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US EPA CSS-HERA Board of Scientific Counselors Chemical Safety Subcommittee Meeting

US EPA CSS-HERA BOSC Meeting – February 2-5, 2021

CSS Session 2 Slides

The work presented within represents US EPA Office of Research and Development research activities. Material includes both peer reviewed, published results and work-in-progress research. Please do not cite or quote slides.

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OCSPP-TSCA Inventory: Prioritization Proof of Concept

Richard Judson, PhD

BOSC Meeting
February 3, 2021

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

Prioritization and Pre-prioritization

- Many organizations face the problem that they have too many chemicals to evaluate given the available resources
- One solution is to use a data-driven approach to prioritize chemicals for detailed assessments
  - OCSPP: TSCA High and low priority chemicals
  - OCSPP: EDSP, potential endocrine disruptors
  - OW: Candidate Contaminant List (CCL)
  - OW: Chemicals in biosolids
  - Health Canada: Domestic Substances List (DSL)
  - Minnesota Department of Health: Chemicals of concern to children

The TSCA Prioritization Problem

- Under the Lautenberg Act, 2016 Amendment to TSCA (*):
  - EPA must establish a risk-based process to determine which chemicals it will **prioritize** for assessment, identifying them as either "high" or low" priority substances.
    - High priority – the chemical may present an unreasonable risk of injury to health or the environment due to potential hazard and route of exposure, including to susceptible subpopulations
    - Low priority – the chemical use does not meet the standard for high-priority
- Assessments for High Priority chemicals must be completed in 3 years, requiring a complete data package at the beginning
- The TSCA Active Inventory contains over 33,000 chemicals
- CompTox resources can provide key inputs to aid this prioritization process

(*) https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/highlights-key-provisions-frank-r-lautenberg-chemical

The CompTox Opportunity

- CCTE staff have been developing resources with data on large numbers of chemicals covering hazard, exposure, toxicokinetics and physico-chemical properties
- Traditional Animal Toxicology: ToxRefDB, ToxValDB
- In Vitro Hazard: ToxCast, specific models for endocrine pathways
- Exposure: ExpoCast (SEEM), CPCat & CPDat, models of use
- Toxicokinetics: HTTK
- PhysChem: OPERA models of physchem and other properties
- Experience building large-scale integrative models

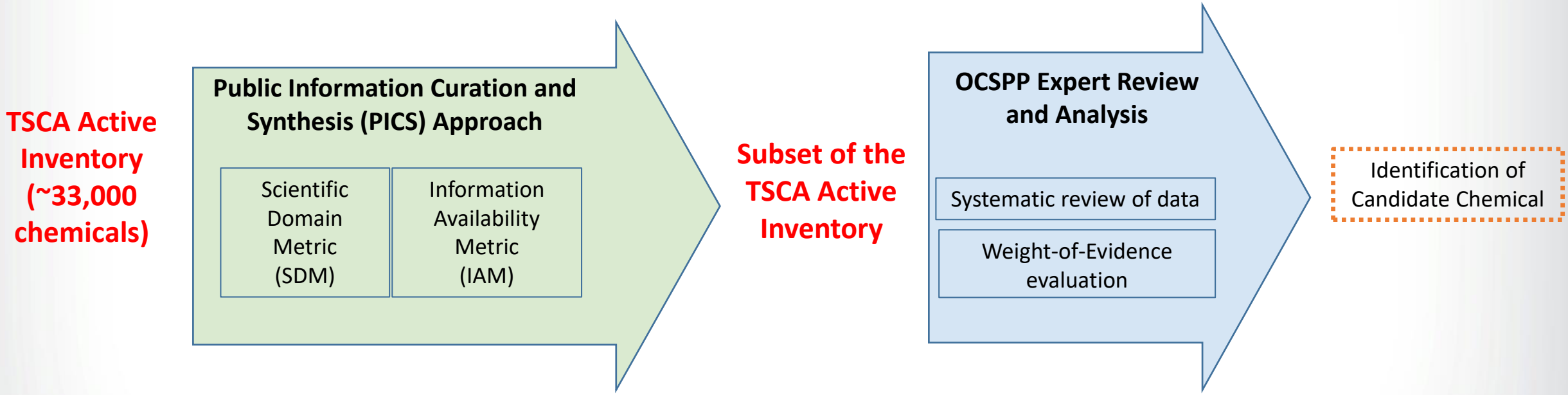
Implementation of the Proof-of-Concept Study

- **Operationalized long-term strategy through development of the <u>Public Information Curation and Synthesis</u> (PICS) approach**
  - Integrates information from a variety of sources to better understand the landscape of publicly available information for large numbers of chemical substances
  - Synthesizes information across key scientific domains used to evaluate chemical risks
  - Consistent with the *Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program to* integrate NAMs to fill gaps when traditional testing data are not available

Defining Intended Application of PICS Approach

- **The PICS approach was intended to:**
  - Understand the landscape of publicly-available information on the over 33,000 substances on the active inventory
  - Provide a transparent and reproducible process for integrating available information and identifying potential information gaps
  - Increase efficiency and manage workload by focusing expert review on substances that may have a greater potential for selection as high- or low-priority candidates
  - Create a flexible and sustainable process that can adapt to scientific advances and continual generation of new safety-related information
  - Organize the process into modular workflows that can be readily updated or adapted to address prioritization needs under other mandates
- **The PICS approach was <u>not</u> intended to:**
  - Replace the formal TSCA prioritization or risk evaluation processes
  - Create a ranked list of substances
  - Signal that the EPA has concerns with particular substances or categories of substances
  - Supplant expert judgment and review
  - Utilize confidential business information
  - Incorporate systematic review of information to address study and data quality

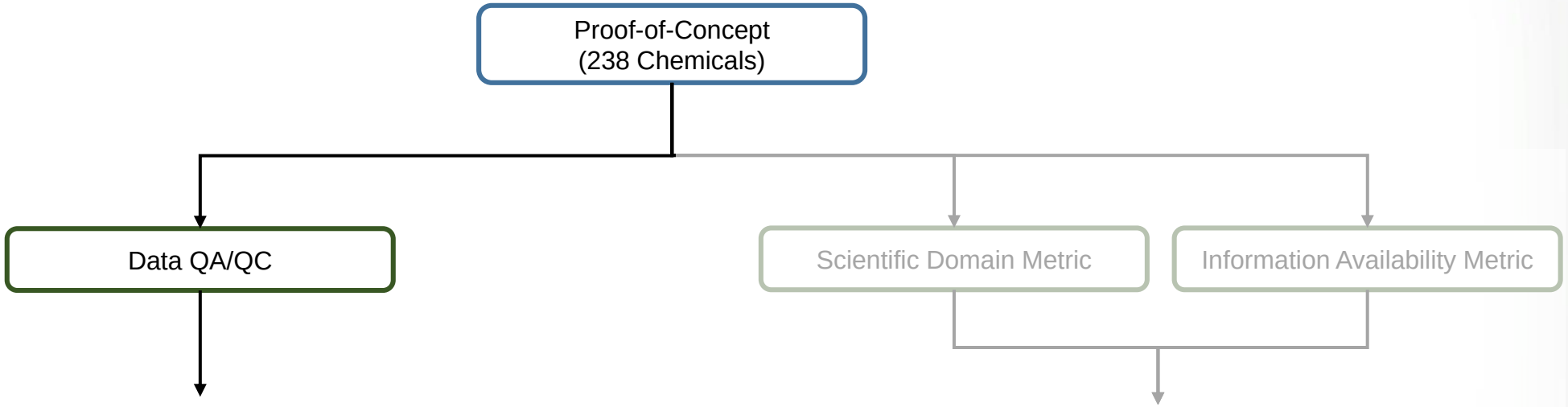
Schematic of PICS Approach Within the Candidate Selection Process



