The Evolution of Toxicology and Chemical Regulation

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Sitlington Lecture
Oklahoma State University
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Outline

- What's the Problem?
- Who's your boss?
- Data, Data, Who's Got Data?
- If you build it, will they use it?
- Nothing is ever perfect

Problem: The Chemical Hazard Universe

United States Environmental Protection Agency

Toxicity Testing

Strategies to Determine Needs and Priorities

Steering Committee on Identification of Toxic and Potentially Toxic Chemicals for Consideration by the National Toxicology Program

Board on Toxicology and Environmental Health Hazards

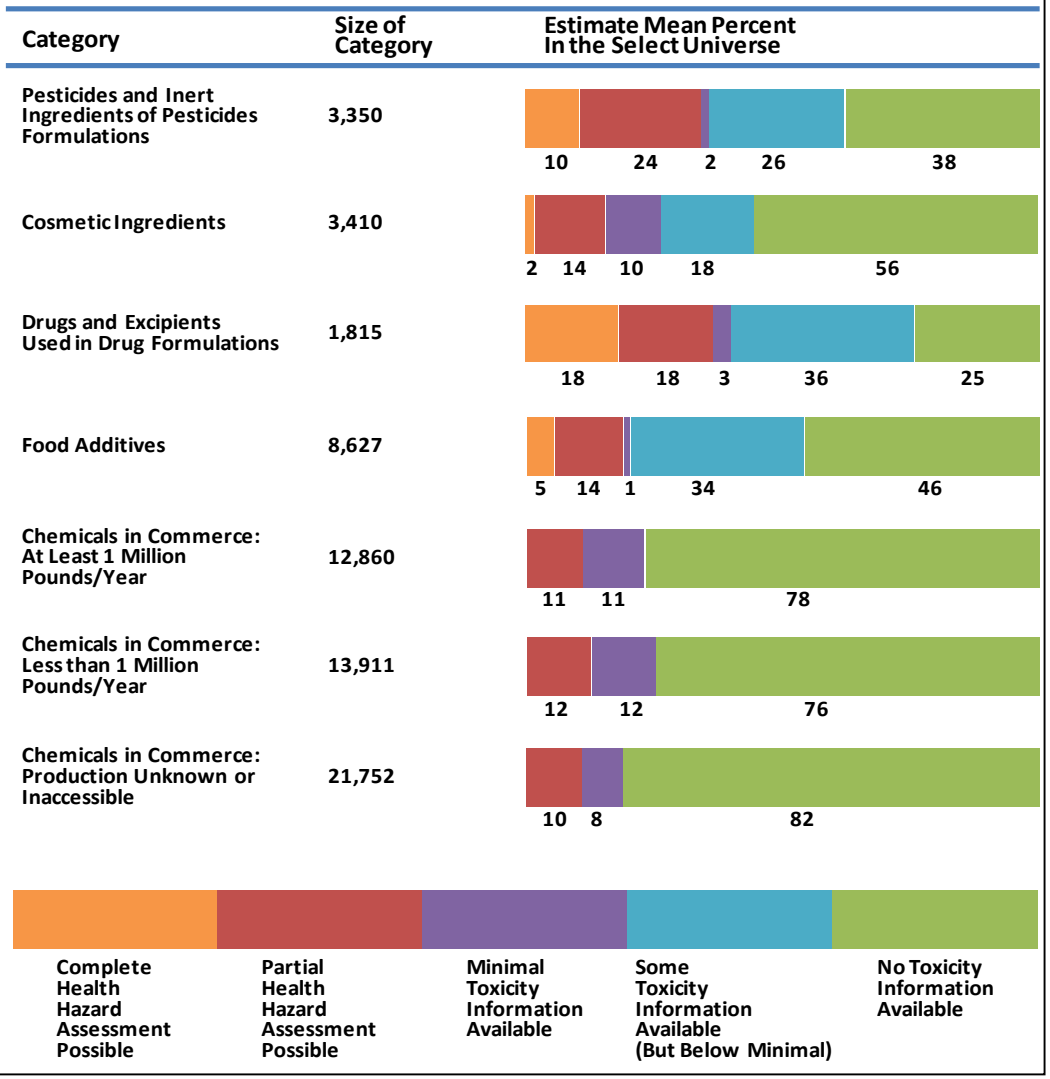
Commission on Life Sciences

National Research Council

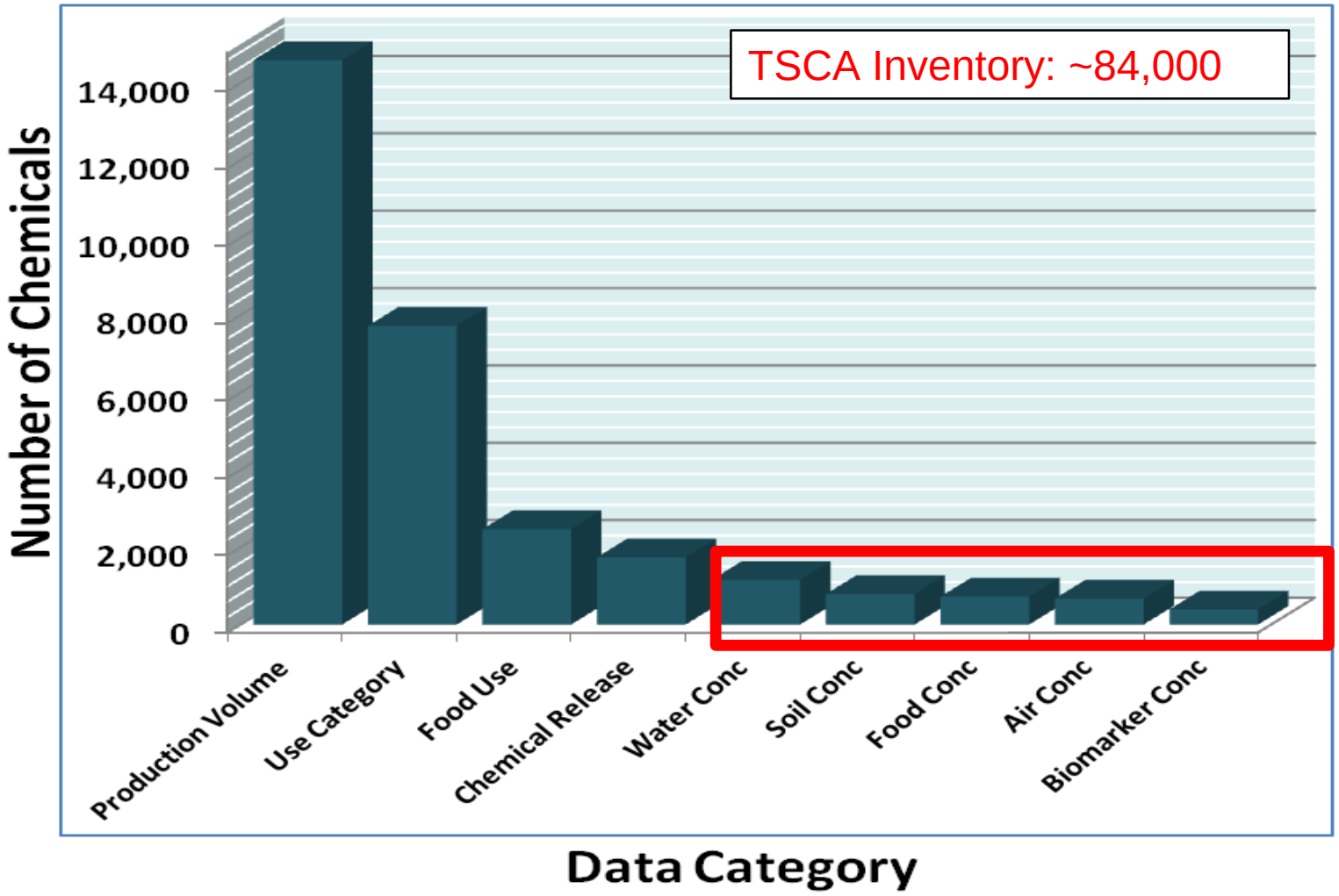
- Major challenge is too many chemicals and not enough data
- Total # chemicals = 65,725
- Chemicals with no toxicity data of any kind = ~46,000

NATIONAL ACADEMY PRESS
Washington, D.C. 1984

US National Research Council, 1984



Problem: The Chemical Exposure Universe

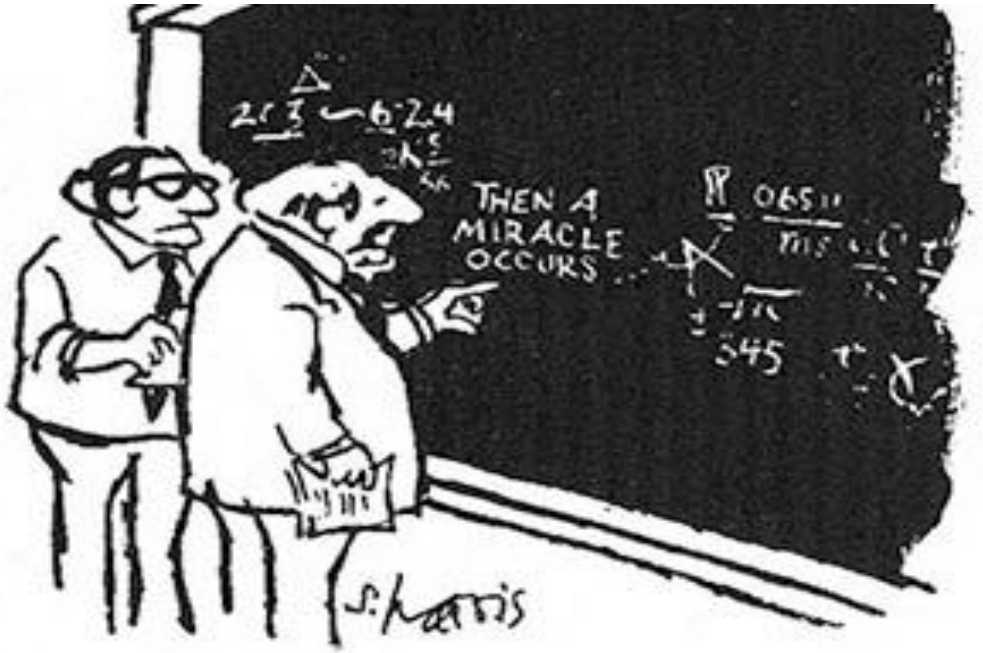


P.P. Egeghy et al. *Sci Total Environ.* 414 (2012) 159–166

Who's Your Boss? Matching Data Type and Uncertainties to Decision Context

It is critical to understand the uncertainties in the data

And match them to the regulatory decision context



"I think you should be more explicit here in step two."

