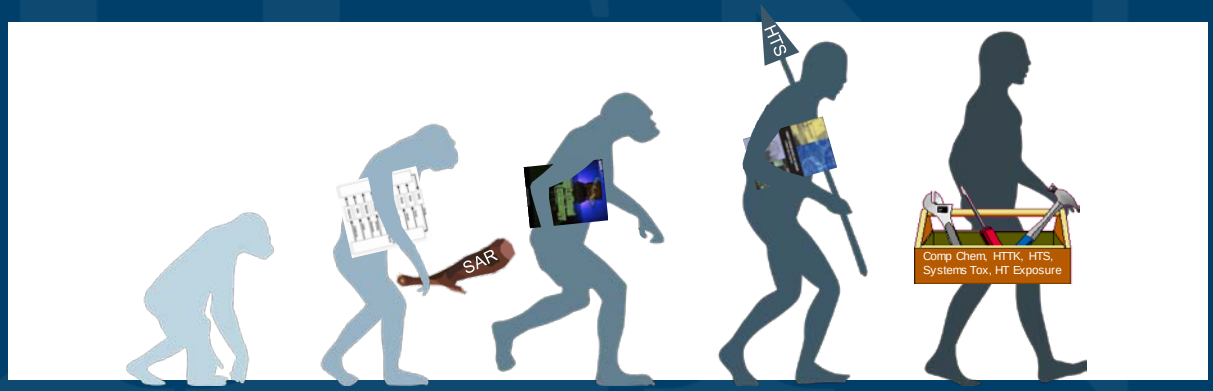
EPA
United States
Environmental Protection
Agency

So Many Chemicals, So Little Time…

Evolution of Computational Toxicology



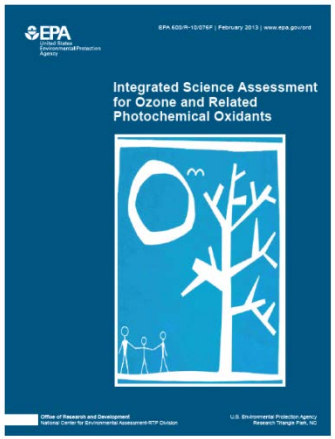
North Carolina State Seminar

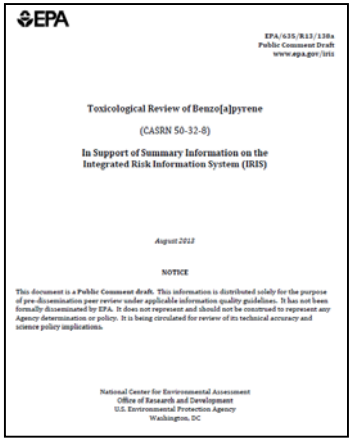
January 17, 2017

Rusty Thomas
Director
National Center for Computational Toxicology

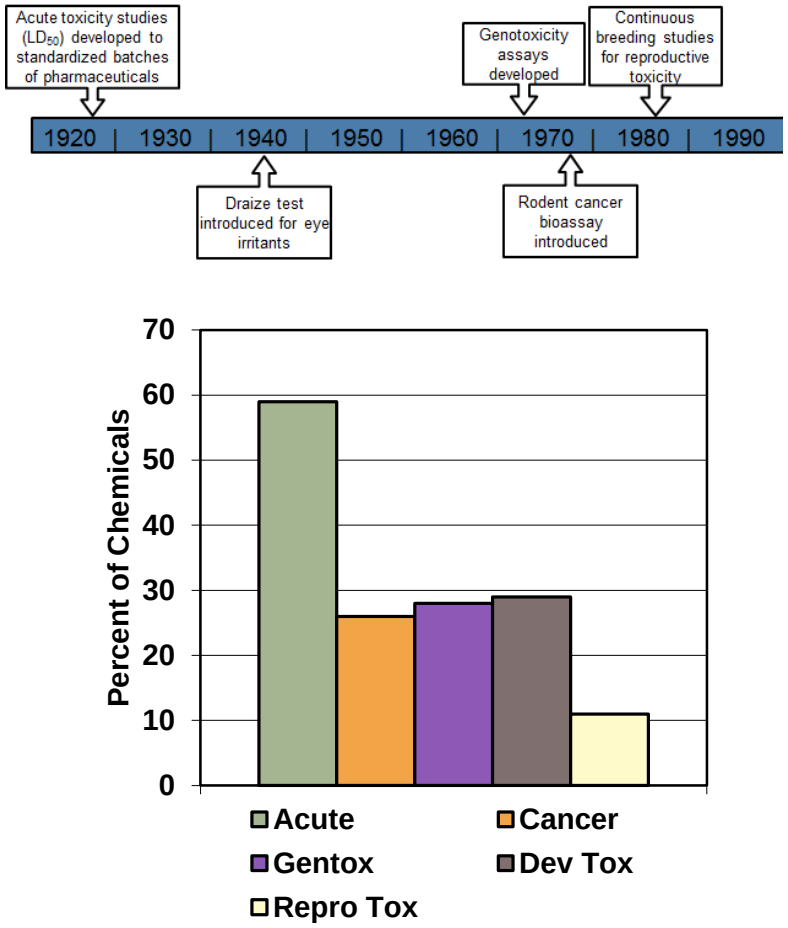
Regulatory Agencies Need to Make A Range of Decisions on Chemicals…

- Multiple drivers shape type of assessment
  - Regulatory demands
  - 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)





Current Testing Limited by Traditional Models and Regulatory System

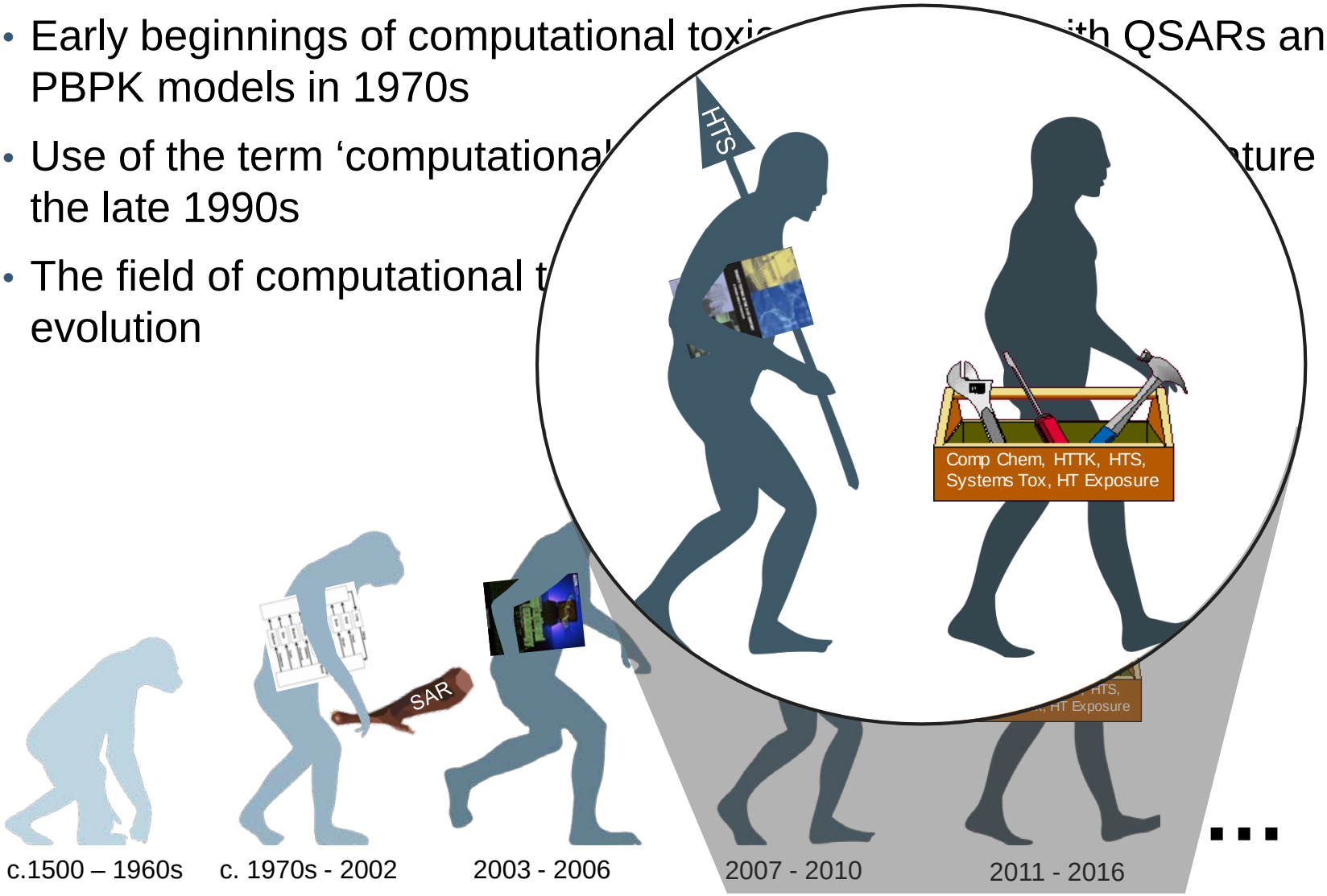


Judson, et al *EHP* (2010)

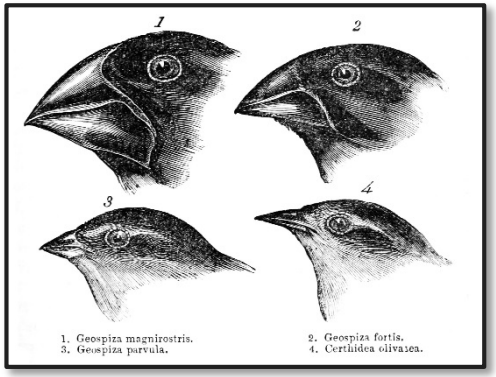
- Traditional toxicity tests have been added as new hazards were recognized
- In 1976, the U.S. Toxic Substances Control Act put burden on EPA to demonstrate "unreasonable risk" for industrial chemicals
- Increased recognition of lack of safety data for many environmental/industrial chemicals
  - 1984 US NRC Report
- Guidance from internal reports and expert committees highlighted need for increased throughput and computational approaches
  - 2005 EPA CompTox Report
  - 2007 US NRC Report

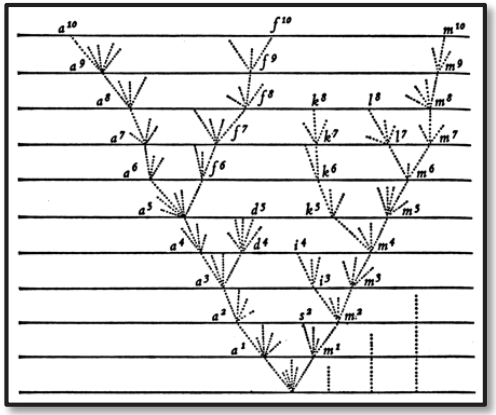
Evolution of Computational Toxicology

- Early beginnings of computational toxi… …ith QSARs and PBPK models in 1970s
- Use of the term 'computational… …ature in the late 1990s
- The field of computational t… evolution



What Traits Have Been Under Selection?