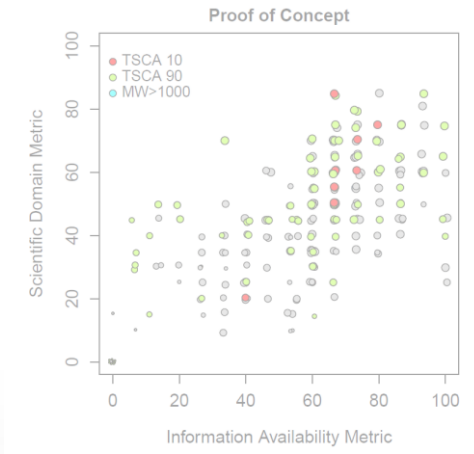
Proof-of-Concept Chemicals (POC 238)

- The process was carried out on the complete TSCA Active Inventory
- For illustration, a total of 238 substances selected from the curated, non-confidential active TSCA inventory
- Selection based on the following:
  - Proposed set of 20 high- and 20 low-priority candidate substances
  - Substances from the 2014 update to the TSCA Work Plan
  - Substances with known relevance to each of the scientific domains
  - Subset of chemical substances listed in the FDA's Substances Added to Food inventory and EPA's Safer Chemical Ingredients List (SCIL)

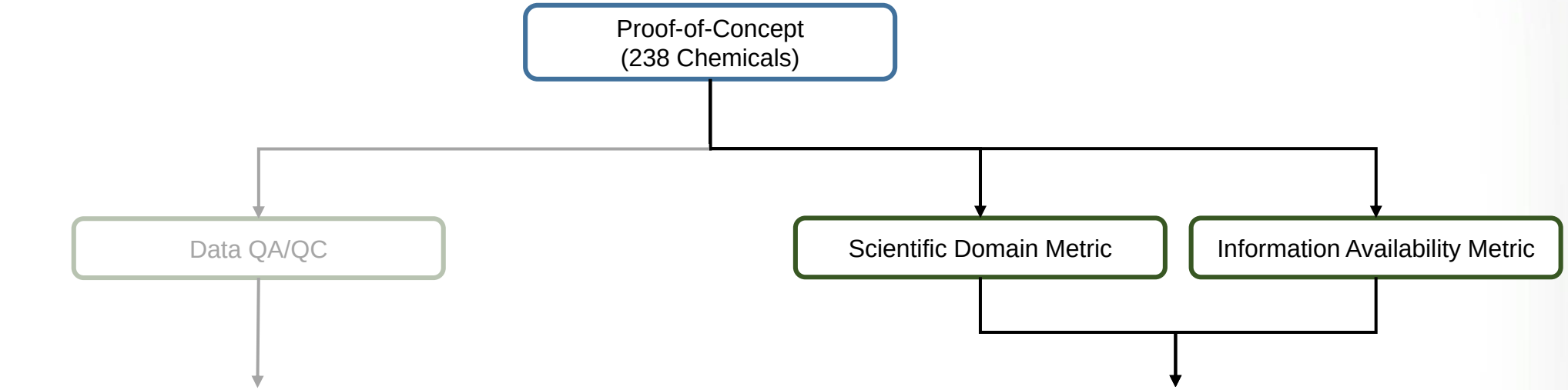
Proof-of-Concept: Data QA/QC



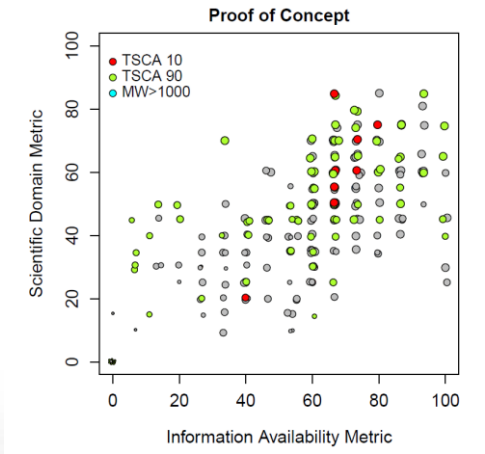
- Specific data domain and data source error rates
- Data QA plan for TSCA active inventory
- FTE estimates for data QC
- QA of massive amounts of data is an ongoing challenge



Proof-of-Concept: Metrics



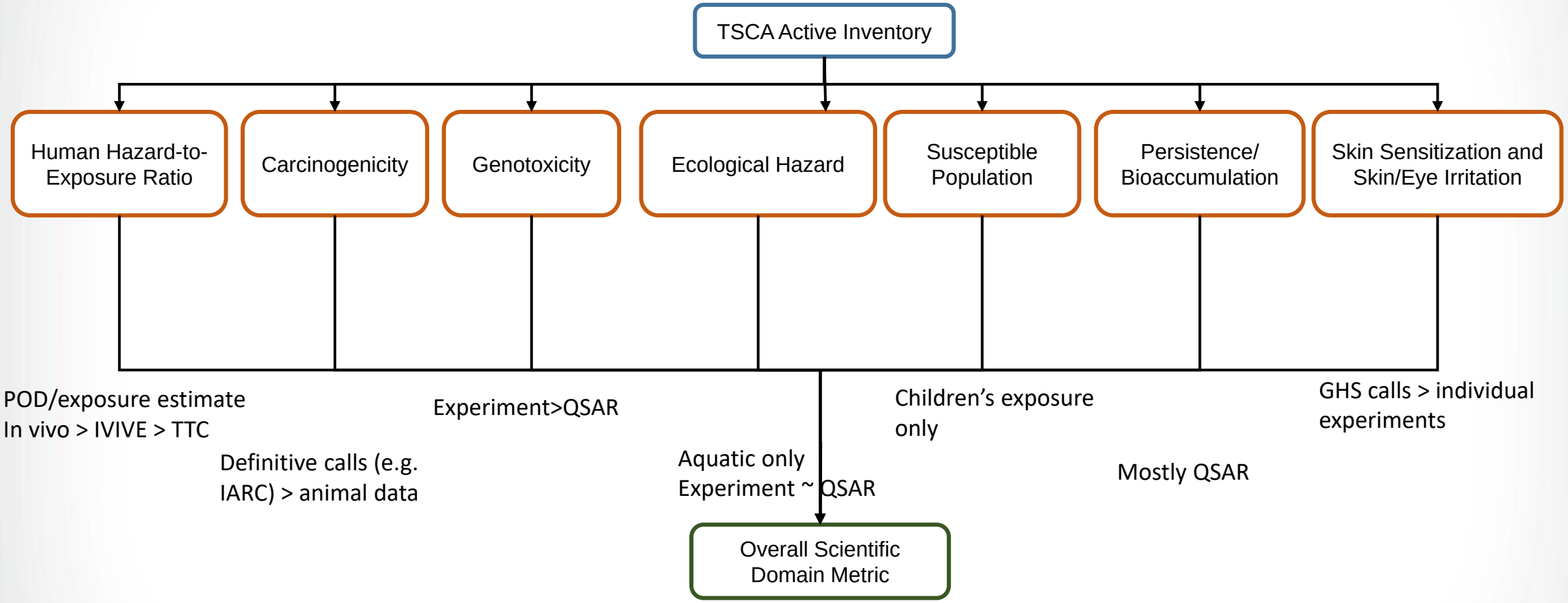
- Specific data domain and data source error rates
- Data QA plan for TSCA active inventory
- FTE estimates for data QC
- QC Tool (beta)