EPA Office	Assessment "Workflows"	Historical Throughput	
OPPTS	Premanufacture Notice (PMN) New chemicals Significant New Use Rule (SNUR) Existing chemicals	~1000/yr 90d/chem ~84,000 total	
	Current Chemical Risk *(new program)*	~10 total	
	DFE / Green Chemistry	~2500	I, II, III
OSCP	Endocrine Screening Program	~10-20/year	
OPP	Pesticide registration (PR)	~10 new/yr ~50 old/yr	I
	Pesticide re-registration	~100 24,576	
OW	Chemical Contaminant List	6y ~6,000	
	Regulatory Actions on CCL	6y 90 to	
	Unregulated Contaminant Monitoring	30/5yr	I
	Drinking Water Health Advisories (MCLs)	~80 total	II, III
ORD NCEA	IRIS	~3/yr ~540 total	I
	PPRTV	400-500	II,III

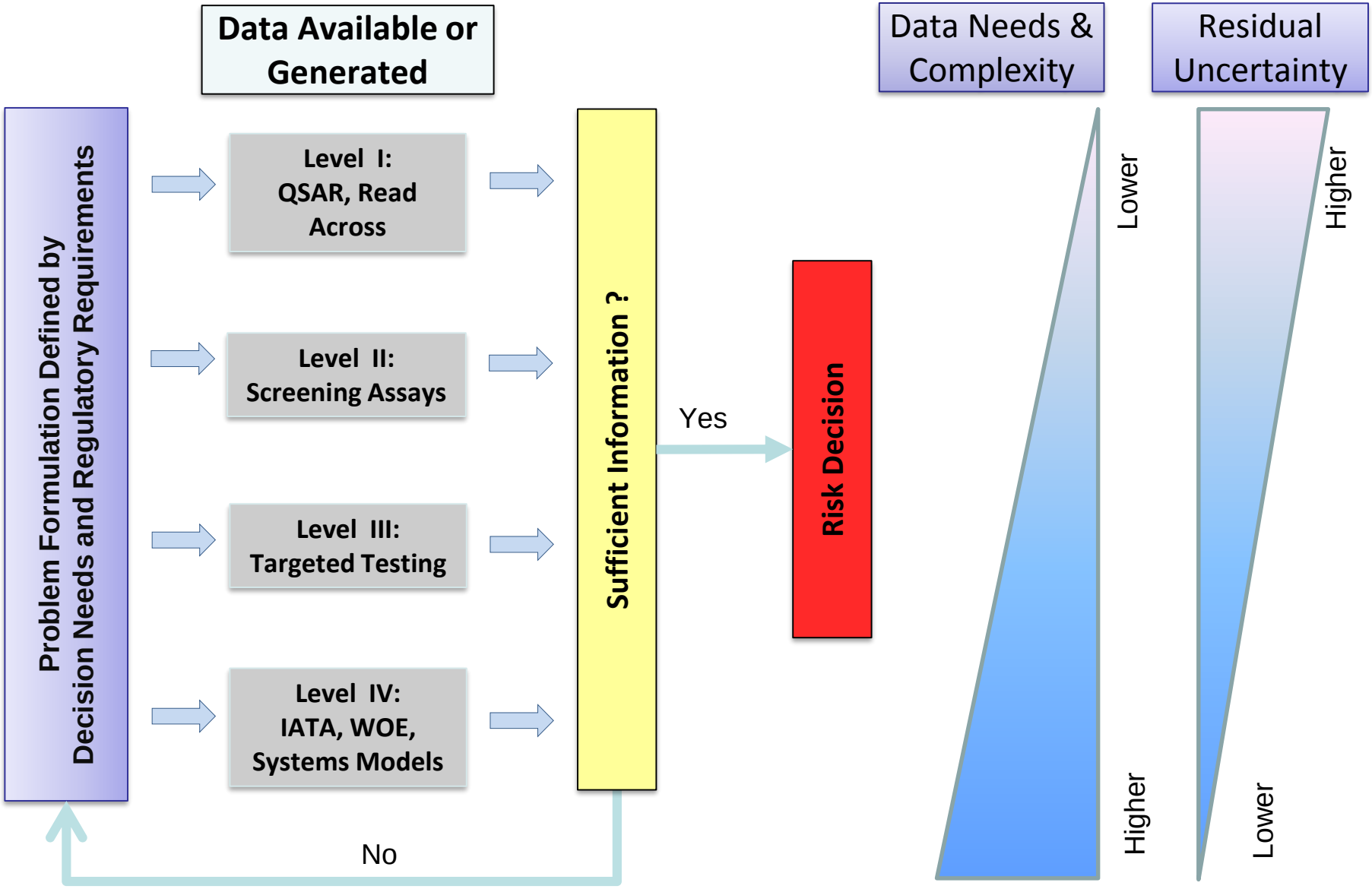
~1000/year
90 days/chemical
Very limited data.
eg structure, LogP

~10 chemicals/year
Lots and lots of data
$ millions/chemical

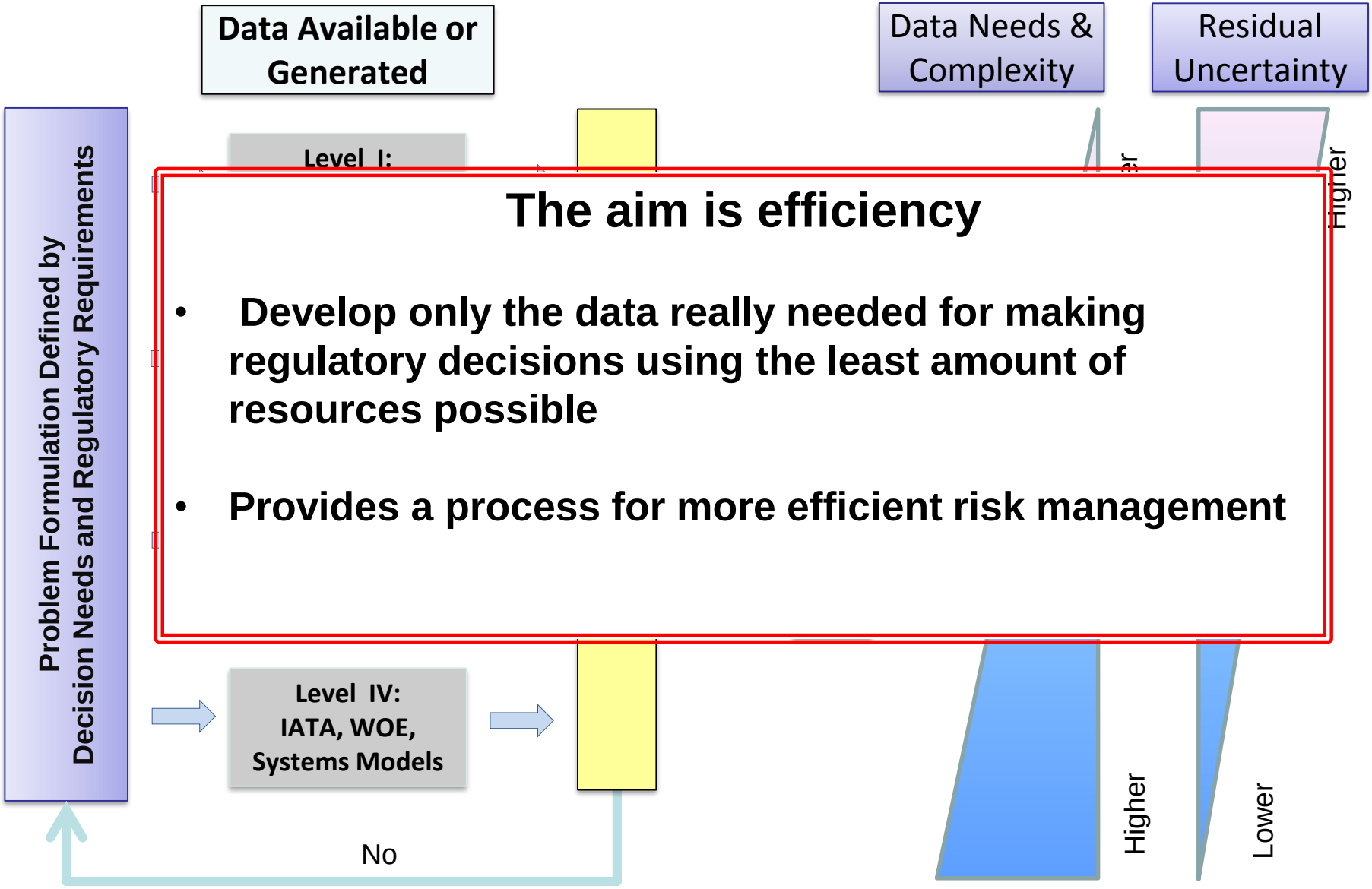
I. Data rich – Extensive guideline studies
II. Data partial – Some acute in vivo and in vitro data, SAR and exposure modeling
III. Data minimal to none – only chemical structure, SAR and exposure modeling

Slide courtesy of I. Shah

Matching Data Type and Uncertainties to Decision Context

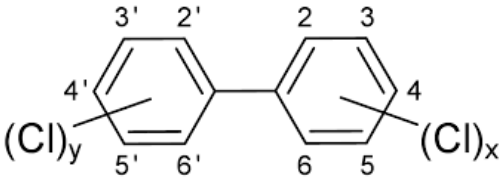


Matching Data Type and Uncertainties to Decision Context

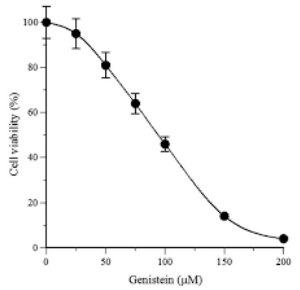


The Beginnings of a Solution

What's Necessary to Begin Solving the Problem of Too Many Chemicals With No Exposure or Hazard Information