- Increased throughput and biological coverage
- Interrogation of effects at the molecular and pathway level
- Increasingly relevant test systems
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- Characterization of uncertainty
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- Delivery of data and models through decision support tools
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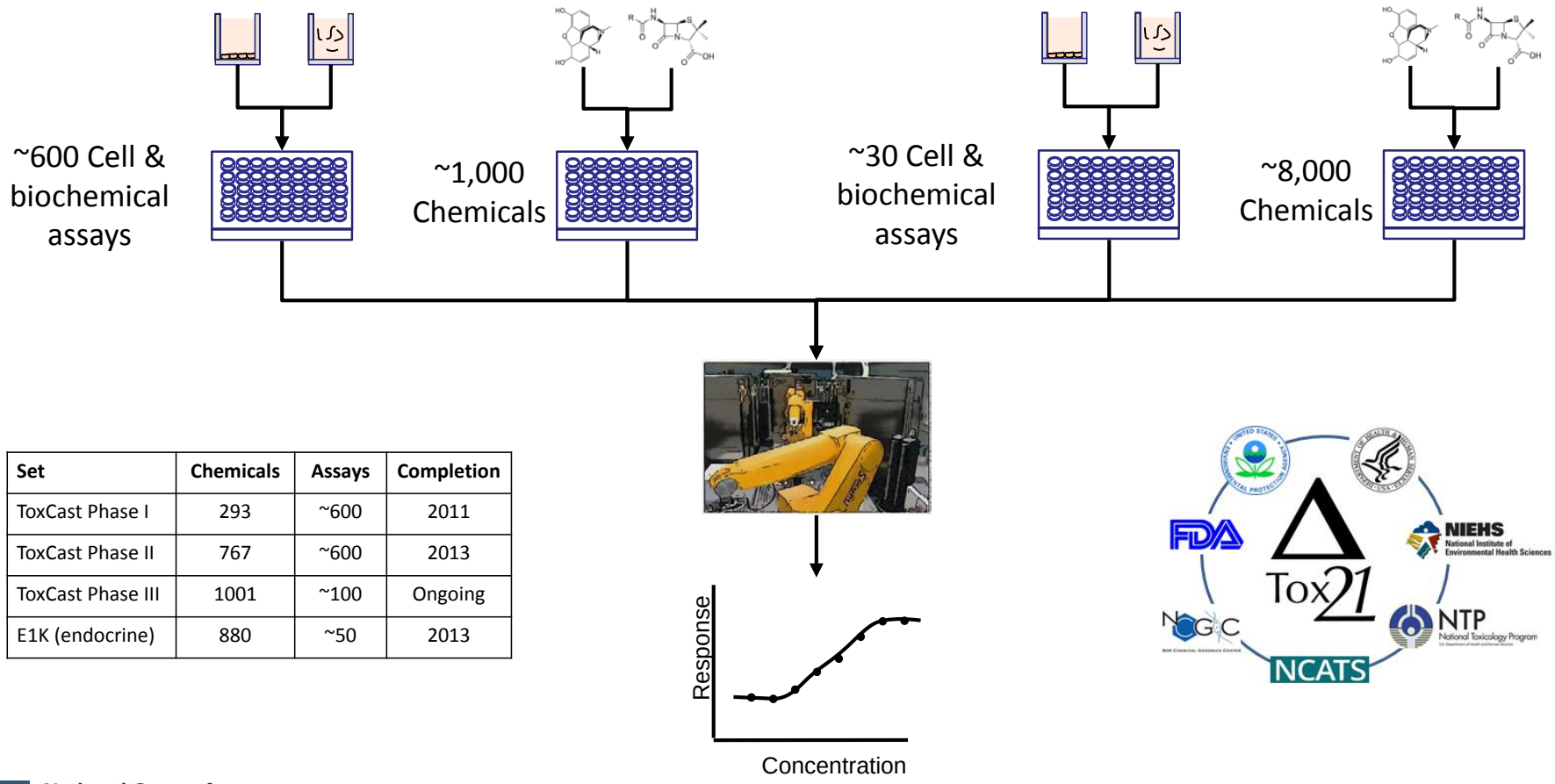
Increased Throughput Required Shift to Molecular/Pathway Approaches

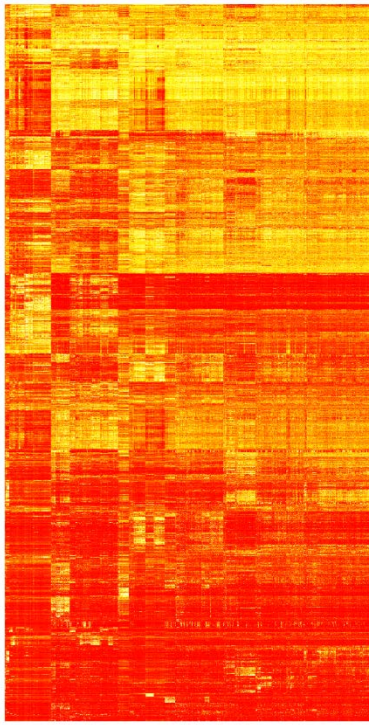
ToxCast | **Tox21**



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

Broad Success Derived from High-Throughput Screening Approaches

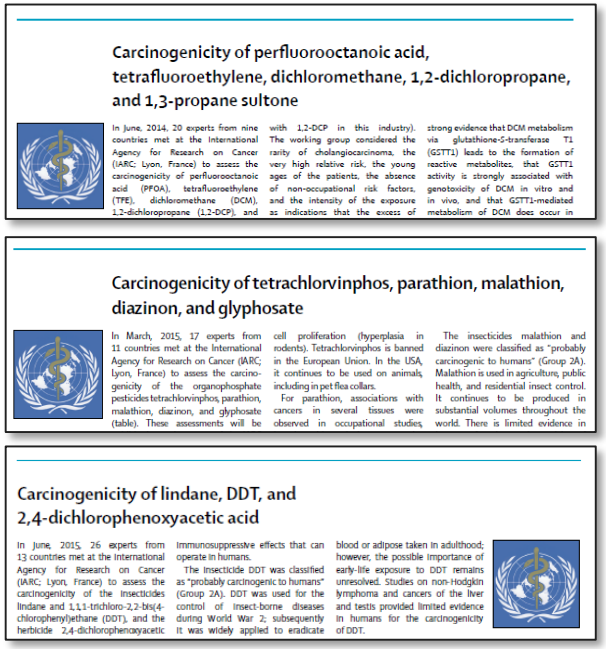
Group Chemicals by Similar Bioactivity and Predictive Modeling



Chemicals

Assays/Pathways

Provide Mechanistic Support for Hazard ID



Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone

In June, 2014, 20 experts from nine countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of perfluorooctanoic acid (PFOA), tetrafluoroethylene (TFE), dichloromethane (DCM), 1,2-dichloropropane (1,2-DCP), and with 1,2-DCP in this industry). The working group considered the rarity of cholangiocarcinoma, the very high relative risk, the young ages of the patients, the absence of non-occupational risk factors, and the intensity of the exposure as indications that the excess of strong evidence that DCM metabolism via glutathione-S-transferase T1 (GSTT1) leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity of DCM in vitro and in vivo, and that GSTT1-mediated metabolism of DCM does occur in

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be cell proliferation (hyperplasia in rodents). Tetrachlorvinphos is banned in the European Union. In the USA, it continues to be used on animals, including in pet flea collars.

For parathion, associations with cancers in several tissues were observed in occupational studies, The insecticides malathion and diazinon were classified as "probably carcinogenic to humans" (Group 2A). Malathion is used in agriculture, public health, and residential insect control. It continues to be produced in substantial volumes throughout the world. There is limited evidence in

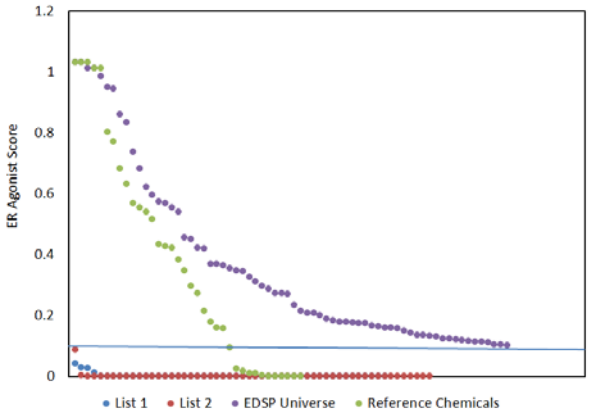
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

In June, 2015, 26 experts from 13 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the insecticides lindane and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), and the herbicide 2,4-dichlorophenoxyacetic Immunosuppressive effects that can operate in humans.

The insecticide DDT was classified as "probably carcinogenic to humans" (Group 2A). DDT was used for the control of insect-borne diseases during World War 2; subsequently it was widely applied to eradicate blood or adipose taken in adulthood; however, the possible importance of early-life exposure to DDT remains unresolved. Studies on non-Hodgkin lymphoma and cancers of the liver and testis provided limited evidence in humans for the carcinogenicity of DDT.

IARC Monographs 110, 112, 113

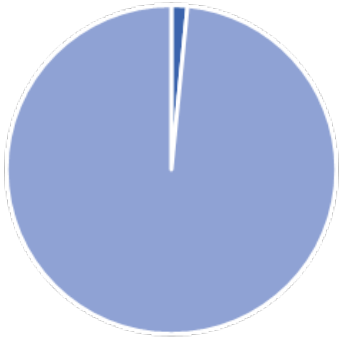
Prioritization of Chemicals for Further Testing



