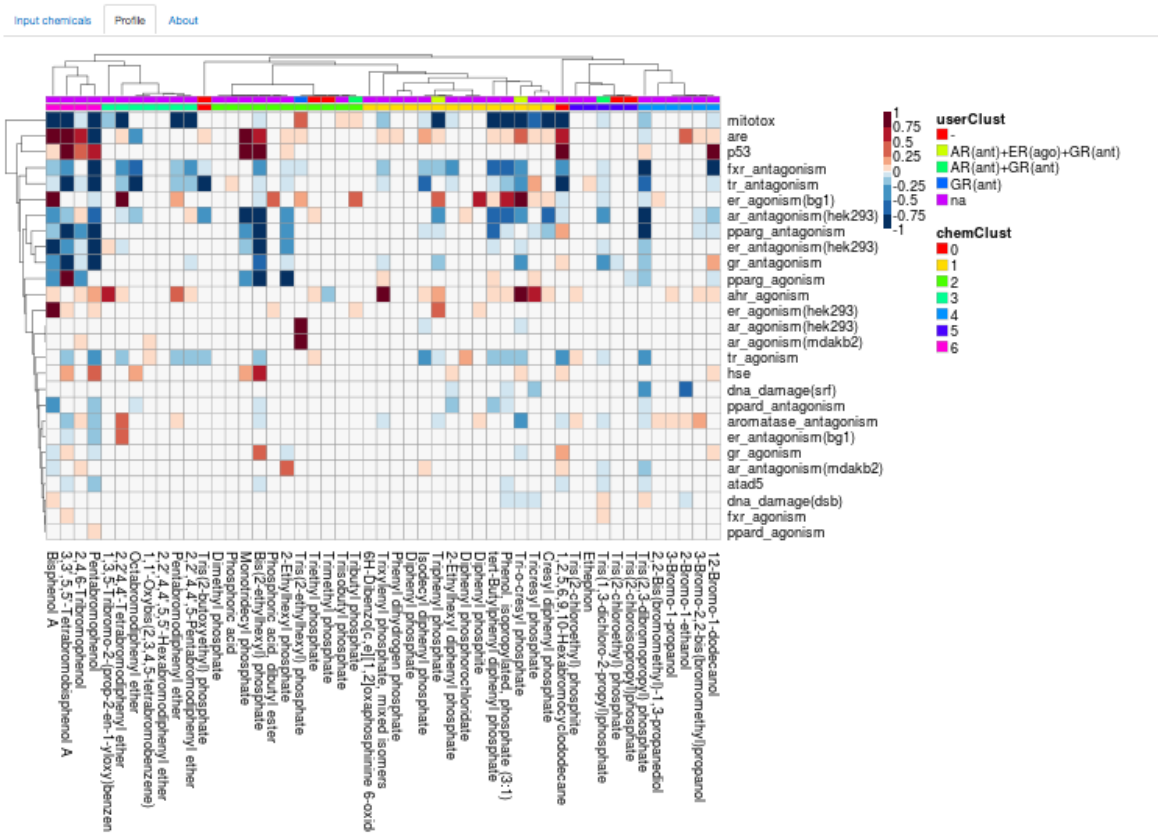
Tox21 Graphical User Interface for Concentration Response Data



