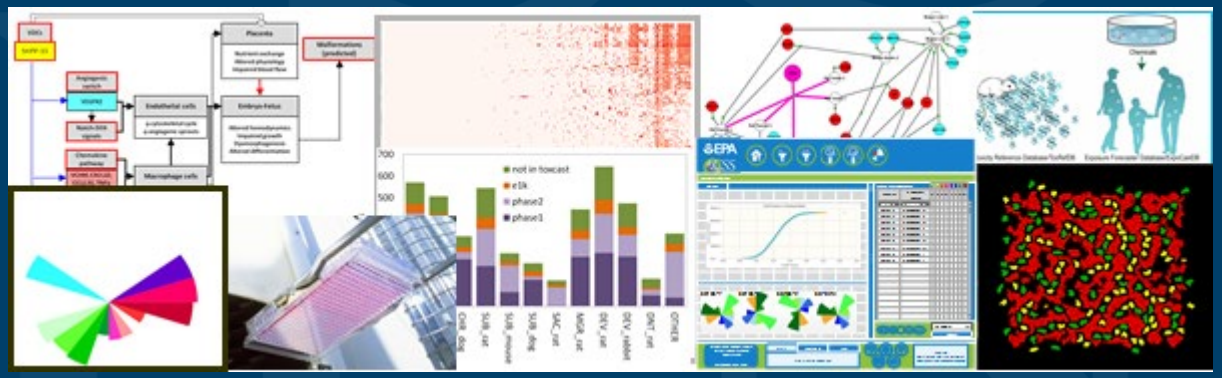
EPA
United States
Environmental Protection
Agency

High Throughput *in vitro* Assay Testing in Hazard Assessment

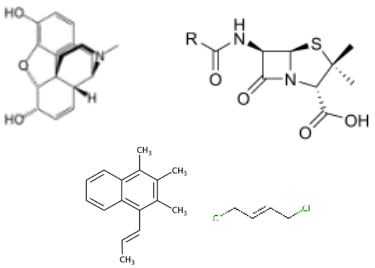


ToxScholar Visit
Trinity University
Washington, DC
October 22, 2018

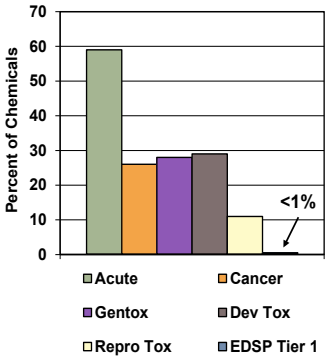
Maureen R. Gwinn
National Center for Computational Toxicology
Office of Research and Development
US Environmental Protection Agency

Regulatory Agencies Make a Broad Range of Decisions on Chemicals…

Number of Chemicals /Combinations



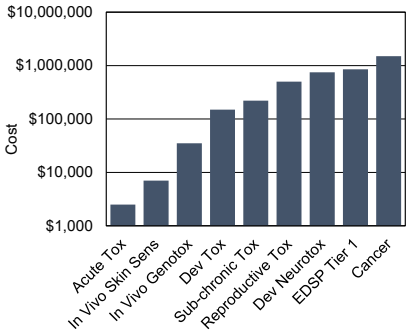
Lack of Data



Modified from Judson *et al.*, EHP 2010

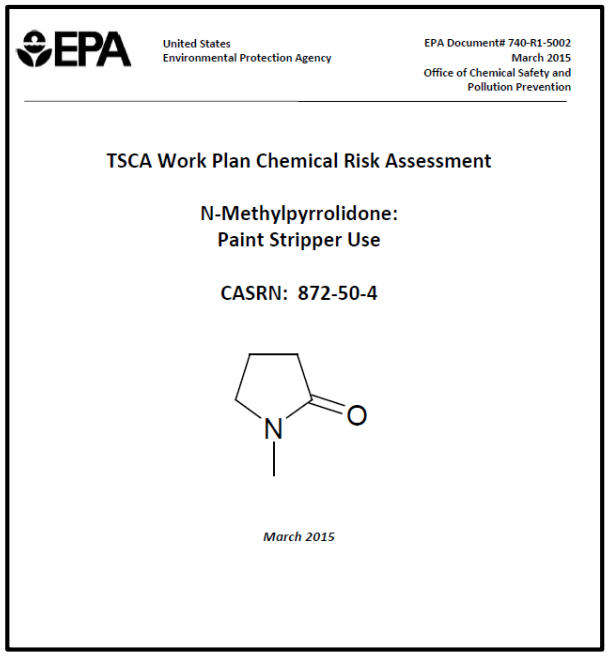
Ethics/Relevance Concerns

Economics



- Number of chemicals and combinations of chemicals is extremely large (>20,000 substances on active TSCA inventory)
- Traditional toxicity testing is expensive and time consuming
- Traditional animal-based testing has issues related to ethics and relevance
- Role of New Approach Methods (NAMs) to inform these decisions

