Harnessing High-Throughput Monitoring Methods to Strengthen 21st Century Risk-Based Evaluations

National Environmental Monitoring Conference (NEMC) 2016
Orange County, CA, Aug 8 - 12, 2016

Jon R. Sobus,
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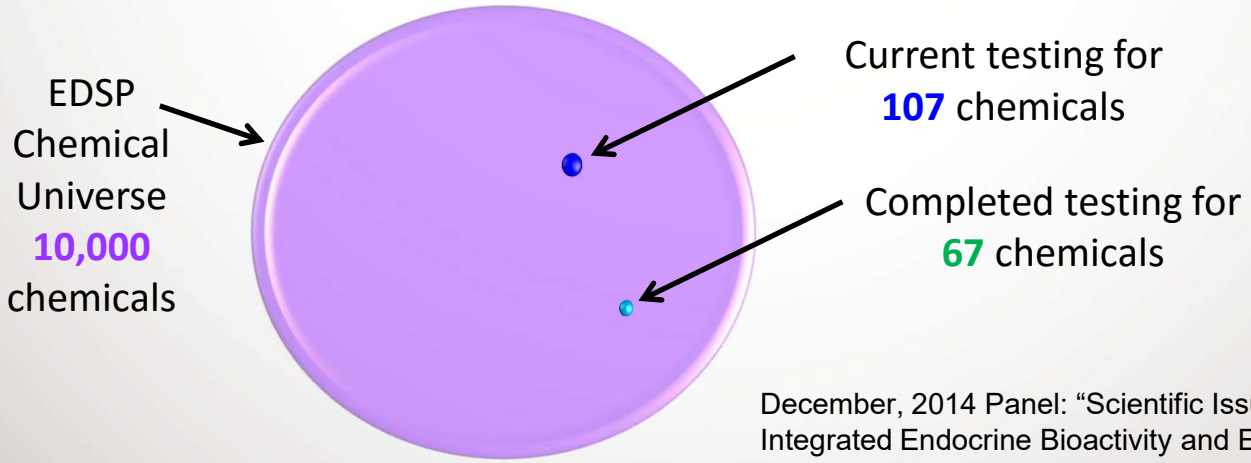
US EPA Office of Research and Development

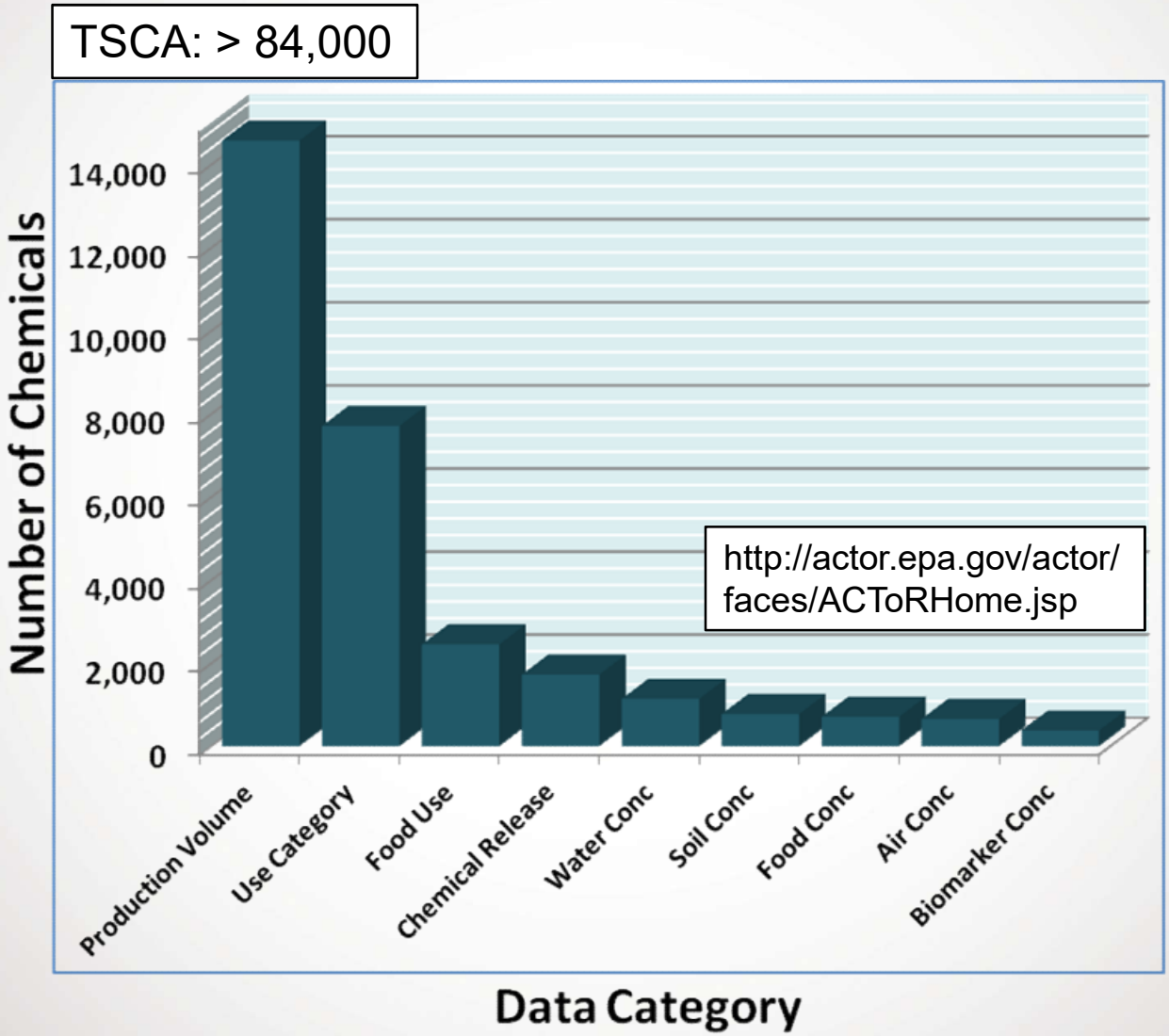
The Vast Number of Chemicals in Commerce Presents Regulatory Challenges

EPA's Endocrine Disruptor Screening Program (EDSP) Chemical List	# of Compounds
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	**10,341**