Scientific Domain Metric

- Seven scientific domains were selected based on:
  - Previous use in TSCA prioritization activities (i.e., TSCA workplan)
  - Statutory language in the amended TSCA
  - Consultation with OCSPP management and staff
- Tiered workflows for each scientific domain designed based on the current state of the science
- The overall scientific domain metric is determined by summing the results from the individual scientific domain workflows

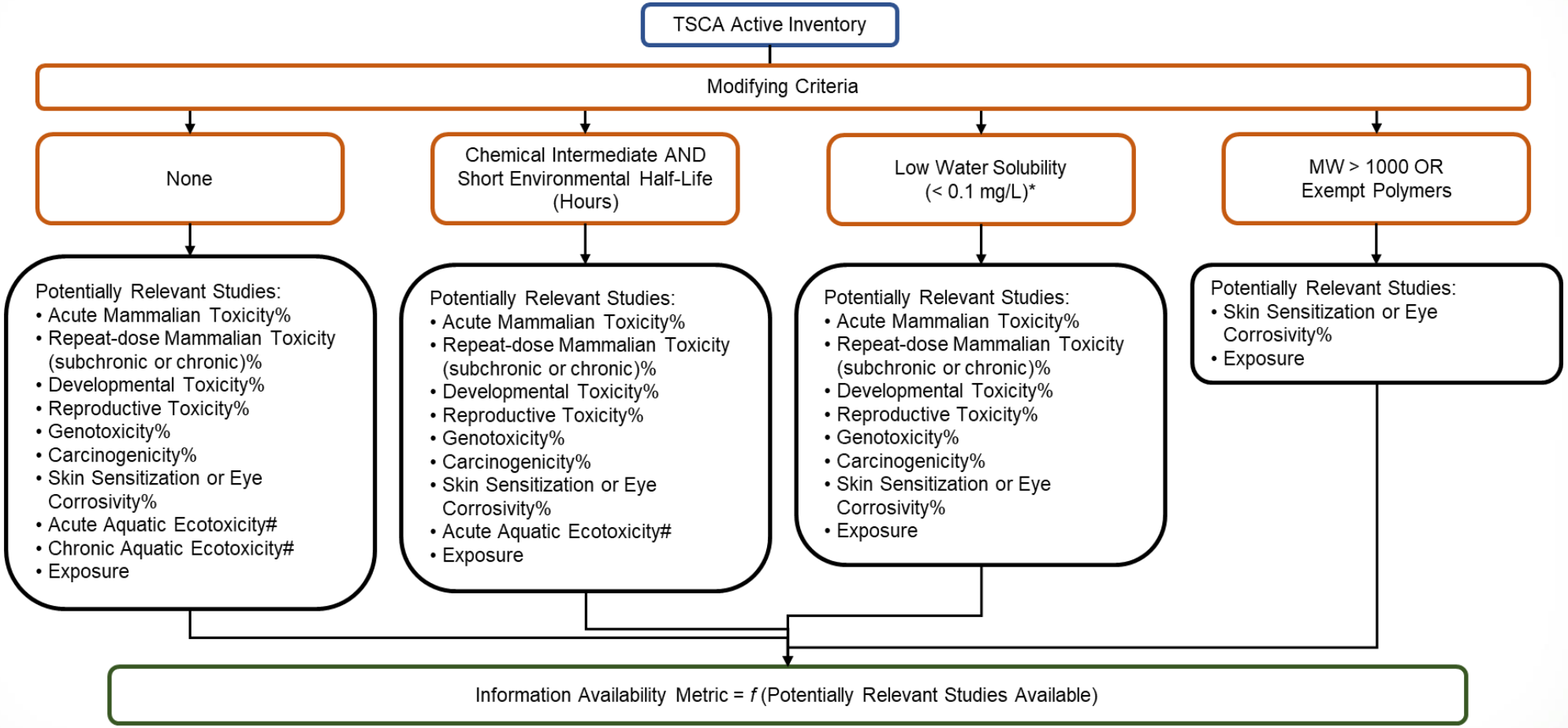
Overall Scientific Domain Metric



Information Availability Metric

- Included in PICS approach to evaluate the amount of information available for use in any future chemical substance risk evaluation
- Needed because detailed risk assessments cannot be carried out without sufficient data
- Based on the potentially relevant information for exposure, human health and ecological toxicity
- Modifying criteria (based on OPPT new chemicals program and consultation with OPPT technical staff) applied to make the score context-specific
- Incorporates “information gathering flags” to highlight data types used in specific scientific domain metrics as well as possible data gaps