FIFRA SAP, Dec 2014

Continuing Pressure Towards Increased Biological Coverage

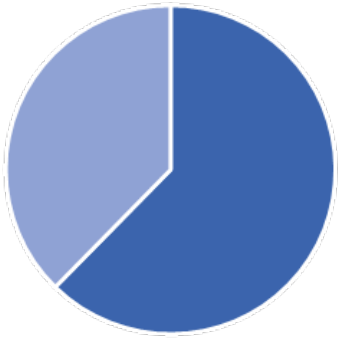
Gene Coverage



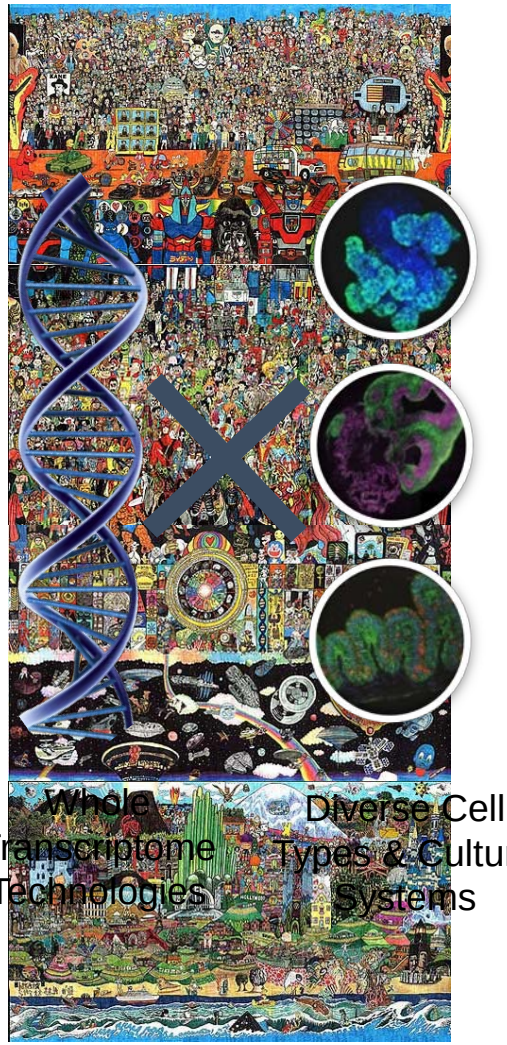
ToxCast

Not in ToxCast

Pathway Coverage*



*At least one gene from pathway represented

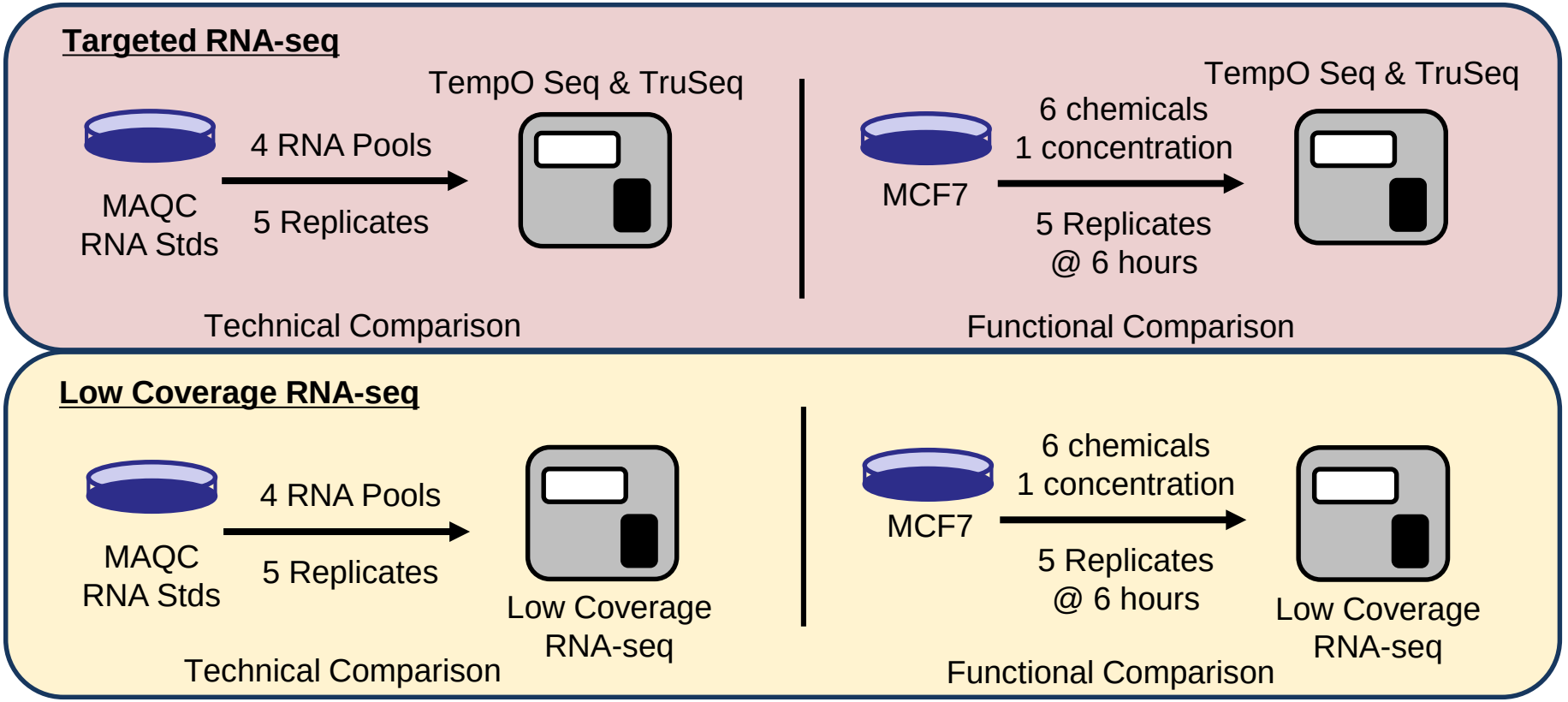


Picture of Everything - Howard Hallis

Searching for a Platform

Requirements:

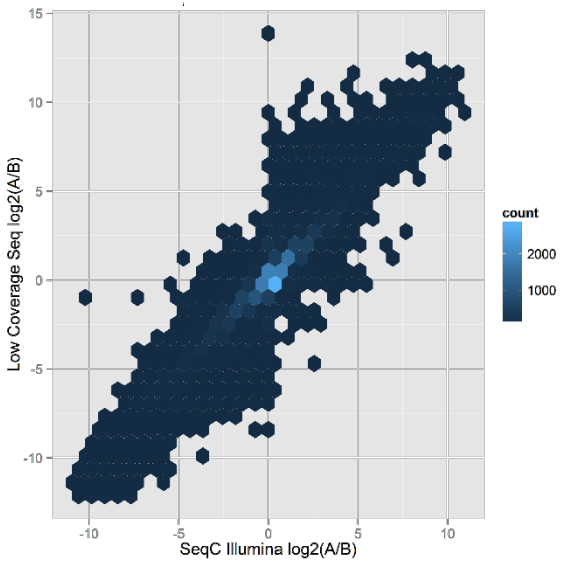
- Low cost
- Whole genome
- 384 well
- Automatable



Technical Performance of the Three Sequencing Platforms

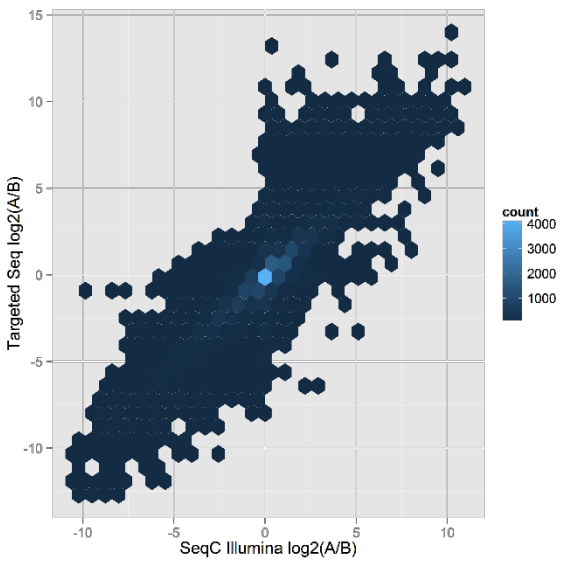
Low Coverage

r^2 0.83



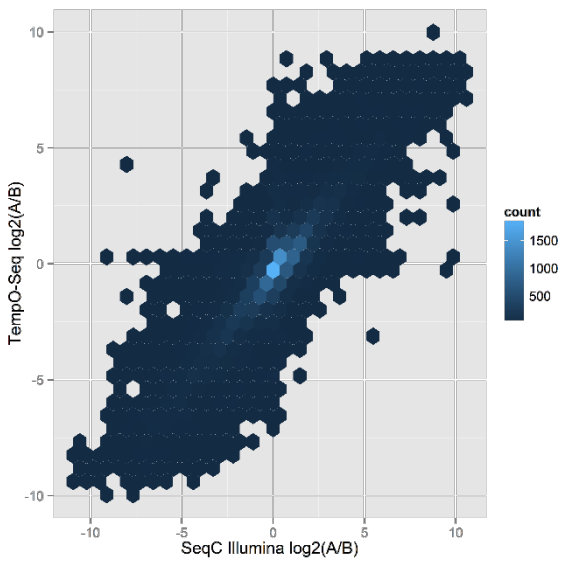
TruSeq

r^2 0.74



TempO-Seq

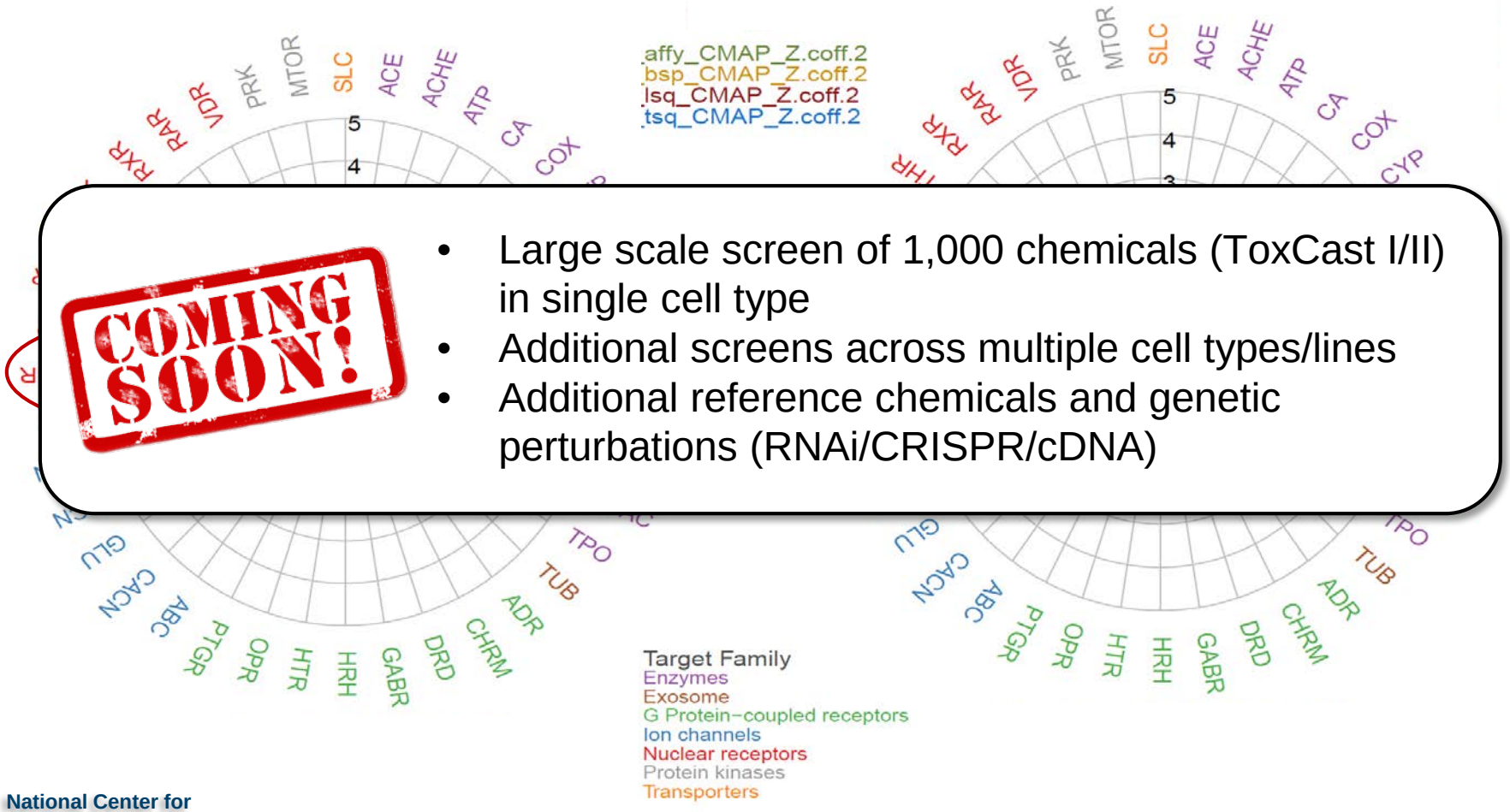
r^2 0.75



Functional Performance of the Three Sequencing Platforms

Genistein

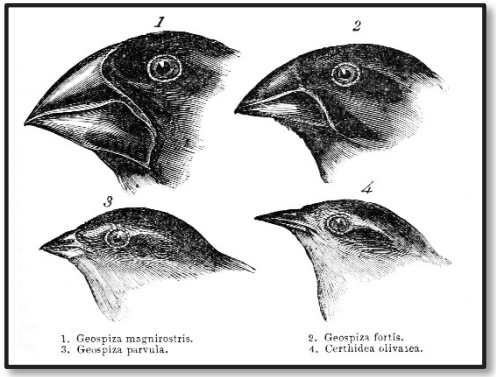
Trichostatin

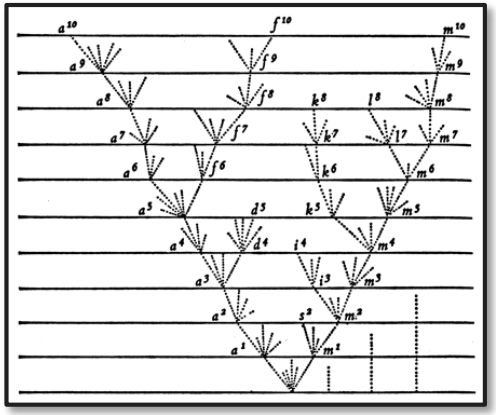


COMING SOON!

