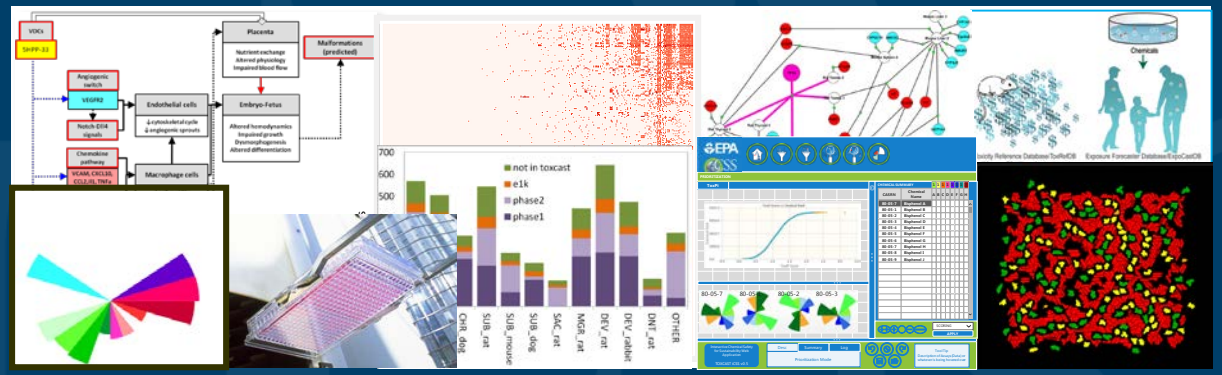
Predictive Toxicology: Current Status and Future Outlook



EBI-EMBL Industry Programme Workshop
Predictive Toxicology

November 16-17, 2016

Imran Shah

National Center for Computational Toxicology

The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

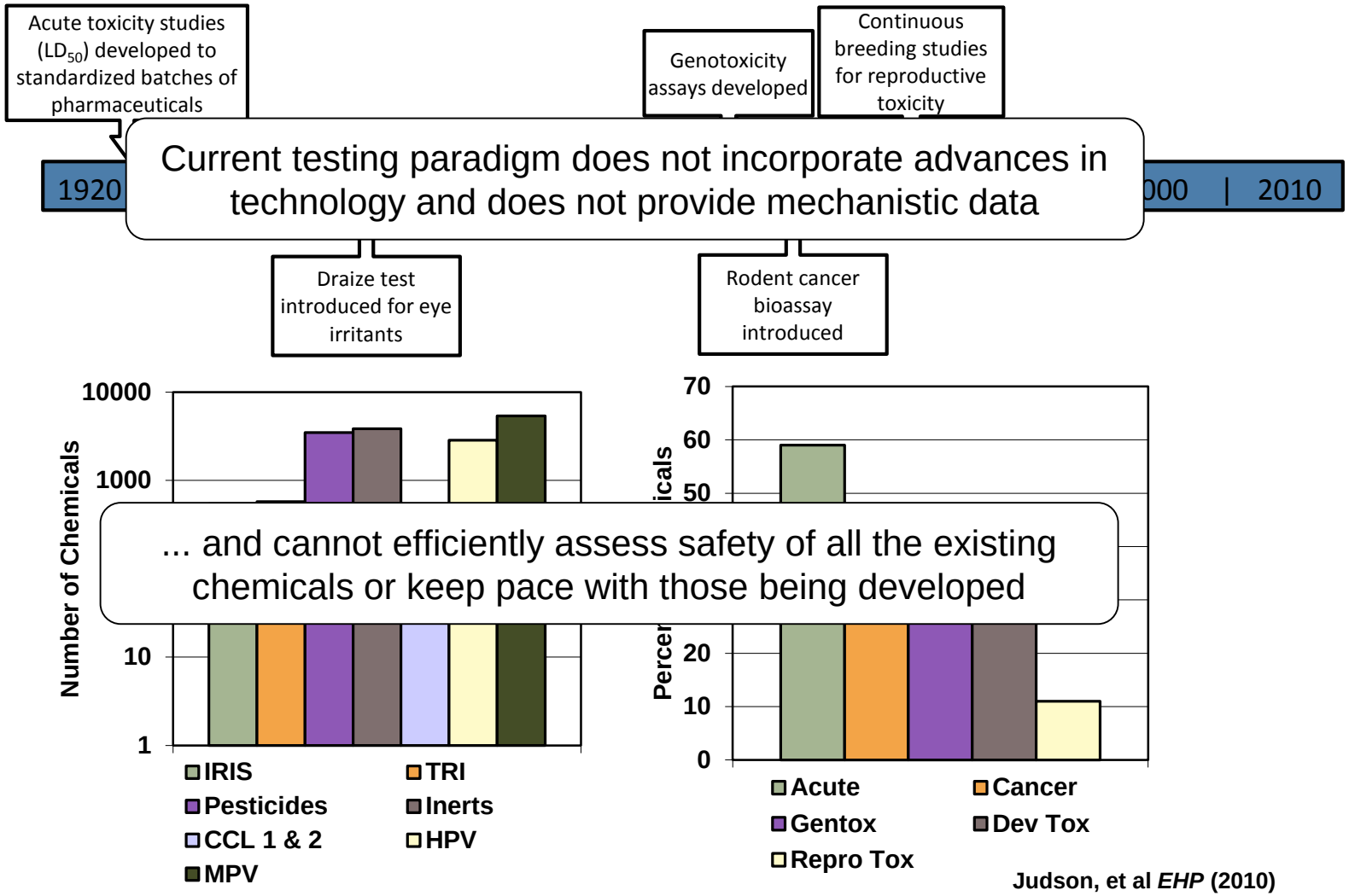
Regulatory Agencies Need to Make A Range of Decisions on Chemicals…

- Multiple drivers shape type of assessment
  - Regulatory scope
  - Economic considerations
  - Multiple applications
- Chemical assessments are “fit-for-purpose”
  - Prioritization (e.g., EDSP, PMN, SNUR)
  - Screening-level assessments (e.g., CCL, GreenChem)
  - Provisional assessments (e.g., PPRTVs)
  - Toxicity assessments (e.g., IRIS)
  - Risk assessments (e.g., MCLs, pesticides)

EPA	Workflow	Throughput	Data
OPPT	New chemicals: Premanufacture Notice (PMN) Existing chemicals: Significant New Use Rule (SNUR)	~1000/yr (90d/chem) ~84,000 total	**III (II)**
	Current Chemical Risk Assessments	~10 total	**I**
	DFE / Green Chemistry	~2500	**I, II, III**
OPP	Pesticide registration (PR)	~10 new/yr ~50 old/yr	**I**
	Pesticide re-registration	~1000/yr 24,576 total	**I**
OW	Chemical Contaminant List	6yr / ~6,000 total	**I,II,III**
	RegDet on CCL	Every 6yr / 90 total	**I**
	Unregulated Contaminant Monitoring	30/5yr	**I**
	Drinking Water Health Advisories		**II, III**
OLEM	**Spills** **Brownfields** **Super Fund**		

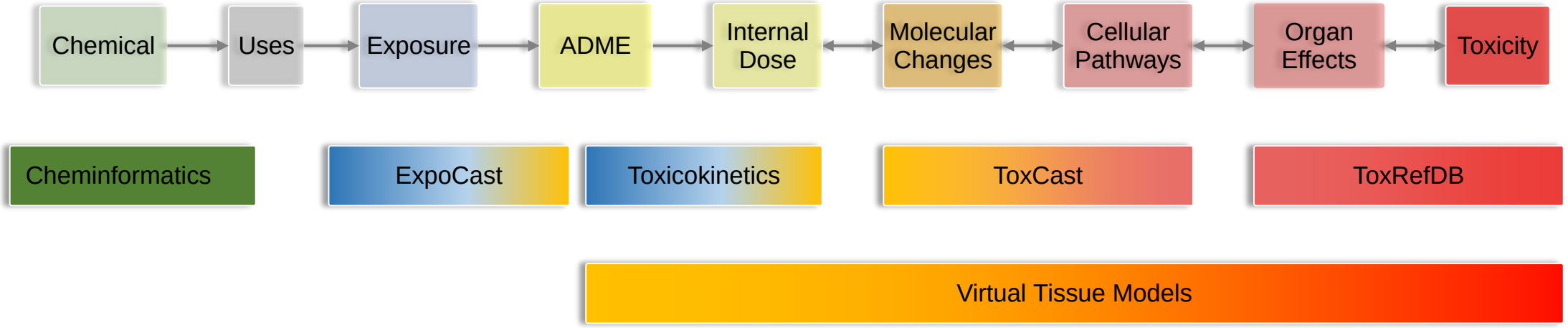
I Guideline animal testing data
II Some *in vitro* bioactivity
III Chemical structure data

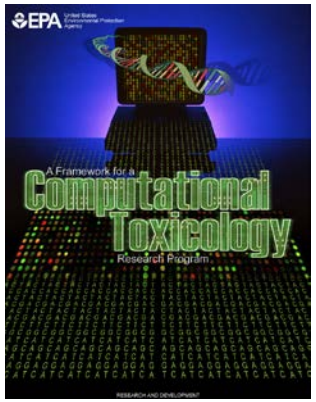
Current System to Evaluate Chemicals is Antiquated and Inefficient



Judson, et al *EHP* (2010)

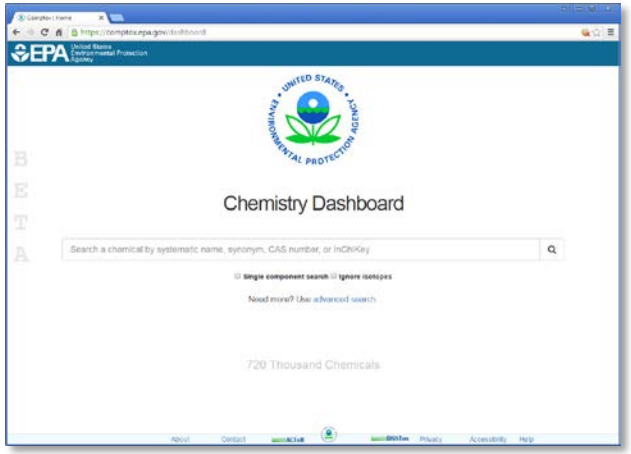
Requires an Integrated and Multidisciplinary Solution



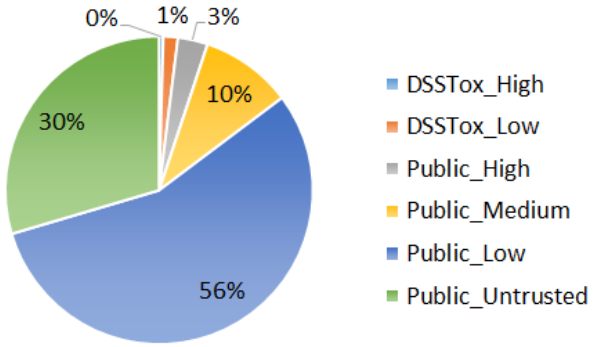


- National Center for Computational Toxicology established in 2005
- Currently staffed by ~60 employees
- Exists within the EPA's Office of Research and Development
- Home of the ToxCast and ExpoCast research efforts
- Key partner in U.S. Tox21 federal consortium