Risk Assessments Generally Contain a Standard Set of Components



EPA United States Environmental Protection Agency

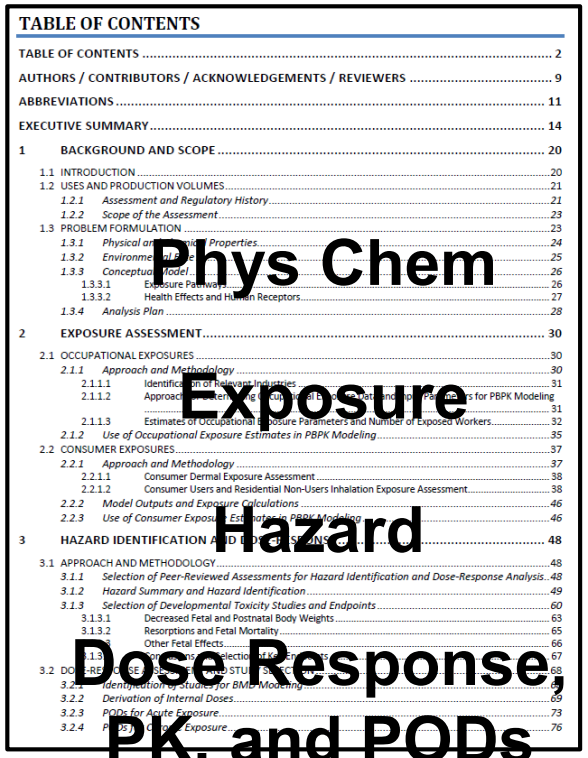
EPA Document# 740-R1-5002
March 2015
Office of Chemical Safety and Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

N-Methylpyrrolidone: Paint Stripper Use

CASRN: 872-50-4

March 2015



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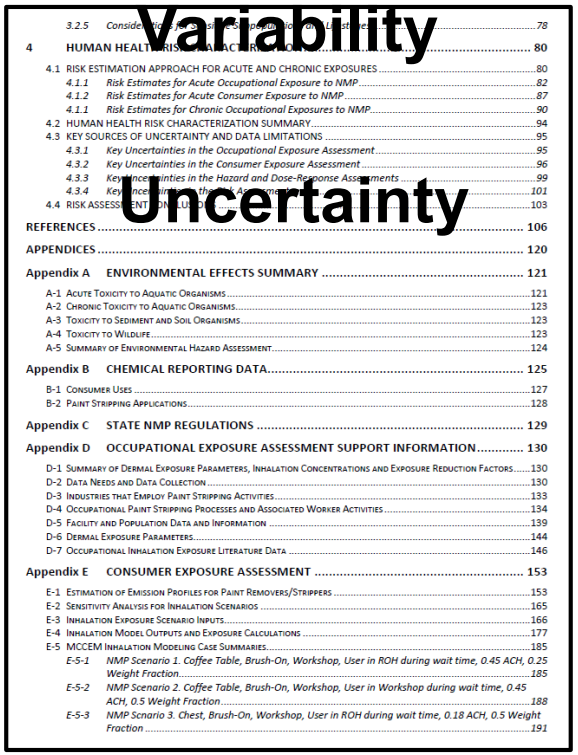