- Large scale screen of 1,000 chemicals (ToxCast I/II) in single cell type
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)

What Traits Have Been Under Selection?





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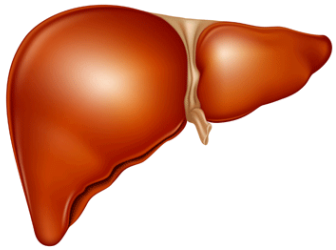
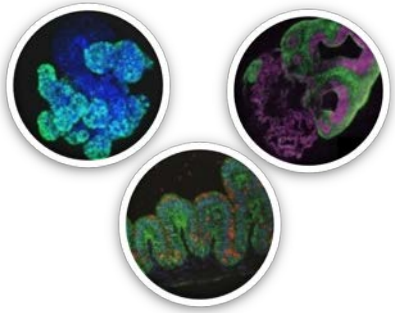
Increasing Relevance of Test Systems Necessary at Multiple Levels

Relevant Species Responses



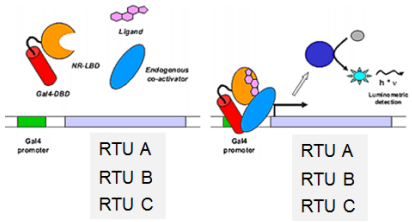
Relevant Metabolism

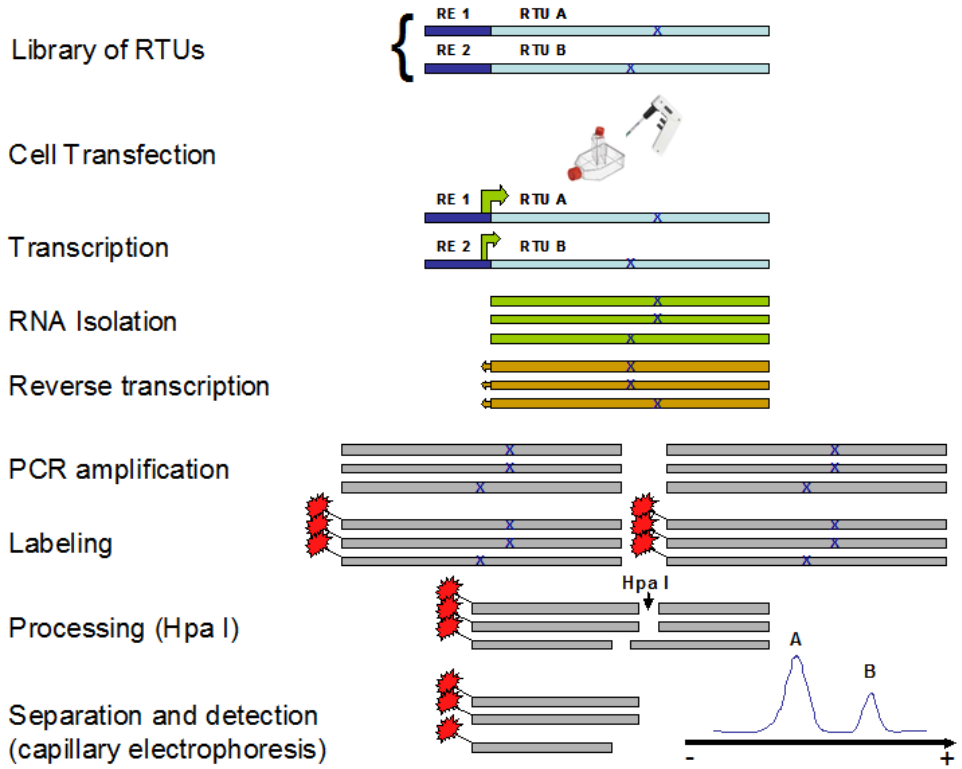


Relevant Tissue and Organ Responses

Assessing Cross-Species Differences in Response

Multispecies Attagene *Trans* Reporter Assay



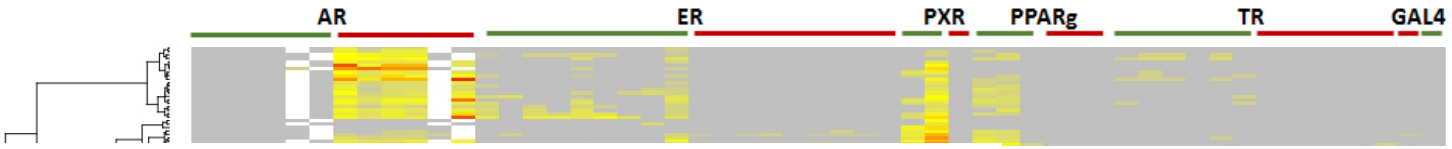


Receptor Family	Receptor Name	Species
Estrogen Receptor	ERa	Human
Estrogen Receptor	ERb	Human
Estrogen Receptor	ER1	Zebrafish
Estrogen Receptor	ER2a	Zebrafish
Estrogen Receptor	ER2b	Zebrafish
Estrogen Receptor	ERa	Chicken
Estrogen Receptor	ER1	Frog
Estrogen Receptor	ER2	Frog
Estrogen Receptor	ERa	Turtle
Estrogen Receptor	AR	Human
Estrogen Receptor	AR	Chicken
Estrogen Receptor	AR	Turtle
Estrogen Receptor	AR	Frog
Estrogen Receptor	AR	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Mouse
Peroxisome Proliferator Activated Receptor γ	PPARg	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Human
Pregnane X Receptor	PXR	Mouse
Thyroid Receptor	TRa	Turtle
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRa	Frog
Thyroid Receptor	TRa	Human
Thyroid Receptor	TRb	Human
Controls	M-06	NA
Controls	GAL4	NA
Controls	M-19	NA
Controls	m-32	NA
Controls	m-61	NA

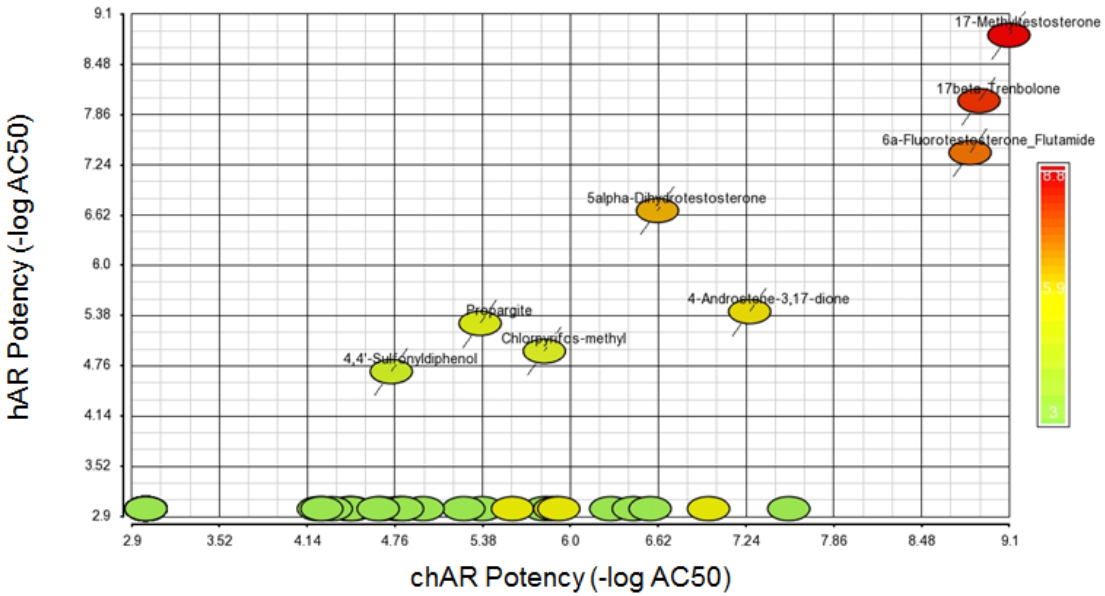
- Host cell: human HepG2
- Stimulation with EC20 of 6a-fluorotestosterone for detection of androgen receptor antagonists
- 100 chemicals with ER, AR, PPAR activity tested in concentration-response
- Data calculated as fold-change over control (6a-fluorotestosterone/DMSO)

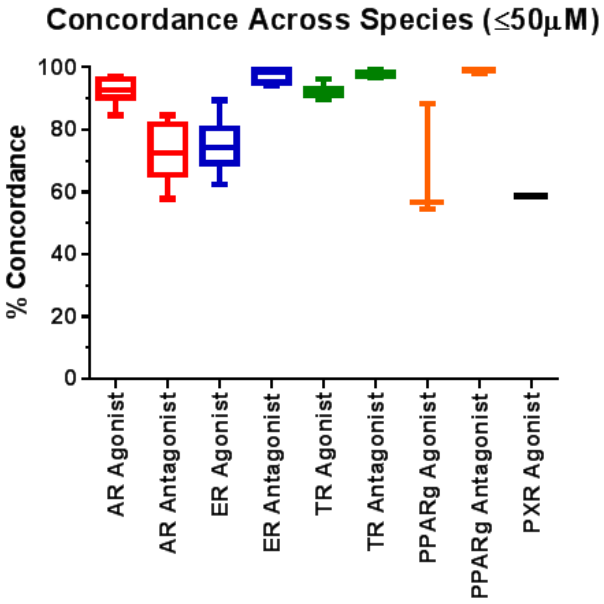
Houck et al., Unpublished

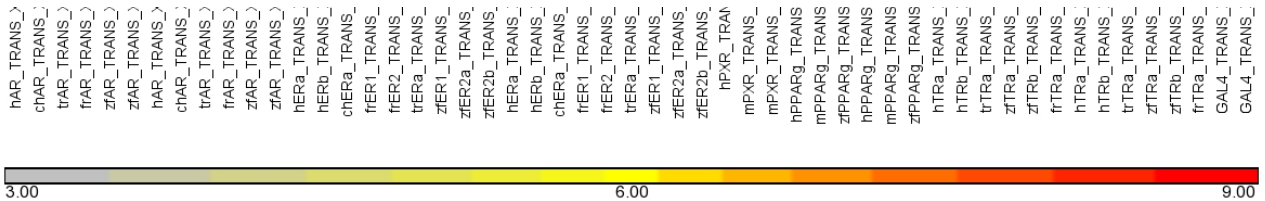
Cross-Species Differences in Nuclear Receptor Responses