1. **Chemical curation**
   - Everything starts with chemical structure

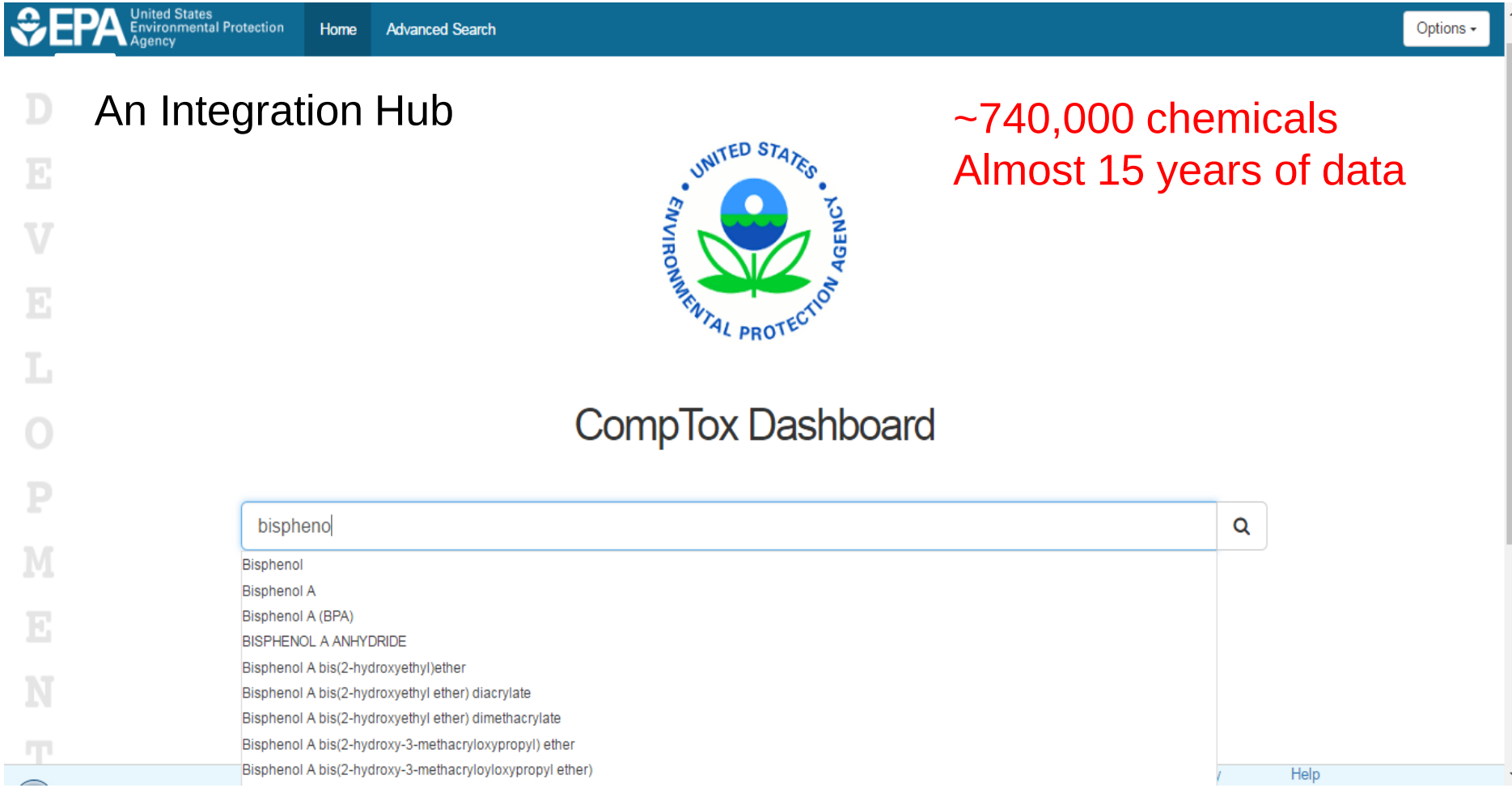


2. **Prediction of hazard (or bioactivity)**
   - *Need fast efficient testing methods*

3. **Predictions of exposure**
   - Need new models that predict or measure exposures

4. **Putting it all together**
   - Models that integrate this into estimates of risk
   - Tools that can be used by risk managers

CompTox Dashboard - Chemistry

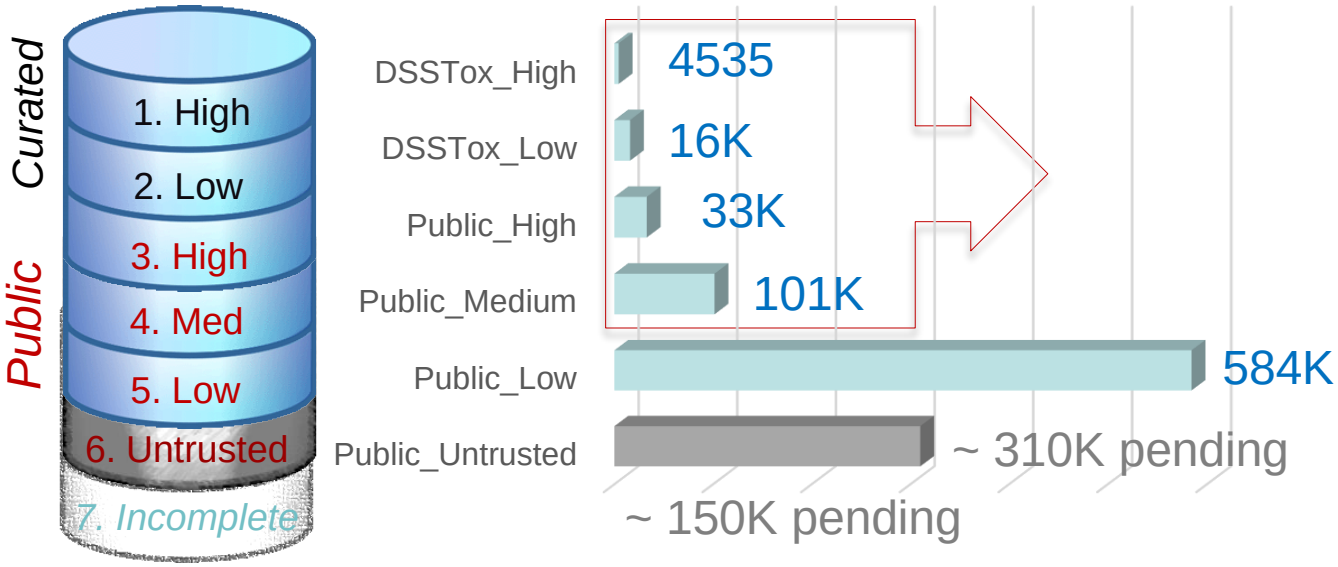


~740,000 chemicals
Almost 15 years of data

https://comptox.epa.gov/dashboard

Even in Chemistry there is Uncertainty

Chemicals Information Ranked on "Confidence"



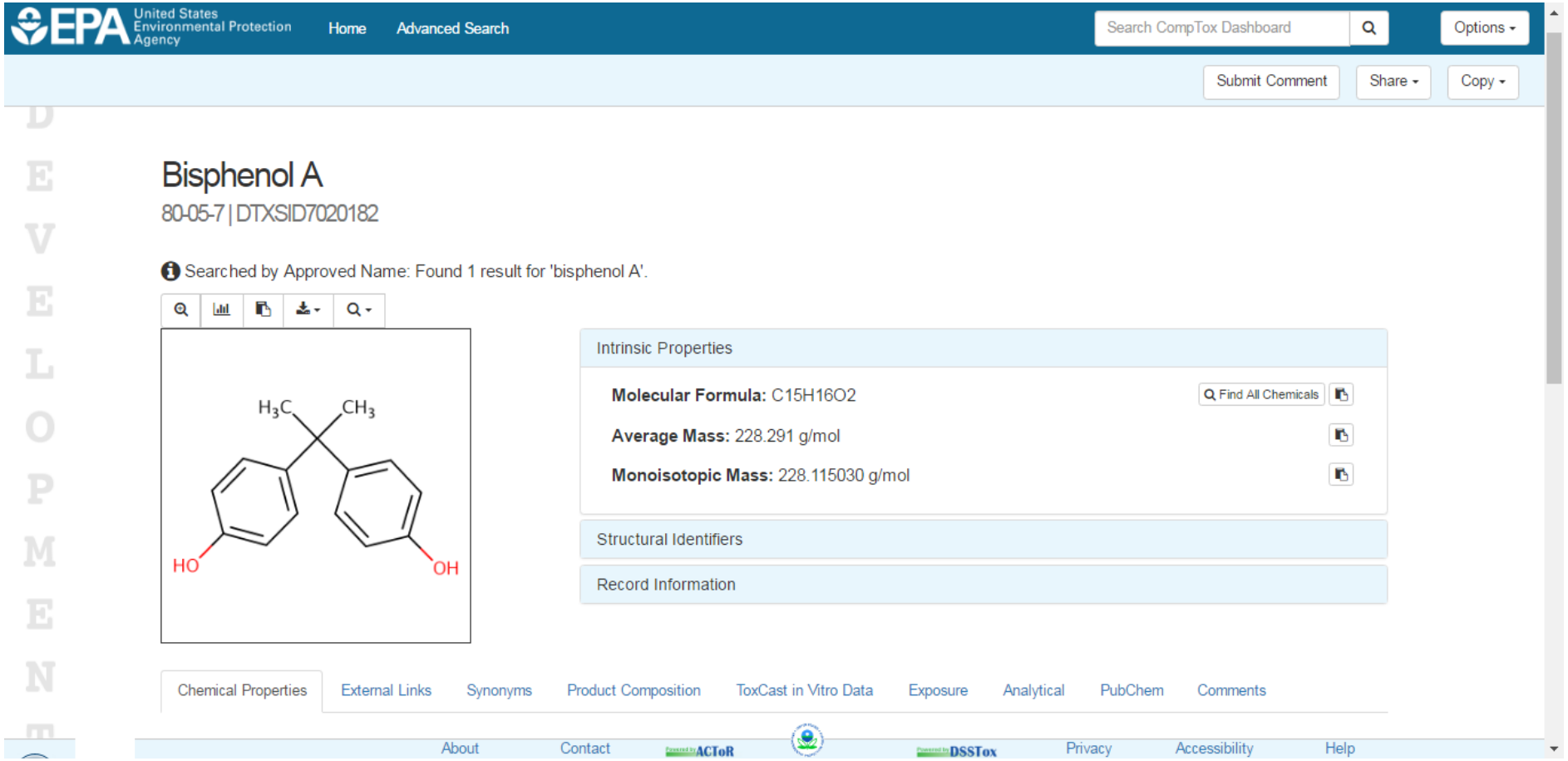
QC Levels

DSSTox_High:	Hand curated and validated
DSSTox_Low:	Hand curated and confirmed using multiple public sources
Public_High:	Extracted from EPA SRS and confirmed to have no conflicts in ChemID and PubChem
Public_Medium:	Extracted from ChemID and confirmed to have no conflicts in PubChem
Public_Low:	Extracted from ACToR or PubChem
Public_Untrusted:	Postulated, but found to have conflicts in public sources