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Variability

Uncertainty

Phys Chem

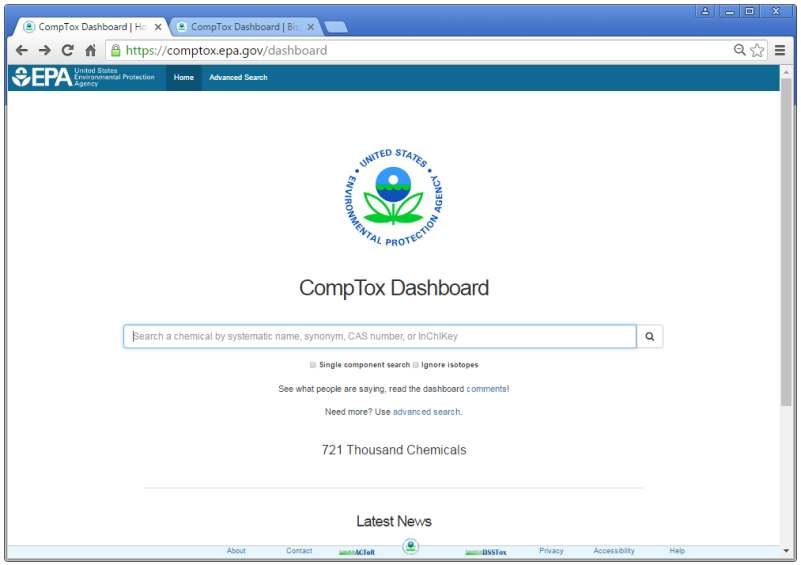
Exposure

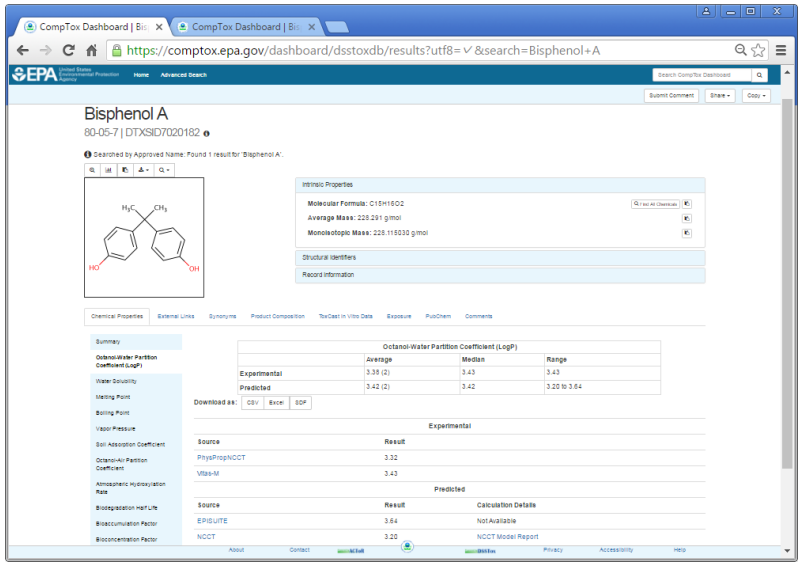
Hazard

Dose Response, PK, and PODs

New technologies and approaches will also have to cover these basic components

It All Starts With Chemistry…





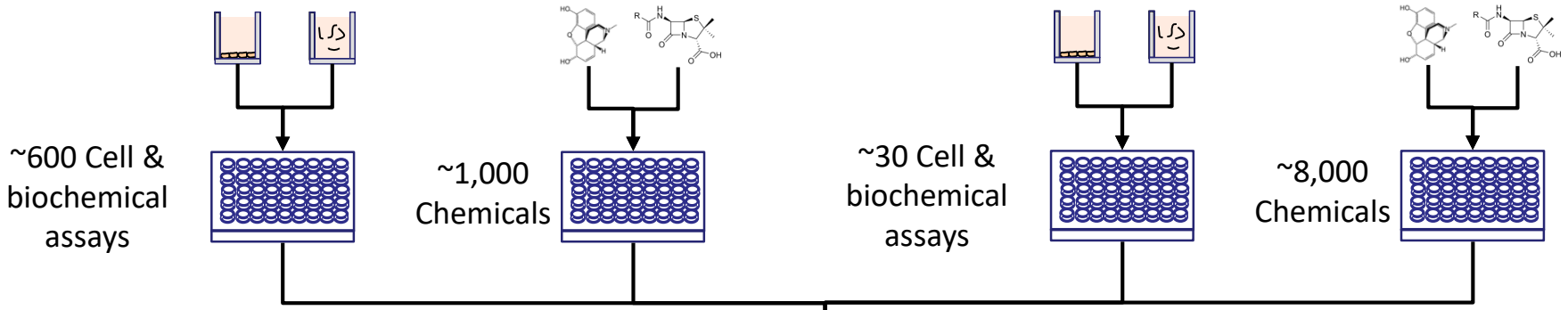
https://comptox.epa.gov/dashboard

- Chemical structure database of >700,000 unique substances with QC flags to link chemical structure with names and identifiers
- Consensus QSAR models for a range of physical chemical properties, environmental fate, and hazard characteristics
- Comprehensive physical-chemical property database (experimental and predicted)

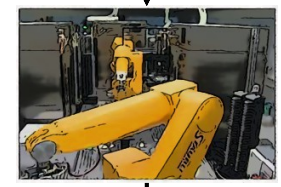
ToxCast and Tox21: Adding the High-Throughput Hazard Screening Component

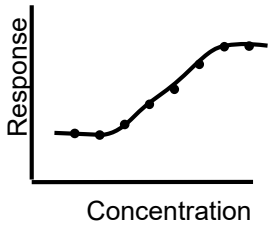
United States Environmental Protection Agency

ToxCast | **Tox21**



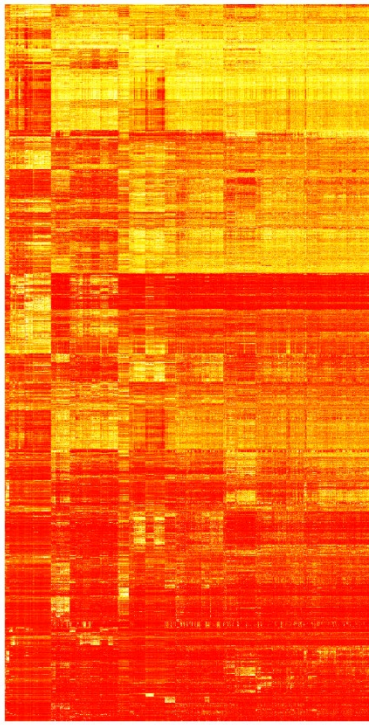
Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