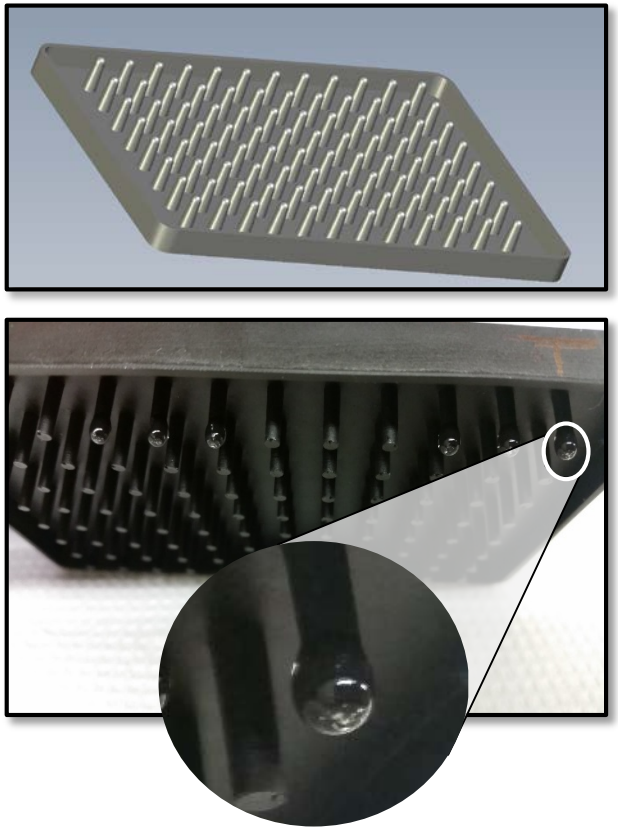
= agonist
= antagonist

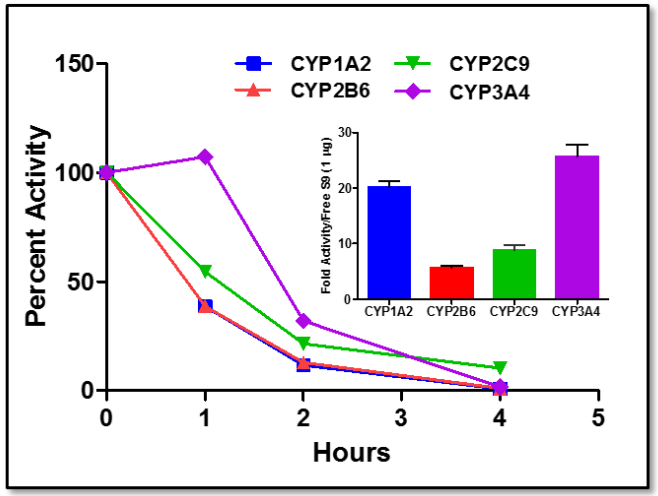


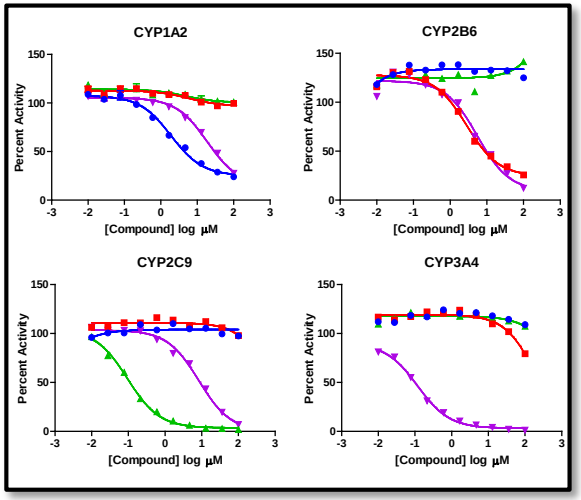




Adding Relevant Metabolic Activity



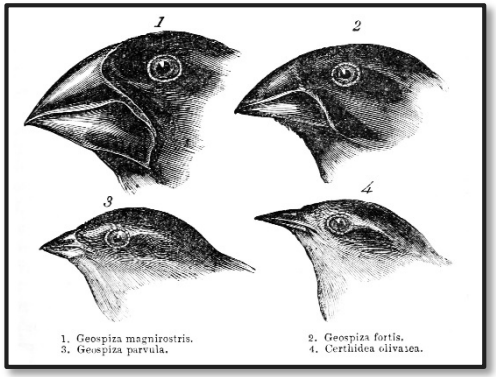


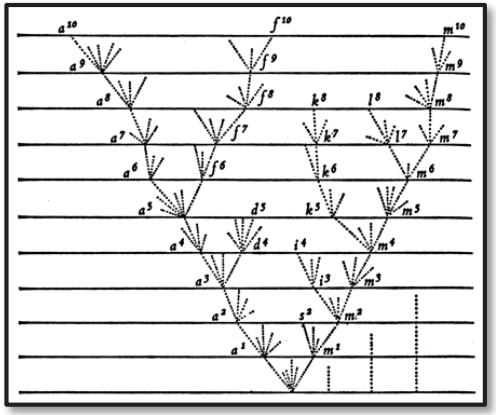


Furafylline
Thio-TEPA
Tienilic Acid
Ketoconazole

DeGroot and Simmons, Unpublished

What Traits Have Been Under Selection?





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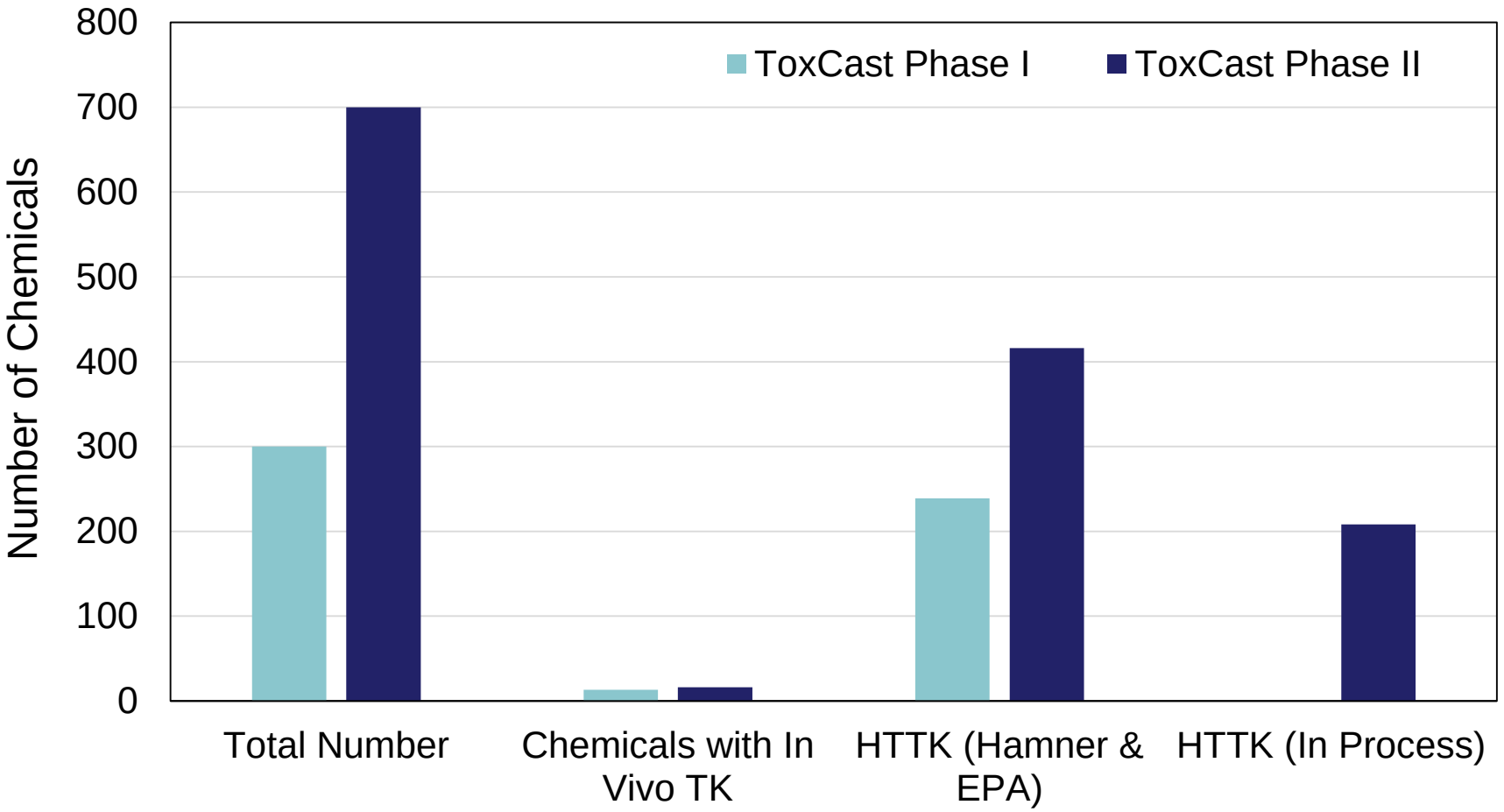
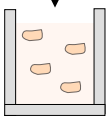
The Need for High(er) Throughput TK Approaches



Incorporating a High-Throughput Toxicokinetic Approach

EPA ToxCast Phase I and II Chemicals



Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

ToxCast AC_{50} Value

Plasma Concentration

Oral Exposure

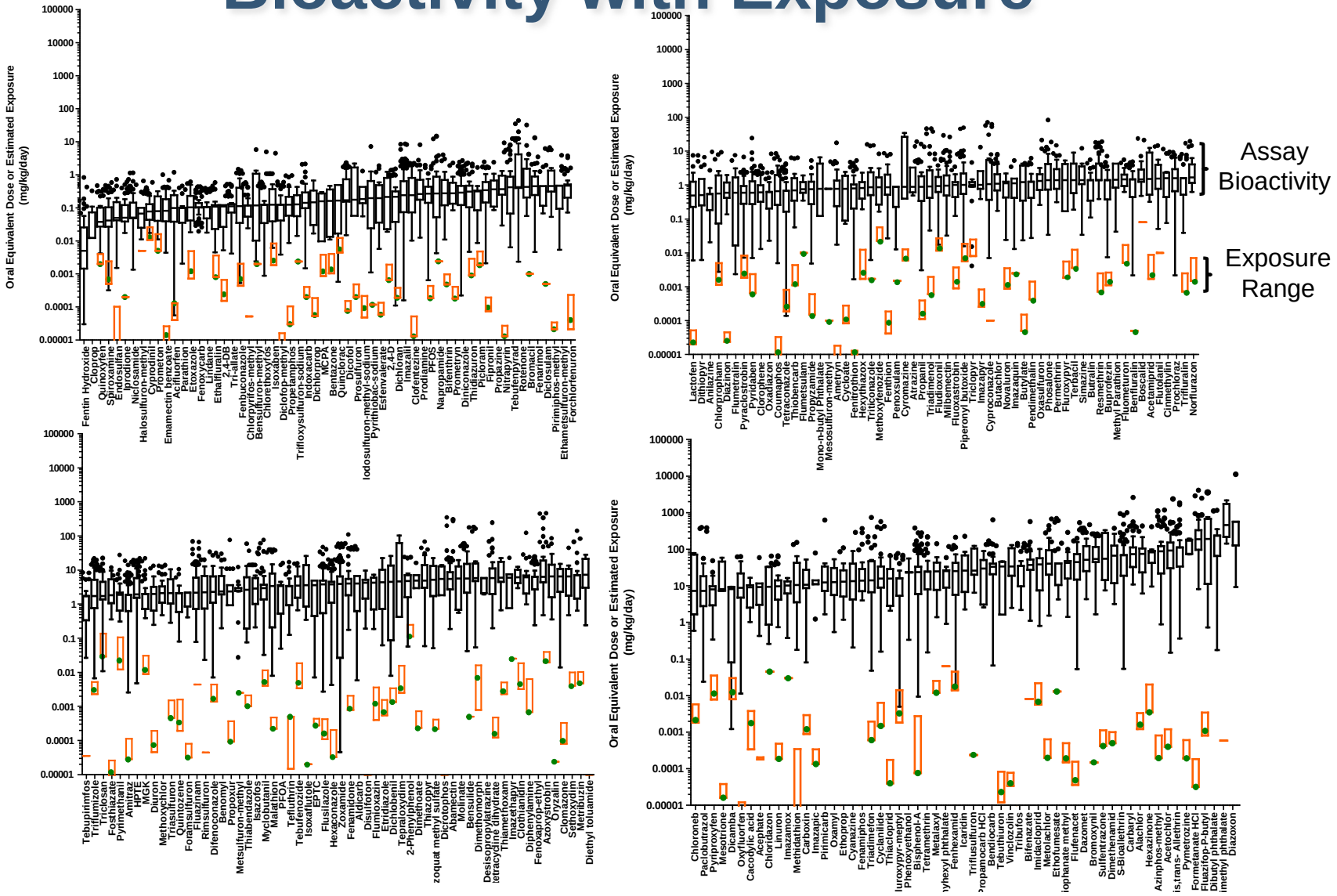
Reverse Dosimetry

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

- Models available through '"httk" R package (https://cran.r-project.org/web/packages/httk/)