Richard et al. Chem Res Toxicol. 29:1225-1251, 2016

EPA United States Environmental Protection Agency

Dashboard Example Bisphenol A



Chemical Properties | External Links | Synonyms | Product Composition | ToxCast in Vitro Data | Exposure | PubChem | Comments

Generating Bioactivity Data

ToxCast and Tox21 Programs

- **ToxCast** – EPA program
  - Multi-year research program started in 2007
  - Use automated in vitro chemical screening technologies to expose living cells or isolated proteins to chemicals where changes in biological activity may suggest potential toxic effects
  - Chemical library
    - ~3400 environmentally relevant chemicals

      http://www.epa.gov/ncct/toxcast/
- **Tox21** – Collaborative project
  - US EPA, NIH/NCATS, NIH/NIEHS/NTP and FDA
  - aimed at developing better toxicity assessment methods using HTS.
  - Chemical library
    - ~8,500 chemicals, including environmental chemicals, food additives and pharmaceuticals
      http://www.ncats.nih.gov/research/reengineering/tox21/tox21.html

Increased Throughput Required Shift to Molecular/Pathway Approaches

ToxCast

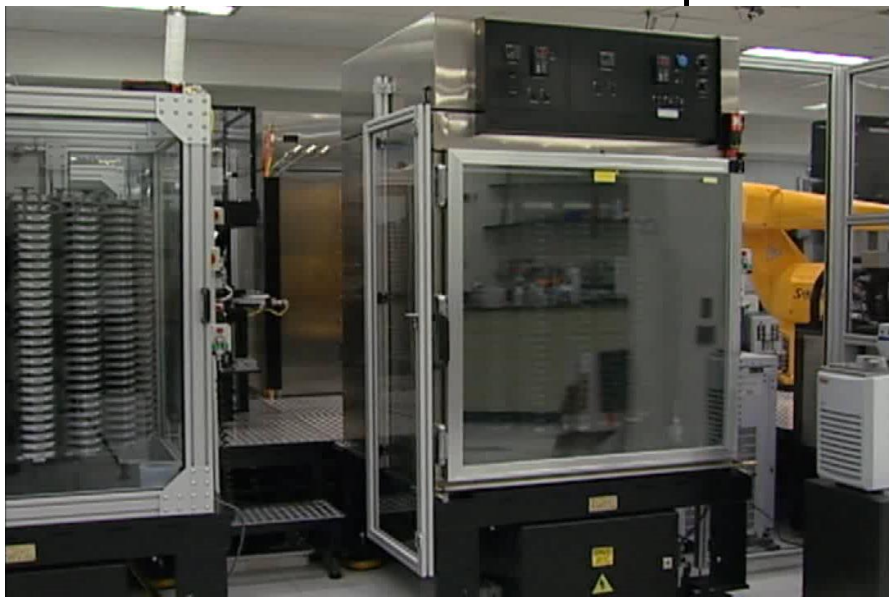
~600 Cell & biochemical assays

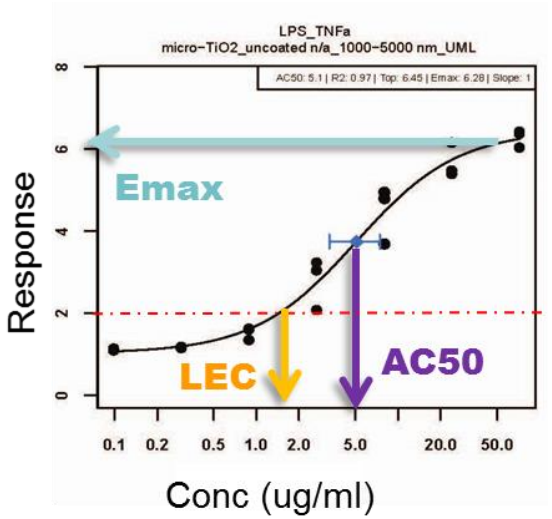
~1,000 Chemicals

Tox21

~30 Cell & biochemical assays

~8,000 Chemicals

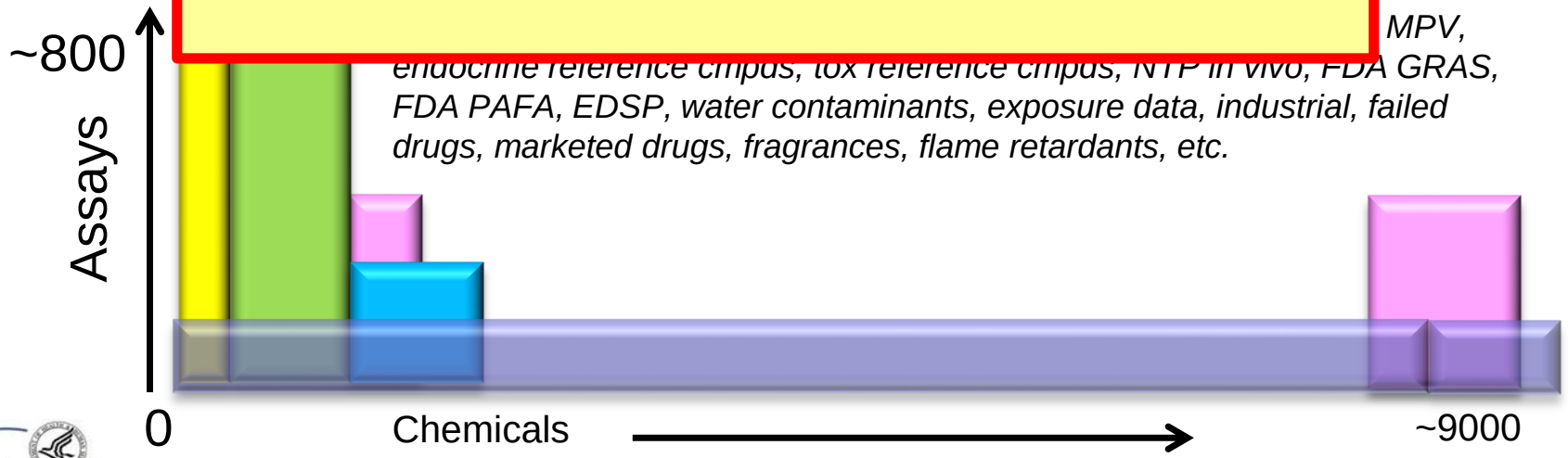




ToxCast & Tox21: Chemicals, Data and Release Timelines

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	Now
ToxCast E1K					Now
Tox21					ngoing
ToxCast Phas					2016

72 million data points
2.8 million conc response curves

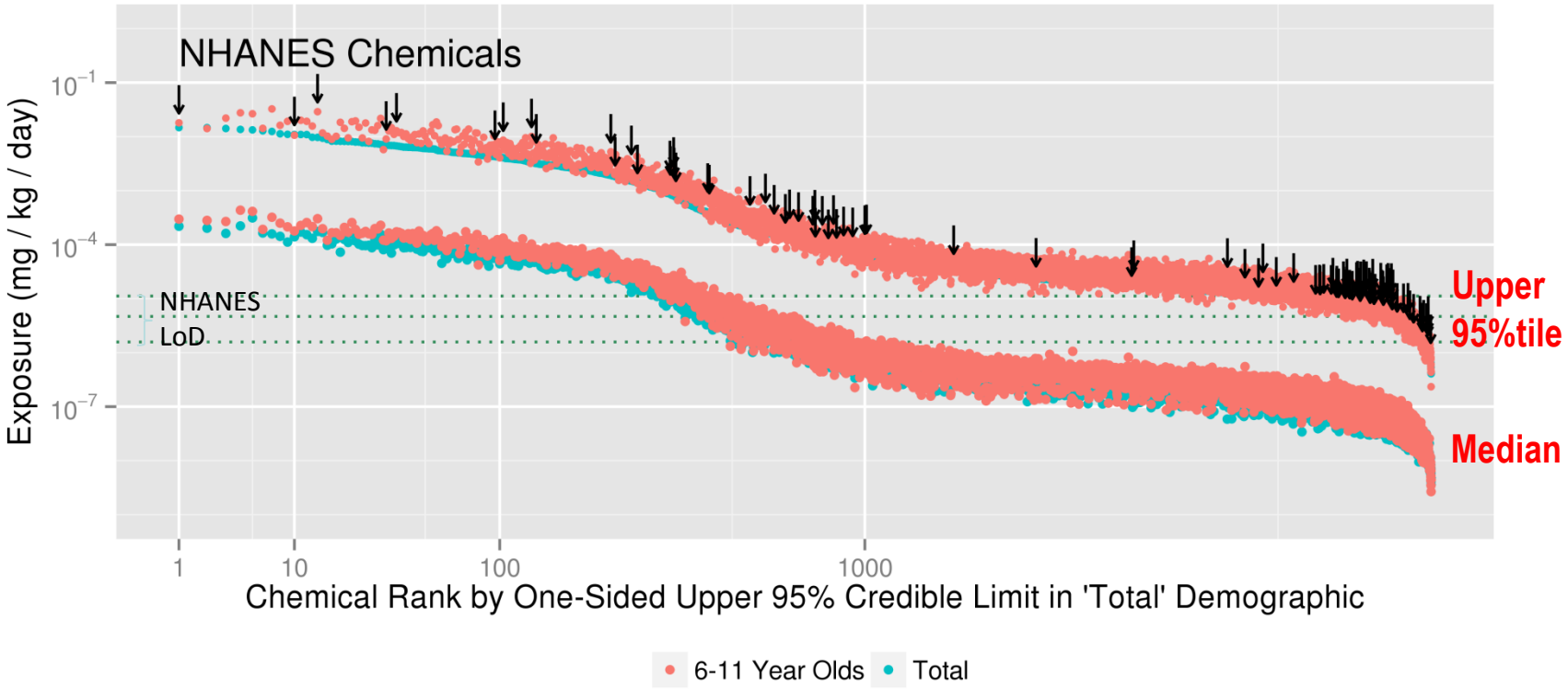


ExpoCast
HTP Exposure Predictions

- **For years exposure science has lagged behind**
  - Most models require extensive information on production, use, fate and transport and rely on empirical data *(no measurement = no exposure?)*