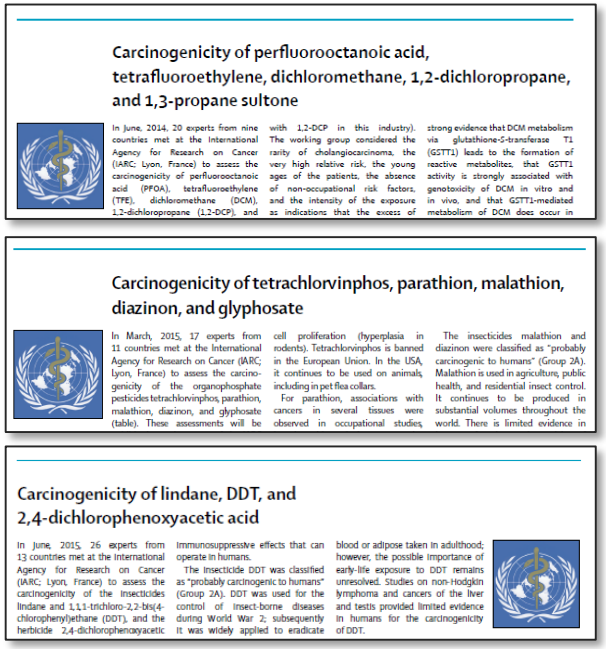


Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling



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

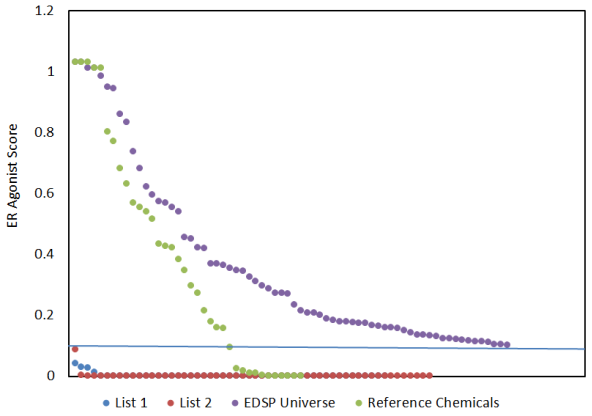
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

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.

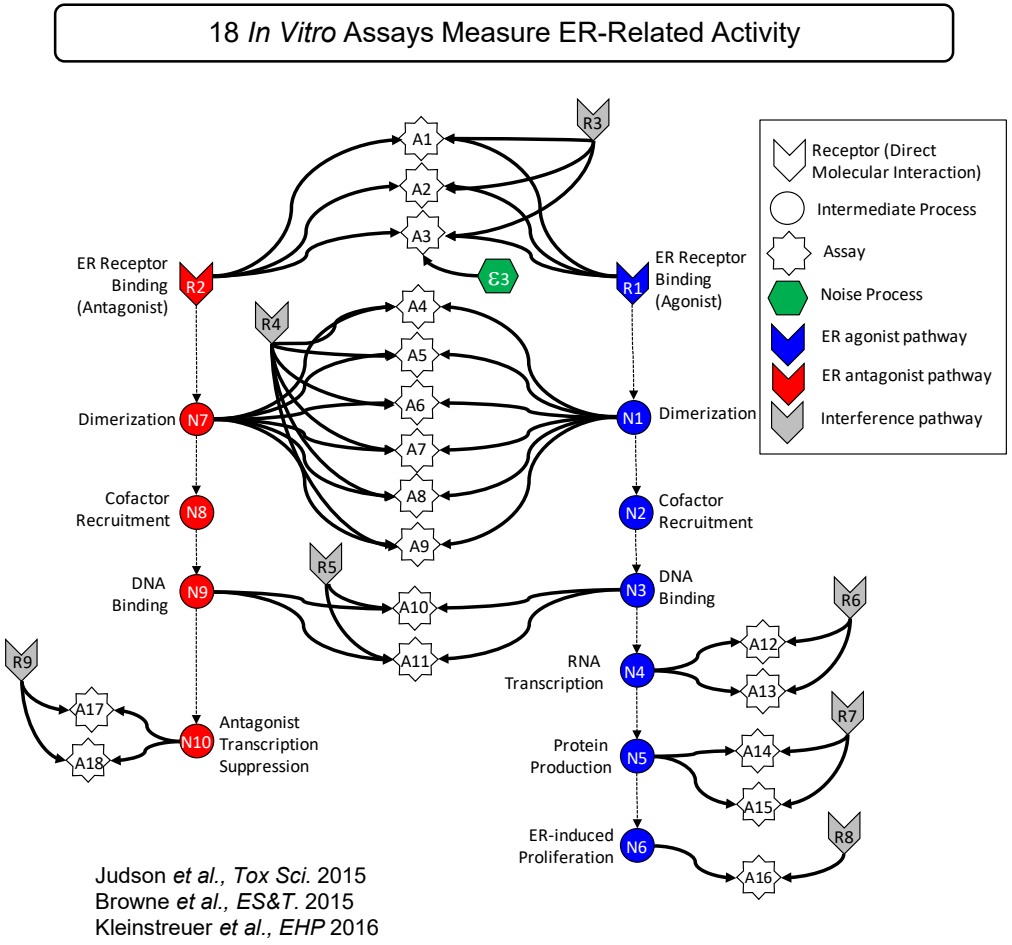
IARC Monographs 110, 112, 113

Prioritization of Chemicals for Further Testing



FIFRA SAP, Dec 2014

Application of High-Throughput Assays to Identify Potential Endocrine Disrupting Chemicals