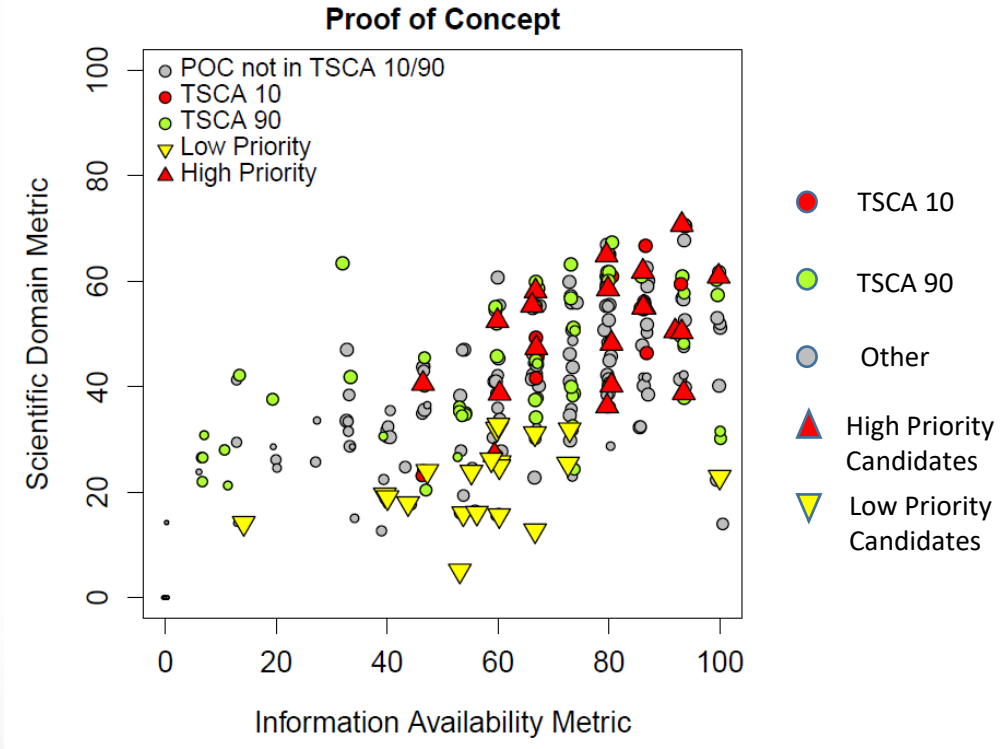
Information Availability Metric



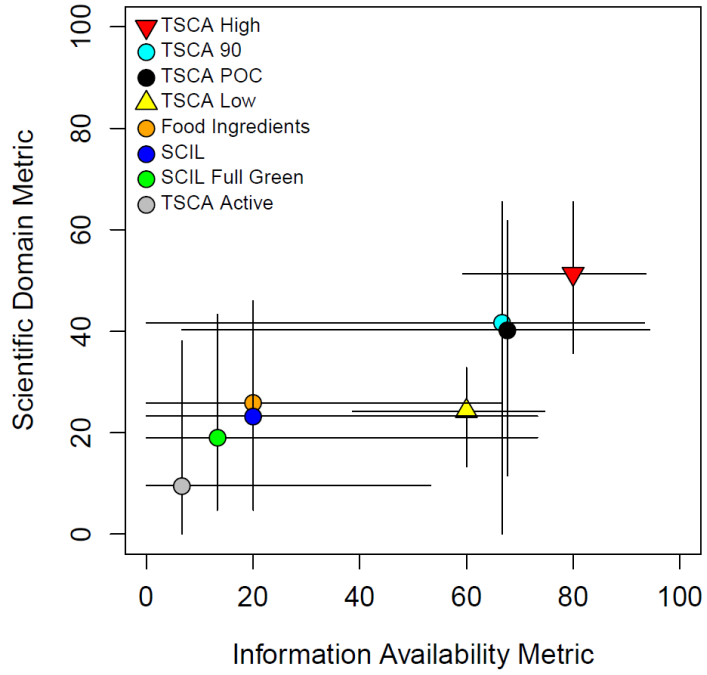
*Criteria based on Sustainable Futures Manual (EPA-748-B12-001); #includes multiple trophic level data; %Not required if chemical has an authoritative human hazard assessment

Proof-of-Concept Results

- High priority chemicals have larger scientific domain scores than the low priority
- “Safe” Chemical sets (e.g. food ingredients) tend to have low scientific domain scores
- The POC chemicals have larger than average information availability



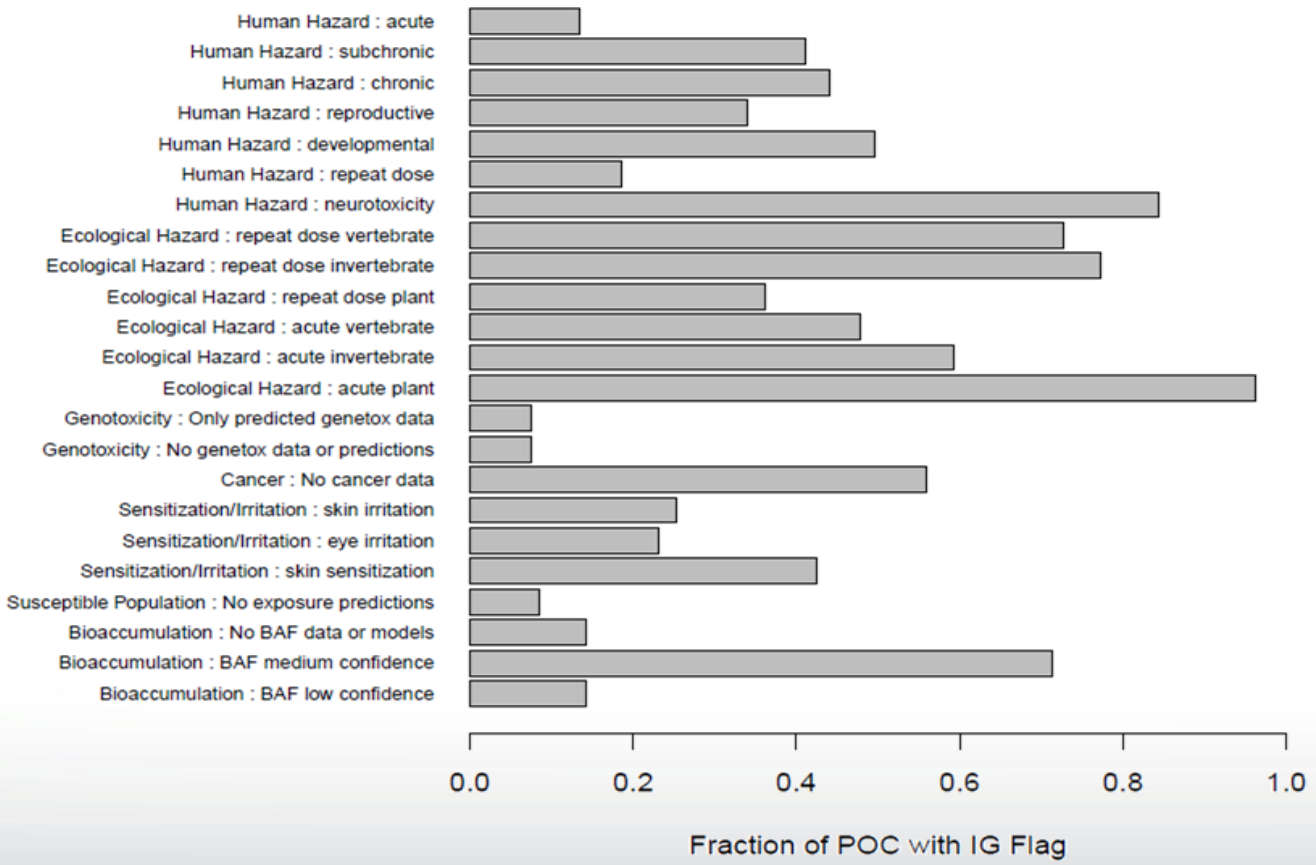
Information availability vs. scientific domain metrics for the POC238 set of chemical substances. Positions of points are staggered for ease of visualization.