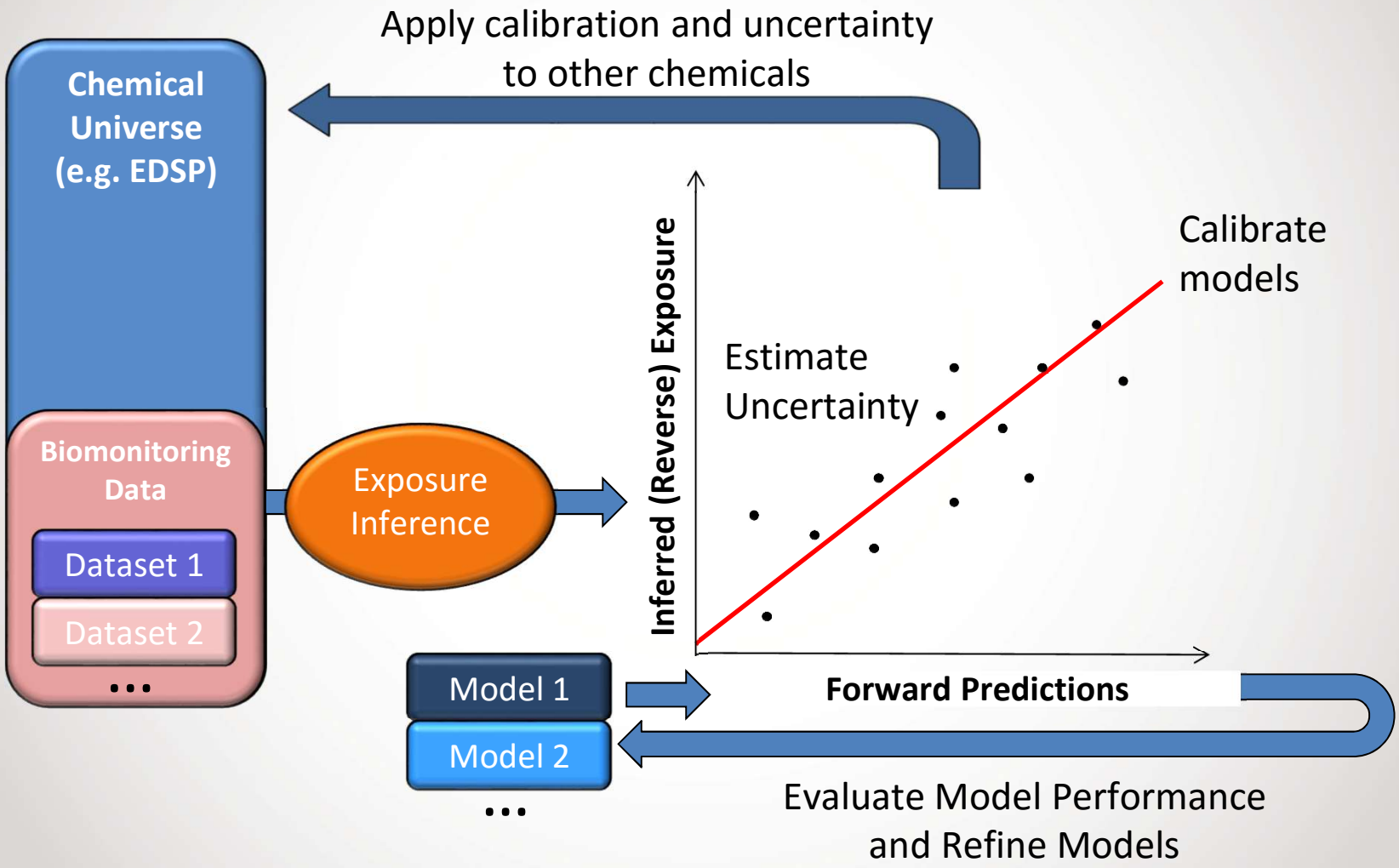
December, 2014 Panel: "Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening" DOCKET NUMBER: EPA–HQ–OPP–2014–0614

Exposure Data Can't Keep Pace with Regulatory Needs



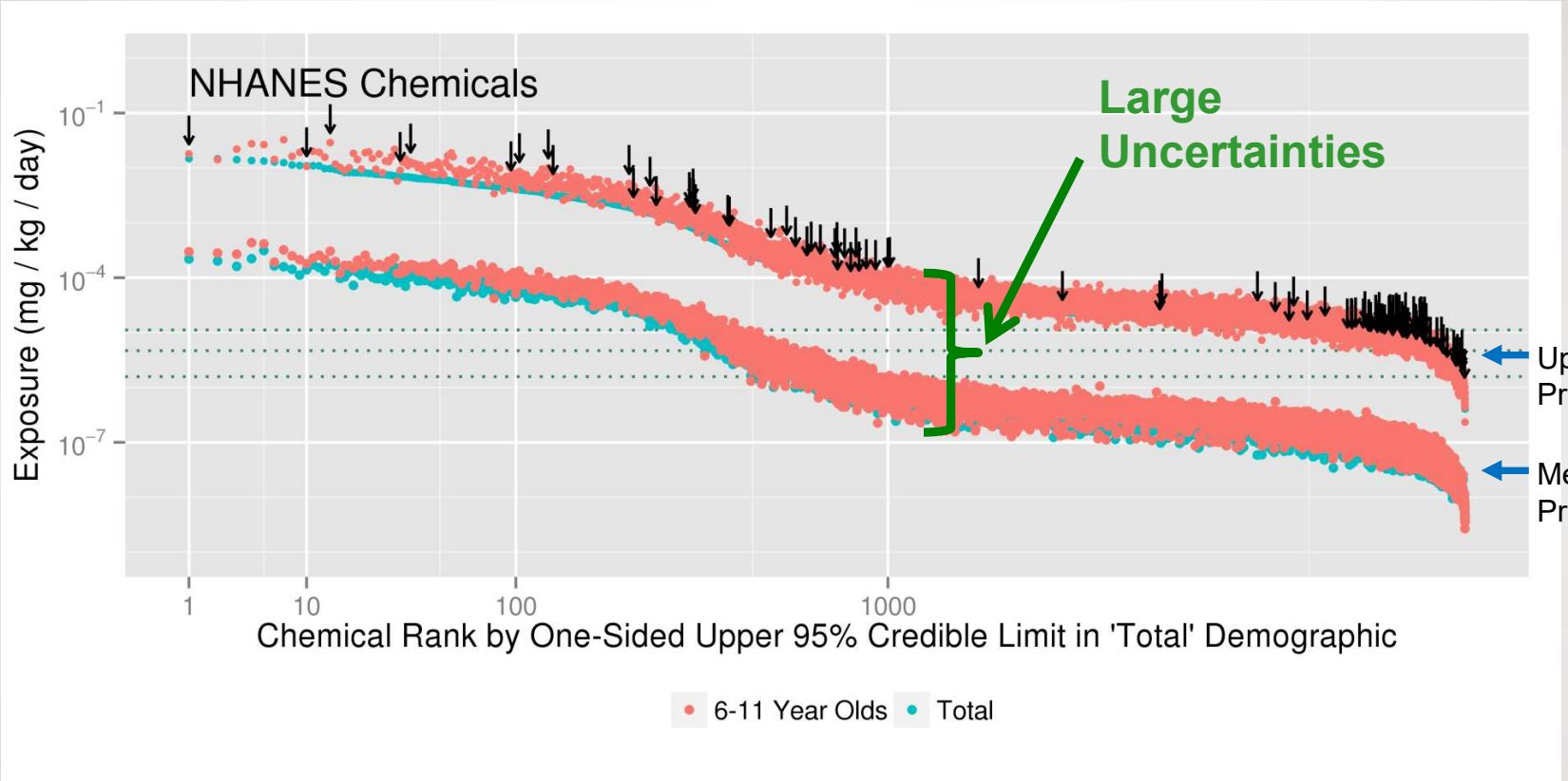
P.P. Egeghy et al. *Sci Total Environ.* 414 (2012) 159–166

Exposure Forecasting→ ExpoCast



Slide from John Wambaugh

Calibrated Exposure Estimates

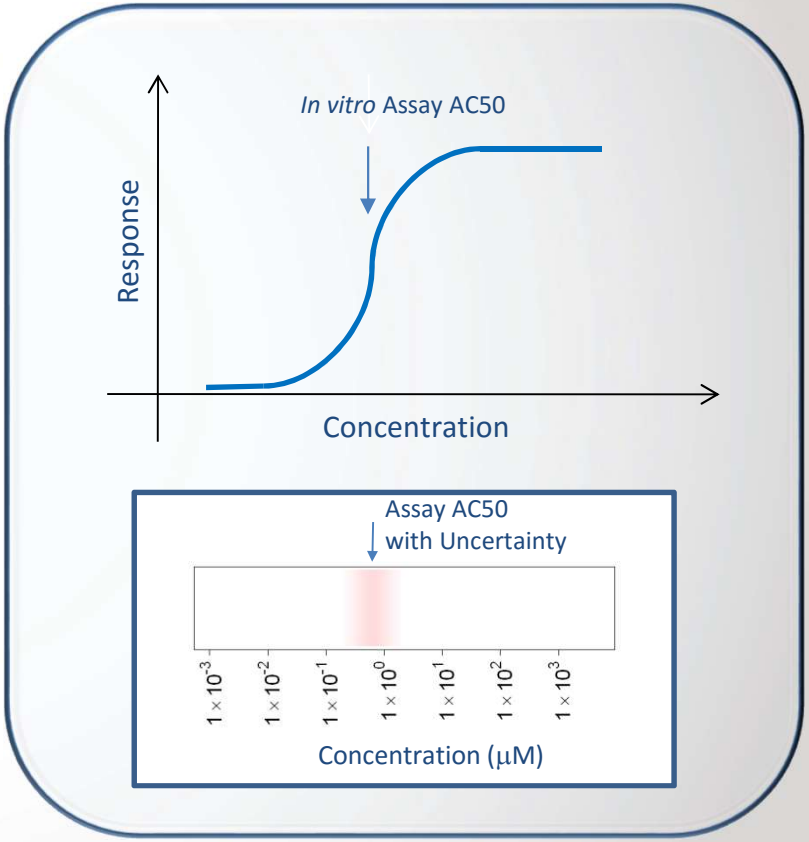


Predictions for ~8000 chemicals of interest to EPA's Endocrine Disruptor Screening Program (EDSP)