Need to Start with a High Quality Chemistry Foundation

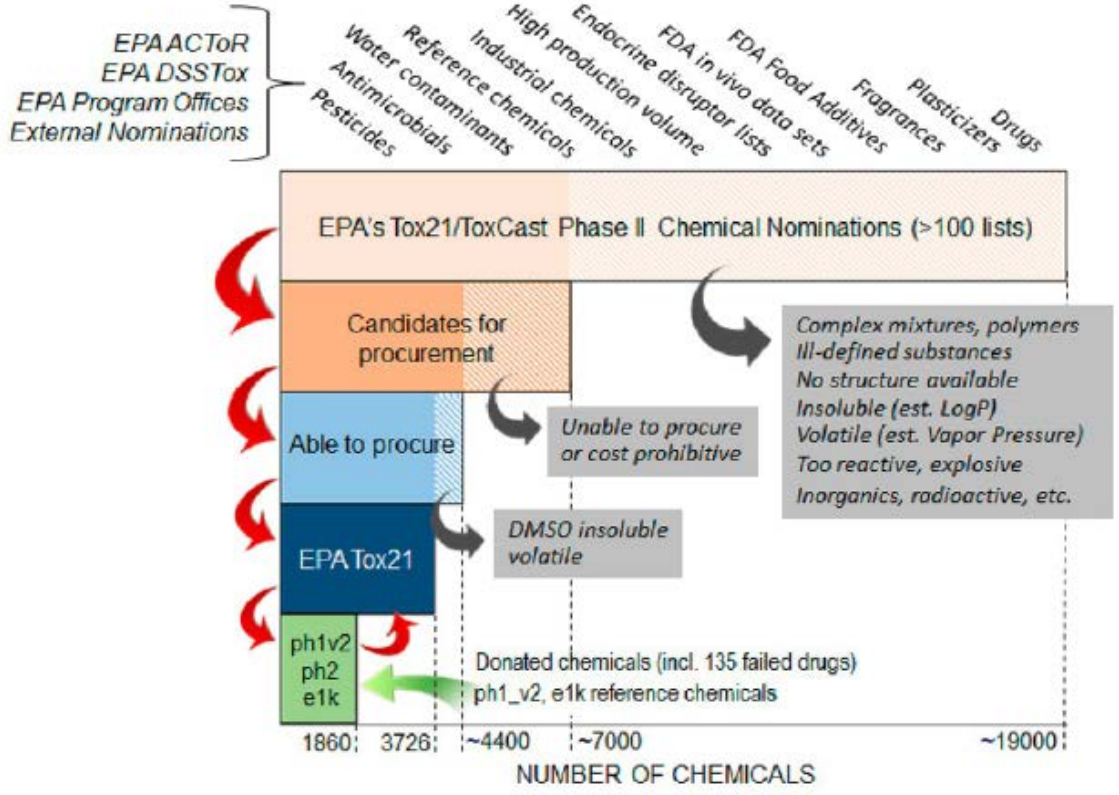


https://comptox.epa.gov



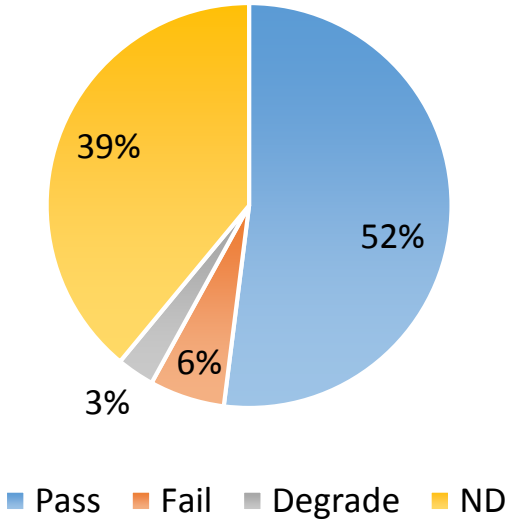
- Developing a centralized resource for curated chemical structure, identifier, and physical chemical properties of >700K unique substances with data quality flags
- Expand and curate training sets for QSAR models for phys-chem, environmental fate, and toxicological properties
- Use the centralized chemical resource as the foundation for an integrated hazard, bioactivity, pharmacokinetics, and exposure information

Need to Start with a High Quality Chemistry Foundation



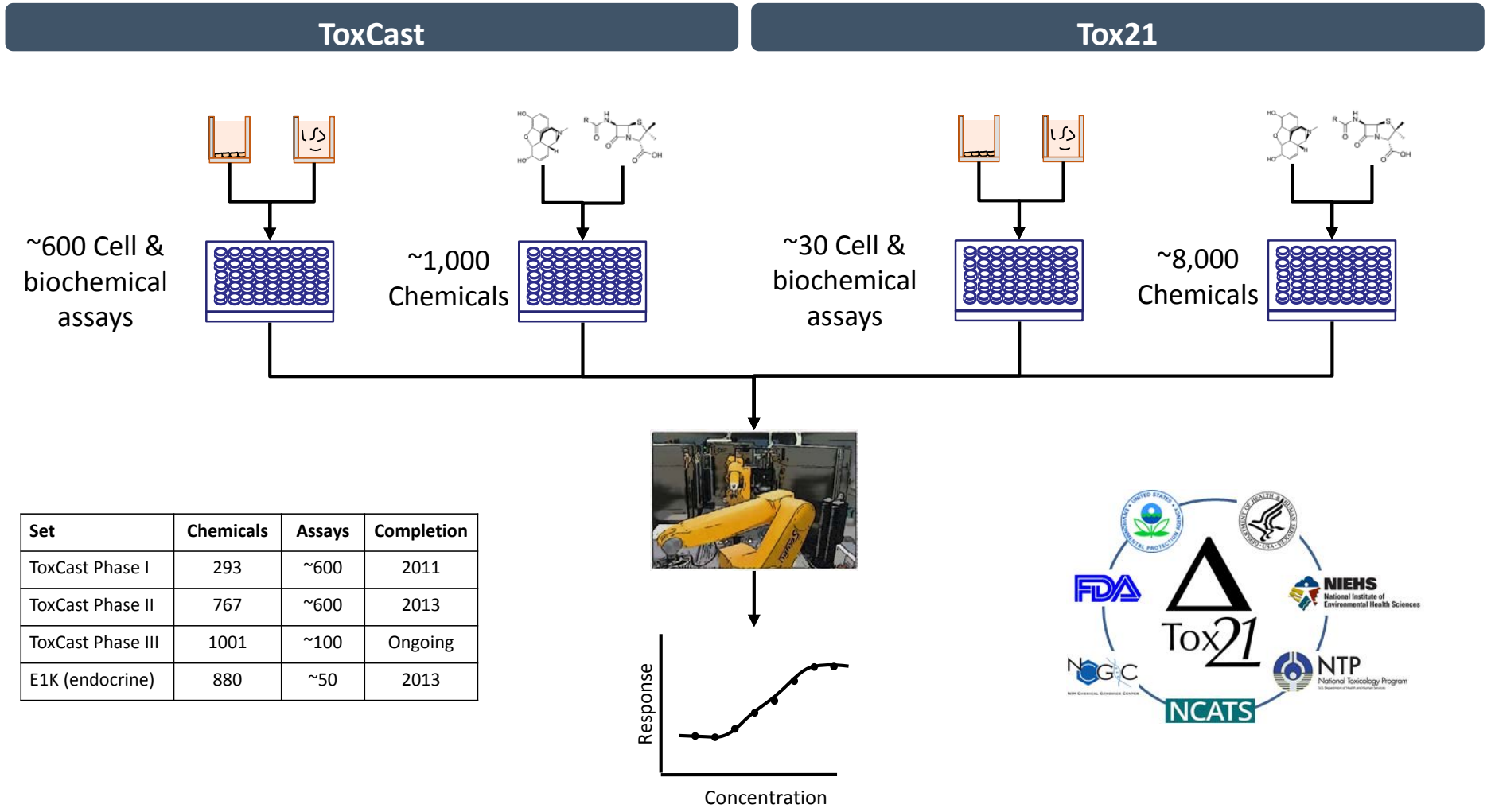
Richard et al., Chem Res Toxicol, 2016

Analytical QC of Chemical Library



Pass = C (75%) or greater
Fail = D, F, Ac, Bc, Cc

High-Throughput Bioactivity Screening



Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013

ToxCast Incorporates a Diverse Array of High-Throughput *In Vitro* Assays

United States Environmental Protection Agency

Assay Provider
- ACEA
- Apredica
- Attagene
- BioReliance
- BioSeek
- CeeTox
- CellzDirect
- Tox21/NCATS
- NHEERL MESC
- NHEERL Zebrafish
- NovaScreen (Perkin Elmer)
- Odyssey Thera
- Vala Sciences

Biological Response
- cell proliferation and death
- cell differentiation
- Enzymatic activity
- mitochondrial depolarization
- protein stabilization
- oxidative phosphorylation
- reporter gene activation
- gene expression (qNPA)
- receptor binding
- receptor activity
- steroidogenesis

Target Family
- response Element
- transporter
- cytokines
- kinases
- nuclear receptor
- CYP450 / ADME
- cholinesterase
- phosphatases
- proteases
- XME metabolism
- GPCRs
- ion channels

Assay Design
- viability reporter
- morphology reporter
- conformation reporter
- enzyme reporter
- membrane potential reporter
- binding reporter
- inducible reporter

Readout Type
- single
- multiplexed
- multiparametric

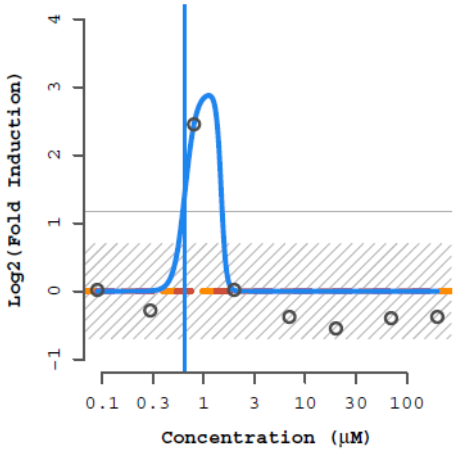
Cell Format
- cell free
- cell lines
- primary cells
- complex cultures
- free embryos

Species
- human
- rat
- mouse
- zebrafish
- sheep
- boar
- rabbit
- cattle
- guinea pig

Tissue Source
- Lung
- Liver
- Skin
- Cervix
- Uterus
- Intestinal
- Bladder
- Pancreas
- Inflammatory
- Breast
- Vascular
- Kidney
- Testis
- Brain
- Spleen
- Ovary
- Prostate
- Bone

Detection Technology
- qNPA and ELISA
- Fluorescence & Luminescence
- Alamar Blue Reduction
- Arrayscan / Microscopy
- Reporter gene activation
- Spectrophotometry
- Radioactivity
- HPLC and HPEC
- TR-FRET