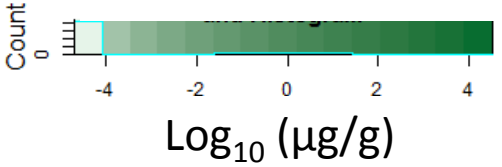
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

Comparing Dosimetry Adjusted Bioactivity with Exposure



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

Improving Exposure Estimates and Characterization



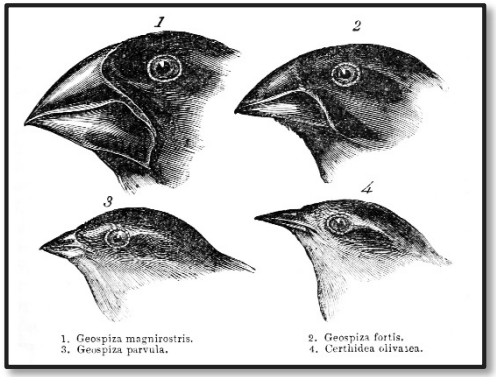
- Air freshener
- Baby soap
- Carpet
- Carpet padding
- Cereals
- Cotton clothing
- Deodorant
- Fabric upholstery
- Glass cleaners
- Hand soap
- Indoor house paint
- Lipstick
- Plastic children's toys
- Shampoo
- Shaving cream
- Shower curtain
- Skin lotion
- Sunscreen
- Toothpaste
- Vinyl upholstery

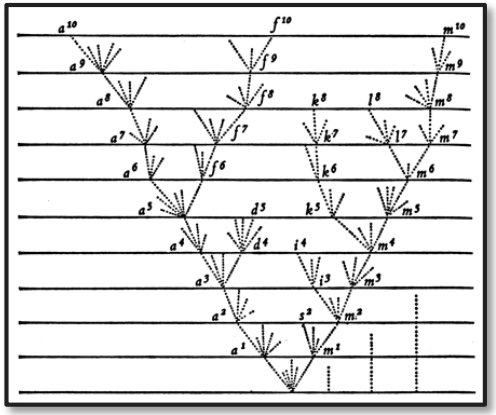
- GCXGC-MS with DCM Extraction
- 1606 tentative and confirmed chemical identifications

- Common Chemical (n>19)
- ToxCast
- Flame Retardant
- Potent ER

Wambaugh et al. Unpublished

What Traits Have Been Under Selection?





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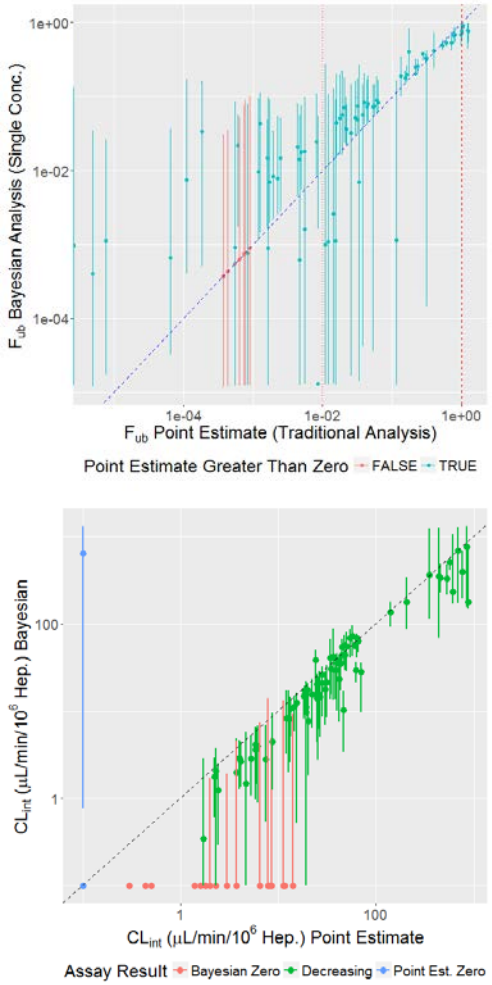
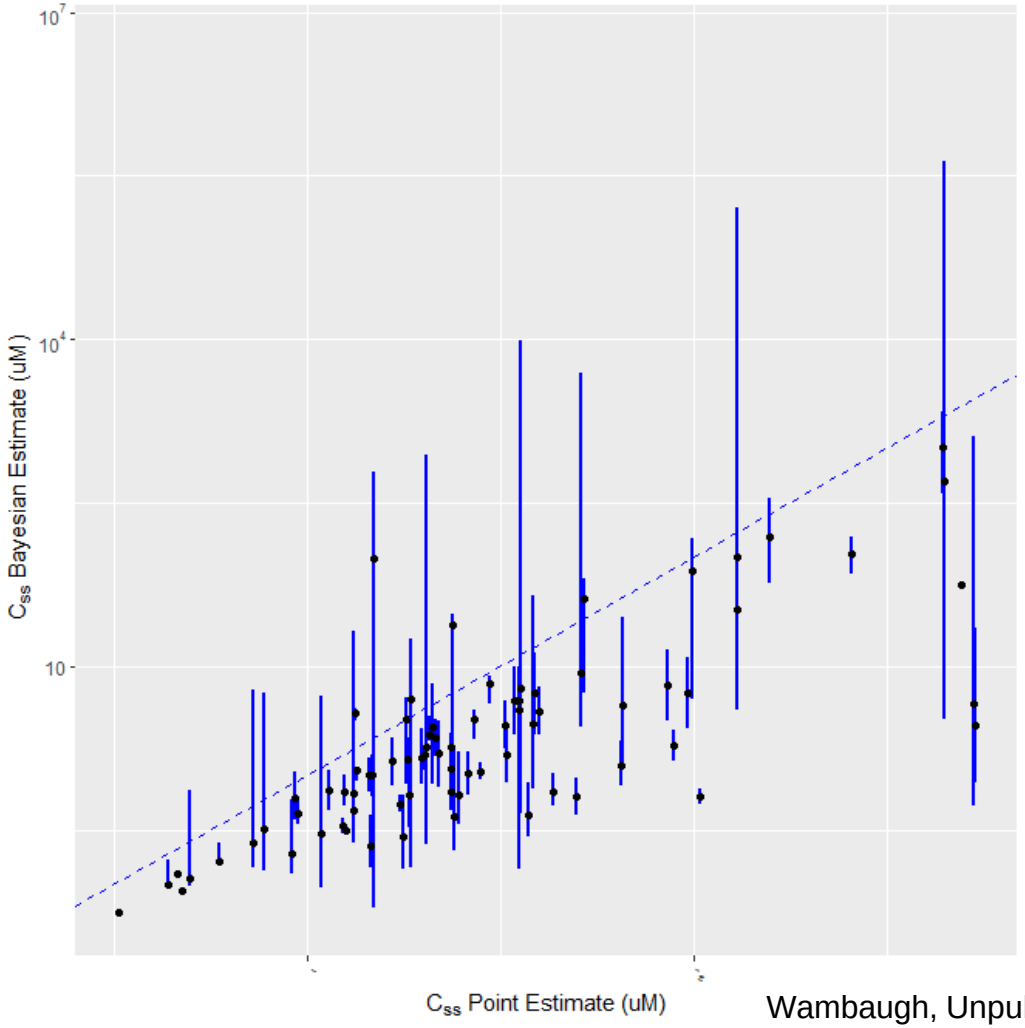
Incorporating Uncertainty Into the Pharmacokinetic Modeling