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

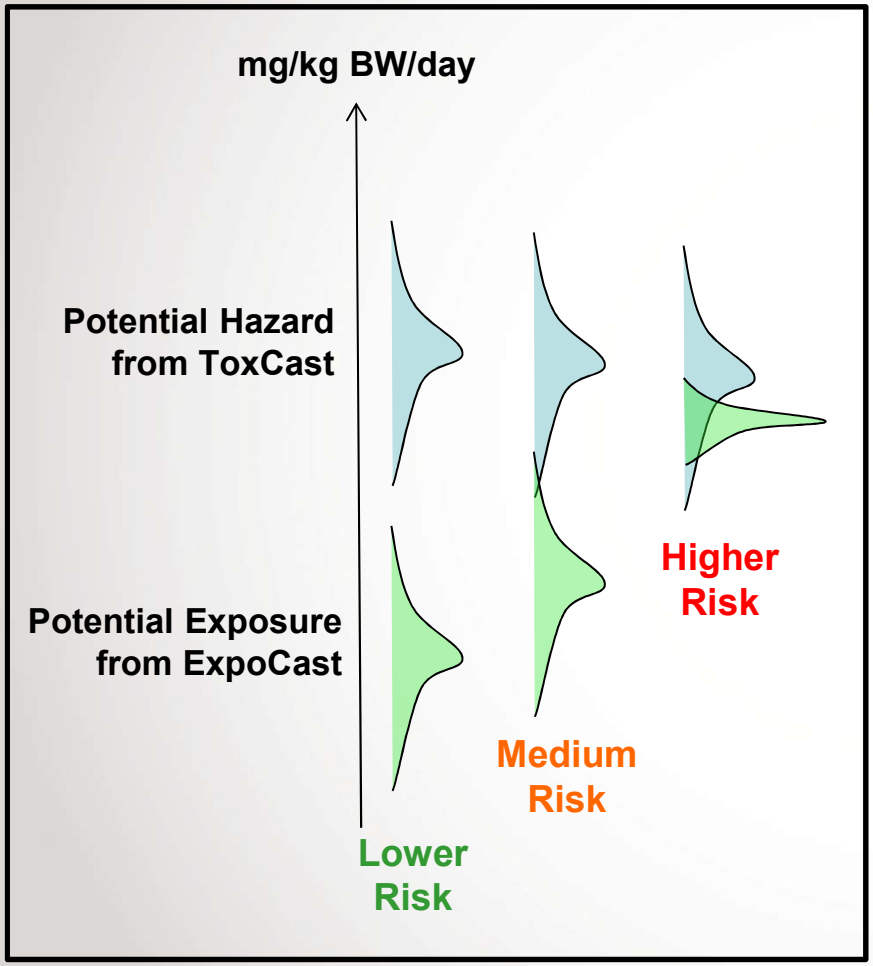
Toxicity Forecasting: Tox21/ToxCast

- **Tox21**: Screened ~8,000 chemicals using ~50 assays intended to identify interactions with biological pathways
- **ToxCast**: Screened a subset (~2,000) of Tox21 chemicals across ~700 assays
- Reverse toxicokinetics used to estimate exposure rate consistent with AC50



Slide from John Wambaugh

High Throughput (HT) Risk Assessment



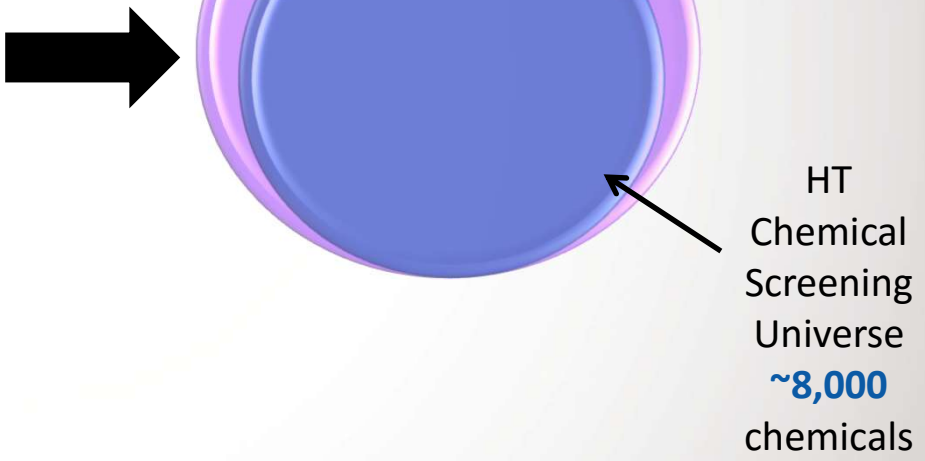


Figure from John Wambaugh

High Throughput Screening Methods

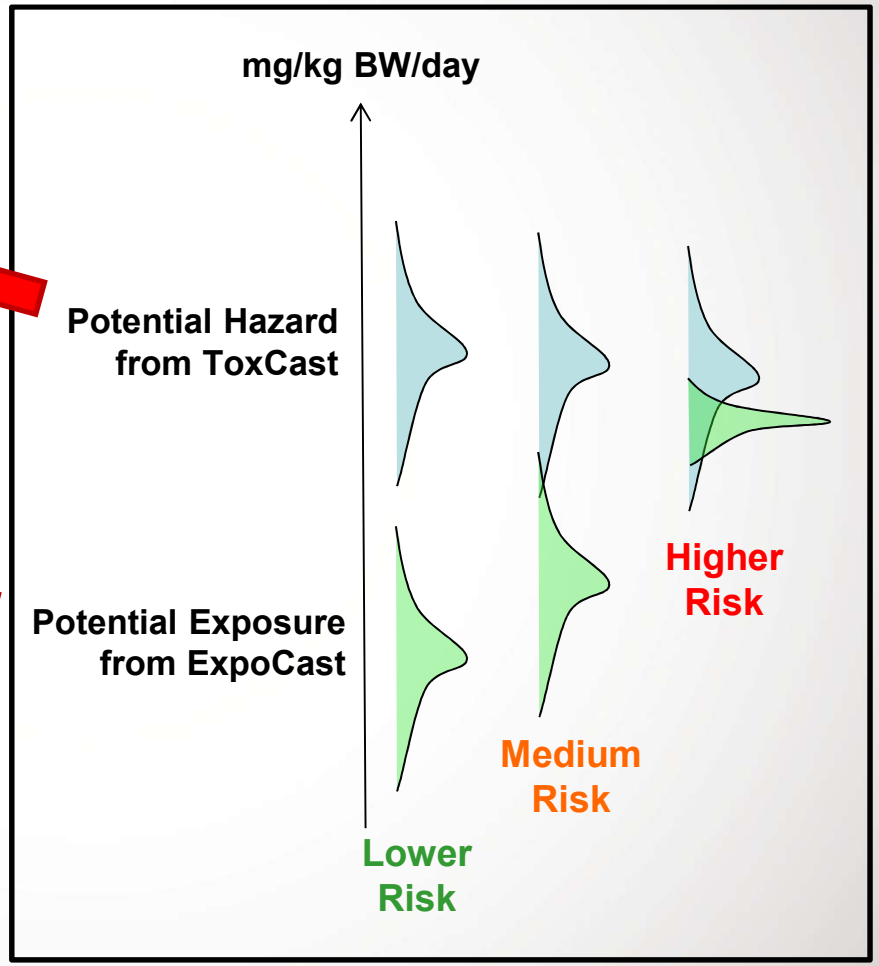
Research and Testing Needs

Nominations for:

1. Parent chemicals
2. Mixtures
3. Metabolites/Degradates

Measurement data for:

1. Model inputs
2. Model evaluation
3. Model refinement



Currently ~8000 chemicals

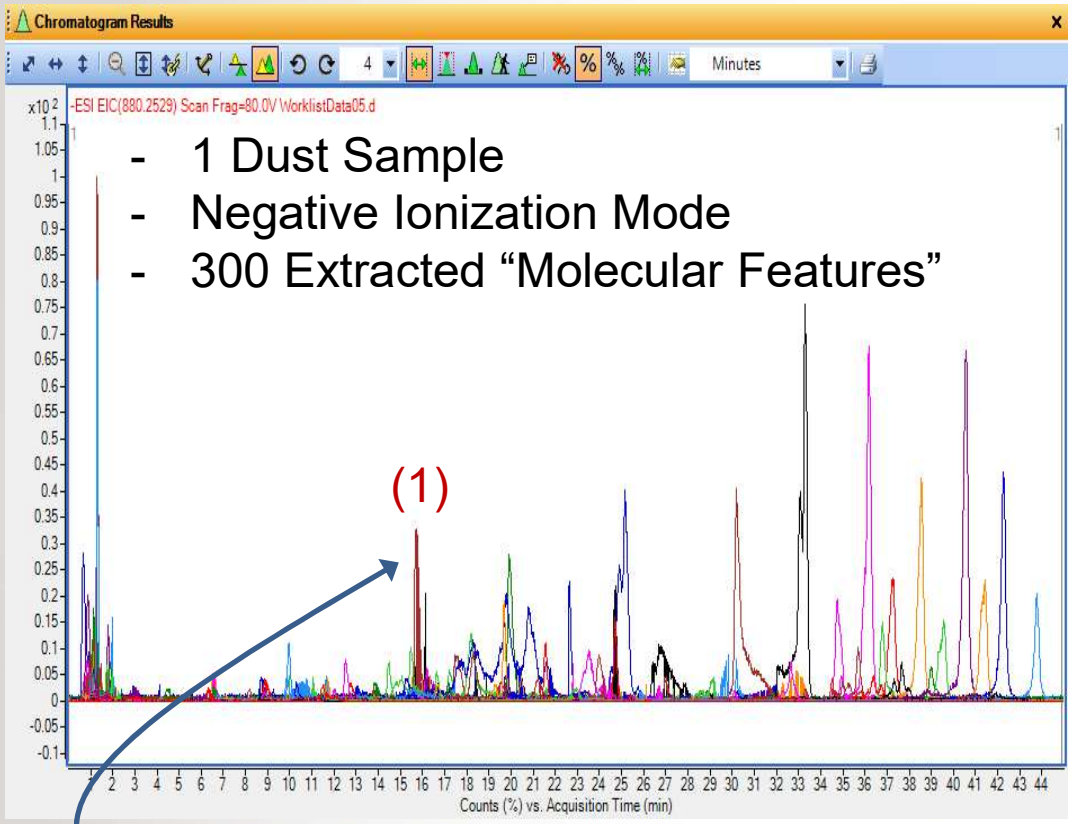
Comparing Analysis Approaches

- Targeted Analysis:
  - We know exactly what we're looking for
  - 10s – 100s of chemicals

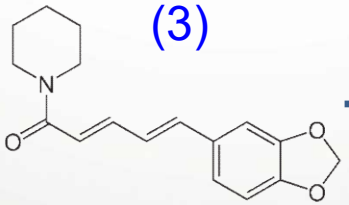
- Suspect Screening Analysis (SSA):
  - We have chemicals of interest
  - 100s – 1,000s of chemicals

- Non-Targeted Analysis (NTA):
  - We have no preconceived notions or lists
  - 1,000s – 10,000s of chemicals
    - In dust, soil, food, air, water, products, plants, animals, and us!!

General Goals of SSA/NTA



1) Prioritize "Molecular Features"
2) Correctly assign formulas
3) Correctly assign structures
4) Determine chemical sources
5) Predict chemical concentrations

(2) C17H19NO3 → (3)  → (4)  → (5) 12 µg/g

Previous Work with SSA

Environment International 88 (2016) 269–280

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring

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SSA Workflow

Dust samples
(<150 um) (n=56)
Taken from National
Survey

Extract and Analyze Samples
(LC-TOF/MS)

Identify Molecular Features
(User-defined criteria)

Agilent Technologies

Assign Formulas to Molecular Features
(DSSTox-MSMF Database)

EPA DSSTox Structure-Browser GO v 2.0

Link Assigned Formulas to Chemicals/Structures
(DSSTox_v2 Database)

Estimate Average **Abundance (A)** and **Number of Samples (N)** Associated with Each Chemical

Group Chemicals Into **Exposure (E)** Categories Using ExpoCast

Assess Chemical **Bioactivity (B)** Using Tox21

Group A
For Chemicals with **E** and **B**,
Prioritization Score = f(**A**+**N**+**E**+**B**)

Group B
For Chemicals without **E** and **B**,
Prioritization Score = f(**A**+**N**)

Confirm Chemicals with High Priority Scores
Using Standards

Molecular Features in Dust

~3000 features identified per sample

Number of features identified varied between samples

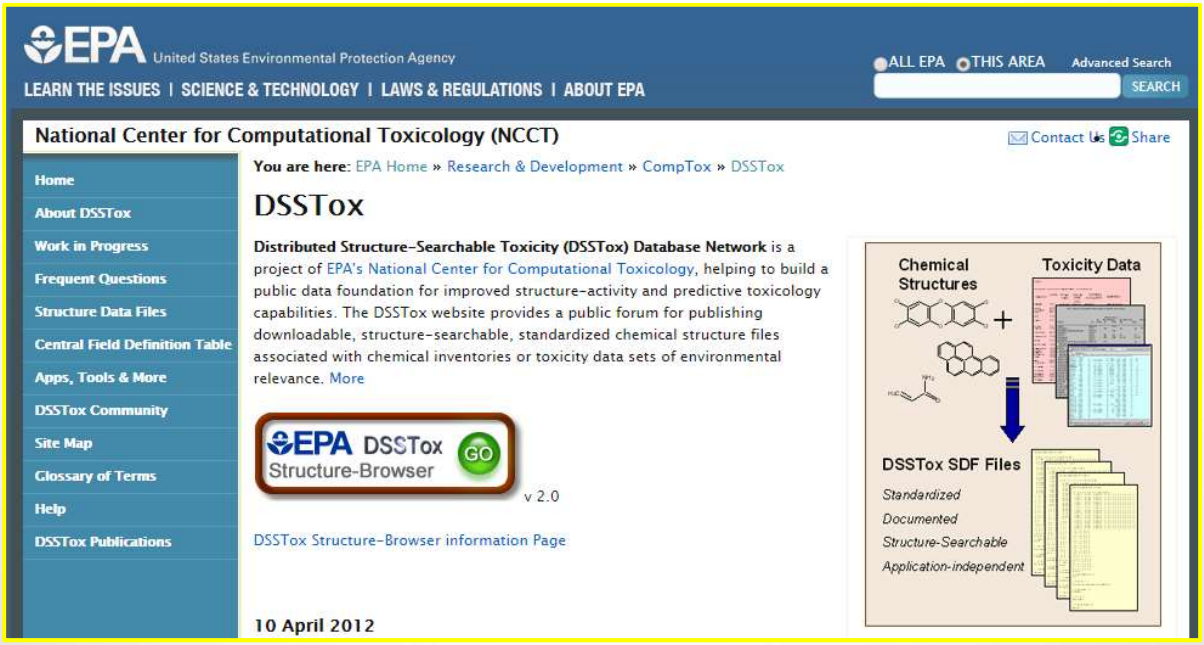
- 10-fold range (max/min) in positive mode
- 15-fold range (max/min) in negative mode

Positive Ionization Mode					
	Mean	SD	Min	Med	Max
Abundance	9.32×10^5	3.94×10^6	1.46×10^4	2.61×10^5	2.33×10^8
Number of Features per Sample	3185	1023	632	3262	5477
Number of Formula Matches per Sample	45	14	4	45	77
Negative Ionization Mode					
	Mean	SD	Min	Med	Max
Abundance	1.26×10^6	7.87×10^6	1.61×10^4	2.58×10^5	6.06×10^8
Number of Features per Sample	2236	646	260	2169	3739
Number of Formula Matches per Sample	44	27	10	38	116

Chemical Database (DSSTox)

- Carefully curated database
- Standardized chemical mass, formula, structure
- One-to-one mapping of CAS-to-chemical name
- Environmental contaminants, pharmaceuticals, industrial chemicals, etc.