Distributions of metric scores for selected chemical substance lists. For each list, the point shows the median scientific domain and information availability metrics. The whiskers span 90% of the distributions. Data here is taken from the lists across the TSCA Active Inventory. Uses data from the complete TSCA active inventory.

Proof-of-Concept Results

- The larger the value, the fewer the number of chemicals with that type of information
- Ecotoxicology, neurotoxicology BAF medium confidence have largest amount of missing data



Example: Compare Two Chemicals

CASRN	4435-53-4	71-43-2
Name	**3-Methoxybutyl acetate**	**Benzene**
Scientific Domain Metric	15.9	70.5
Information Availability Metric	60	93
IG flag human hazard (missing mammalian hazard data)	subchronic, chronic, developmental	developmental, reproductive
IG flag ecological hazard (missing eco hazard data)	acute plant, repeat dose invertebrate, repeat dose vertebrate	acute plant, acute invertebrate
Human hazard-to-exposure ratio metric	2.3	2.7
Ecological hazard metric	2.0	2.0
Carcinogenicity metric	0 (no data)	4
Genotoxicity metric	1	4
Susceptible population metric	2	4
Persistence bioaccumulation metric	1	2
Sensitization / irritation metric	1	3
HER repeat dose	13253000	11374
POD in vivo oral repeat dose	100 mg/kg-day	0.015 mg/kg-day
Human exposure (SEEM3)	0.0000075 mg/kg-day	0.0000013 mg/kg-day
Ecological min POD	0.71 mg/L	0.49 mg/L
Genotoxicity call	non-genotoxic	genotoxic
Carcinogenicity call		Group I: carcinogenic to humans
Skin sensitization metric		L
Eye irritation metric	L	H
Skin irritation metric	L	H
Volatile	No	Yes

Challenges

- Data sources are limited
  - Many chemicals do not have data in any source
  - Only public data was used, i.e., no CBI data
  - Largely only use data from other compilations, i.e., do not carry out targeted literature search and data extraction
- Manual data QA/QC is time and resource intensive for thousands of chemicals
  - CCTE is developing automated pipelines and web-based manual QC tools
- Apples and oranges tradeoffs
  - How to weigh relative concerns of hazard, exposure, physchem properties?
  - This is finally a policy decision

Summary

- The PICS approach was developed to better understand the landscape of publicly available information for large numbers of chemical substances
- It combines results from domain-specific workflows that reflect the overall degree of potential concern related to human health and the environment with the amount of relevant information
- It is intended to focus expert review on substances that may have a greater potential for selection as high- or low-priority candidates
- The proof-of-concept case study demonstrated that the PICS approach generally resulted in higher metrics for the high-priority candidates as compared to the low-priority candidates and identified areas for potential information gathering
- The method and software are flexible and can be customized for other prioritization applications

Data Curation and QC Tiger Team

- **General –** John Cowden (NCCT), Richard Judson (NCCT), Amar Singh (NCCT)
- **QC Data Integration and QA Automation Workgroup** - Richard Judson (NCCT), Jeremy Dunne (NCCT), Amar Singh (NCCT), Chris Grulke (NCCT)
- **Human Health Hazard/Risk Assessment Workgroup** - Johanna Congleton (NCEA), Urmila Kodavanti (NHEERL), Chris Lau (NHEERL), Mary Gilbert (NHEERL), Yu-Sheng Lin (NCEA), Dan Vallero (NHEERL), Kelly Garcia (NCEA), Carolyn Gigot (NCEA), Andrew Greenhalgh (NCEA), Allison Eames (NERL)
- **Ecological Toxicity Data Workgroup** - Dale Hoff (NHEERL), Colleen Elonen (NHEERL), Leslie Hughes (NHEERL), Anita Pascocello (NHEERL)
- **Exposure Data Workgroup** - Katherine Phillips (NERL), Janet Burke (NERL), Abhishek Komandur (NERL), Ashley Jackson (NERL), Lauren Koval (NERL)
- **Genotoxicity Data Workgroup** - David DeMarini (NHEERL), Maureen Gwinn (NCCT), Catherine Gibbons (NCEA), Sarah Warren (NHEERL), Jeff Dean (NCEA), Anita Simha (NCCT), Nagu Keshava (NCEA)
- **Chemistry Data Workgroup** - Kent Thomas (NHEERL), Michael Gonzalez (NRMRL), Doug Young (NRMRL), Chris Grulke (NCCT)

Proof-of-Concept Tiger Team

- **General** - Maureen Gwinn (NCCT), Richard Judson (NCCT), Amar Singh (NCCT)
- **Information availability** - Tony Williams (NCCT), Jeremy Dunne (NCCT), Jason Lambert (NCCT)
- **Human Hazard-to-Exposure Ratio** - Katie Paul-Friedman (NCCT), John Wambaugh (NCCT), Elaina Kenyon (NHEERL), Kristin Isaacs (NERL), Jason Lambert (NCCT)
- **Susceptible Population Exposure** - Kathie Dionisio (NERL), Kristin Isaacs (NERL), John Wambaugh (NCCT)
- **Carcinogenicity/Genotoxicity** - Grace Patlewicz (NCCT), David DeMarini (NHEERL), Catherine Gibbons (NCEA), Jeffry Dean (NCEA), Anita Simha (NCCT), Nagu Keshava (NCEA), Todd Martin (NRMRL), Sarah Warren (NHEERL)
- **Eco Hazard** - Dan Villeneuve (NHEERL), Carlie LaLone (NHEERL), Todd Martin (NRMRL)
- **Persistence/bioaccumulation** - John Nichols (NHEERL), Lawrence Burkhard (NHEERL), Eric Weber (NERL)
- **Skin sensitization/irritation and Eye irritation** - Todd Martin (NRMRL), Leora Vegosen (NRMRL)

Developmental Neurotoxicity (DNT) in vitro Battery as an Alternative to DNT in vivo Guideline Studies Used by OPP