- **ExpoCast**
  - Exposure predictions based on:
    - pChem, production values, fate and transport, and product use categories (e.g., industrial, pesticide use, consumer personal care)
    - Industrial vs consumer use
  - Yields *predictions of exposure estimates* and Baysian confidence

Exposure Predictions for 7968 Chemicals & Comparison to NHANES

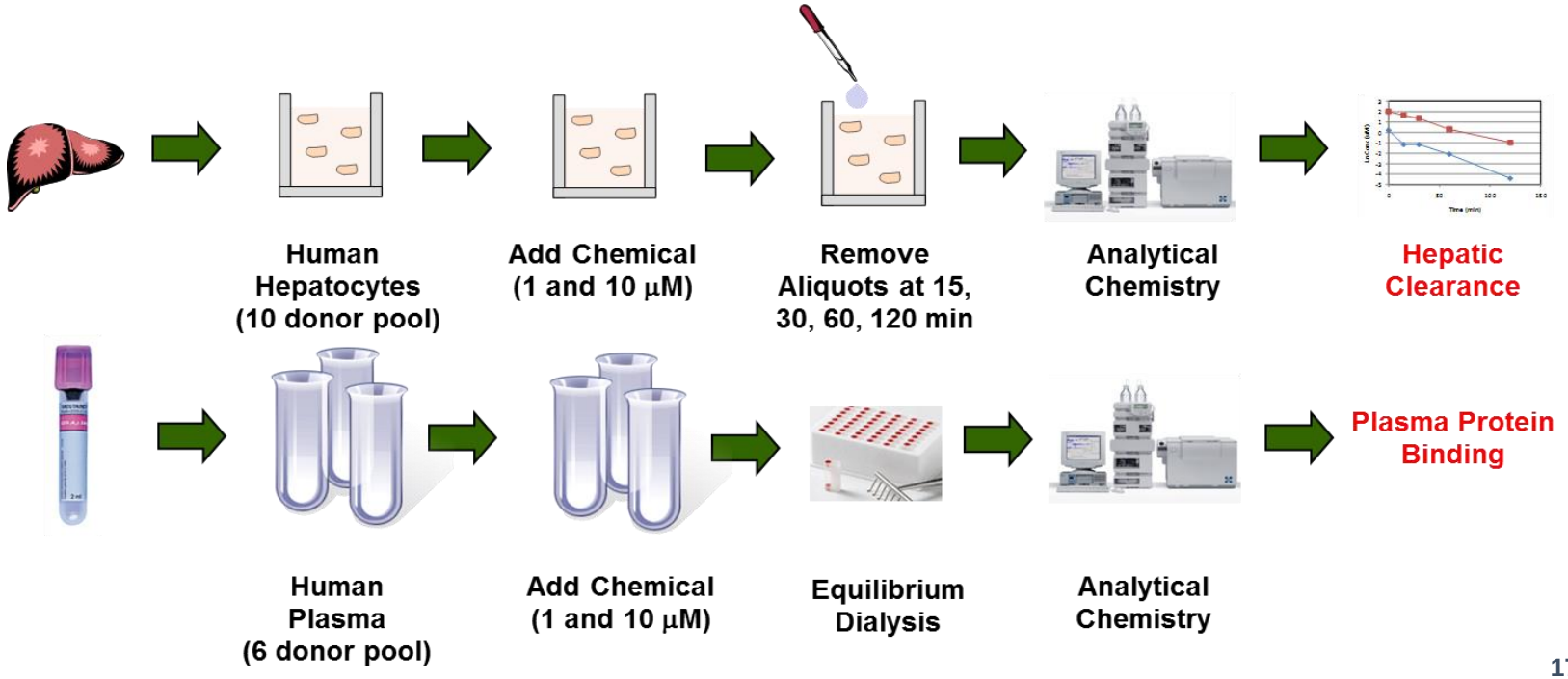


- NHANES – US National Study – measures exposures in human serum and urine
- Chemicals currently monitored by NHANES are distributed throughput the predictions
- Shows accuracy of the prediction model

***Wambaugh et al Environ. Sci. Technol.*, 2014, *48* (21), pp 12760–12767**

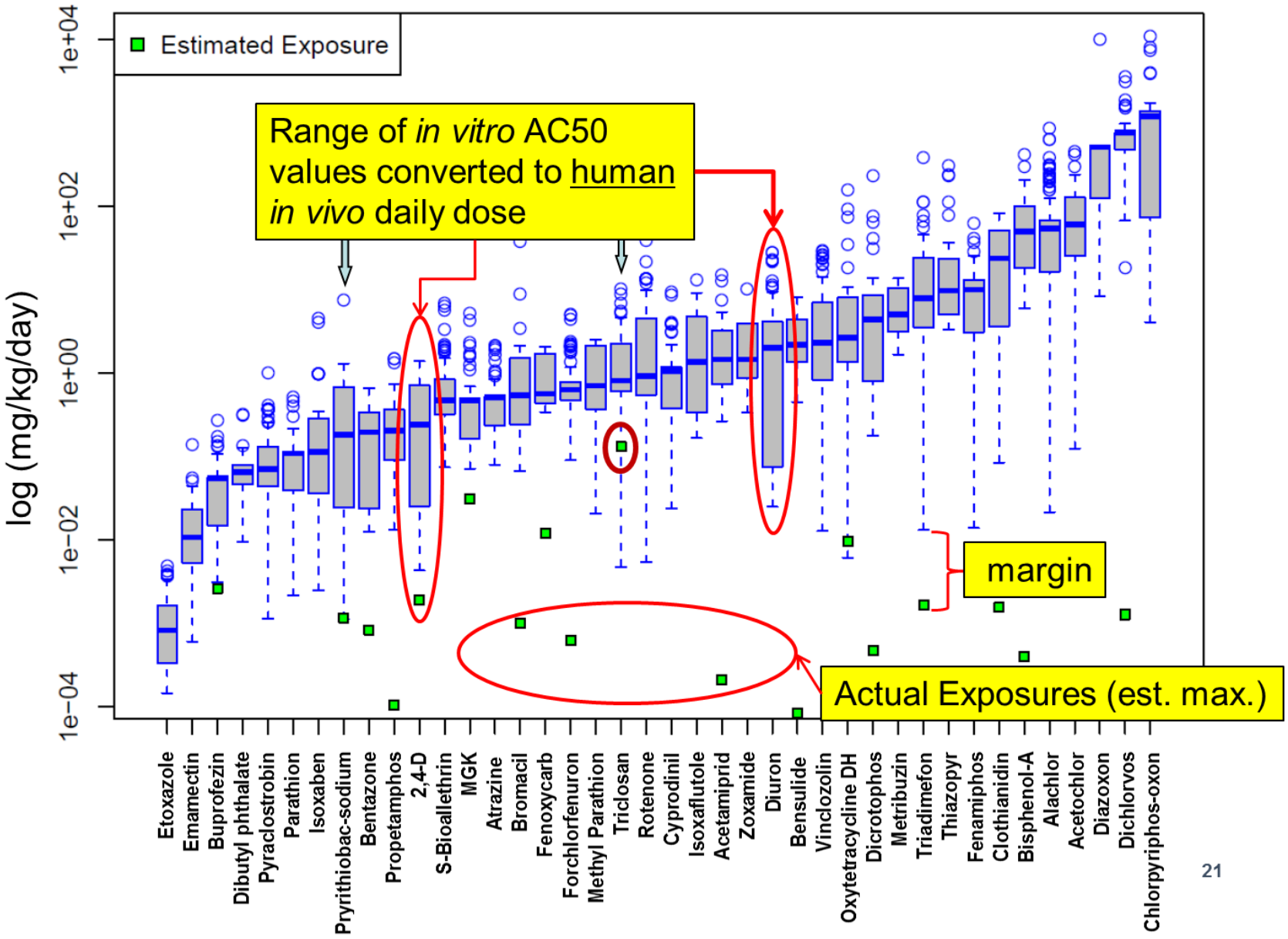
Estimating Daily Dose with Reverse ToxicoKinetics (rTK)

- VERY SIMPLE biokinetics models – *measure only 2 parameters*
  - in vitro hepatic clearance disappearance of parent compound
  - serum protein binding values
- Provides scaling from concentration in which there is in vitro biological activity to in vivo activity dose (mg/kg/day)



(Rotroff et al, ToxSci 2010, Wetmore et al, ToxSci 2012)