•~33K chemicals in DSSTox at time of dust SSA analysis



Formulas Identified in Dust

Required strict match score of ≥ 90

~45 formulas tentatively identified per sample, per mode, on average

Represents < 2% of the total # of observed features

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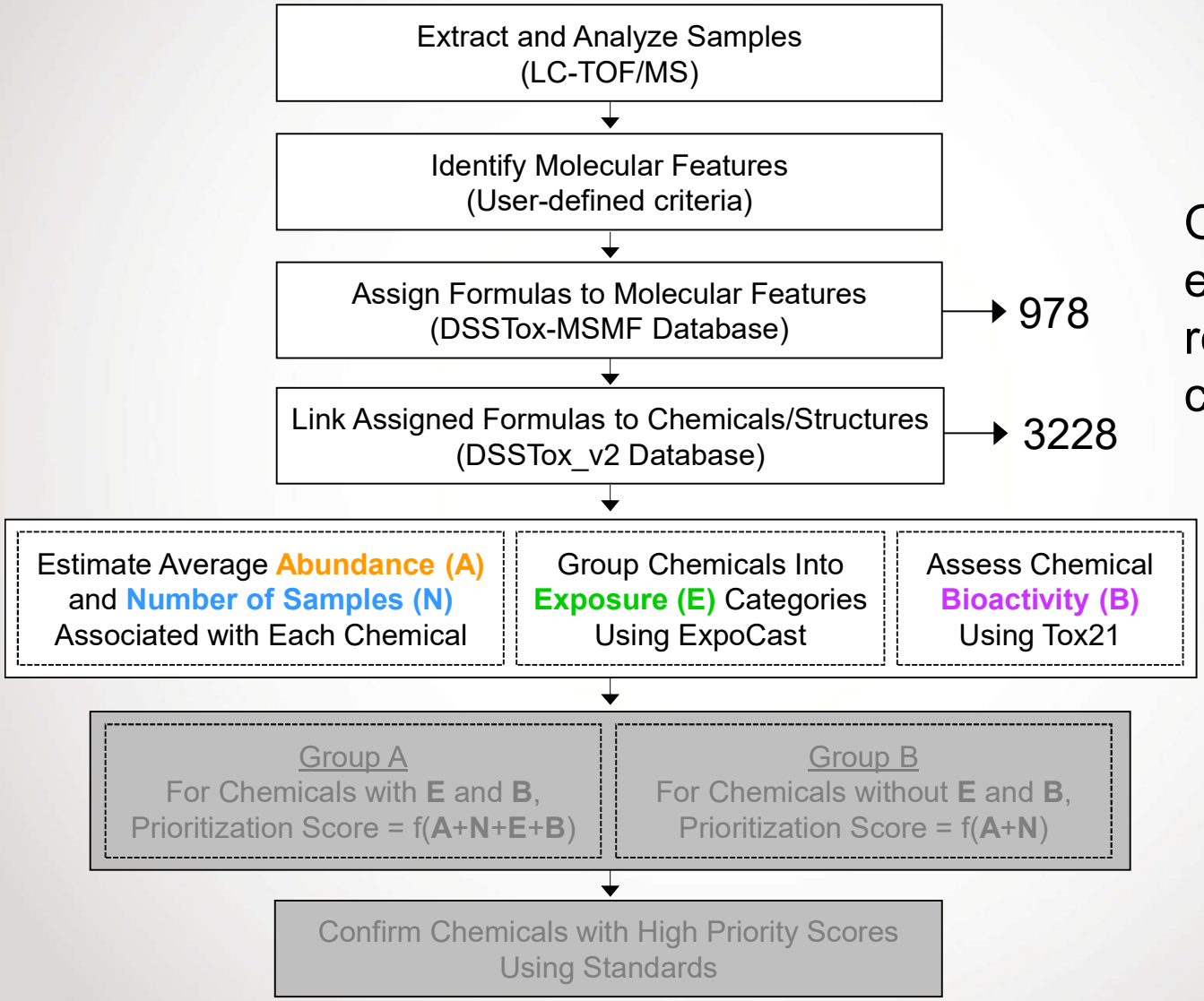
SSA Workflow



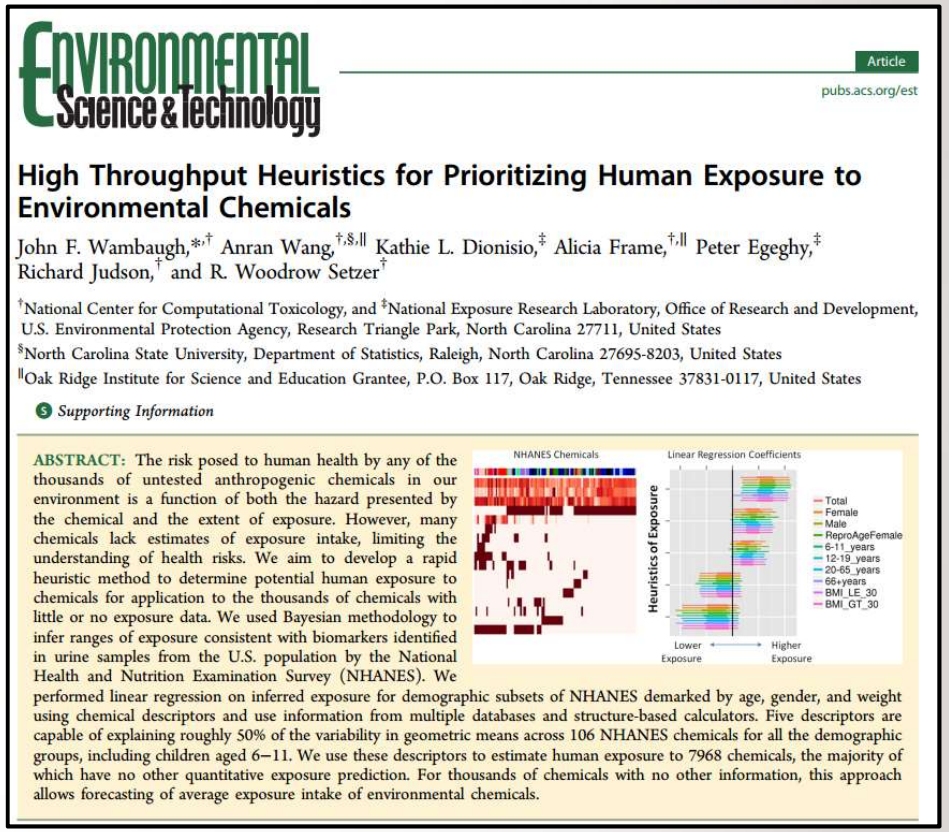
On average every formula represents 3 chemicals

Exposure Estimates from ExpoCast

- 5 exposure descriptors used to estimate exposure to ~8000 chemicals

- Exposure rates grouped into categories (based on estimated median values for U.S. population):