Tim Shafer

Board of Scientific Counselors Subcommittee

Chemical Safety for Sustainability and

Health and Environmental Risk Assessment National Research Programs

Virtual Meeting

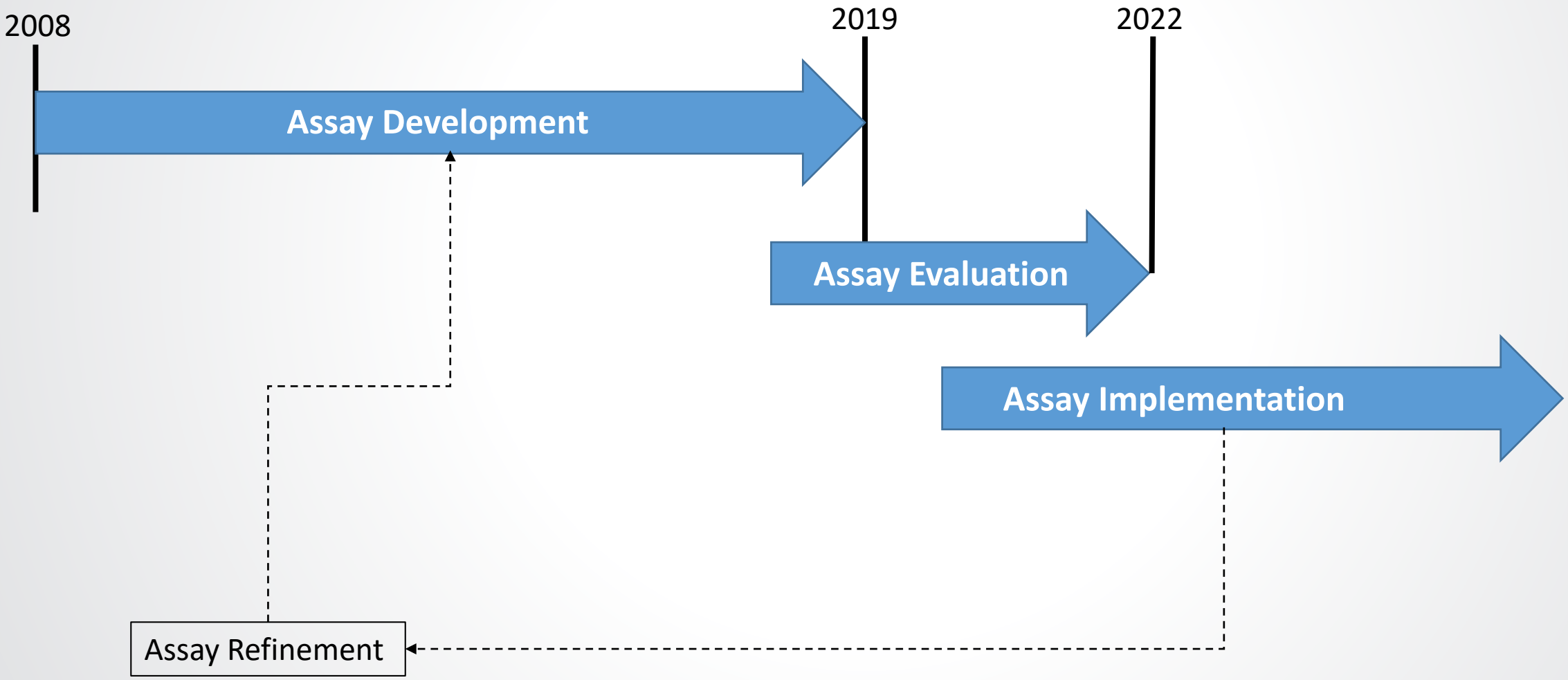
February 3, 2021

The subsequent presentation has been cleared by the Office of Research and Development but is not Agency Policy. This presentation contains unpublished data.

Outline

I. (Re)-Introduction to CSS Research on alternative approaches for developmental neurotoxicity (DNT) hazard assessment
II. International Efforts on use of NAMs for DNT hazard assessment
III. Application of NAMs to OCSPP issues.
IV. Future Directions

Status of DNT NAMs Research in CSS



Issues with in vivo DNT studies

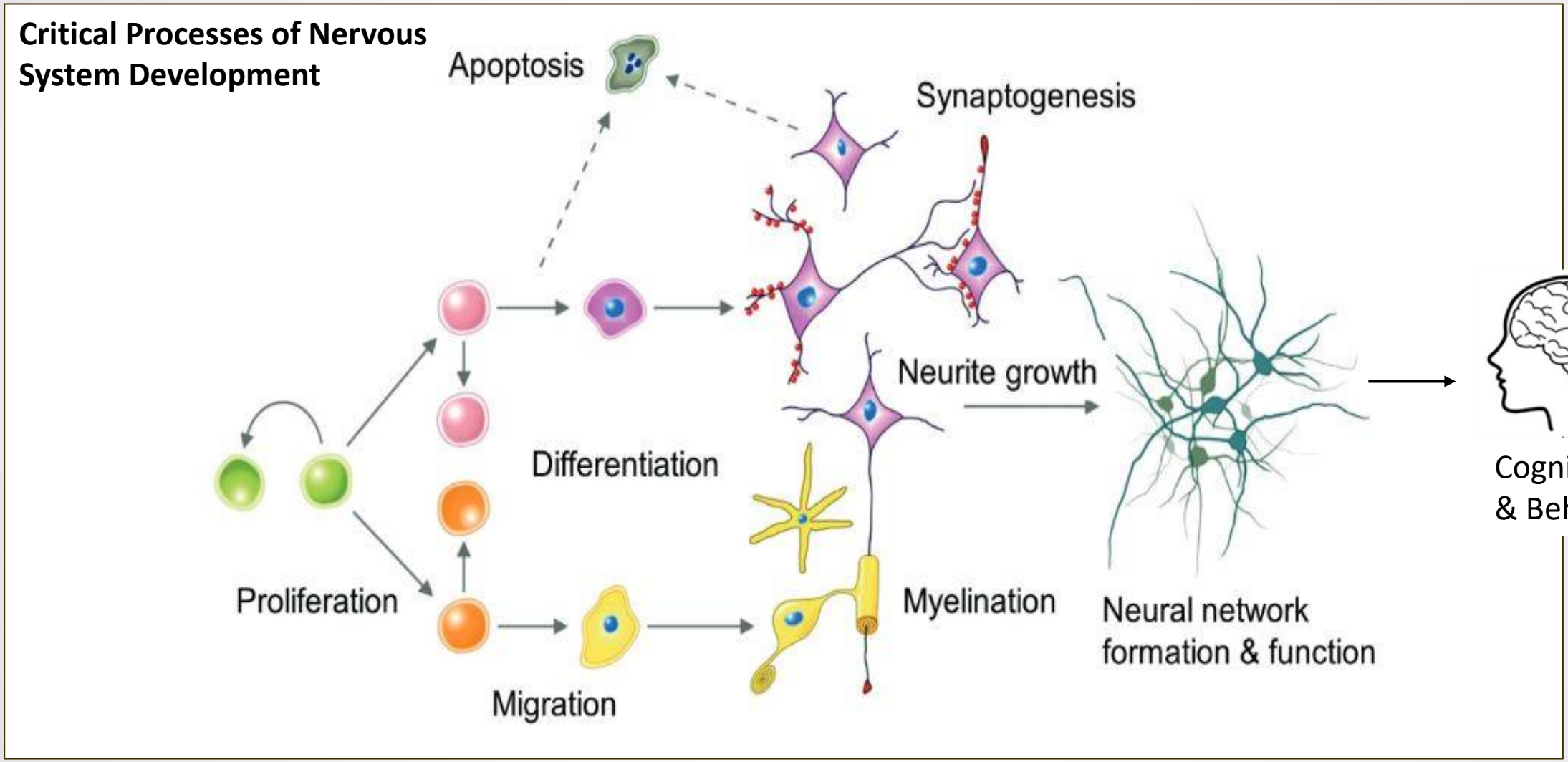
- "Triggered" test- Only requested if concern for neurotoxicity
- Expensive- ~$1,000,000/chemical
- Time-consuming- takes 1-2 years to complete
- Ethically questionable- Estimated ~1000 animals/test
- Value of Information
  - High variability; low precision
  - Not often used (~25%) for point of departure values for risk assessment*