J-H Hsieh (NTP): https://hsiehjh.shinyapps.io/tox21curvebrowser

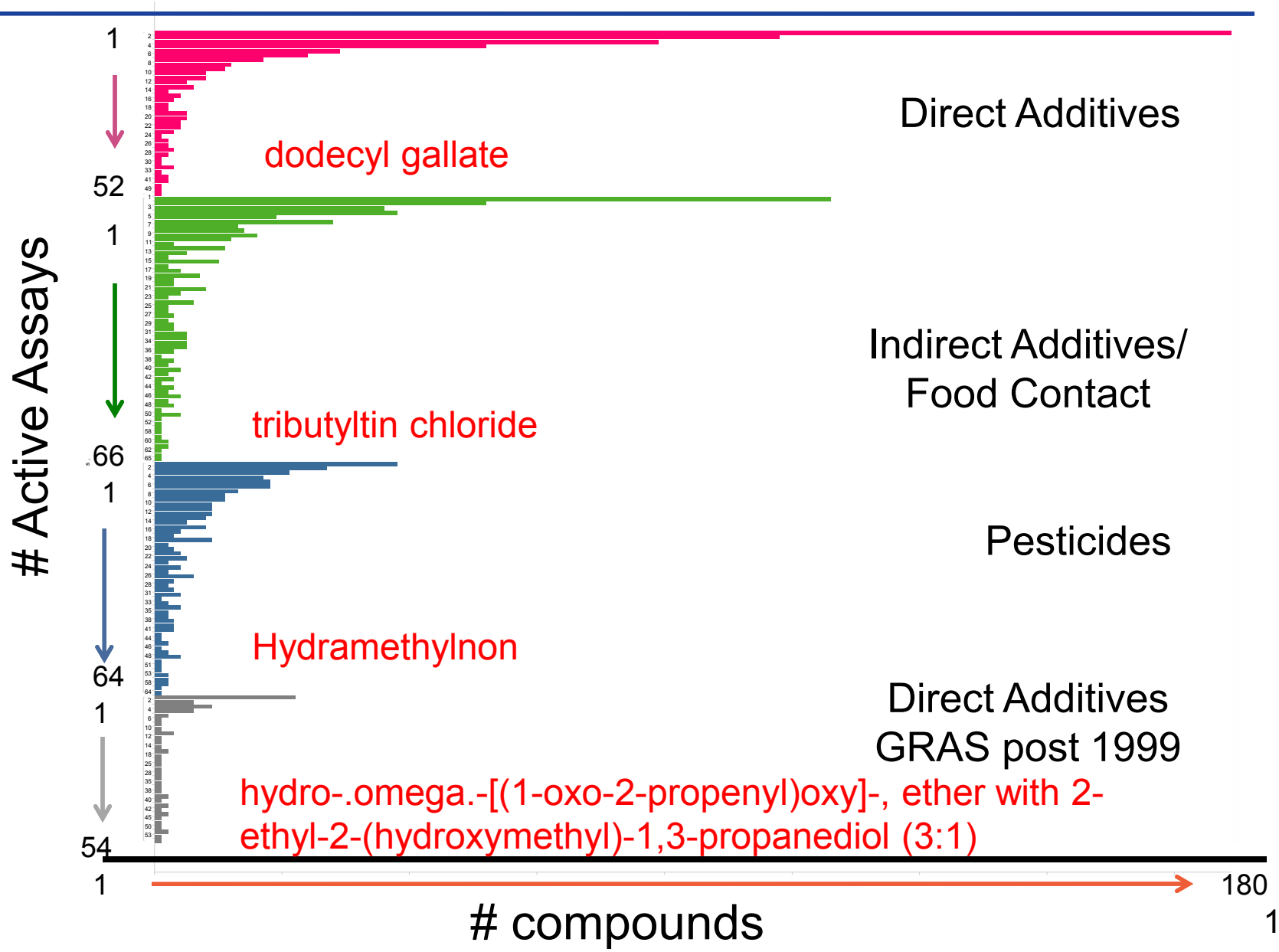
Tox21 Graphical User Interface For Compound Profiling



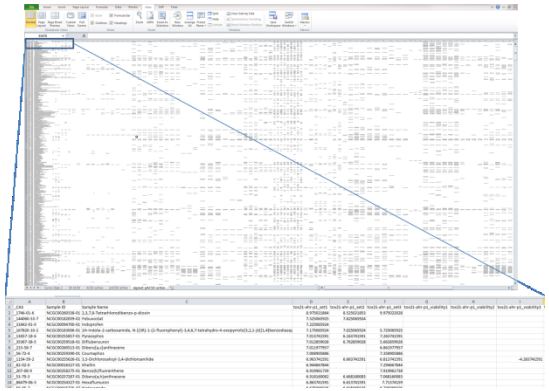


[J-H Hsieh (NTP): https://hsiehjh.shinyapps.io/tox21actprofiler](https://hsiehjh.shinyapps.io/tox21actprofiler)

Hit Rate of the ~1700 "Food-Relevant" Compounds in the Tox21 10K Library



Scott Auerbach/Dan Svoboda
10K qHTS BioActivity Network



Data

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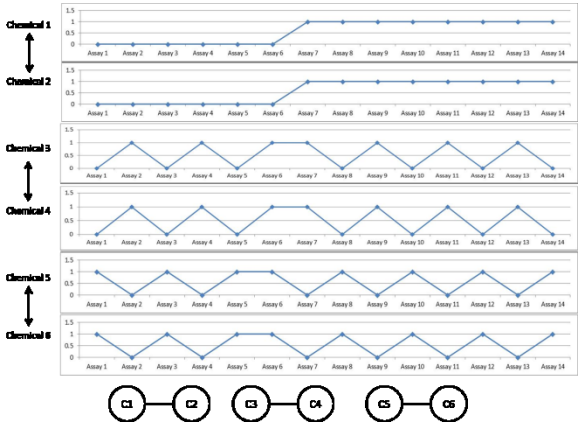