Category 1 < $1x10^{-8}$ mg/kg/day;
Category 2 > $1x10^{-8}$ and < $1x10^{-7}$ mg/kg/day;
Category 3 > $1x10^{-7}$ and < $1x10^{-6}$ mg/kg/day;
Category 4 > $1x10^{-6}$ and < $1x10^{-5}$ mg/kg/day;
Category 5 > $1x10^{-5}$ and < $1x10^{-4}$ mg/kg/day;
Category 6 > $1x10^{-4}$ and < $1x10^{-3}$ mg/kg/day;
Category 7 > $1x10^{-3}$ and < $1x10^{-2}$ mg/kg/day



ENVIRONMENTAL Science & Technology

Article

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High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals

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Supporting Information

ABSTRACT: The risk posed to human health by any of the thousands of untested anthropogenic chemicals in our environment is a function of both the hazard presented by the chemical and the extent of exposure. However, many chemicals lack estimates of exposure intake, limiting the understanding of health risks. We aim to develop a rapid heuristic method to determine potential human exposure to chemicals for application to the thousands of chemicals with little or no exposure data. We used Bayesian methodology to infer ranges of exposure consistent with biomarkers identified in urine samples from the U.S. population by the National Health and Nutrition Examination Survey (NHANES). We performed linear regression on inferred exposure for demographic subsets of NHANES demarked by age, gender, and weight using chemical descriptors and use information from multiple databases and structure-based calculators. Five descriptors are capable of explaining roughly 50% of the variability in geometric means across 106 NHANES chemicals for all the demographic groups, including children aged 6−11. We use these descriptors to estimate human exposure to 7968 chemicals, the majority of which have no other quantitative exposure prediction. For thousands of chemicals with no other information, this approach allows forecasting of average exposure intake of environmental chemicals.

Bioactivity Data from Tox21

High-throughput toxicity screening data on >8,000 chemicals

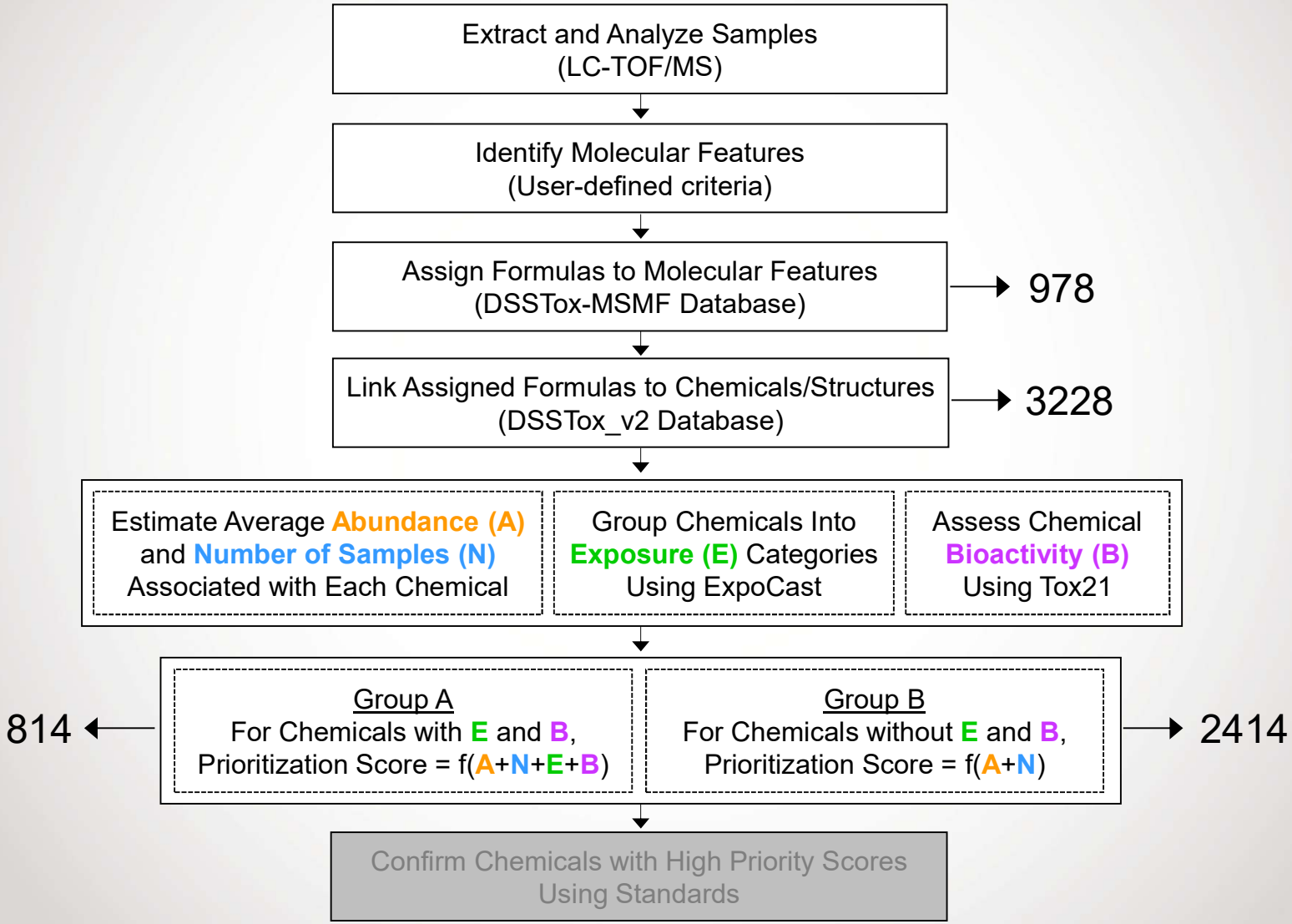
http://www.epa.gov/ncct/Tox21/

Tox21 data used here:

Hit calls (0=inactive, 1=active) for:

- AhR (aryl hydrocarbon receptor)
- AR (androgen receptor)
- ERα (estrogen receptor 1)
- NFκB1 (nuclear factor of kappa light polypeptide gene enhancer in B cells 1)
- PPARγ (peroxisome proliferator-activated receptor gamma)

SSA Workflow

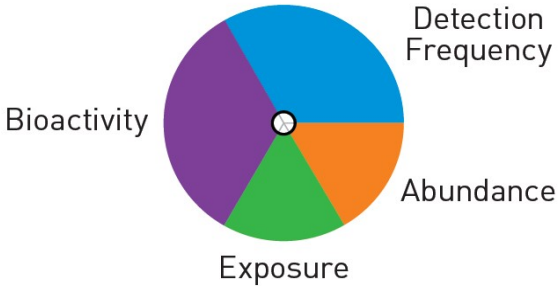


Prioritization Scoring with ToxPi

$$\text{ToxPi Score}_i = w_A \frac{A_i - A_{min}}{A_{max} - A_{min}} + w_N \frac{N_i - N_{min}}{N_{max} - N_{min}} + w_E \frac{E_i - E_{min}}{E_{max} - E_{min}} + w_B \frac{B_i - B_{min}}{B_{max} - B_{min}}$$

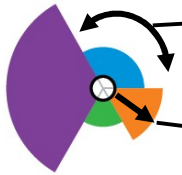
$w_A = w_E = 1;\ w_N = w_B = 2$

ToxPi Legend



Individual components of a unit circle are scaled and represented as "slices"