Efforts to Ensure HTS Data Quality and Increase Transparency

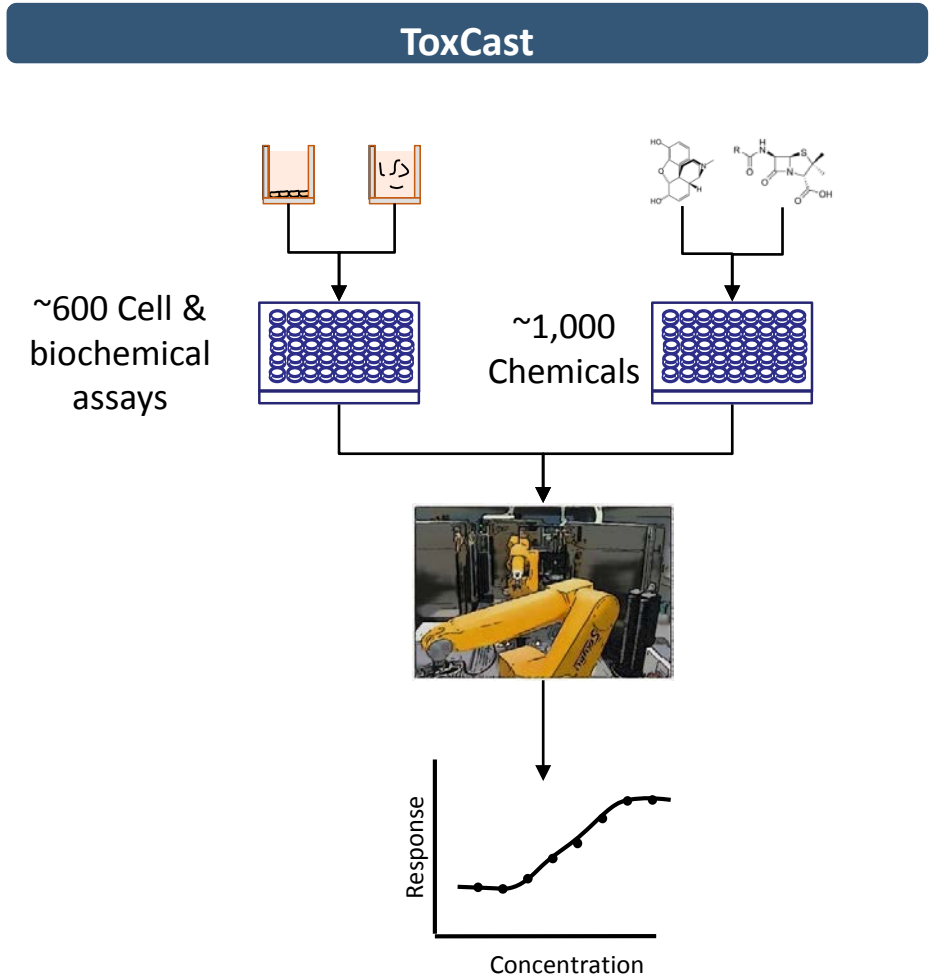


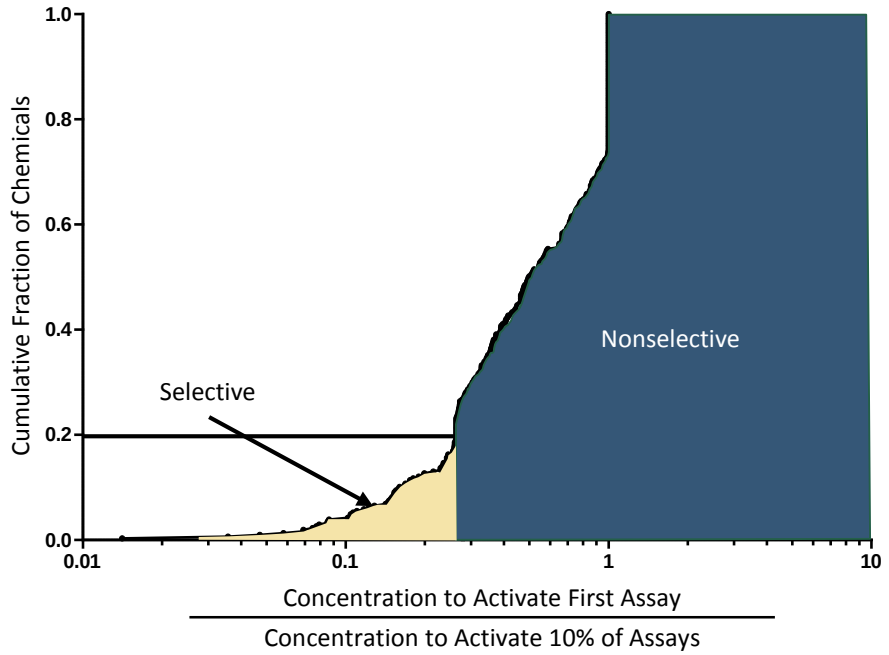

```
ASSAY:   AEID117 (ATG_ERa_TRANS)

NAME:    Thioglycolic acid
CHID:    26141   CASRN: 68-11-1
SPID(S): TX007664
L4ID:    420385

HILL MODEL (in red):
        tp        ga        gw
val:  3.1e-11   -2.15     0.416
sd:   NaN       NaN       NaN

GAIN-LOSS MODEL (in blue):
        tp        ga        gw        la        lw
val:  2.93      -0.184    8         0.173     18
sd:   3.56      0.334     9.48      5.82      814

        CNST        HILL        GNLS
AIC:  20.14         26.14       17.79
PROB: 0.23          0.01        0.76
RMSE: 0.92          0.92        0.32

MAX_MEAN: 2.45      MAX_MED: 2.45      BMAD: 0.233

COFF: 1.17   HIT-CALL: 1    FITC: 50   ACTP: 0.77
```

```
FLAGS:
Only one conc above baseline, active
Borderline active
```

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Transparent ToxCast data analysis pipeline
  - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
  - Publicly available as an R package
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
  - Chemical Procurement and QC
  - Data Analysis
  - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline

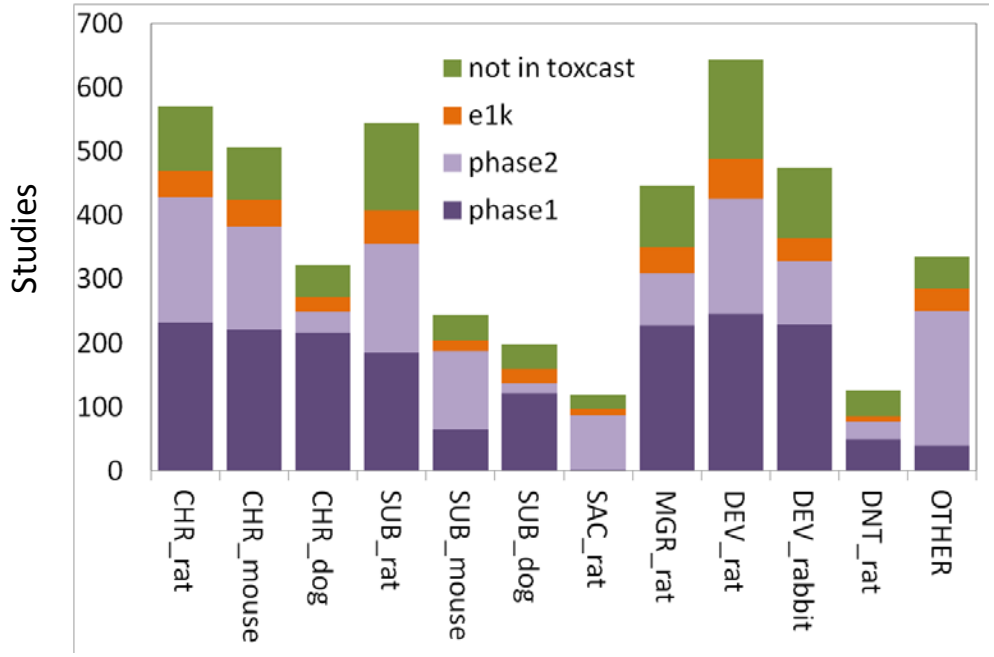
Most Environmental Chemicals are Nonselective for Biological Targets





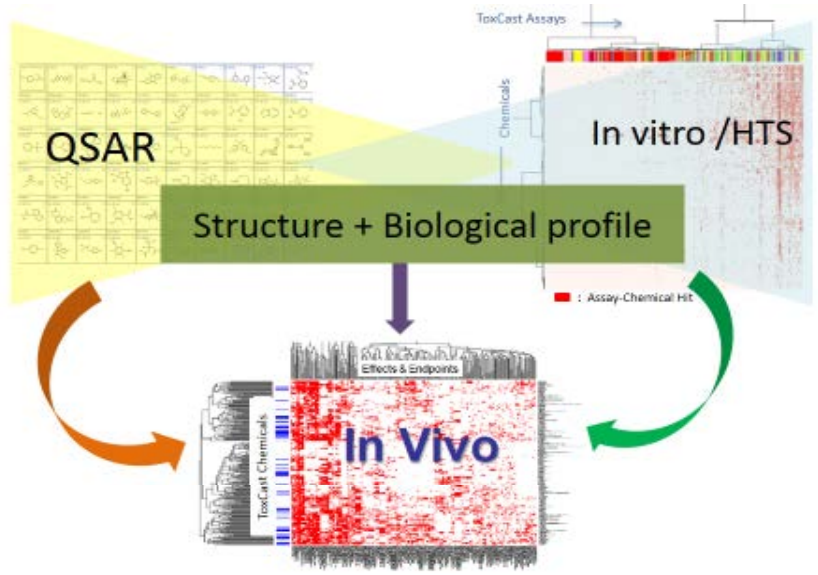
ToxRefDB: Digitizing Legacy *in vivo* Toxicology Data

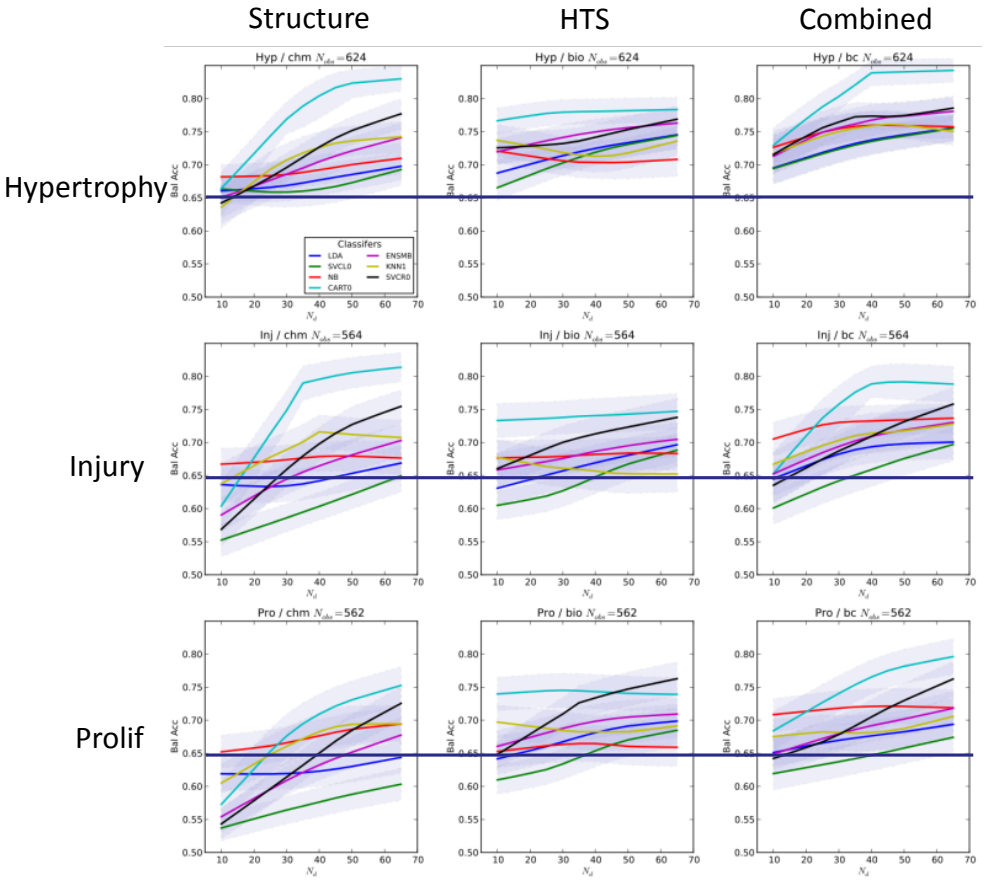
- ToxRefDB holds in vivo endpoint data from animal toxicology studies (DERs, NTP, open literature, pharma)
- Currently at 5567 studies on 1049 unique chemicals