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Cytoscape

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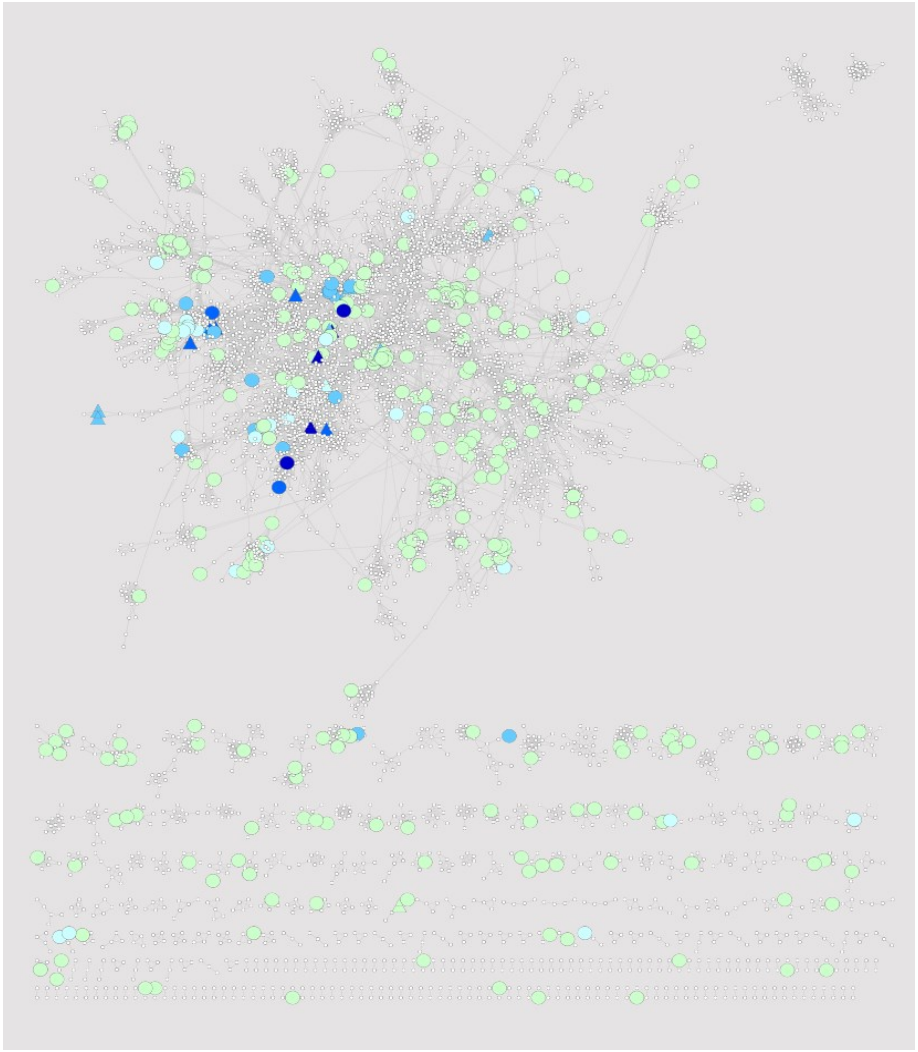
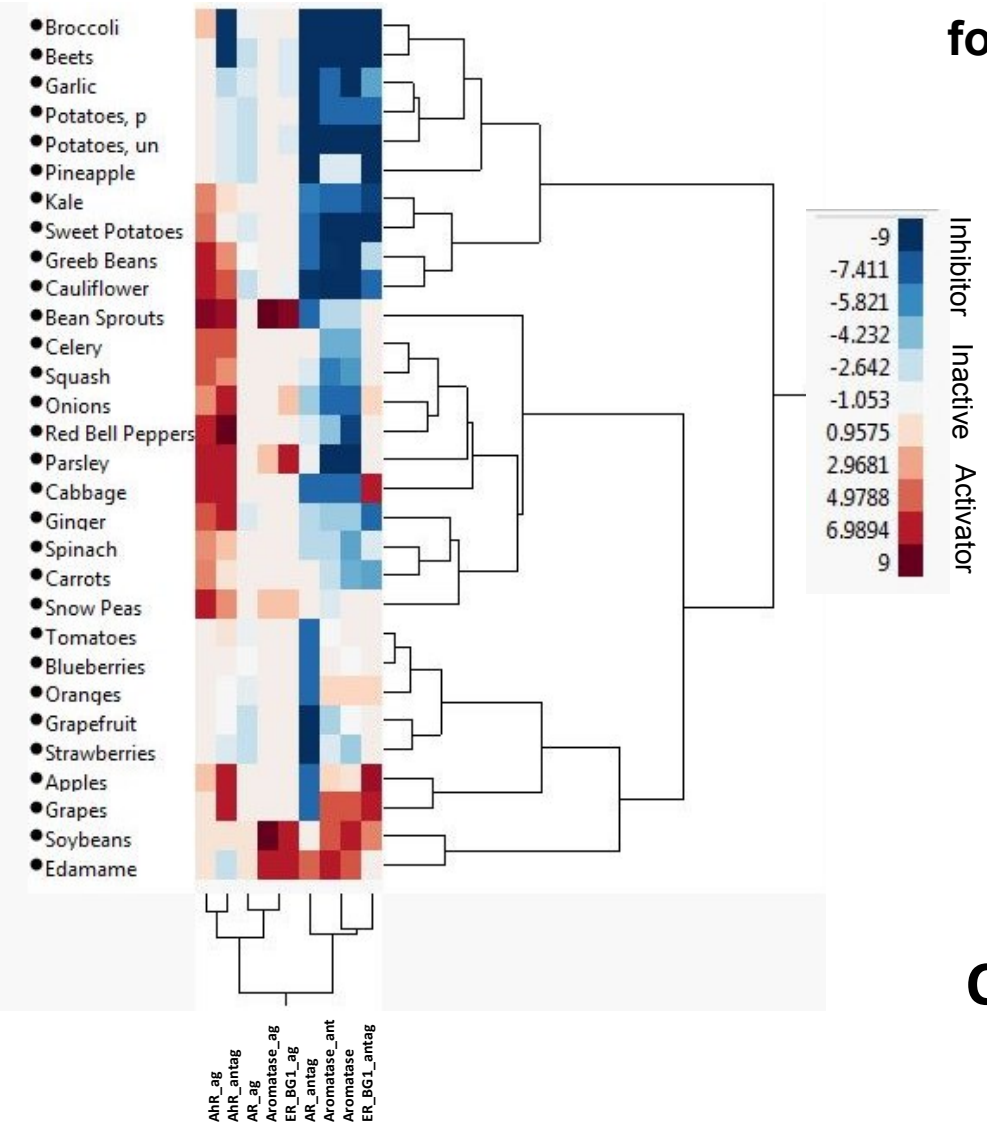