Estimating Exposures for *in vitro bio*activity



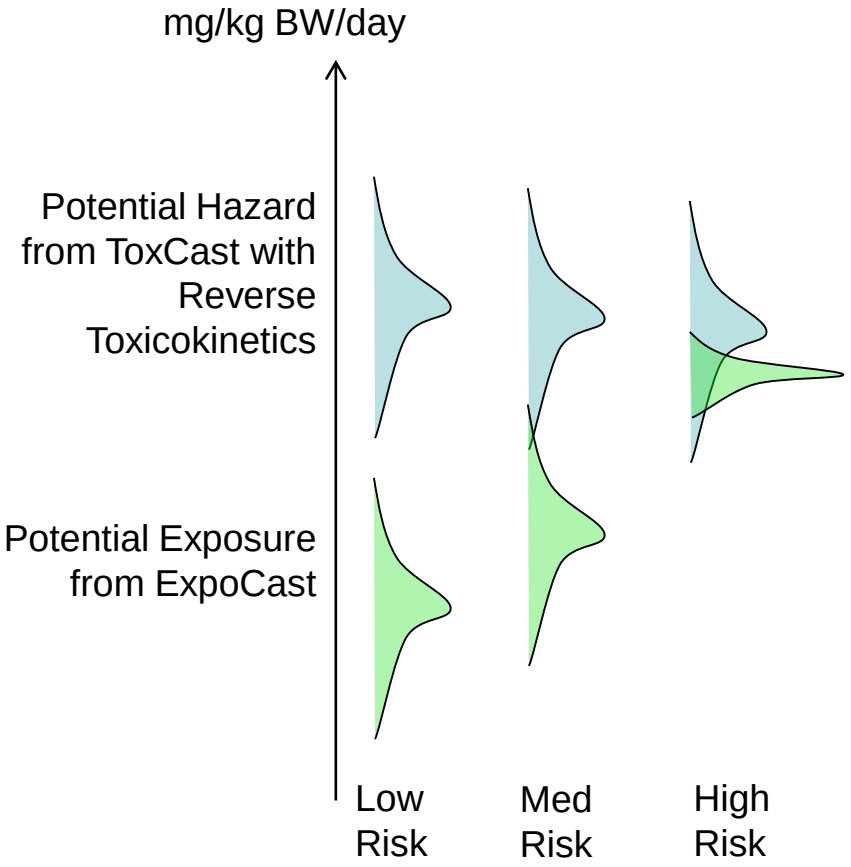
Wetmore et al Tox Sci 2012

Putting It All Together
HT Prioritization

Risk is the product of hazard and exposure

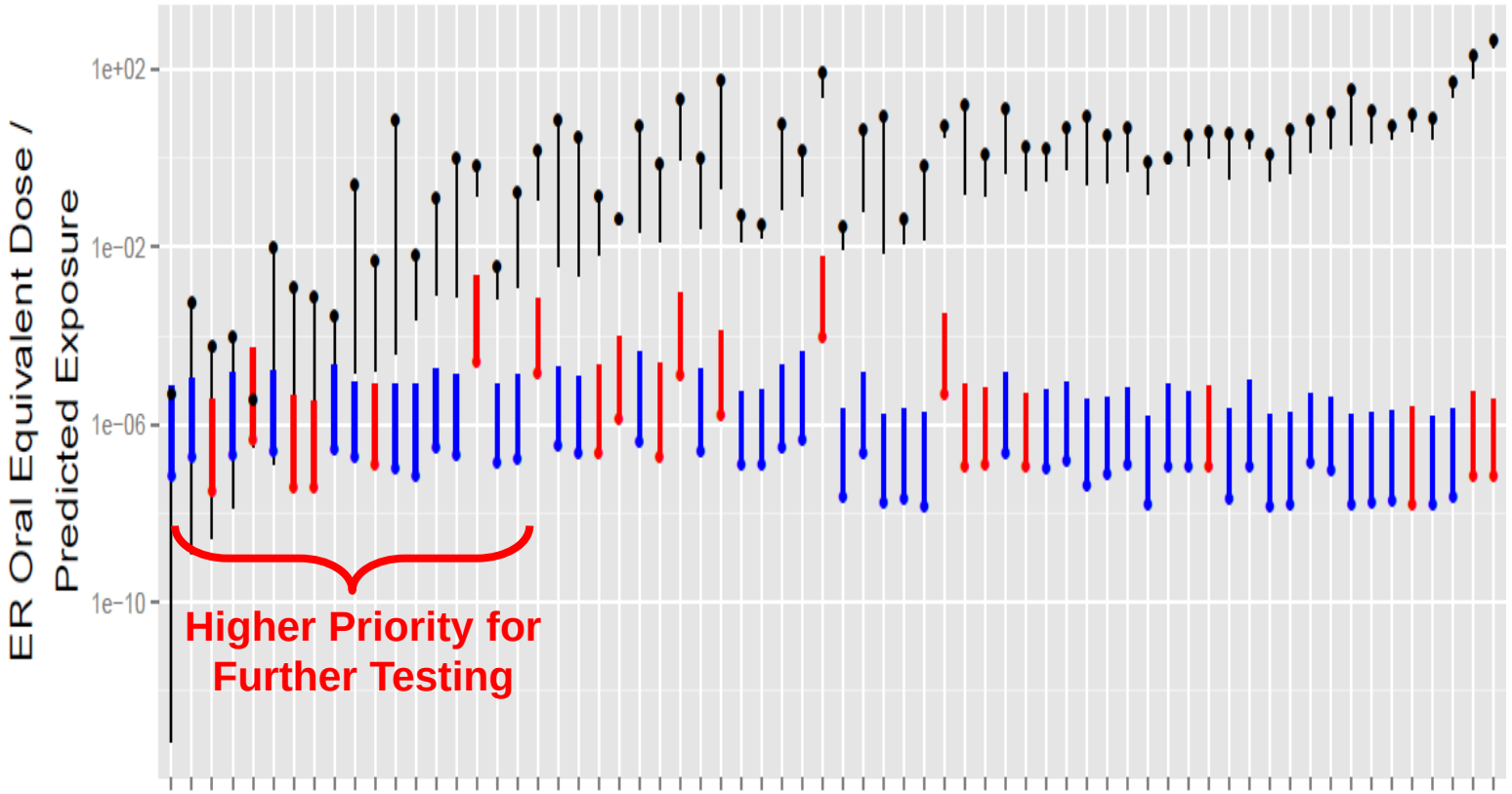
Use rTK convert bioactive concentrations to daily dose

Combine with exposure prediction



Judson *et al.*, (2011)
Chemical Research in Toxicology

Combining Bioactivity-Base Dose and Exposure Estrogen Active Chemicals



Prioritization = test the chemicals that might be the worst, first!

If You Build It, Will They Use It?

United States Environmental Protection Agency

Peer Review of New Approaches by FIFRA Science Advisory Panel and Public Led to Adoption by Regulatory Partners

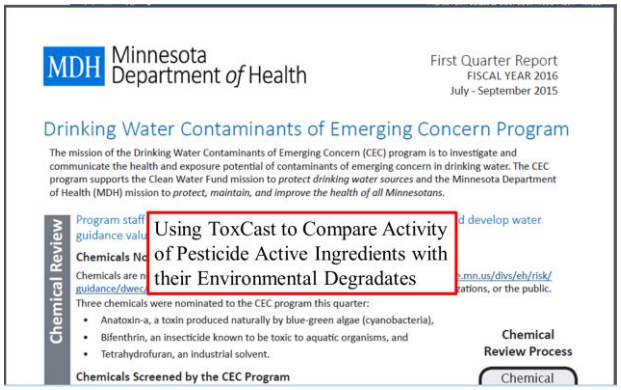
Federal Register Notice - Dec 2014

Proposal for use:

- **Prioritization**
- **And for first time replacement!**

If You Build It, Will They Use It?

Minnesota Dept Health Risk Assessments for Water Contaminants


MDH Minnesota Department of Health

First Quarter Report
FISCAL YEAR 2016
July - September 2015

Drinking Water Contaminants of Emerging Concern Program

The mission of the Drinking Water Contaminants of Emerging Concern (CEC) program is to investigate and communicate the health and exposure potential of contaminants of emerging concern in drinking water. The CEC program supports the Clean Water Fund mission to *protect drinking water sources* and the Minnesota Department of Health (MDH) mission to *protect, maintain, and improve the health of all Minnesotans.*

Chemical Review

Program staff … d develop water guidance valu…

Chemicals No…

Chemicals are n… …c.mn.us/divs/eh/risk/guidance/dwec… …rations, or the public.

Three chemicals were nominated to the CEC program this quarter:

- Anatoxin-a, a toxin produced naturally by blue-green algae (cyanobacteria),
- Bifenthrin, an insecticide known to be toxic to aquatic organisms, and
- Tetrahydrofuran, an industrial solvent.

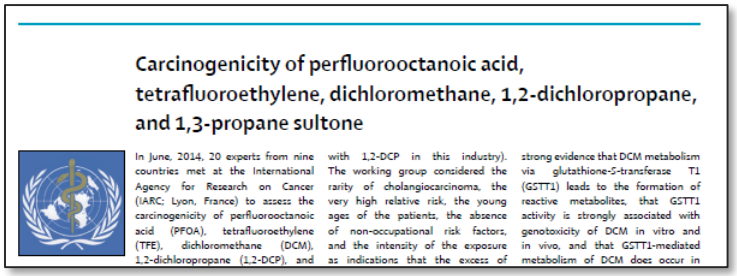
Chemicals Screened by the CEC Program

Chemical Review Process

Chemical

Using ToxCast to Compare Activity of Pesticide Active Ingredients with their Environmental Degradates

IARC Monographs


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 …

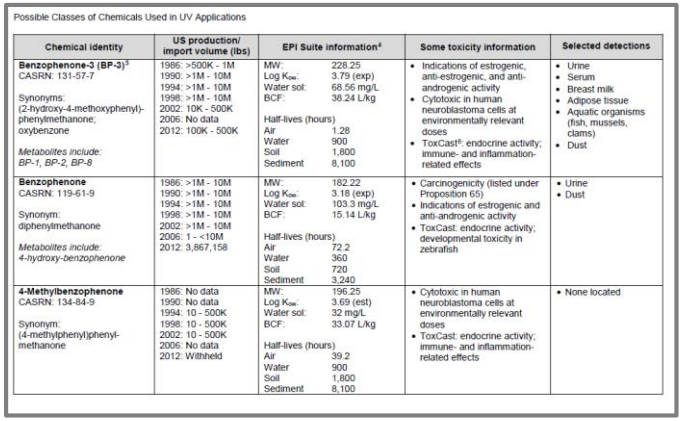
Australian IMAP


Australian Government
Department of Health
National Industrial Chemicals Notification and Assessment Scheme