Data Source	Study Count
EPA OPP DER	3279
Open Literature	731
National Toxicol Program	666
Sanofi_Pharma	222
Unpublished Submissions	50
GSK Pharma	38
Health Canada PMRA DER	23

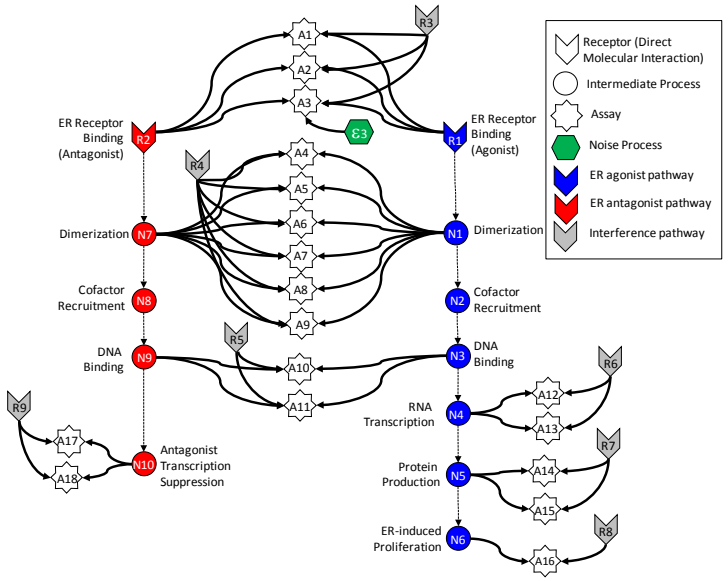
Predicting Target Organ Toxicities by Machine Learning





Liu *et al.*, CRT 2015

Developing a Pathway Model to Predict Endocrine Activity



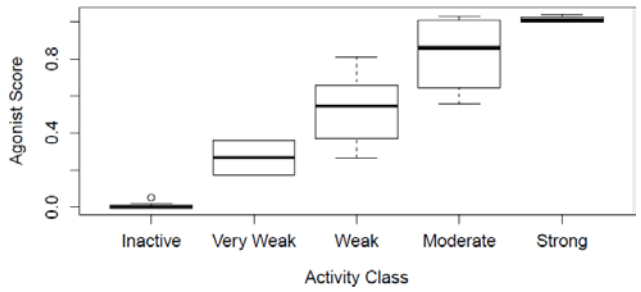
ER Pathway Model Integrating 18 *In Vitro* Assays

Performance for *In Vitro* Reference Chemicals

# True Pos	28
# True Neg	12
# False Pos	0
# False Neg	4
PPV	1.0
NPV	0.75
BA	0.94
Sensitivity	0.88
Specificity	1.0

Performance for *In Vivo* Uterotrophic Studies

# True Pos	28
# True Neg	12
# False Pos	1
# False Neg	1
PPV	0.97
NPV	0.92
BA	0.95
Sensitivity	0.97
Specificity	0.92

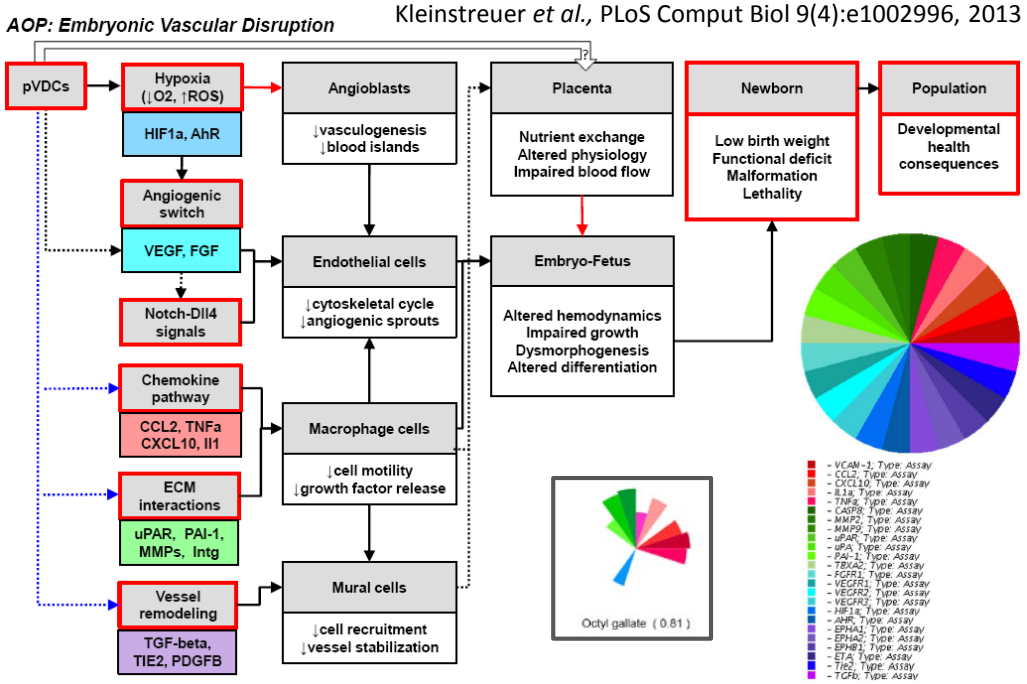


Judson *et al.,* ToxSci (in press)

ER Active Hit Rate

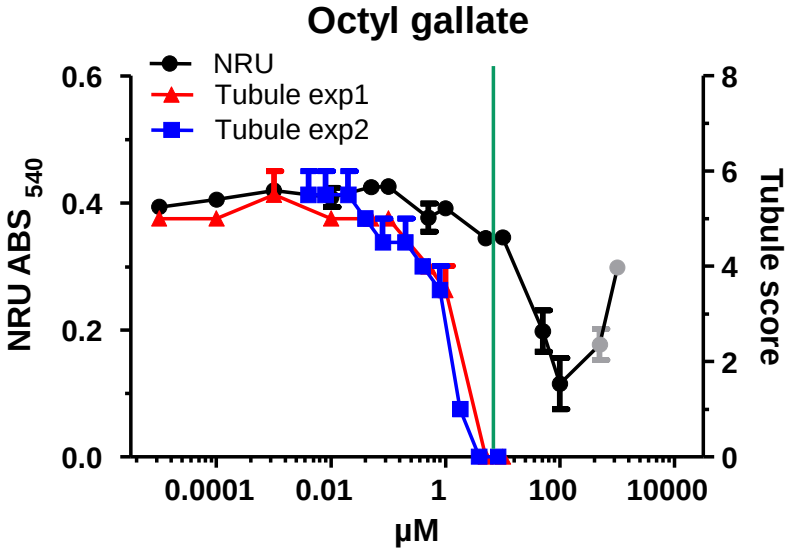
1431 EDSP chemicals run *in vitro*
71 (5%) have a significant ER score

Systems Biology Models To Scale Targets to Pathways and Networks – Virtual Tissues



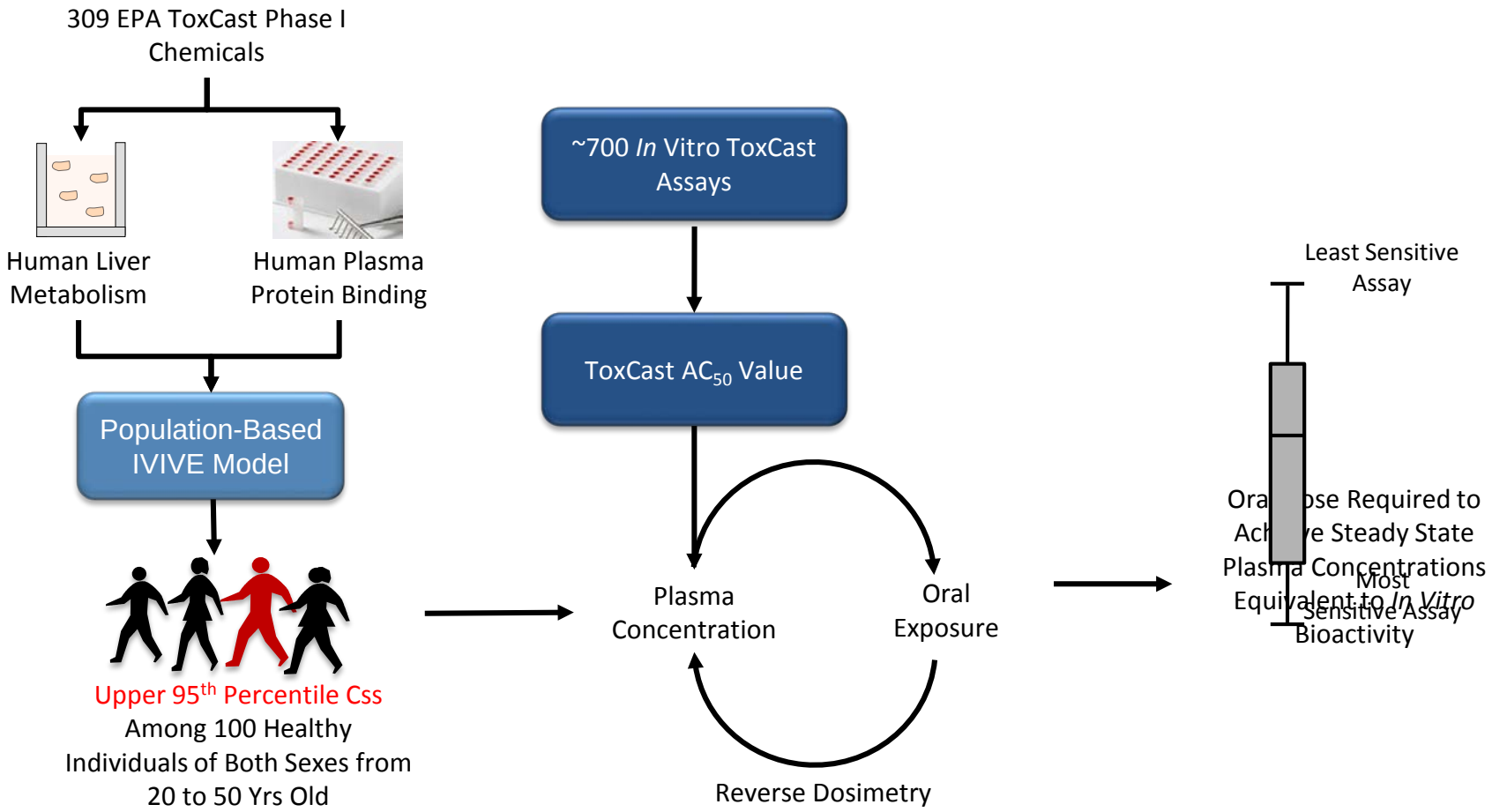
Kleinstreuer *et al.,* PLoS Comput Biol 9(4):e1002996, 2013