Only ~150 compounds have DNT Guideline Studies

Problem for OPPTS and OPP

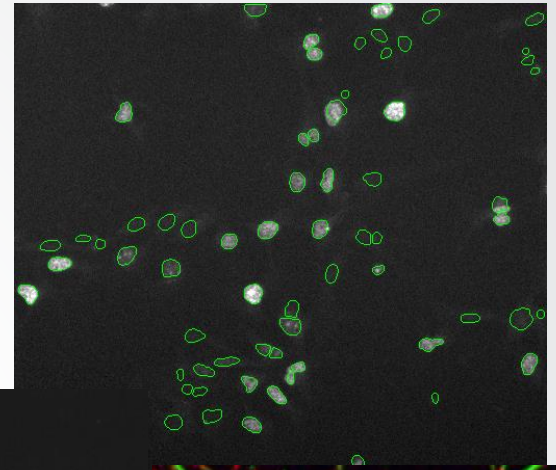
*Raffaele et al. The use of **developmental neurotoxicity** data in pesticide risk assessments. Neurotoxicol Teratol. 2010 Sep-Oct;32(5):563-72.

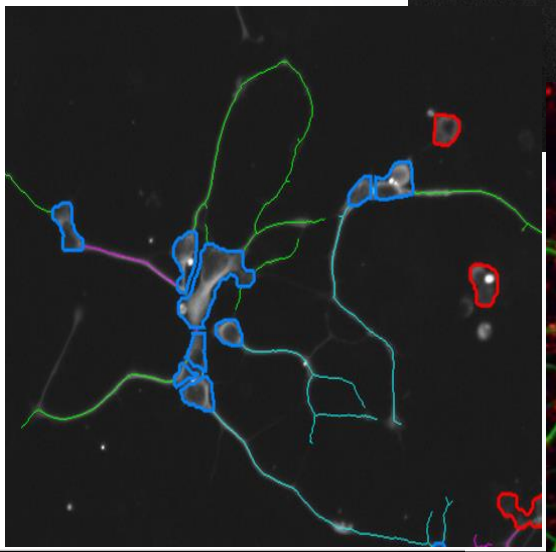
Addressing the limitations of the DNT Guideline Study by using Phenotypic Screens

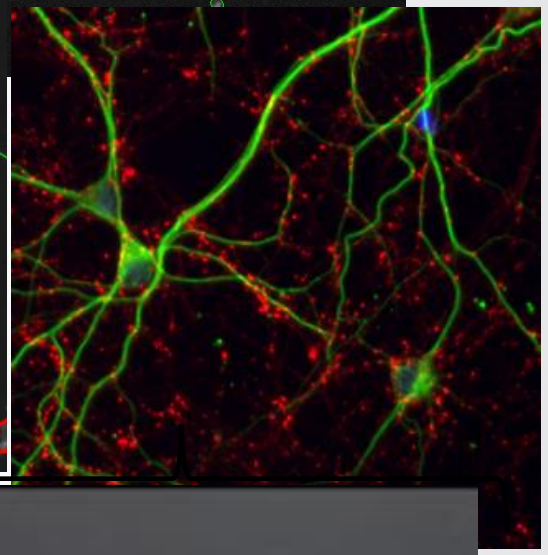


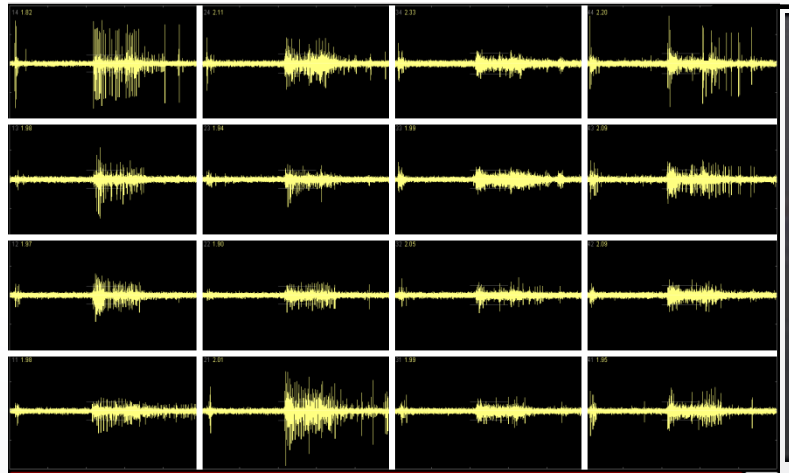
The EPA Assay Battery

Proliferation	-human neuroprogenitors (hNP1)
Apoptosis	-human neuroprogenitors (hNP1)
Neurite initiation	-human neurons (hN2, $iCell_{gluta}$)
Neurite initiation	-rat primary neural culture
Neurite maturation	-rat primary neural culture
Synaptogenesis	-rat primary neural culture
Network formation (MEA)	-rat primary neural culture
Behavior/Anatomy	-zebrafish









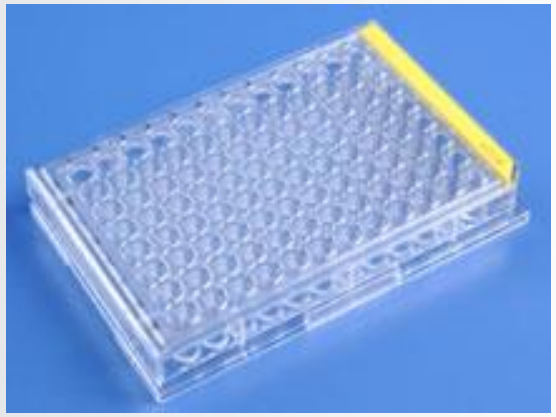
Each assay has concurrent assessments of cell health/viability and has been vetted with assay positive controls as well as by testing DNT reference compounds.

High Content Imaging: Overview

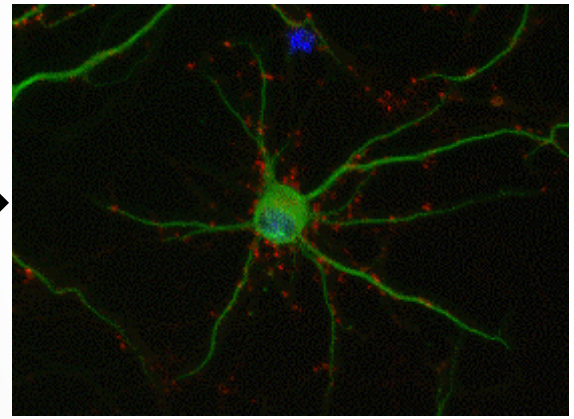
Automated microscopy providing data at the level of the individual cell

High throughput : automated data acquisition and analysis in multi-well plates

High content : large amounts of data from a single image.