Inventory Multi-tiered Assessment and Prioritisation (IMAP) Framework Review

April 2016

Prioritization for Biomonitoring CA


Possible Classes of Chemicals Used in UV Applications

Chemical identity	US production/ import volume (lbs)	EPI Suite information[4]	Some toxicity information	Selected detections
Benzophenone-3 (BP-3)[5] CASRN: 131-57-7 Synonyms: (2-hydroxy-4-methoxyphenyl)-phenylmethanone; oxybenzone *Metabolites include:* *BP-1, BP-2, BP-8*	1986: >500K - 1M 1990: >1M - 10M 1994: >1M - 10M 1998: >1M - 10M 2002: 10K - 500K 2006: No data 2012: 100K - 500K	MW: 228.25 Log K_{ow}: 3.79 (exp) Water sol: 68.56 mg/L BCF: 38.24 L/kg Half-lives (hours) Air 1.28 Water 900 Soil 1,800 Sediment 8,100	• Indications of estrogenic, anti-estrogenic, and anti-androgenic activity • Cytotoxic in human neuroblastoma cells at environmentally relevant doses • ToxCast[6]: endocrine activity; immune- and inflammation-related effects	• Urine • Serum • Breast milk • Adipose tissue • Aquatic organisms (fish, mussels, clams) • Dust
Benzophenone CASRN: 119-61-9 Synonym: diphenylmethanone *Metabolites include:* *4-hydroxy-benzophenone*	1986: >1M - 10M 1990: >1M - 10M 1994: >1M - 10M 1998: >1M - 10M 2002: >1M - 10M 2006: 1 - <10M 2012: 3,867,158	MW: 182.22 Log K_{ow}: 3.18 (exp) Water sol: 103.3 mg/L BCF: 15.14 L/kg Half-lives (hours) Air 72.2 Water 360 Soil 720 Sediment 3,240	• Carcinogenicity (listed under Proposition 65) • Indications of estrogenic and anti-androgenic activity • ToxCast: endocrine activity; developmental toxicity in zebrafish	• Urine • Dust
4-Methylbenzophenone CASRN: 134-84-9 Synonym: (4-methylphenyl)phenyl-methanone	1986: No data 1990: No data 1994: 10 - 500K 1998: 10 - 500K 2002: 10 - 500K 2006: No data 2012: Withheld	MW: 196.25 Log K_{ow}: 3.69 (est) Water sol: 32 mg/L BCF: 33.07 L/kg Half-lives (hours) Air 39.2 Water 900 Soil 1,800 Sediment 8,100	• Cytotoxic in human neuroblastoma cells at environmentally relevant doses • ToxCast: endocrine activity; immune- and inflammation-related effects	• None located

Health Canada


Government of Canada / Gouvernement du Canada — Canada

Chemical Substances
www.chemicalsubstances.gc.ca

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Home > Chemicals Management Plan

Main Menu
- Canada's approach on chemicals
- Chemicals Management Plan
- Canada-United States Regulatory Cooperation Council
- Initiatives
- Monitoring & Surveillance
- Resources
- Science Committee

Chemicals Management Plan

Chemicals are part of everyday life, essential to our economy, our communities and our homes. While chemicals provide benefits, they may also have harmful effects if not properly managed.

The Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. The next phase of the CMP, launched in May 2016, will address the remaining 1550 priority chemicals out of the original 4300 chemicals identified as priorities during the categorization. The Minister of Health and the Minister of Environment and Climate Change have committed to addressing these chemicals by 2020. The CMP builds on previous initiatives by assessing chemicals used in Canada and by taking action on chemicals found to be harmful to human health and/or the environment.

Nothing is Ever Perfect

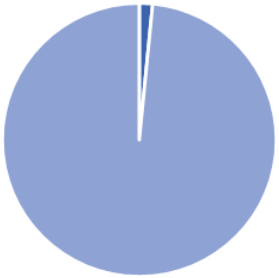
- You don't include metabolism in you in vitro assays
- You don't measure my favorite endpoint (or all of biology)
- In vitro assays are not normal biology
- Assay (x) in your battery did not get the right answer for my chemical
- My assay disagrees with your assay (x), so your approach is flawed
- You can't test my favorite chemicals because of limitations in your methods (e.g., solvents, high LogP)
- You can't possibly do RTK modeling with those simple models!
- You can't do HT Exposure predictions based on simple use models!

- If not this, then what? What other methods could we use for prioritization of thousands of chemicals?

Challenges: Increasing Biological Coverage

Next Generation Chemical Testing with New Sequencing Technologies

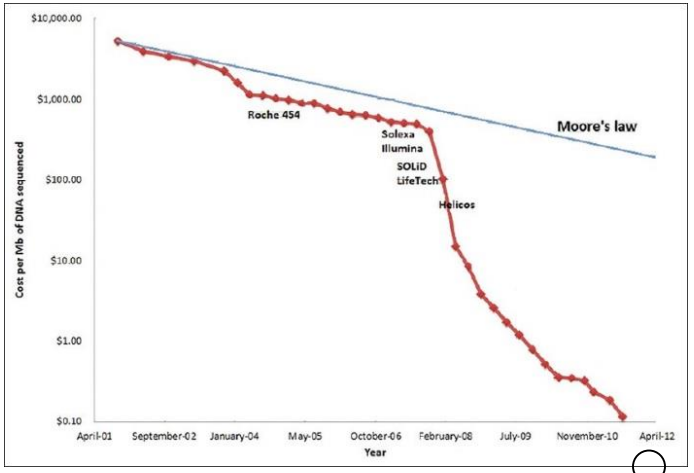
Gene Coverage

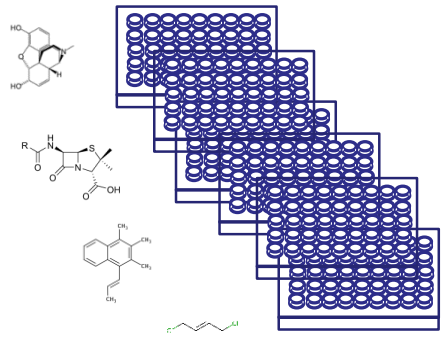


ToxCast

Not in ToxCast

Sequencing Costs 2001-present





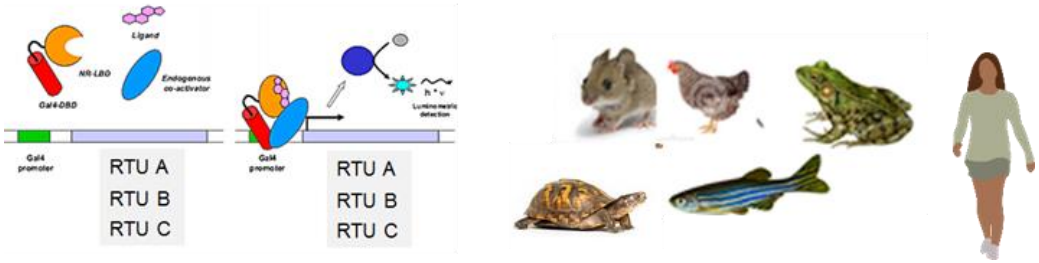
Ongoing pilot projects using TempOSeq – a more **cost-efficient** whole gene sequencing platform

Assessing the effects of thousands of chemicals on all the genes in the genome

Challenges: Increasing Species Coverage

- ToxCast & Tox21 are mostly human based
- Pilot project using Attagene system – insert receptor ligand binding domains from multiple species
- Multiple readouts of nuclear receptor hits from one cell

Multispecies Attagene *Trans* Reporter Assay



- Host cell: human HepG2
- 100 chemicals with ER, AR, TR, PPAR activity tested in concentration-response
- Pilot data using positive and negative reference chemicals is promising

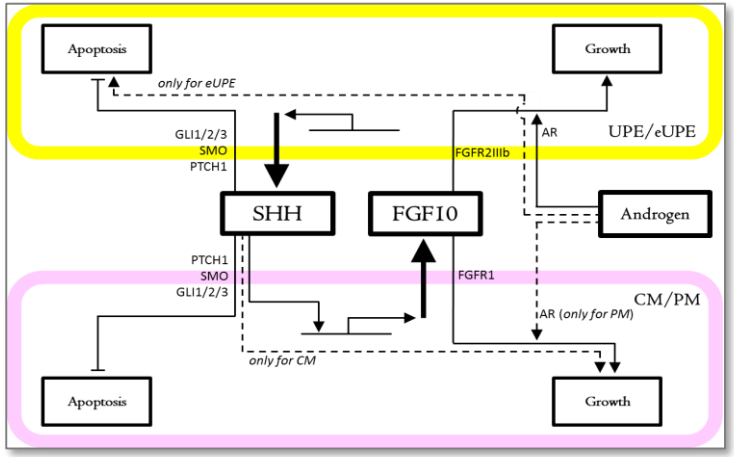
Receptor Family	Receptor Name	Species
Estrogen Receptor	ERa	Human
Estrogen Receptor	ERb	Human
Estrogen Receptor	ER1	Zebrafish
Estrogen Receptor	ER2a	Zebrafish
Estrogen Receptor	ER2b	Zebrafish
Estrogen Receptor	ERa	Chicken
Estrogen Receptor	ER1	Frog
Estrogen Receptor	ER2	Frog
Estrogen Receptor	ERa	Turtle
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

Houck, unpublished

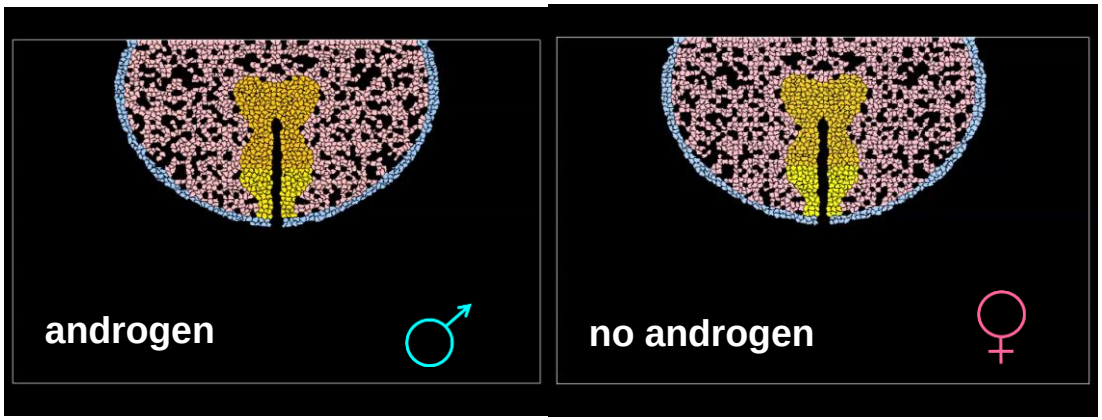
Challenges: Recapitulating Complex Biology in In Vitro and In Silico

An Example of Complex Systems Modeling

Signaling Network Underlying Virtual Genital Tubercle Model (Mouse)