Human Tubulogenesis Assay (FICAM: T Heinonin)



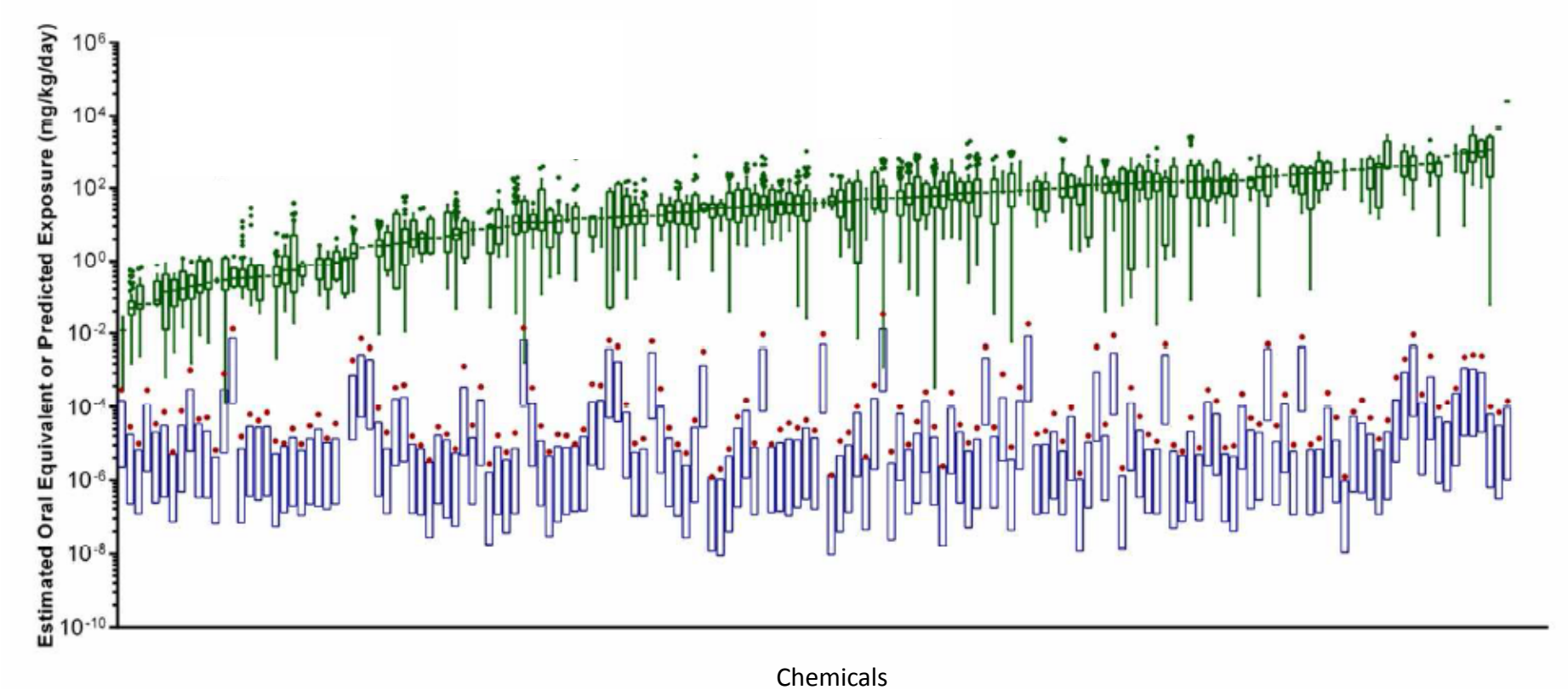
Cell ABM of Octyl Gallate (NCCT: G Nagaraj)

Providing Context by Incorporating Toxicokinetics and Exposure



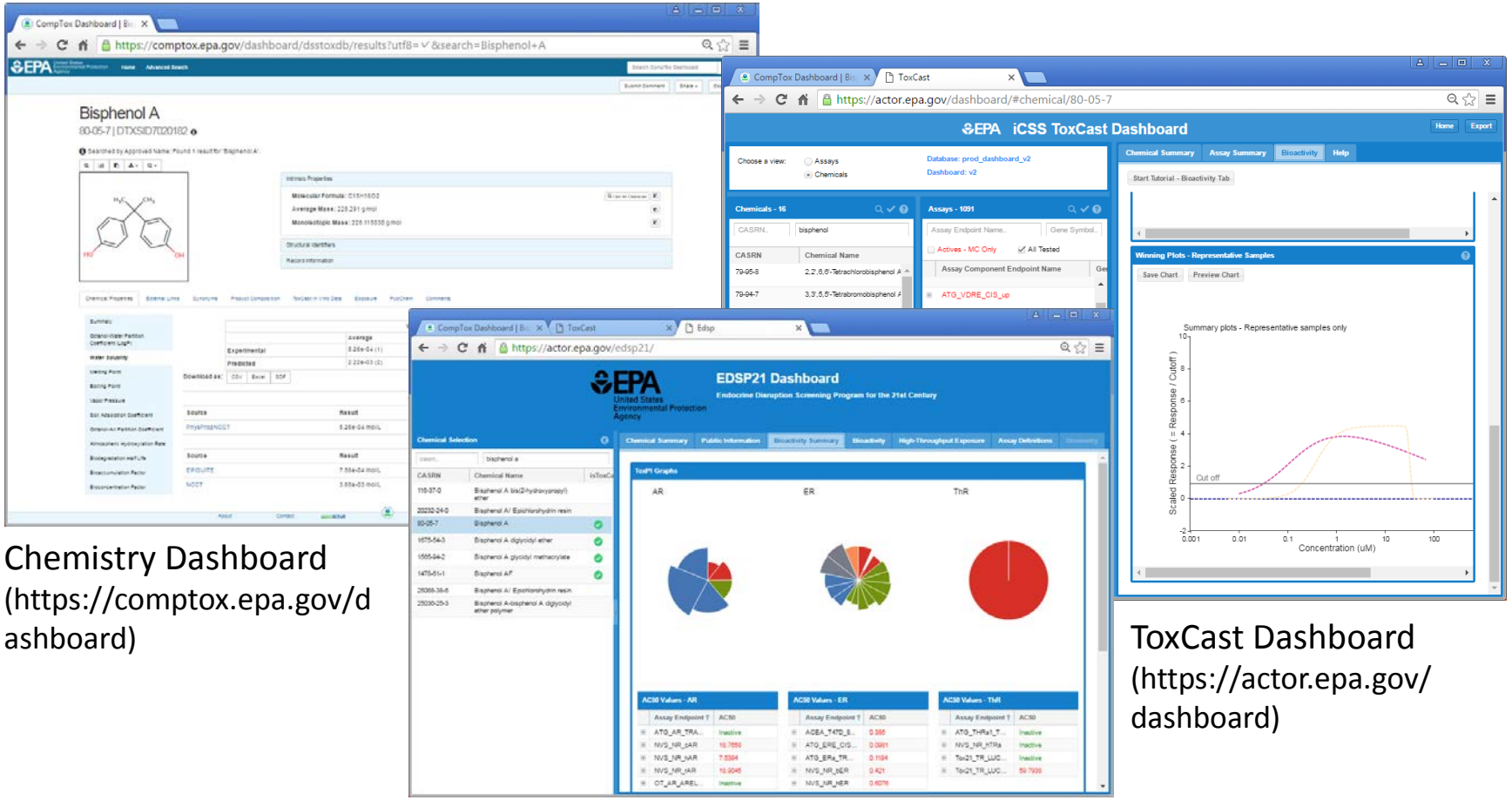
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

Comparing Bioactivity with Exposure Predictions for Risk Context



Wetmore *et al., Tox Sci.,* 2015

Delivering Data to Stakeholders and Scientific Community

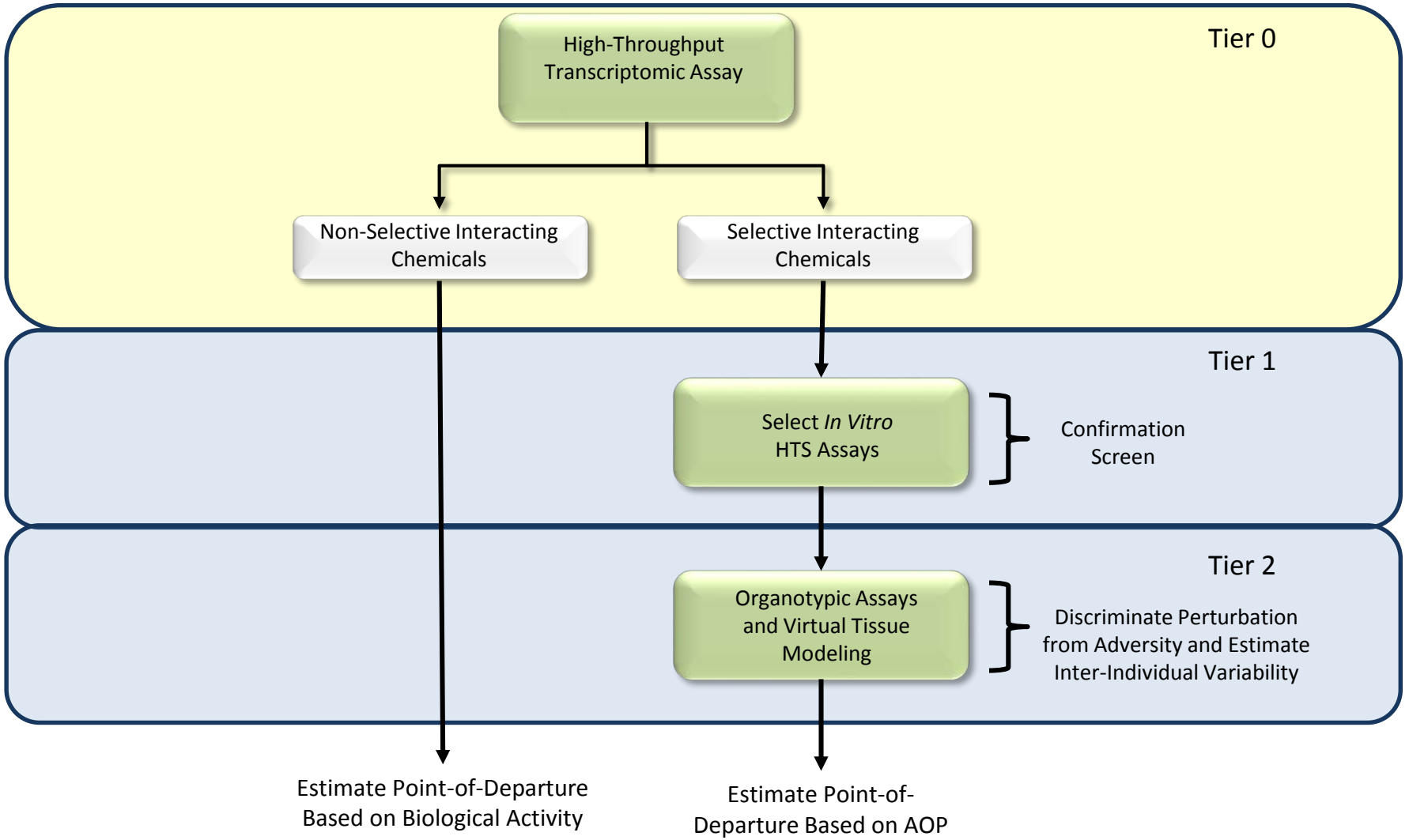


Chemistry Dashboard (https://comptox.epa.gov/dashboard)