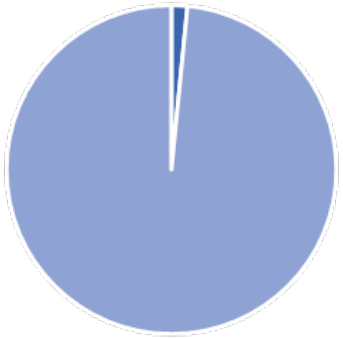
Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T.* 2015
Kleinstreuer *et al.*, *EHP* 2016

- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Model creates a composite dose-response curve for each chemical to summarize results from all assays

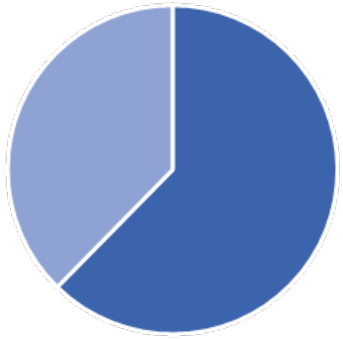
Beginning to Address Concerns for Increased Biological Coverage

United States Environmental Protection Agency

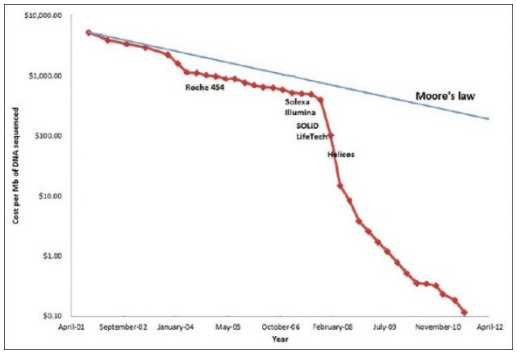
Gene Coverage



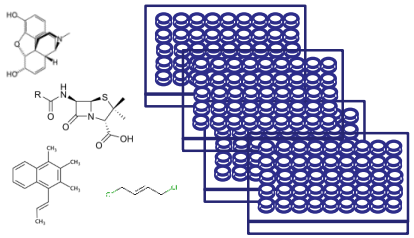
Pathway Coverage*



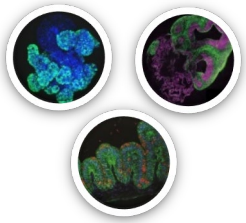
*At least one gene from pathway represented



Thousands of chemicals



Multiple Cell Types



Requirements:

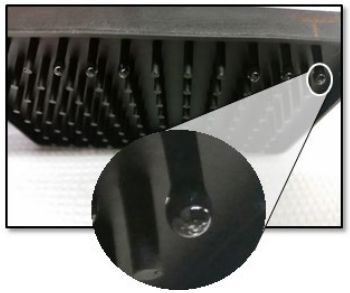
- Low cost
- Whole genome
- 384 well
- Automatable

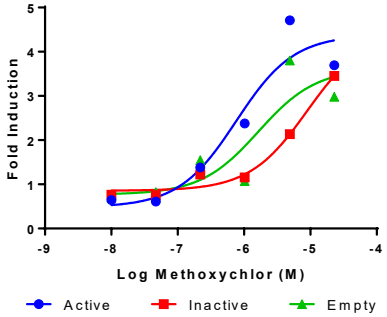
Beginning to Address Metabolic Competence

United States Environmental Protection Agency

"Extracellular" Approach

Chemicals metabolism in the media or buffer of cell-based and cell-free assays

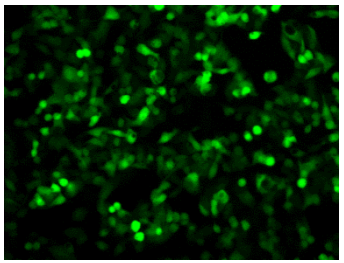


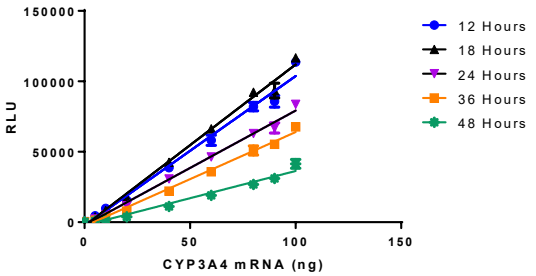


More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

Capable of metabolizing chemicals inside the cell in cell-based assays





More closely models effects of target tissue metabolism

Integrated approach to model *in vivo* metabolic bioactivation and detoxification