4-(1,1,3,3-Tetramethylbutyl)phenol (D. Svoboda)

Compound	CASRN	Pearson's Correlation
4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	0.96
4-Nonylphenol, branched	84852-15-3	0.88
Menthyl anthranilate	134-09-8	0.83
Lysergide	50-37-3	0.79
o,p'-DDD	53-19-0	0.79
Pyrrolnitrin	1018-71-9	0.77
Zearalanol	26538-44-3	0.77
Ethynodiol diacetate	297-76-7	0.73
Estradiol valerate	979-32-8	0.72
meso-Hexestrol	84-16-2	0.72
Bisphenol A	80-05-7	0.72
Diphenan	101-71-3	0.72
Celecoxib	169590-42-5	0.72
Estradiol acetate	4245-41-4	0.72
Dienestrol	84-17-3	0.72
Quinestrol	152-43-2	0.71
Hexadecyltrimethylammonium bromide	57-09-0	0.71
4-Octylphenol	1806-26-4	0.71
Allylestrenol	432-60-0	0.71
Androstenone	18339-16-7	0.70

Assessing Bioactivity-Exposure Profiles of Fruit and Vegetable Juices

Barbara Wetmore, The Hamner Institutes for Health Sciences, RTP, NC



Top 10 Most Active Juices

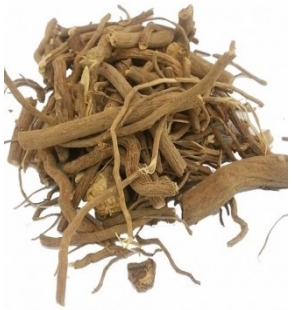
Agent	# Hits	Highly Potent	Potent	Moderately Potent
Beets	7	5	7	7
Garlic	7	1	6	6
Broccoli	7	1	6	6
Sweet Potatoes	6	2	5	6
Bean Sprouts	7	2	2	4
Green Beans	6	1	4	5
Cauliflower	8	0	2	5
Spinach	6	3	3	3
Kale	6	1	1	4
Potatoes (u)	7	0	2	5

Clustering of fruit and vegetable juices in NCATS qHTS assays

NTP High-throughput Screening of Botanicals

- Annato extract (10)
  - Bixin (2)
- Black walnut/Juglone (5)
- Cedarwood oil (2)
- Citral (5)
- Comfrey root (2)
- Corn oil (2)
- Curcumin (1)
- Echinacea purpurea (1)
- Emodin (6)
- Eugenol (1)
- Gallic acid (1)
  - Pyrogallol (3)
- Ginkgo biloba extract (4)
  - Kaempferol (2)
  - Quercetin (3)

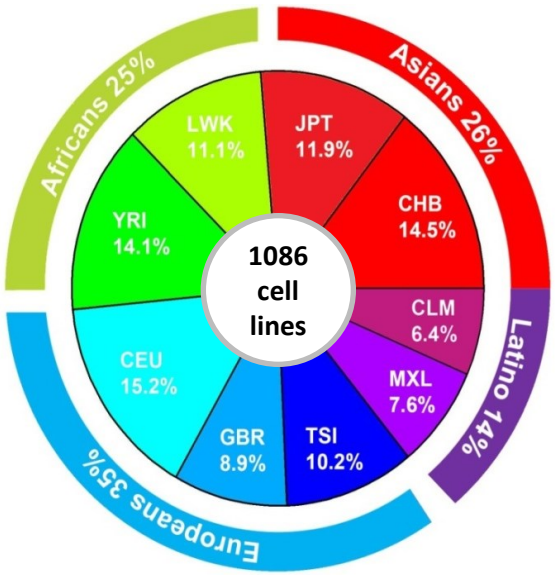
- Goldenseal root powder (9)
  - Berberine (1)
- Grape seed extract (4)
- Gum guggal extract (5)
  - Gugulipid (3)
- Kava Kava extract (6)
- Methyleugenol (4)
- Milk thistle extract (6)
  - Silybin (3)
- Olive oil (1)
- Pine bark extract (2)
- Pulegone (3)
- Resorcinol (3)
- Resveratrol (3)
- Safflower oil (2)
- Turmeric (4)
  - Curcumin (1)

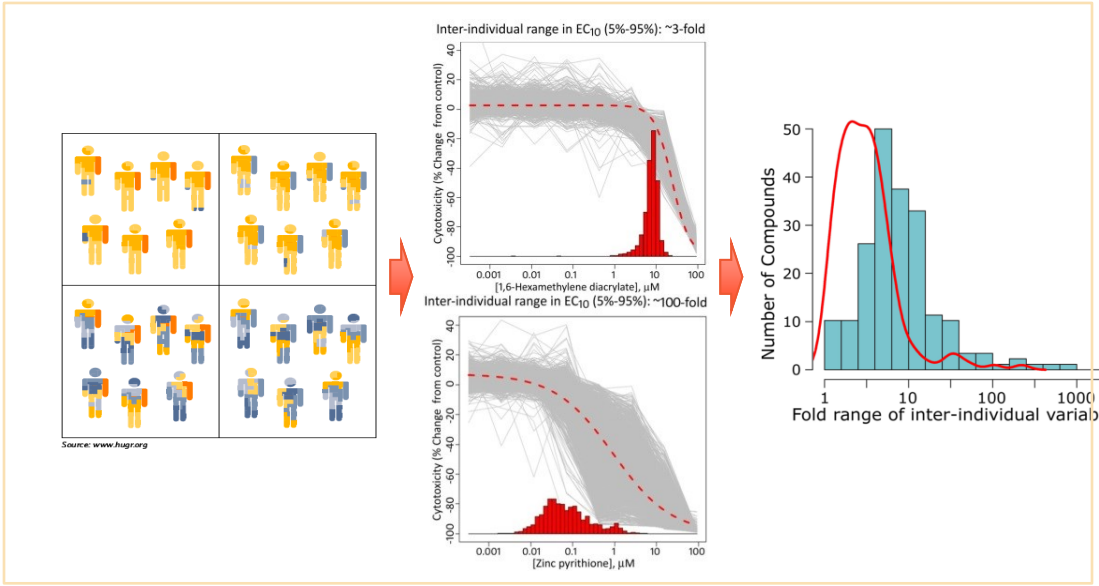


Cynthia Rider (NTP)