ToxCast Dashboard (https://actor.epa.gov/dashboard)

EDSP21 Dashboard (https://actor.epa.gov/edsp1)

Developing a Broad Hazard Screening Platform

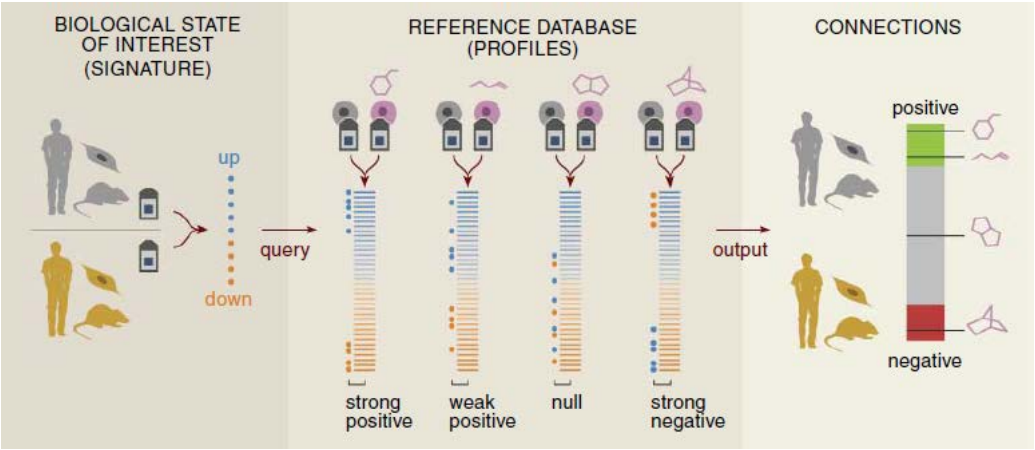


How Would a HT Transcriptomic Platform be Deployed?

Tier 0

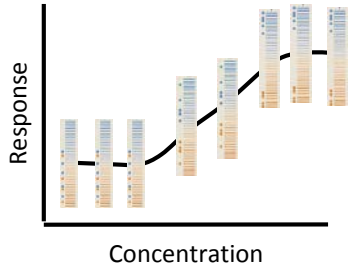
High-Throughput Transcriptomic Assay

- **Identify predominant mechanisms as a function of concentration**
- Group chemicals by similar mechanism/bioactivity
- Identify a concentration that results in no transcriptional effects

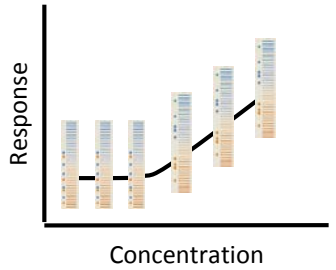


Lamb et al. *Science* (2006)

Broad CMAPdb: 7,000 profiles; 1,309 compounds
NIH LINCs CMAPdb: 9,000 shRNAs, 3,000 over expression ORFs, and 4,000 compounds in 20 cell types/lines (cell lines and primary cells)



Cell Type #1



Cell Type #2

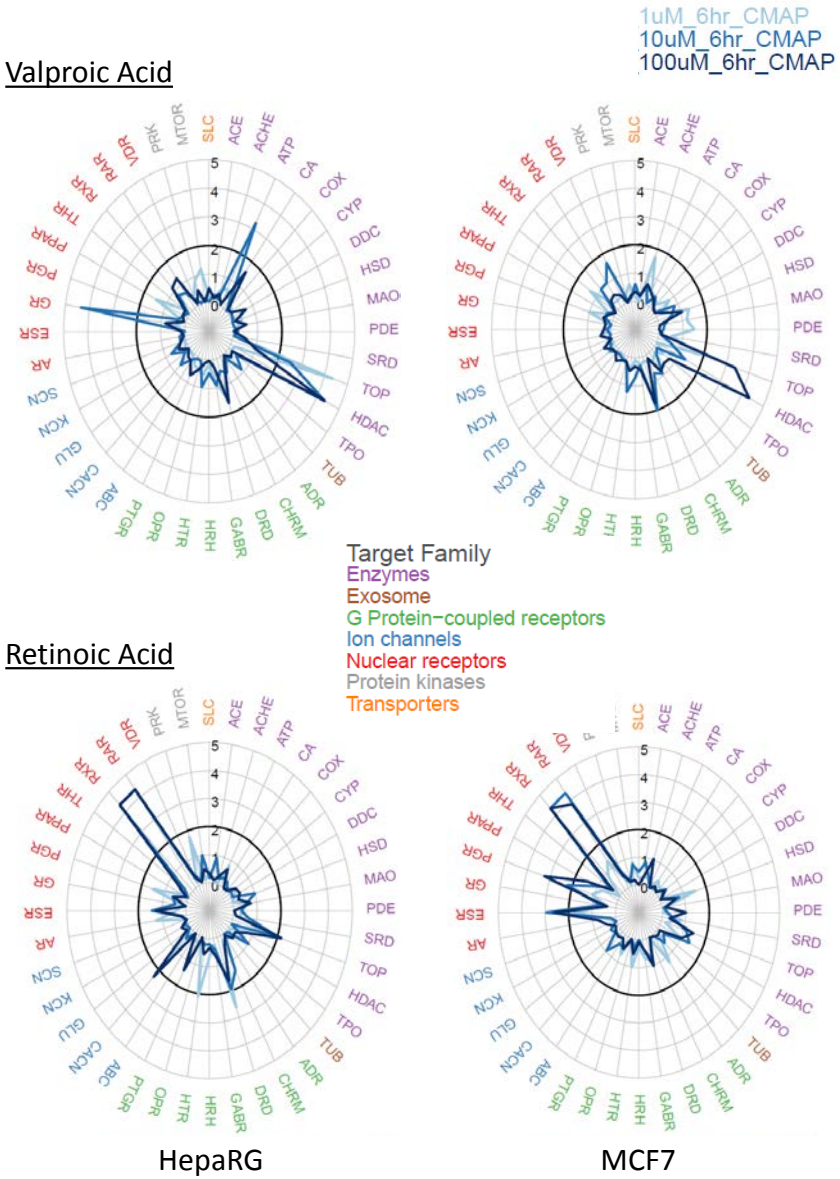
Cell Type #3

...

Using HT Transcriptomics to Identify Mode-of-Action

Target Family	Total Profiles	Target Genes	Chemicals	Cell Lines
Cytokine receptors	3	1	1	3
Enzymes	336	40	112	5
Exosome	14	1	4	4
G protein-coupled receptors	585	16	192	4
Ion channels	194	8	65	3
Nuclear receptors	227	10	71	5
Protein kinases	19	8	6	4
Transporters	102	2	35	3

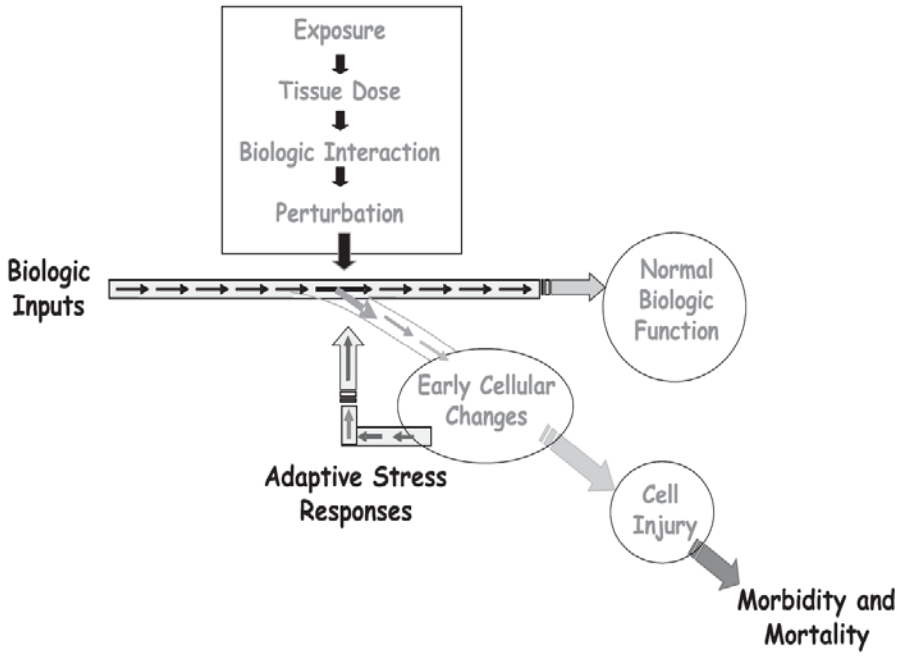
- Developed local database of Broad's CMAP data (~3,000 profiles)
- Annotated targets using KEGG (1,571 profiles)
- Significant genes identified using a z-score cutoff of 2
- Incorporated "JG" scoring method (Jiang and Gentleman 2007)
- Determine significance using a permuted rank approach across target family