Example Chemical

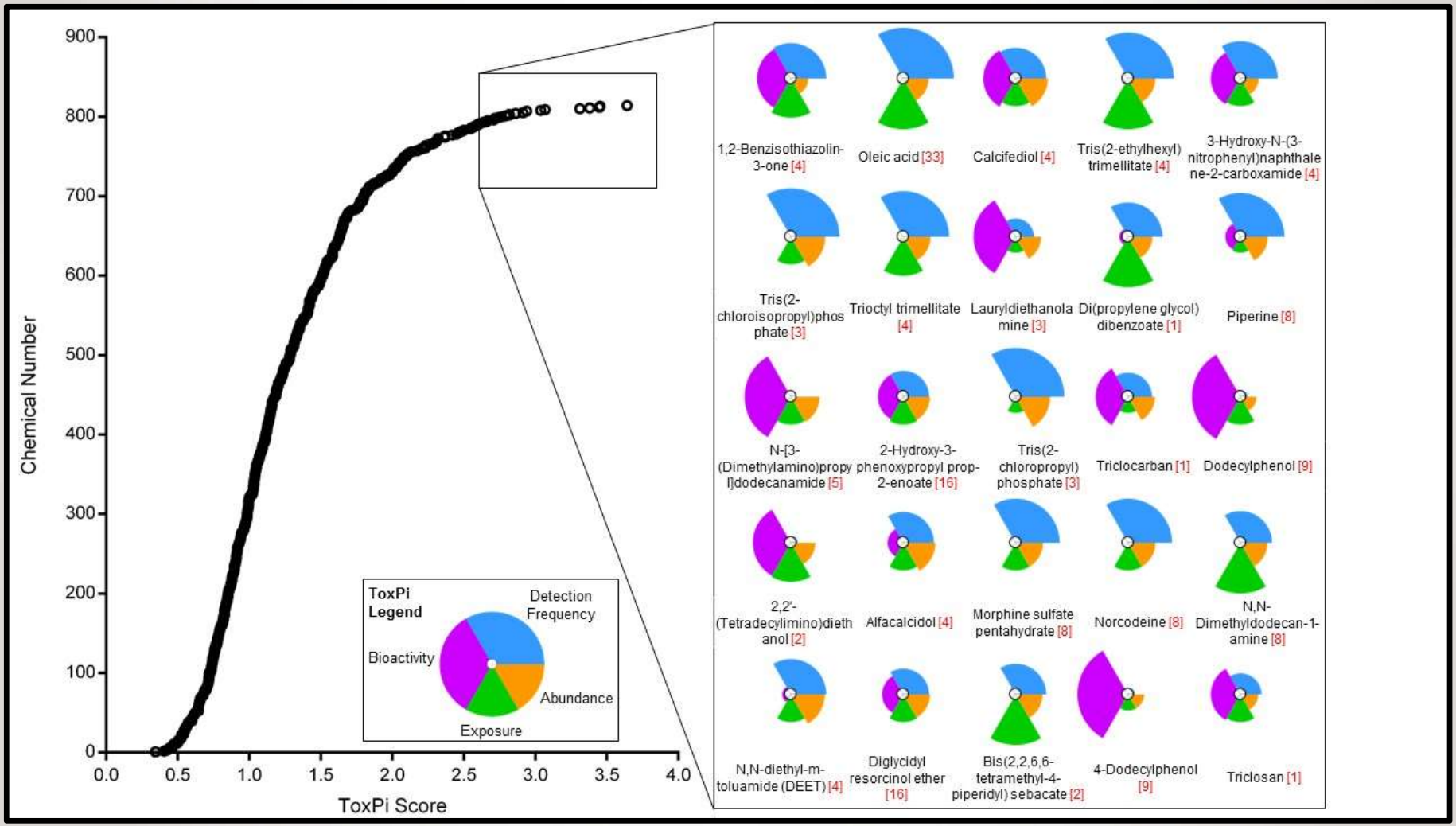


Width indicates the relative weight of the variable

Distance from the origin is proportional to the normalized value of the data

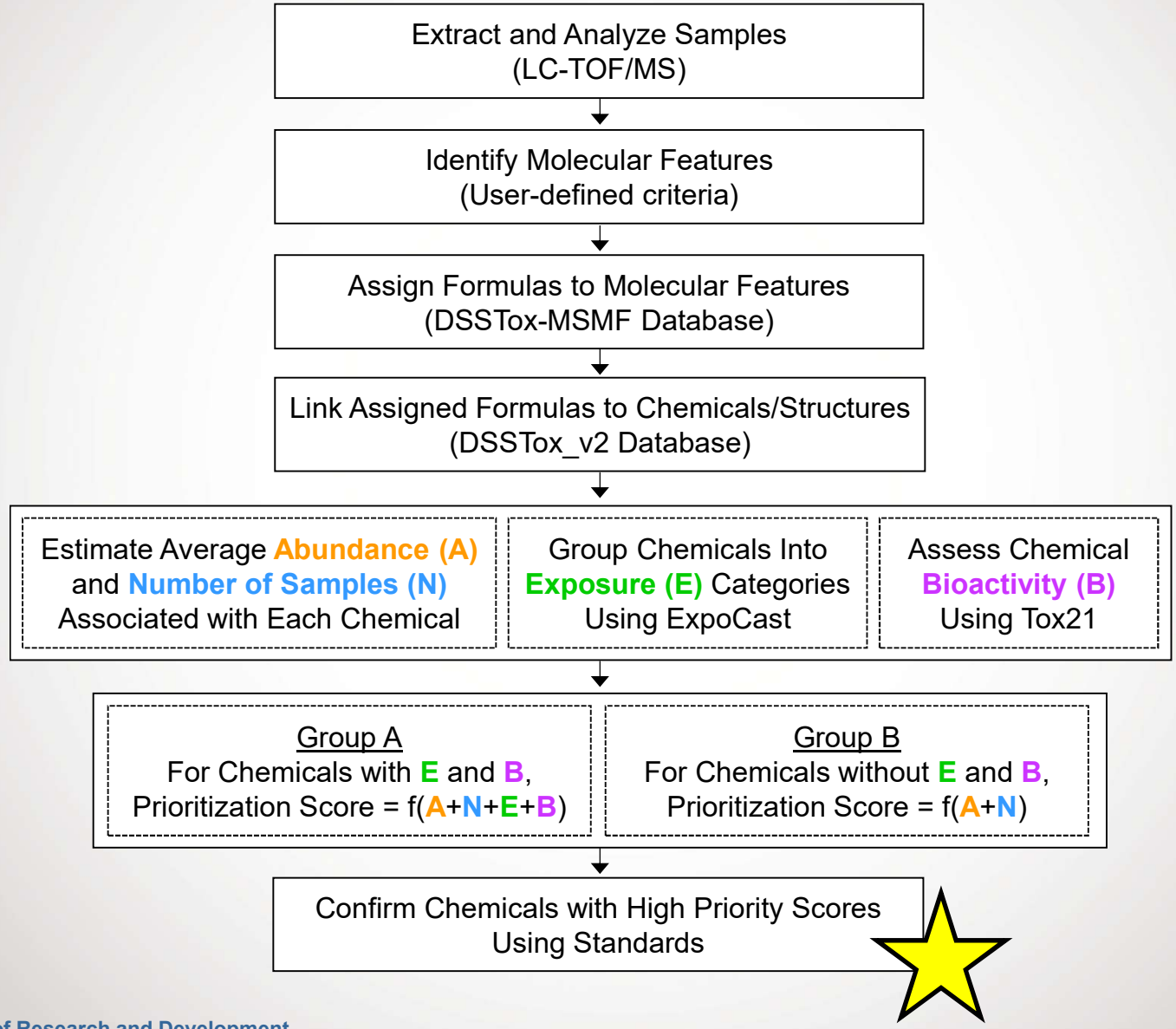
(Reif et al. 2010)

Group A Priority Candidates*



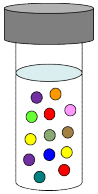
*listed chemicals are not necessarily confirmed

SSA Workflow



Blinded Analysis of 100-Chemical Mixture

ToxCast™



Blinded Analysis: Procedures & Results

- Analyzed at 2 µM and 0.2 µM, neg. and pos. modes
- Logical scheme used to rank features from 0 to 5 stars
  - Present at both concentrations (>3x difference in response)
  - Consistent retention times
  - Match score ≥ 90
  - Peak saturation?
- Matching to dust features using formula, RT & spectra