Bayesian Modeling of Plasma Protein Binding and Intrinsic Clearance

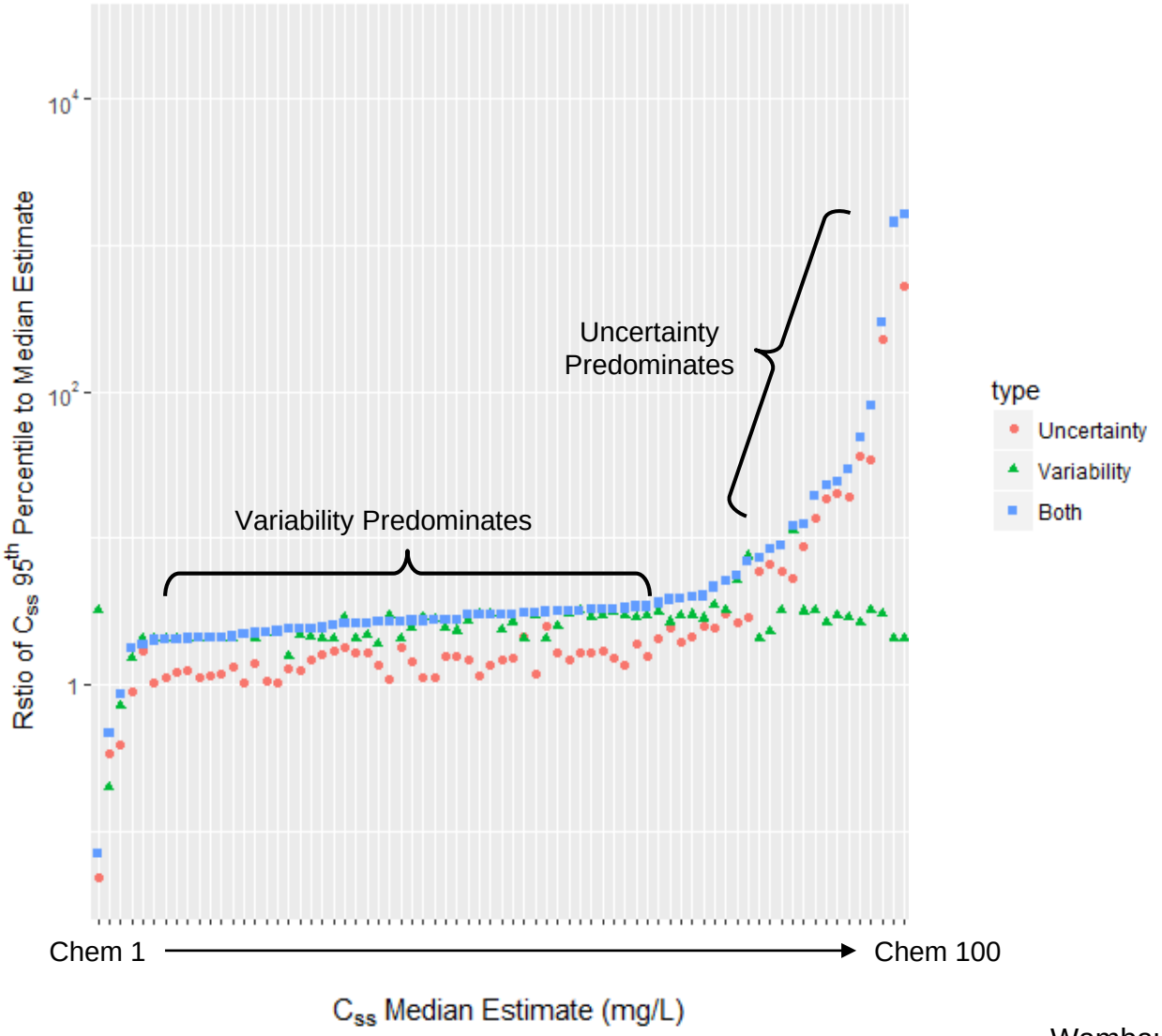


Propagation of Experimental Uncertainty to Steady State Plasma Concentrations



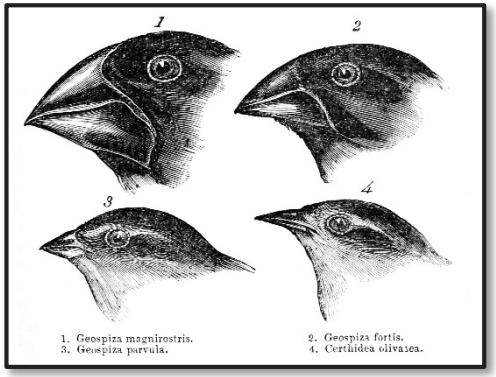
Wambaugh, Unpublished

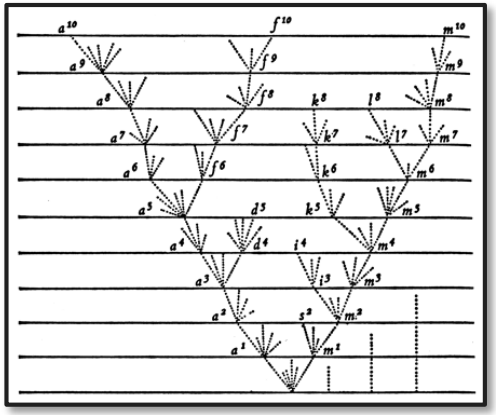
Impact of Incorporating Uncertainty and Variability is Chemical Specific



Wambaugh, Unpublished

What Traits Have Been Under Selection?

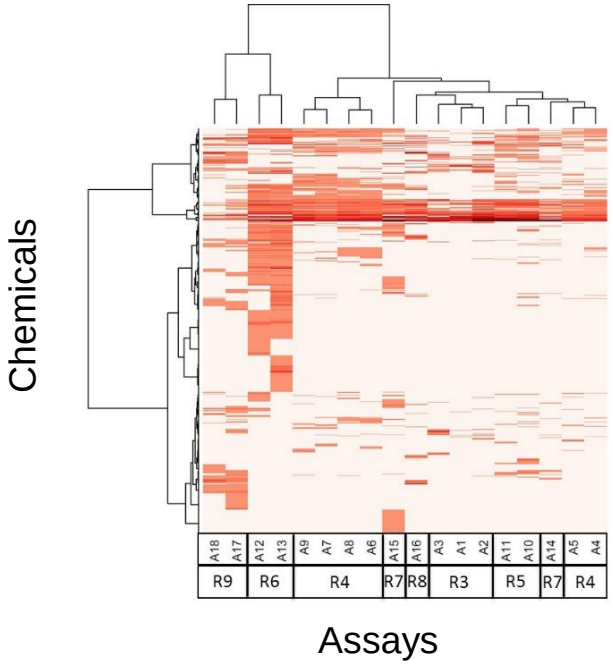




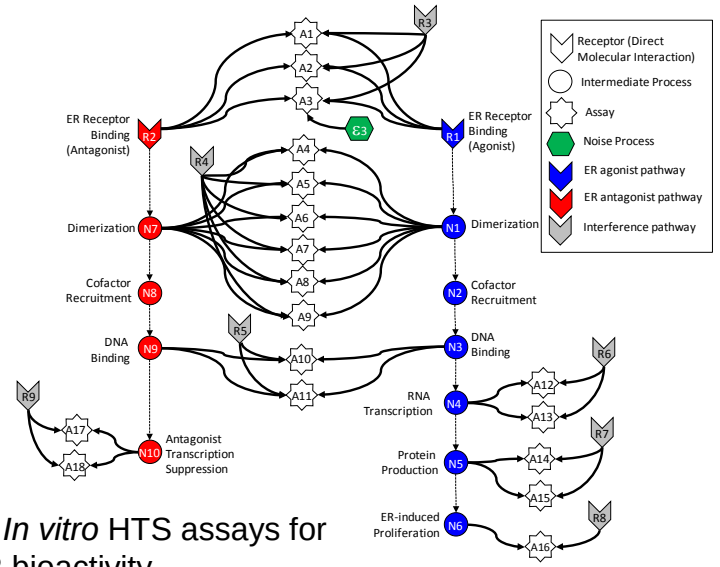
- Increased throughput and biological coverage
- Interrogation of effects at the molecular and pathway level
- Increasingly relevant test systems
- Putting results in a dose/exposure context
- Characterization of uncertainty
- Computational modeling to integrate experimental data
- Delivery of data and models through decision support tools
- Transparency and performance-based validation

Relatively Simple Models Used to Reduce Assay Interference

Assays cluster by technology, suggesting technology-specific non-ER bioactivity



Computational Modeling of Estrogen Receptor Pathway



18 *In vitro* HTS assays for ER bioactivity

In Vitro Reference Chemicals*		*In Vivo* Reference Chemicals*	
Accuracy	**0.93 (0.95)**	**Accuracy**	**0.86 (0.95)**
Sensitivity	**0.93 (0.93)**	**Sensitivity**	**0.97 (0.97)**
Specificity	**0.92 (1.0)**	**Specificity**	**0.67 (0.89)**

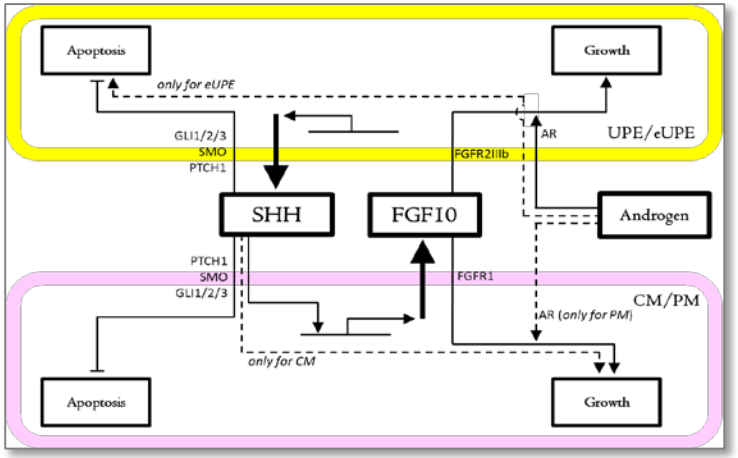
*Values in parentheses exclude inconclusive chemicals

Judson *et al., Tox Sci.* 2015
Browne *et al., ES&T.* 2015

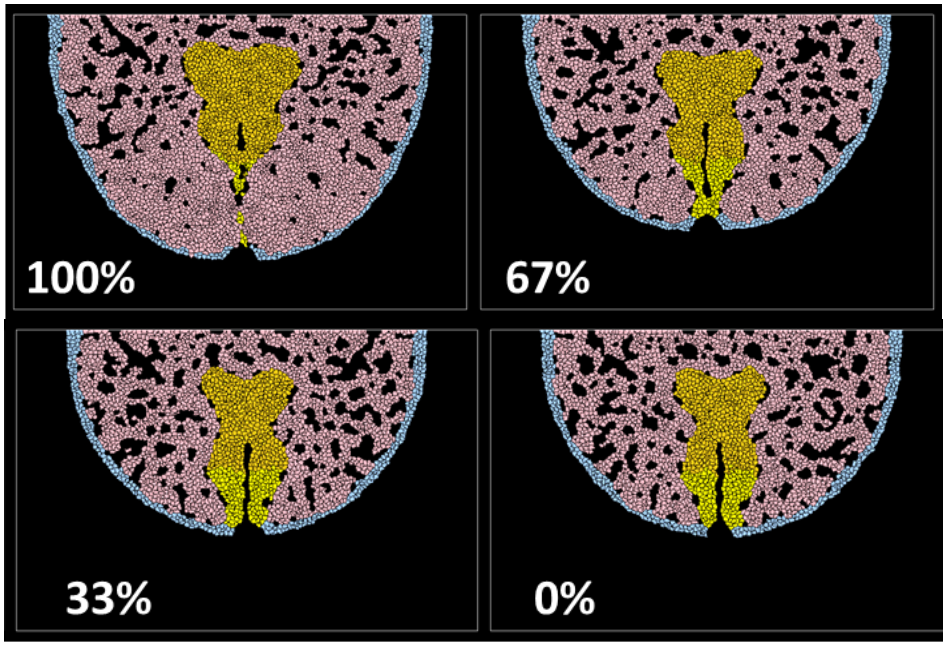
Complex Systems Models to Predict Phenotypic Responses to Chemicals

United States Environmental Protection Agency

Signaling Network Underlying Virtual Genital Tubercle Model (Mouse)



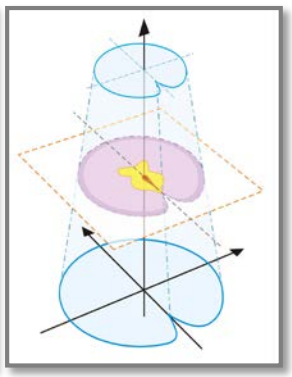
Simulation of Genital Tubercle Closure



GD13.5 – 17.5

Embryonic GT

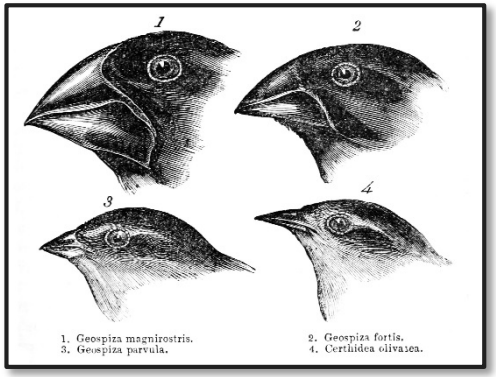
Abstracted GT

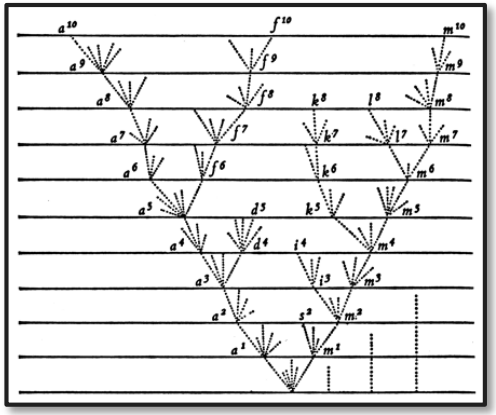


Leung et al., *Repro Toxicol*, 2016

Androgenization (*n = 10 sims*)	*Phenotype (MCS 4000)* Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

What Traits Have Been Under Selection?