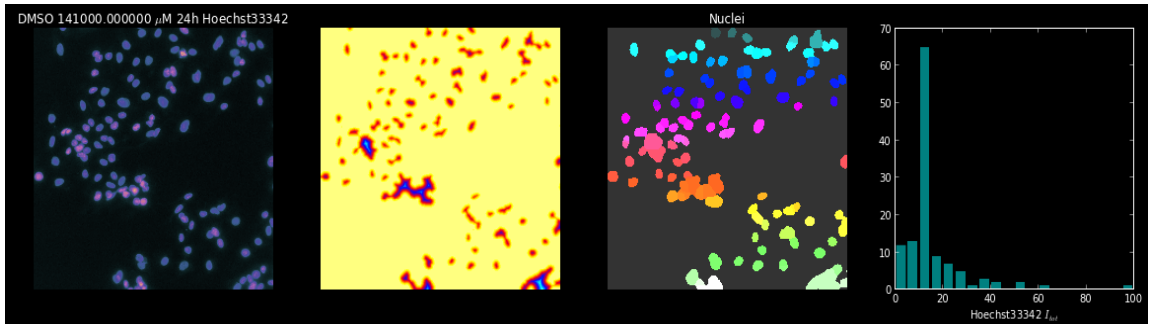
Martin and Karmaus, Unpublished

Distinguishing Adaptation from Adversity

- **Tipping Point**: Threshold between adaptation and adversity



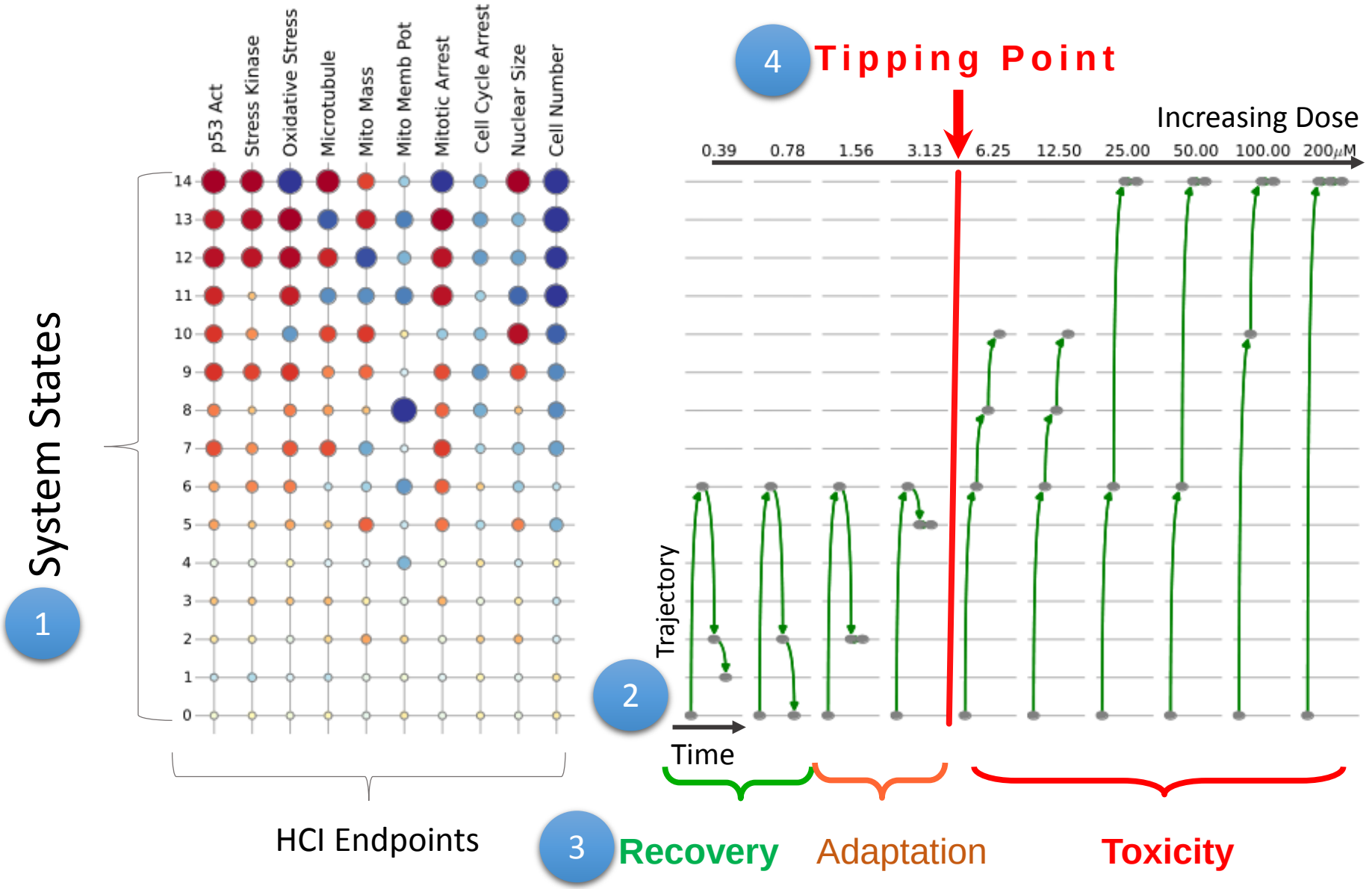
- Use ToxCast High Content Imaging (HCI) data to identify Tipping Points



- 967 chemicals (ToxCast)
- HepG2 cells culture
- 10 concentrations
- 3 Time points
- 10 HCI Assays
- 400 plates
- 100,000 wells
- 2,400,000 images

- Can we use **Tipping Point** to define a point of departure (PoD) for risk assessment ?

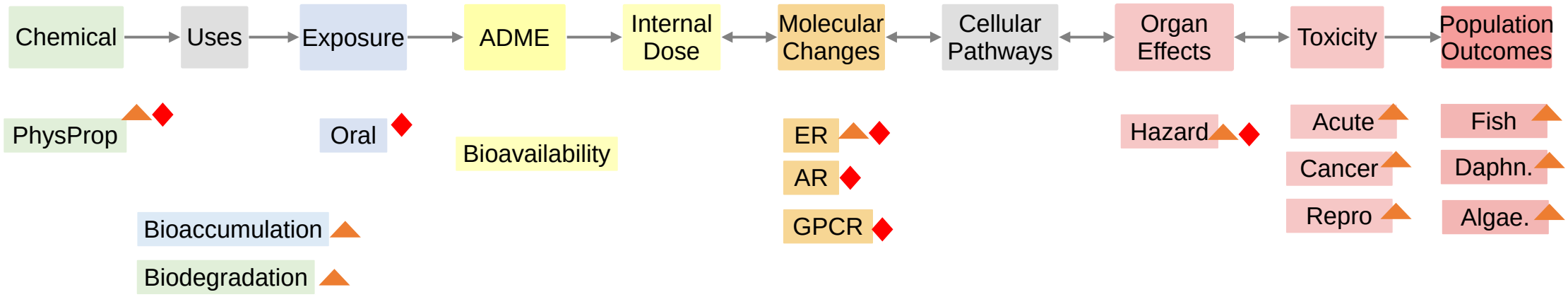
Tipping Point Analysis



Shah et al EHP 2016

Thousands of chemicals have limited data!

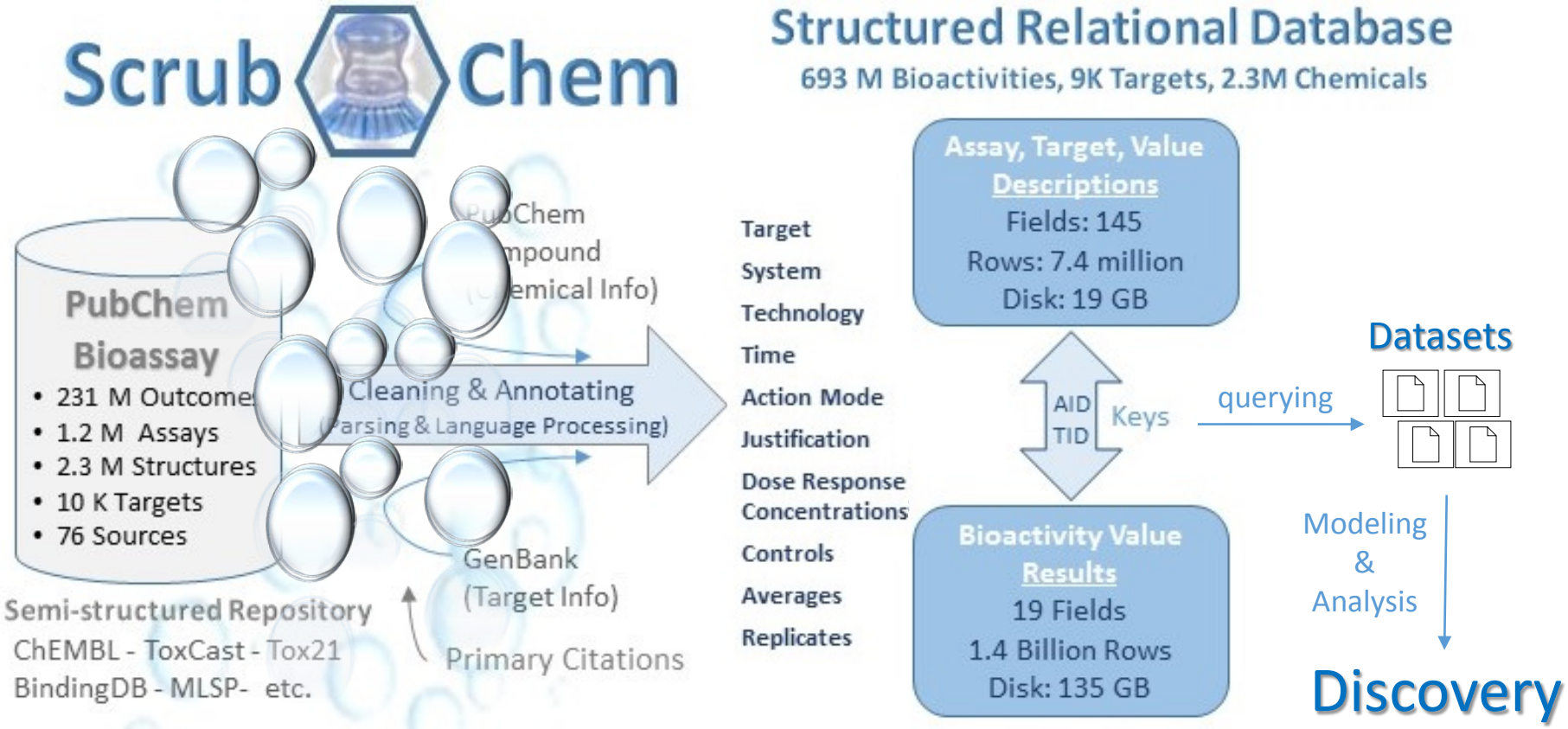
- Tens of thousands of environmental chemicals have very limited exposure/biological data
  - → Need more effective tools to describe chemical properties, effects and linkages
  - → Need predictive models to fill data gaps



▲ existing/legacy tools: TIMES, LeadScope, ECOSAR, EPIWIN

◆ developed internally: Read-across/GenRA, Machine Learning: classification and regression