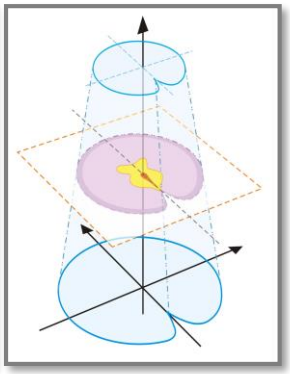
Simulation of Genital Tubercle Closure



GD13.5 – 17.5

Embryonic GT

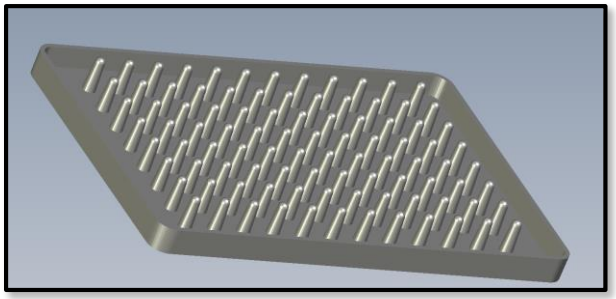
Abstracted GT

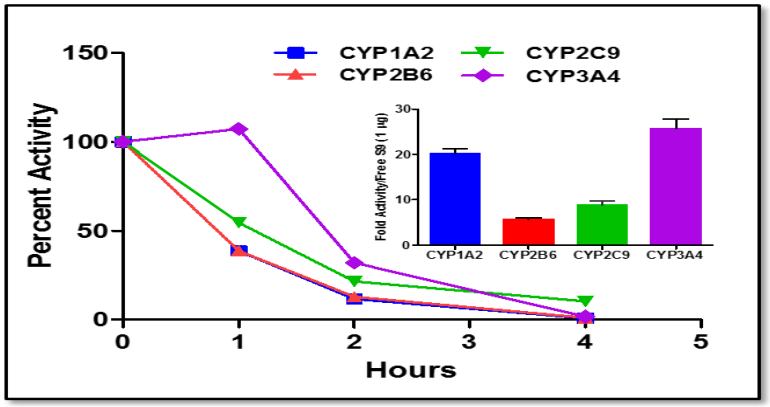


Androgenization	*Phenotype (MCS 4000)*			
(n = 10 sims)	Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

Leung et al. *Repro Toxicol*, 2016

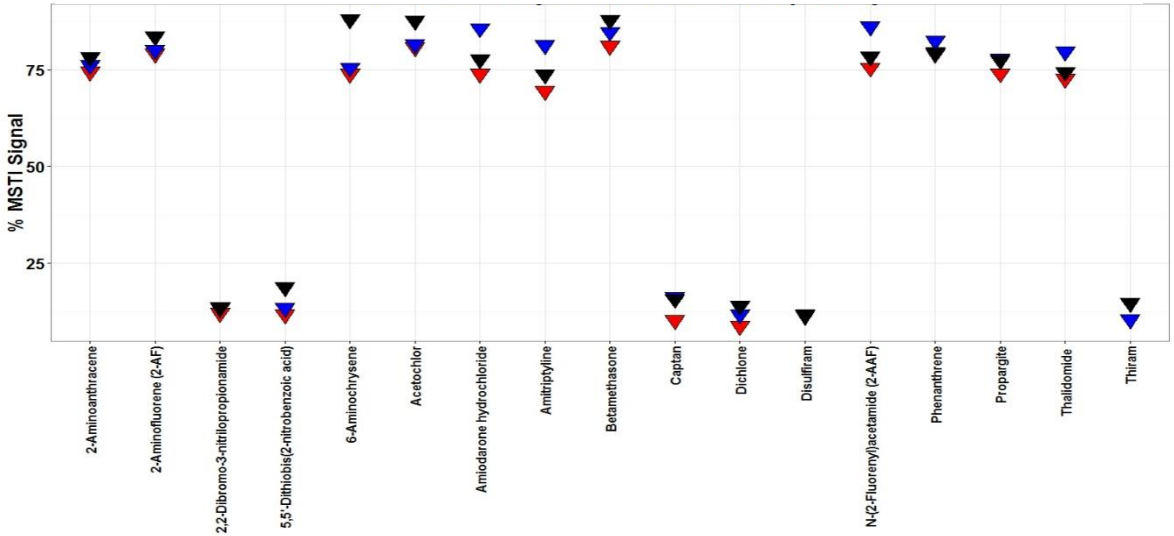
Challenges: Retrofitting Assays with Metabolic Activity





Cyp activity over time of encapsulated S9 fraction

MSTI Assay - An increase in electrophilicity was detected as a decrease in the fluorescent signal.



DeGroot and Simmons, Unpublished

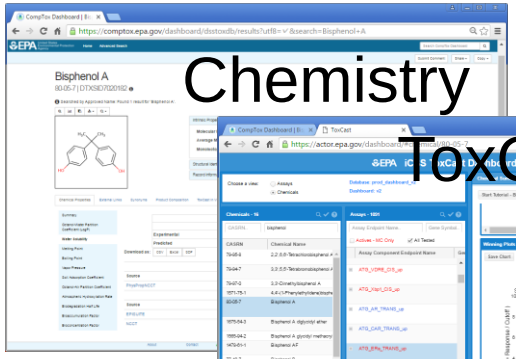
Challenges: Unless data is available and useful it will not be used

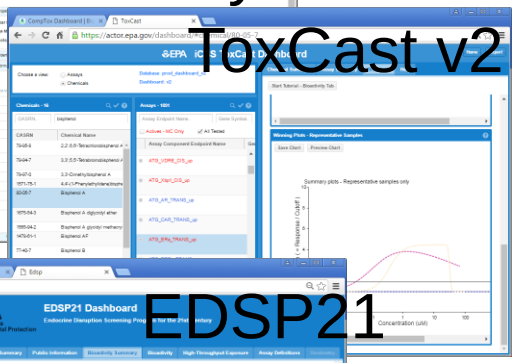
2011 Initial ToxCast Phase I Data Delivered as Flat Files

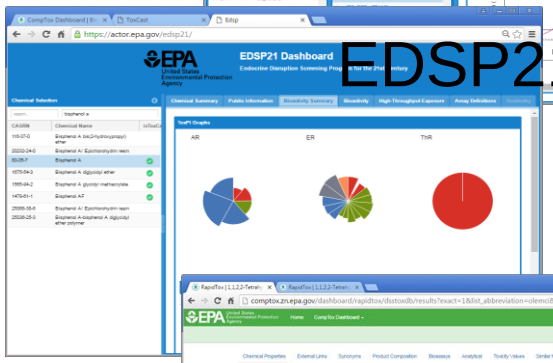
You need a bioinformatics degree for this to useful

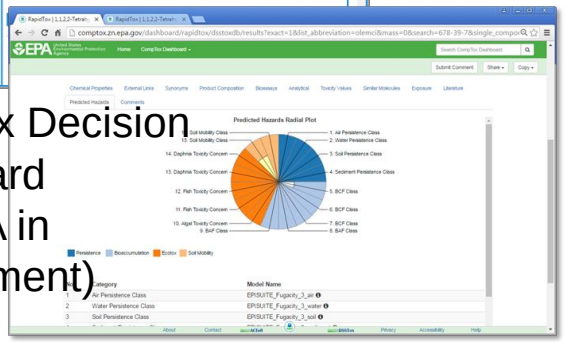
2013 Dashboard with Limited Search, Visualization, and Export Functionality

"Better, but still difficult to really get what you want without help from NCCT"





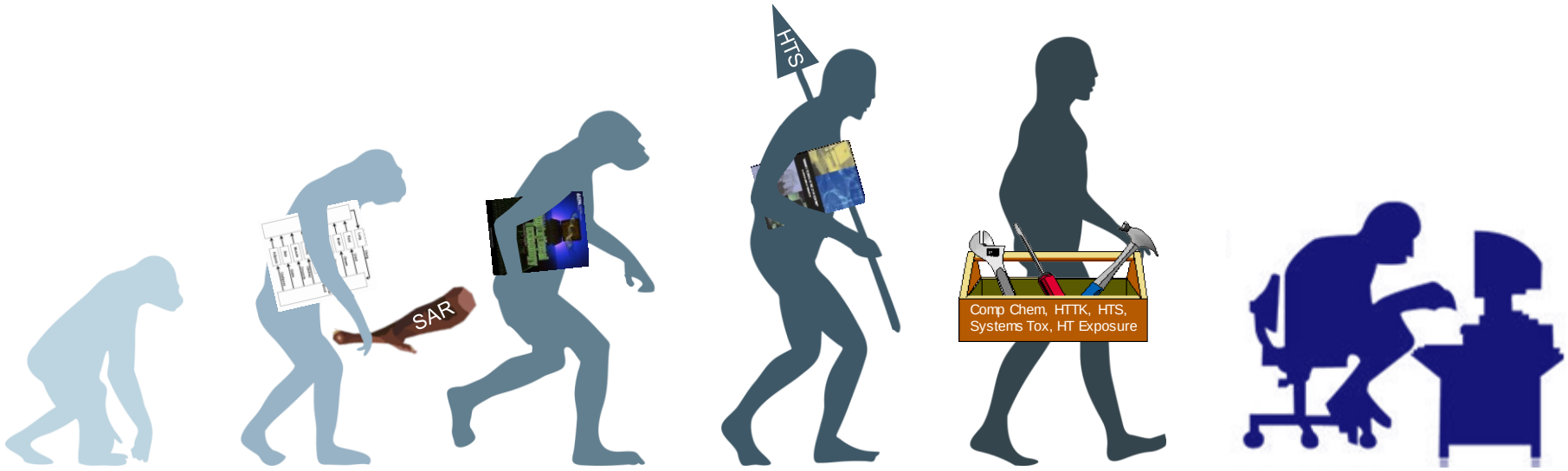




RapidTox Decision Dashboard (HTP RA in development)

Building data, analyses and visualization tools that allow for more rapid development of specific Decision Support Dashboards

Next Phase… Evolution Towards a Truly Predictive Science



c.1500 – 1960s | c. 1970s - 2002 | 2003 - 2006 | 2007 - 2010 | 2011 - 2017 | 2017 - ?

Acknowledgements

Tox21 Colleagues:

- NTP Crew
- NCATS Collaborators
- FDA Collaborators

EPA Colleagues:

- NERL
- NHEERL
- NCEA
- EPA Program Offices

External Stakeholders

- Health Canada
- CalEPA
- EDF
- EU Joint Research Center
- European Chemicals Authority

EPA's National Center for Computational Toxicology