The 1000 Genomes qHTS Toxicity Screening Project

Population-wide study design (Collaboration with I. Rusyn at UNC-CH)





- **1086 Human lymphoblastoid cell lines representing 9 geographical/racial groups**
- 179 compounds **(9 duplicates)**
- 8 concentrations (0.33 nM - 92 µM)
- **1-3 plate replicates**
- 1 assay (CellTiterGLO® - ATP production)

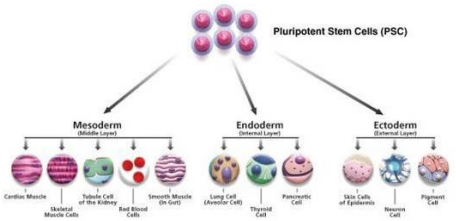
= ~2,400,000 data points + 2-5x10^6 SNPs

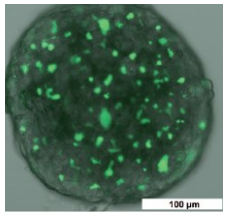
Abdo et al. (2015) EHP 123:458-466; DOI:10.1289/ehp.1408775.

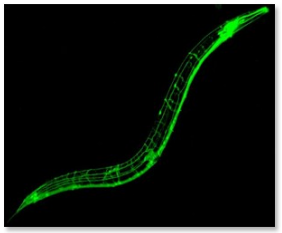
Eduati et al. (2015) Nature Biotech 33: 933–940; DOI:10.1038/nbt.3299

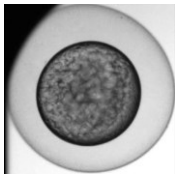
Tox21 Phase III – Improving on Biological Coverage and Relevance (2013 - ?)

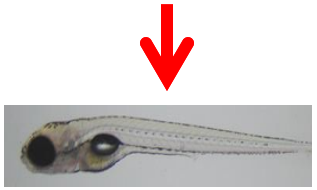
- Include more physiologically-relevant *in vitro* cell systems (e.g., human stem cell derived differentiated cell populations).
- Include cell types (e.g., HepaRG in 2D and 3D models) that incorporate xenobiotic metabolism/allow for longer-term exposures and measuring of metabolites and free concentrations.
- Increased use of *in silico* models and quantitative extrapolation approaches.
- Increased testing of formulated and natural mixtures.
- Increased use of alternative animal models (e.g., zebrafish, *C. elegans,* planaria).
- Development of a high throughput transcriptomics platform for human, rat, mouse, zebrafish, and *C. elegans*.











Development of a Tox21 "Sentinel" Gene Set
(cross agency working group led by Richard Paules, NTP)

- Developed approach & trained on available rat data sets
- Generated a human "S1500+" gene set
  - Draft human S1500+ gene set released to the scientific community for comment in the Federal Register on 4/15/15
  - Revised S1500+ (now 2753 genes) released on 9/23/15 at http://ntp.niehs.nih.gov/results/hts/s1500-gene-set/index.html
- A goal is to develop similar gene sets for rat, mouse, zebrafish, and *C. elegans* that focus on orthogonal pathways
- Currently evaluating various technological platforms (e.g., Luminex Beads, RASL-Seq / TempO-Seq, Agilent Sure-Select, Illumina NextGen Seq) for high throughput at low cost (goal is <$25/sample) with a relatively simple analysis pipeline.

The Future of Toxicology?

- An increased use of computational methods as more data are generated on more compounds in different biological platforms.
- An increased use of *in vitro* 3D models and organoids that better reflect human biology.
- An integrated systems biology approach based on high content screening and 'omics that can be applied to any cell type *in vitro* or *in vivo,* which will allow for an assessment of pathway driven alterations that represent tipping points leading to adverse health outcomes.
- This will:
  - allow for better prediction of human health effects while taking into account variability in sensitivity across cell types, states of differentiation, developmental windows, and individual susceptibility.
  - allow for better extrapolation of results from other species.

Tox21: A Collaboration of Many ……

Biomolecular Screening Branch and NTP Colleagues