Collaboration with Unilever

Adding the High-Throughput Toxicokinetic Component

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

In Vitro Potency Value

Plasma Concentration

Exposure Route

Reverse Dosimetry

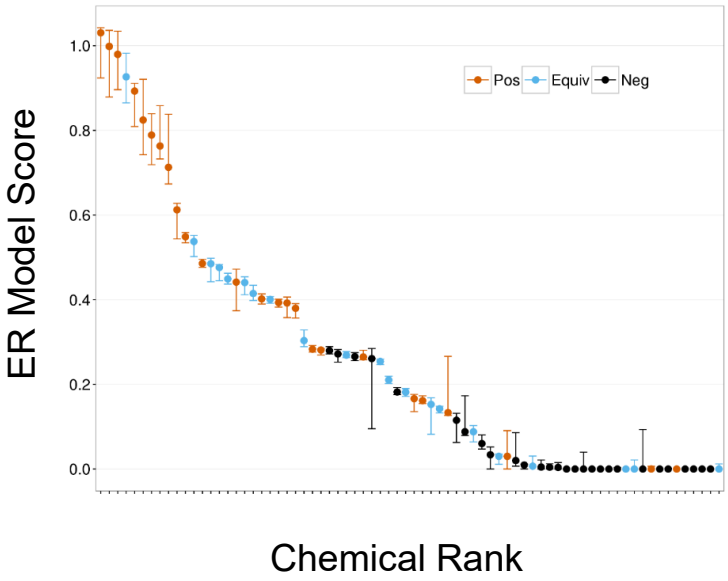
Administered Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through "'httk" R package (https://cran.r-project.org/web/packages/httk/)

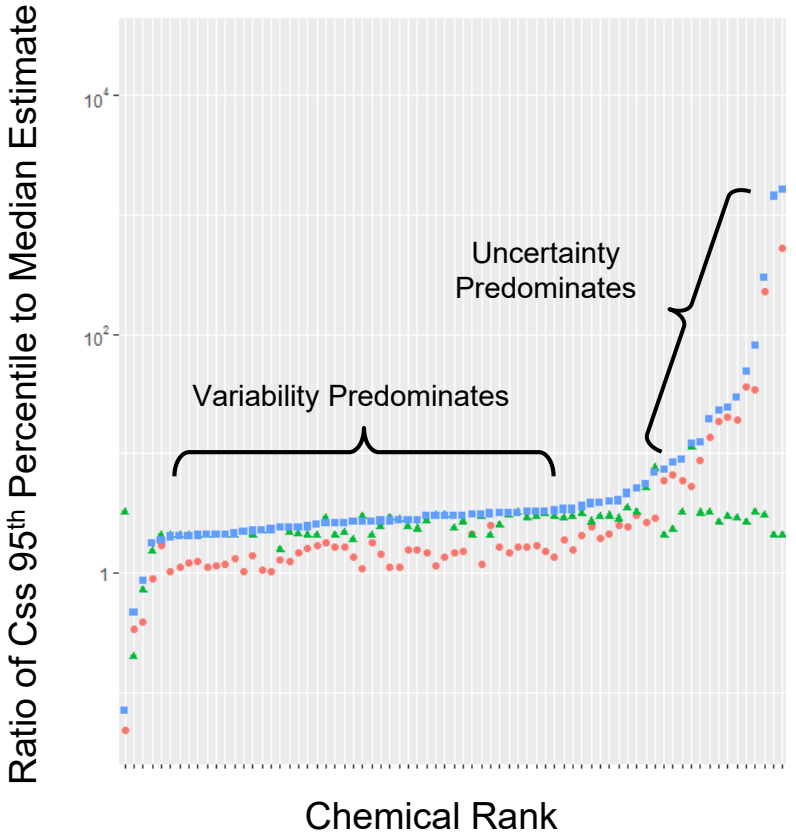
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

Adding in Uncertainty and Variability for PD and PK

Propagation of Experimental Uncertainty in Models of ER Potency

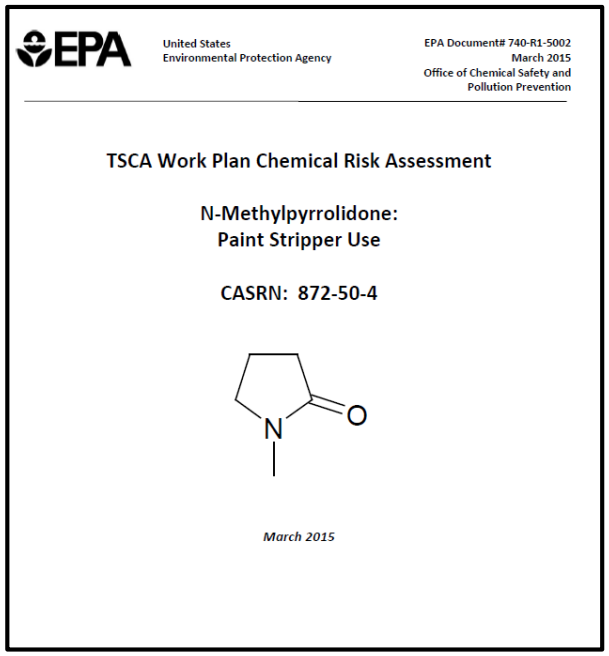


Propagation of Experimental Uncertainty in High-Throughput Toxicokinetic Estimates



Covering All the Components of a 21st Century Risk Assessment

United States Environmental Protection Agency



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EPA Document# 740-R1-5002
March 2015
Office of Chemical Safety and Pollution Prevention