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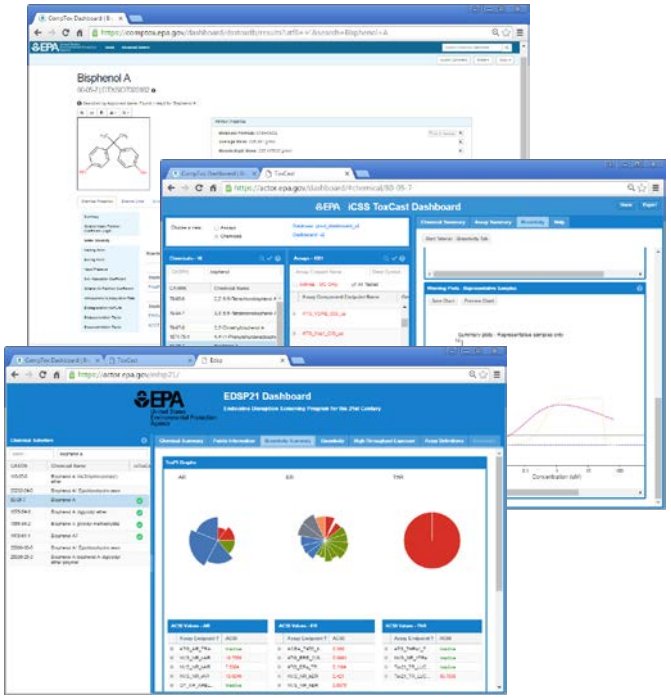
Significant Pressure to Deliver Data and Models in Useful Format

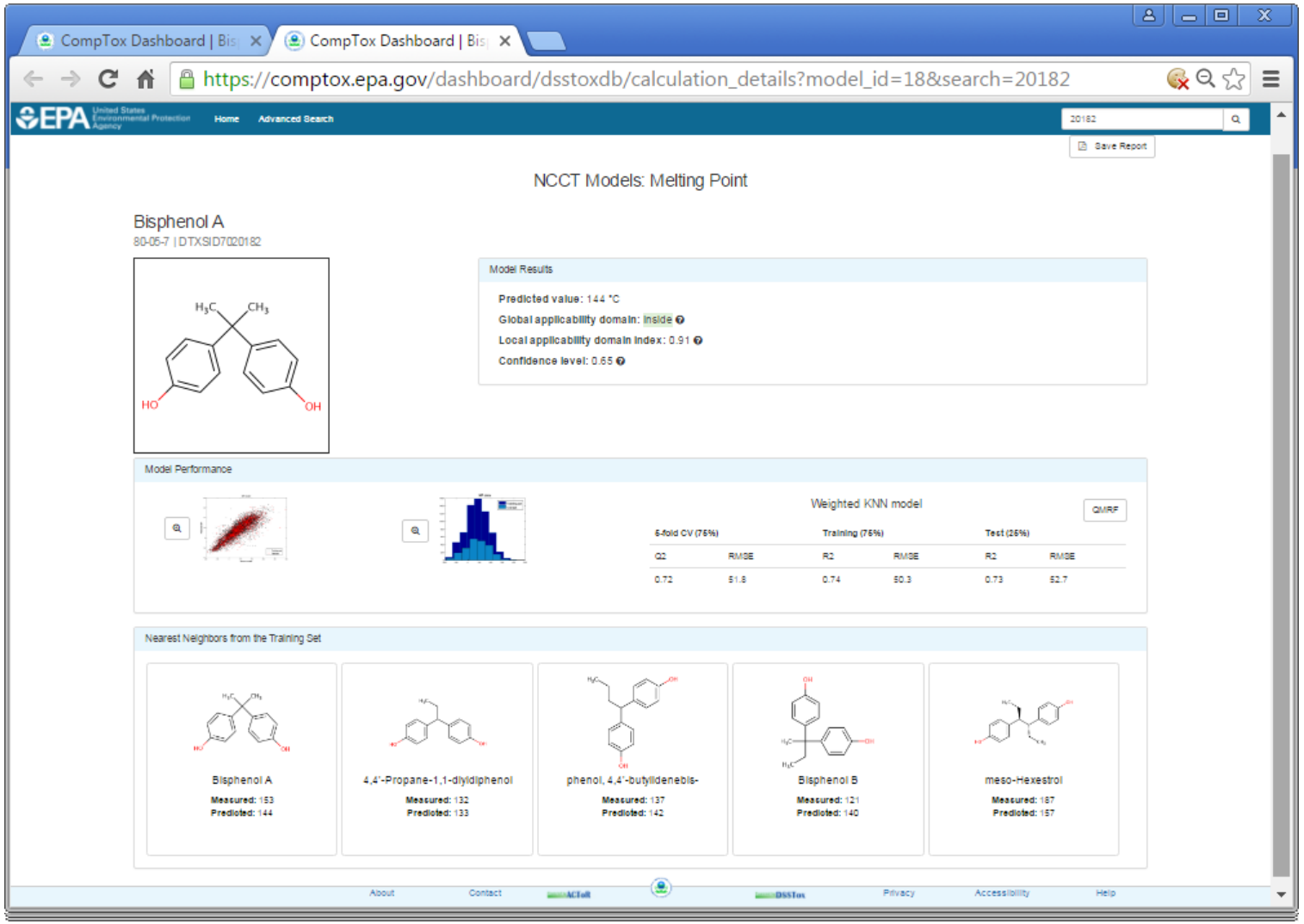
Initial Data Delivered as Flat Files

Progress to Dashboard with Limited Search, Visualization, and Export Functionality



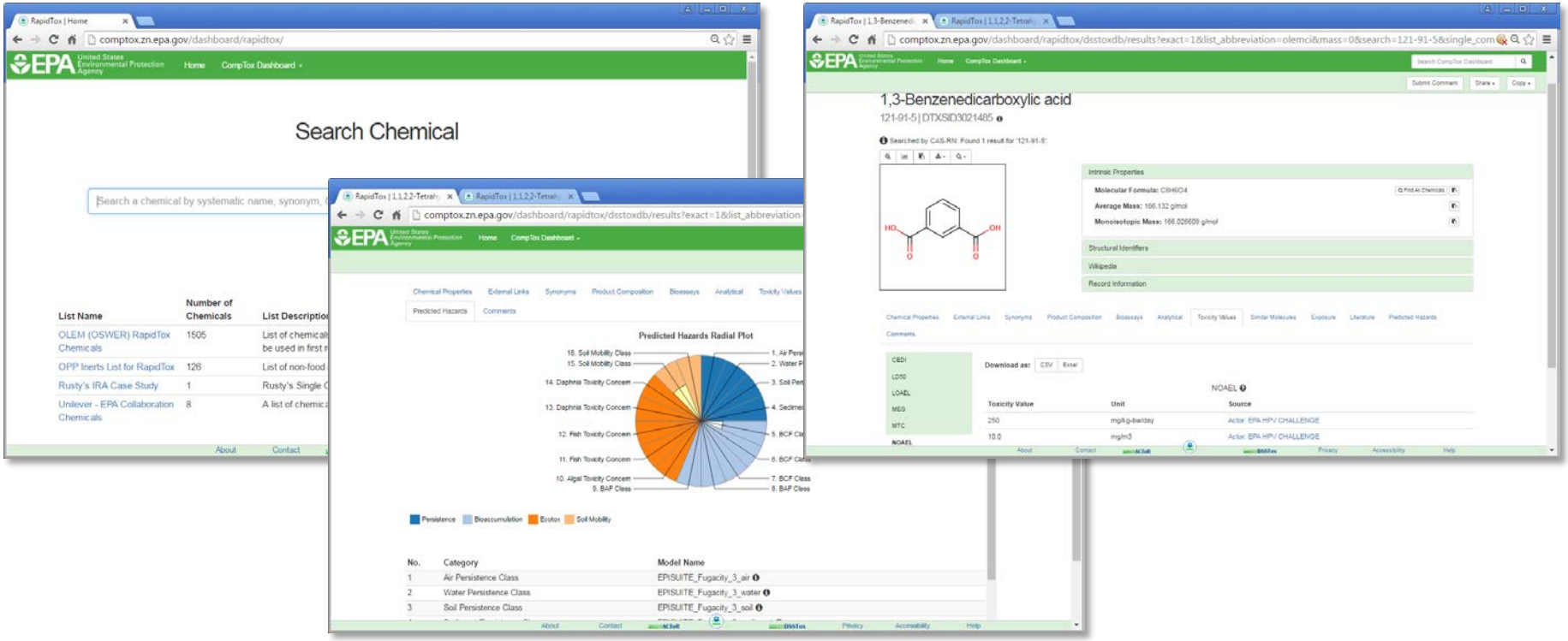
Currently Providing Cross-Functional and Decision Support Dashboards

New Chemistry Dashboard Delivers Structural and Property Data



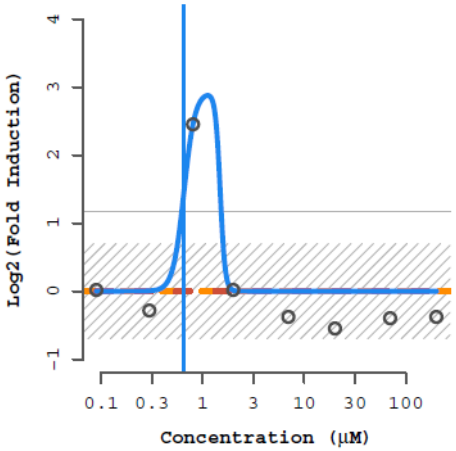
https://comptox.epa.gov/dashboard

RapidTox Decision Support Dashboard in Development



- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Combining diverse data streams into quantitative toxicity values with associated uncertainty

Regulatory Applications Require More Focus on Quality and Transparency

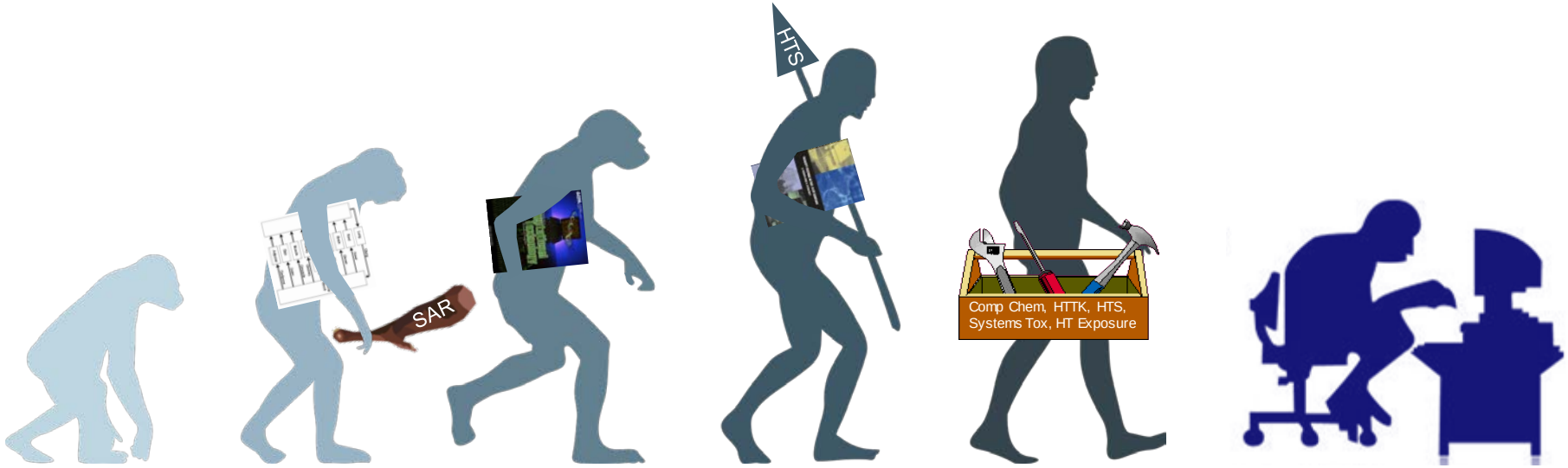



```
ASSAY:    AEID117 (ATG_ERa_TRANS)

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

Next Phase… Evolution Towards a Truly Predictive Science



c.1500 – 1960s | c. 1970s - 2002 | 2003 - 2006 | 2007 - 2010 | 2011 - 2016 | 2016 - ?

Acknowledgements and Questions

Tox21 Colleagues:
- NTP Crew
- FDA Collaborators
- NCATS Collaborators

EPA Colleagues:
- NERL
- NHEERL
- NCEA

EPA's National Center for Computational Toxicology