100 Total Chemicals

→ 70 Detected Across Both Modes

→ 51 of Minimally-Sufficient Quality

→ 33 Matches in House Dust

Chemical Name	ToxPi Rank (%)	N_{true}	SciFinder hits
Di(propylene glycol) dibenzoate	1.1	4	0
Piperine	1.2	42	1
Triclocarban	1.7	21	0
N,N-diethyl-m-toluamide (DEET)	2.6	33	22
Diethyl phthalate (DEP)	4.2	23	36
Propylparaben	5.4	19	7
3,6,9,12-Tetraoxahexadecan-1-ol	5.7	1	0
N-Dodecanoyl-N-methylglycine	6.0	6	0
Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)	6.8	15	38
Methylparaben	8.7	16	10
Carbamazepine	12.0	1	0
Tris(2-ethylhexyl) phosphate (TEHP)	12.4	1	18
2-[2-(2-Butoxyethoxy)ethoxy]ethanol	15.5	2	2
Triethyl citrate	16.8	6	0
Tetradecanoic acid, 2,3-dihydroxypropyl ester	18.3	1	0
Clorophene	25.1	4	0
Nicotine	25.3	10	24
4,4'-Sulfonyldiphenol	33.5	4	1
Perfluoroctylsulfonamide acid (PFOSA)	34.4	1	9
Fluconazole	34.8	1	0
Perfluorooctanoic acid (PFOA)	38.0	3	33
Corticosterone	39.9	1	3
Dibutyl hexanedioate	48.9	1	3
Phosphoric acid, dibutyl ester	51.0	4	1
C.I. Disperse Yellow 3	51.4	3	0
Octyl beta-D-glucopyranoside	51.7	1	0
Perfluorodecanoic acid (PFDA)	54.2	3	13
Carbaryl	55.5	2	15
Rofecoxib	77.1	1	0
Primidone	78.6	3	0
2,4,5-Trichlorobenzenesulfonic acid	82.7	2	0
Lufenuron	89.7	1	0
Diphenyl phosphate	91.4	6	3

Results for Chemicals Confirmed in House Dust

45% of confirmed chemicals not previously studied in house dust?

We're on the Right Path…

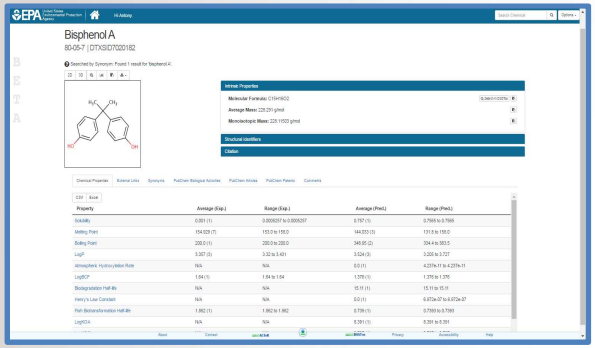
- … but certainly room for improvement
- ~300,000 total molecular features (not unique)
- 33 confirmed chemicals
- State-of-the-art SSA yields <5% confirmed IDs
- So what else is in these (and other) samples??

Planned Work (2016-2017)

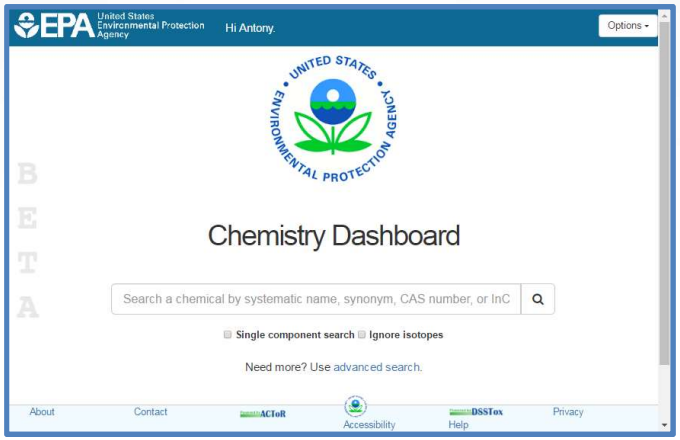
- Apply SSA/NTA workflow for the analysis of:
  - Brita filters *(Strynar presentation)*
  - Consumer products
  - Crumb rubber
- Conduct SSA/NTA research trial
  - ~25 participating laboratories
  - 10 mixtures each containing 100-400 ToxCast chemicals
  - “standard” dust, serum, and silicone wristband extracts
- Expand SSA/NTA workflow
  - Enhanced DSSTox database
  - RT prediction models
  - Functional-use data/models
  - Media occurrence data/models
  - ORD’s iCSS Chemistry Dashboard

Integrating NTA Workflow Components within EPA's iCSS Chemistry Dashboard

https://comptox.epa.gov/dashboard



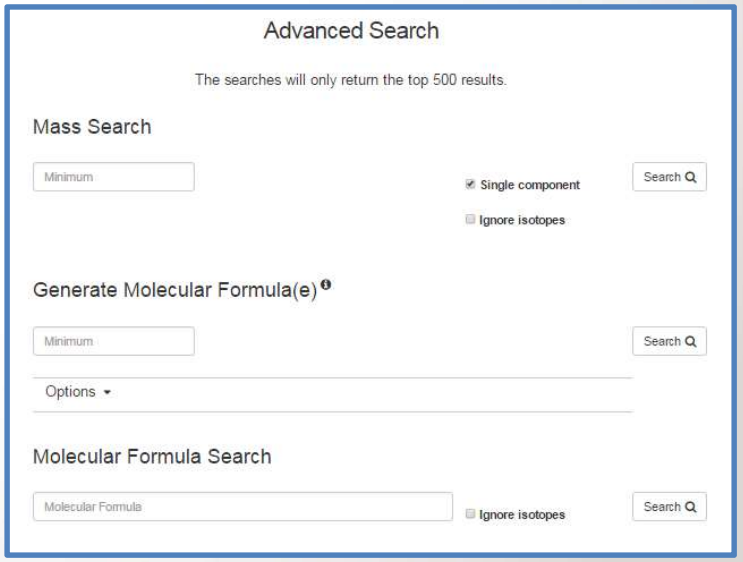
>8 million experimental and predicted physchem properties



Web access >720,000 chemicals

williams.antony@epa.gov

Integration Hub to Public Data



Advanced Searches

SJ1

Acknowledgements

Chemical Safety for Sustainability (CSS)
Rapid Exposure and Dosimetry (RED) Project

EPA NERL

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Paul Price
Jon Sobus
Mark Strynar
Elin Ulrich

* = ORISE Participant

EPA NCCT

Chris Grulke
Richard Judson
Kamel Mansouri*
Andrew McEachran*
Ann Richard
John Wambaugh
Antony Williams

Julia Rager
(ToxStrategies Inc.)

Brandy Beverly
(EPA NCEA)

Slide 29

SJ1 Sobus, Jon, 7/15/2016

Web Art Links

- Forrest vs. Trees: http://tobininvestmentplanning.com/wp-content/uploads/2015/09/do-you-see-forest-or-trees.jpg
- Black Pepper: http://blog.econugenics.com/wp-content/uploads/2014/07/blackpepper_blog_headerimage_featuredarticle-670x443.jpg
- Mad Scientist: https://upload.wikimedia.org/wikipedia/commons/thumb/9/9b/Mad_scientist_transparent_background.svg/513px-Mad_scientist_transparent_background.svg.png
- Brita Filter: https://www.brita.com/wp-content/uploads/faucet-hero1.png
- Soil in Hands: https://contentzone-bonnieplants1.netdna-ssl.com/wp-content/uploads/2011/12/soil-in-hands.jpg
- Soccer Field: http://www.ceh.org/wp-content/uploads/turf-graphic2.jpg
- Dust: http://cdn.skim.gs/images/fncsxggrflcio0qibeud/get-rid-of-dust-in-your-house
- Wastewater Effluent: http://nts-industrie.com/wp-content/uploads/sites/2/2015/09/photo-traitement-de-leaux4-200x300.jpg
- Consumer Products: http://www.findpaidfocusgroup.com/sites/default/files/CONSUMER-PRODUCTS.jpg