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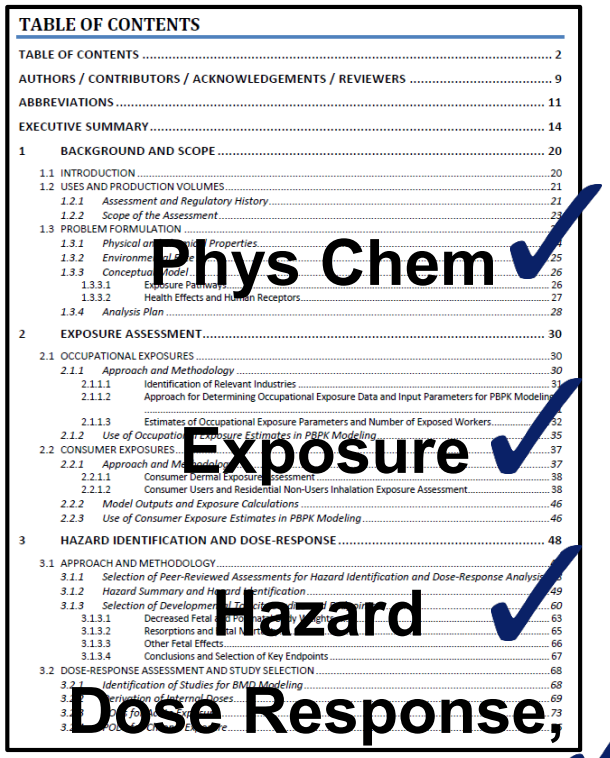
N-Methylpyrrolidone: Paint Stripper Use

CASRN: 872-50-4

March 2015



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Phys Chem ✓

Exposure ✓

Hazard ✓

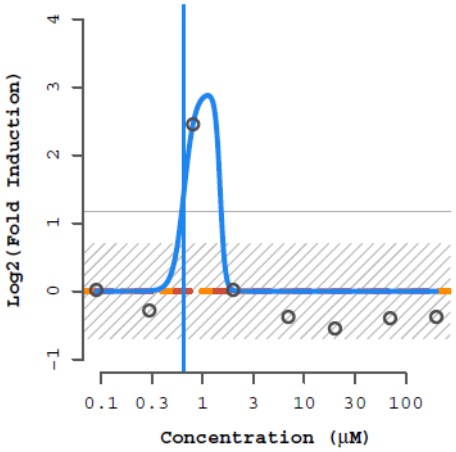
Dose Response, PK, and PODs ✓

Variability ✓

Risk Summary ✓

Uncertainty ✓

Regulatory Applications Require More Focus on Quality and Transparency

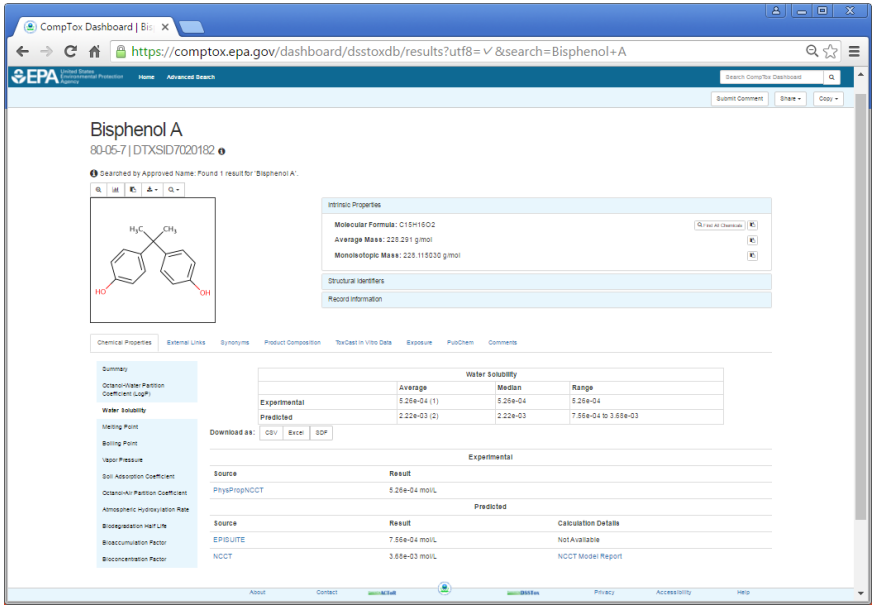



```
ASSAY:    AEID117 (ATG_ERa_TRANS)

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
```