Structuring PubChem Data for Analysis

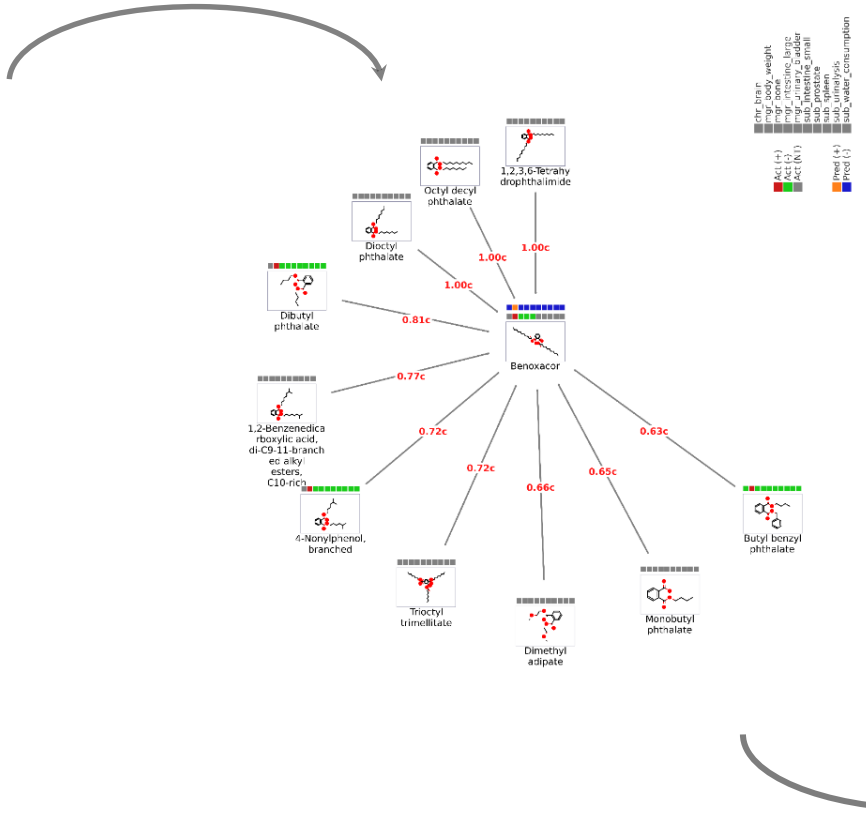


Jason Harris

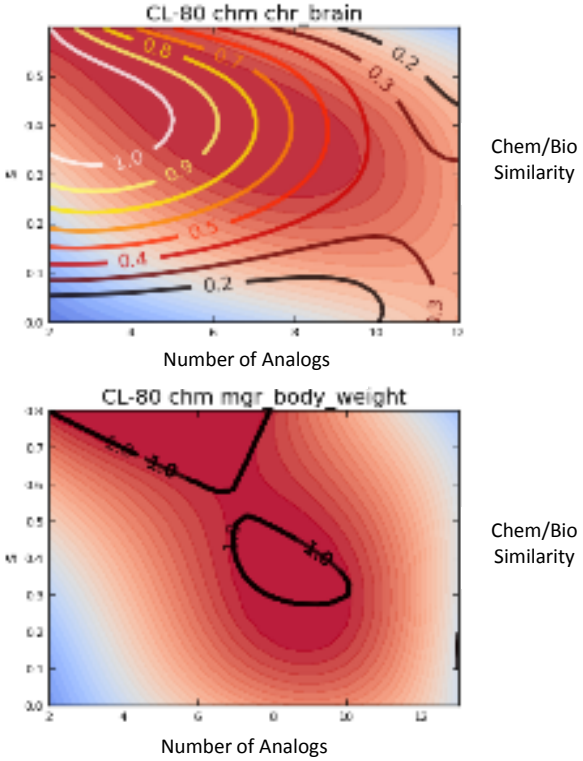
Generalised Read-across (GenRA)

Chemicals are clustered on the basis of chemical descriptors to identify local neighbourhoods

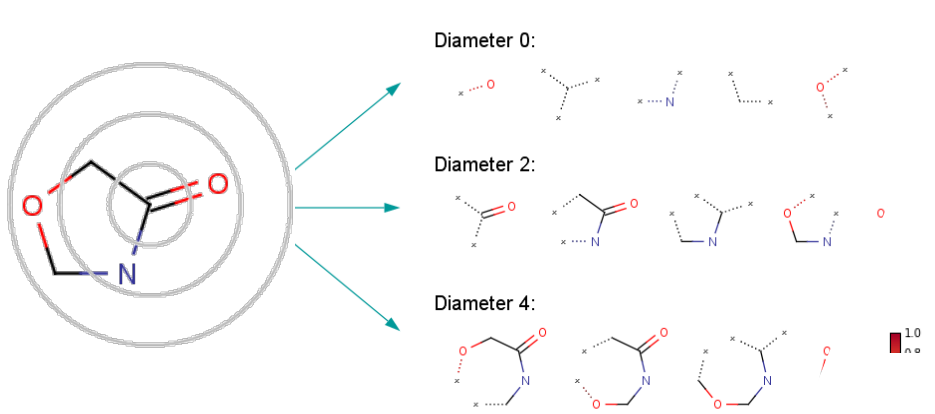
The Read-across toxicity prediction is a similarity-weighted activity of nearest neighbours based on chemistry and bioactivity descriptors

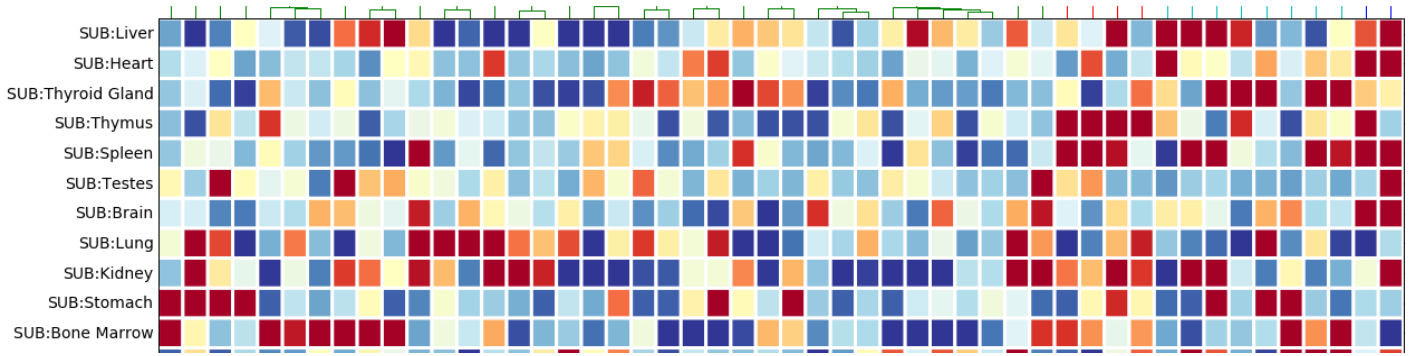


Uncertainties can be evaluated across the local neighbourhoods

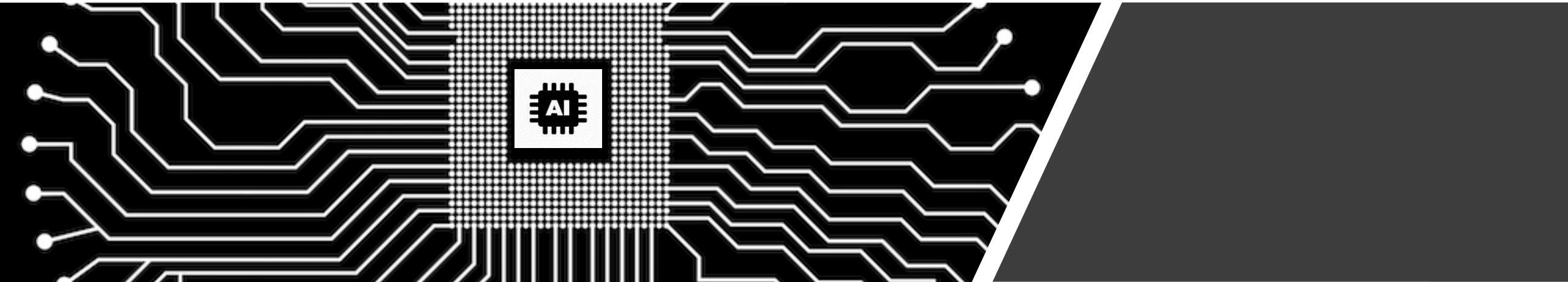


Shah, et al. RTP 2016





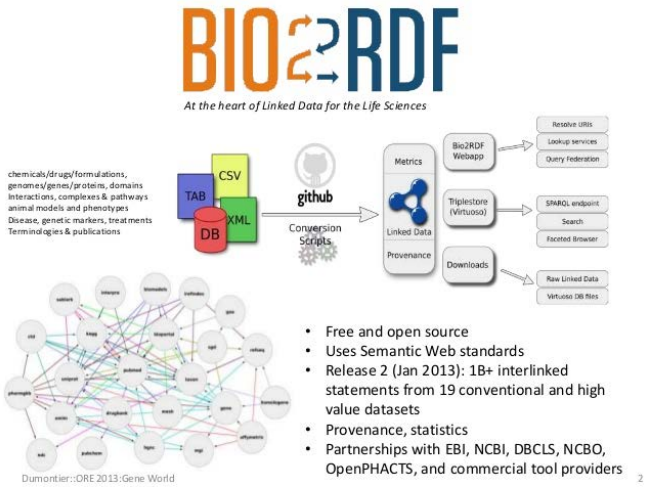
Machine Learning to Predict Chemical Effects

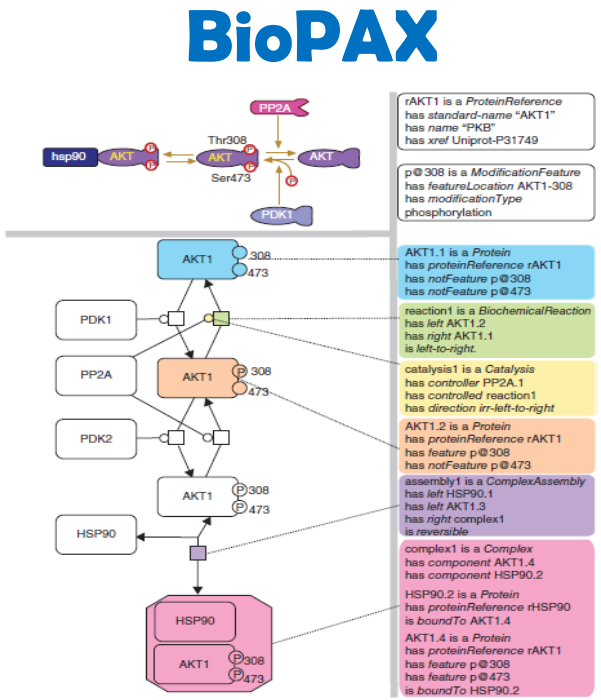


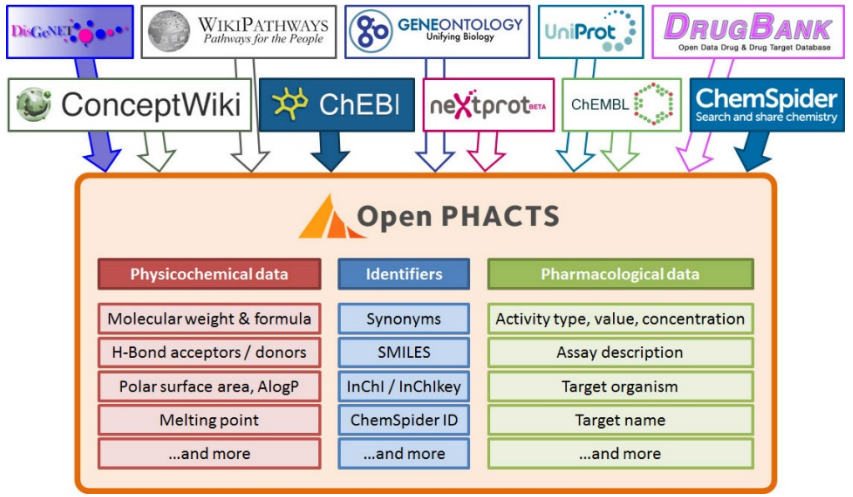
Use Semantic Tools to Link Disparate Data

Chemical → Uses → Exposure → ADME → Internal Dose ↔ Molecular Changes ↔ Cellular Pathways ↔ Organ Effects ↔ Toxicity → Population Outcomes

Can we use semantic tools (OWL/RDF, existing ontologies to meaningfully integrated disparate resources ?







Data Challenges

- Transparently sharing complex data streams– adequately capturing chemical (treatment dose and time), biological (experimental modal, assay, etc.) context to ease re-use
- Systematically integrating disparate data streams– representing linkages across molecular, cellular, tissue, organs. This is vital for relating early molecular changes to adverse (e.g. histopathological) outcomes
- Effectively extracting evidence from unstructured textual data– the literature is one of the largest resource for information about apical outcomes
- Using linked data to better discriminate between adaptation vs adversity– predicting which molecular markers lead to apical outcomes
- Quantifying and incorporating uncertainty and variability in predictions
- Legal defensibility of new methods and assessment products

Acknowledgements and Questions

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