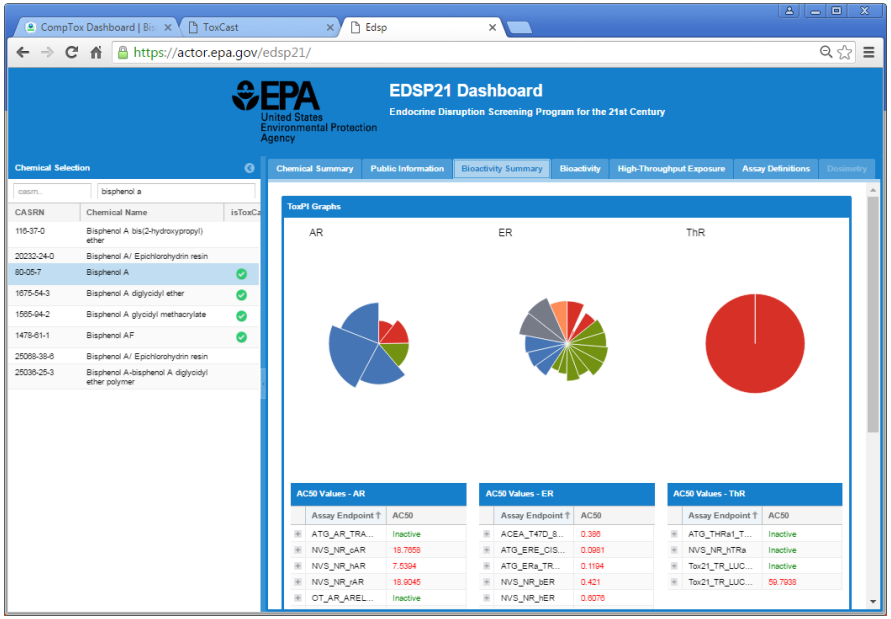
```
FLAGS:
Only one conc above baseline, active
Borderline active
```

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Publicly available ToxCast Owner's Manual
  - A resource to guide users through accessing data, database and software package for data analysis pipeline
- Publicly available ToxCast data analysis pipeline
  - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
- 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
- Migrating ToxCast assay annotations to OECD 211 compliant format

Effort to Provide Data Through Display and Decision Support Dashboards

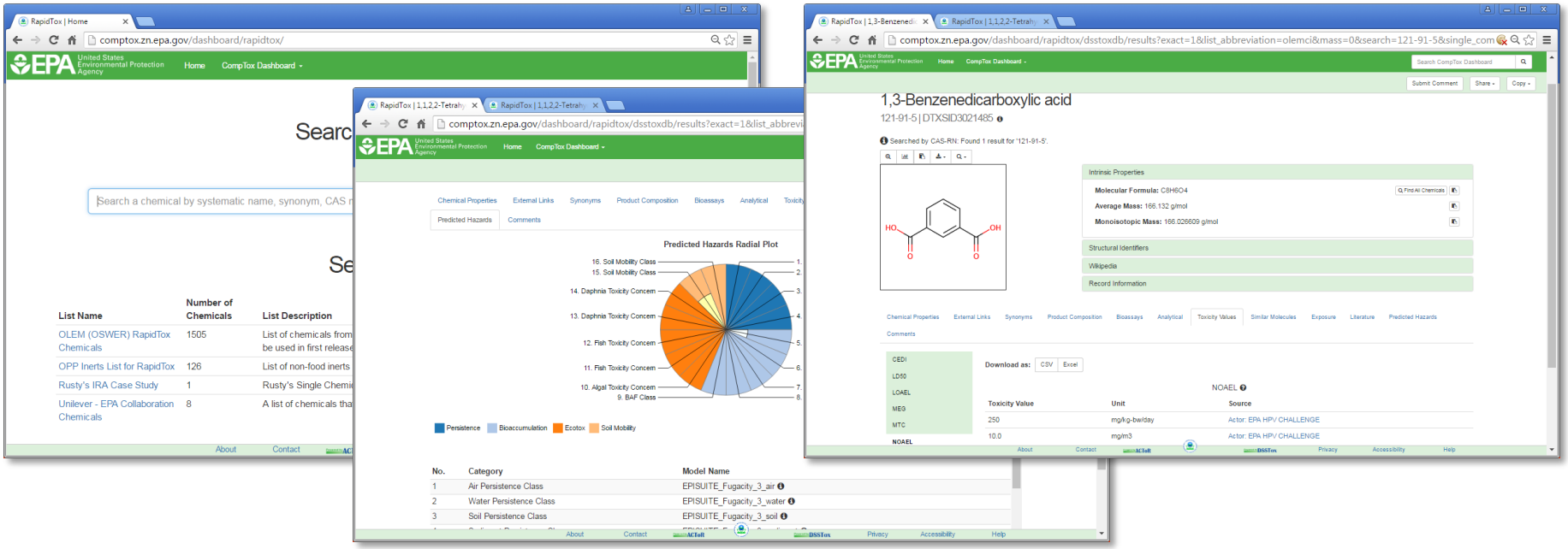


CompTox Dashboard
(https://comptox.epa.gov/dashboard)



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

RapidTox Workflow as a Focal Point for Integrating Components



- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure
- Transparent and interactive enough to enable expert users to review the assumptions made and refine the predictions
- Deliver quantitative toxicity values with associated estimates of uncertainty

Future Directions to Advance Regulatory Application of NAMs

- Using new data for TSCA (e.g., chemical prioritization, strategic plan)
- Working with state agencies to help use of new approaches to evaluate chemicals
- Collaborating with international regulatory agencies through case studies as proof of concept for use of NAMs in chemical risk assessment

EPA United States Environmental Protection Agency

Thank You for Your Attention!

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

EPA Colleagues:
- NERL
- NHEERL
- NCEA

Collaborators:
- Unilever

EPA's National Center for Computational Toxicology