Multiwell Culture



Immunocytochemistry

Image Acquisition

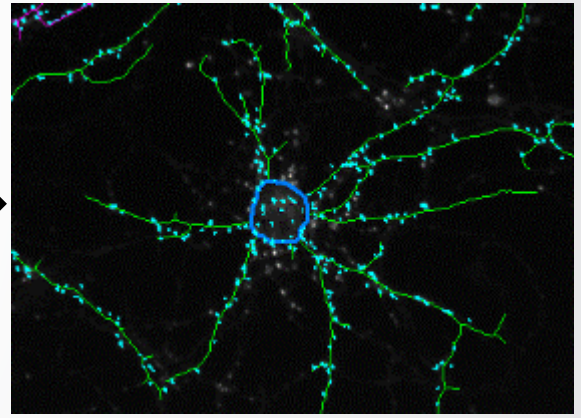
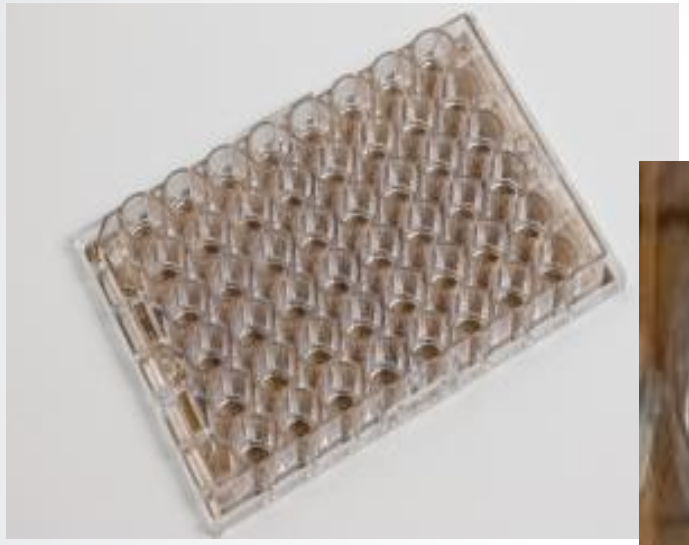
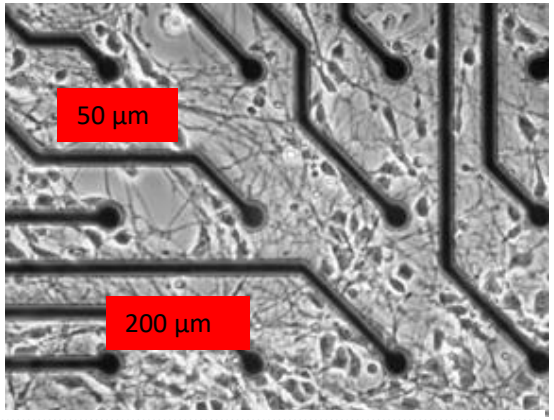


Image Analysis
Feature Extraction

- Epifluorescence microscope and digital camera *in a box*
- Automated stage movement, exposure, and focusing capabilities
- Computer algorithms analyze the images to provide cell-based data (*e.g.* size, shape, location, fluorescence intensity)

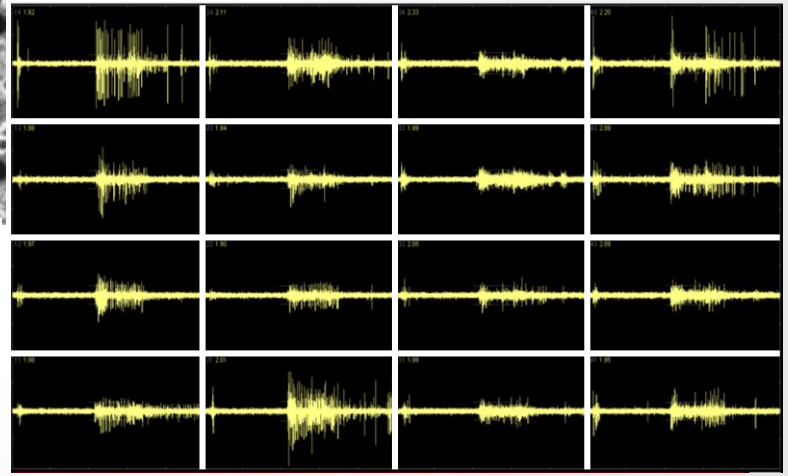
Measurement of Network Formation in vitro using Microelectrode Array (MEA) Recording

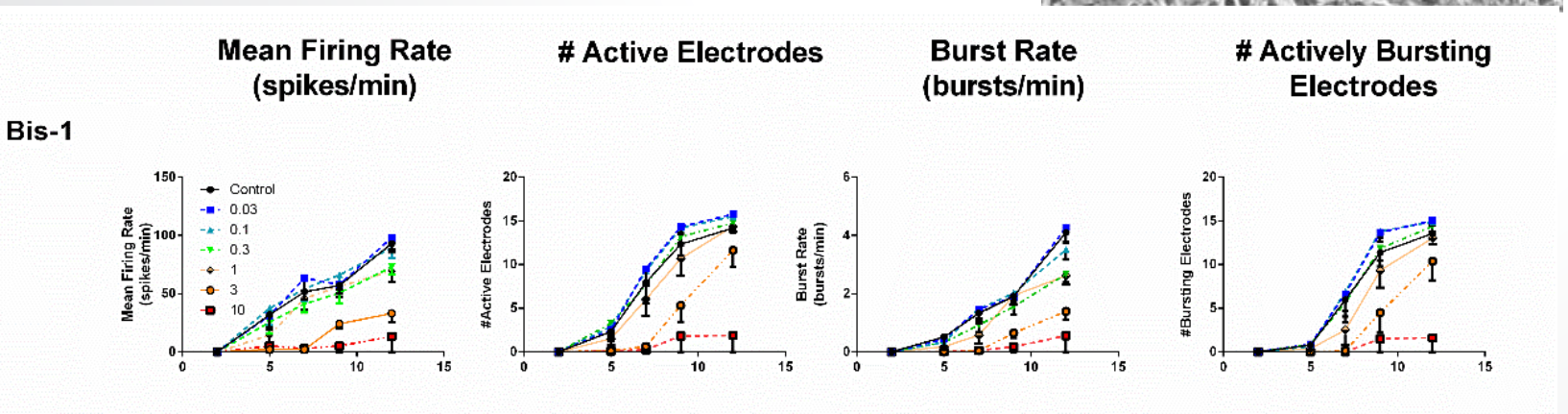




"Brain-on-a-Chip": Complex 2D model

- Rat cortical neural networks
- Contains neurons & glia cells
- Spontaneous activity
- Develops rapidly in vitro
- Follow network development over time
- Integrates activity of multiple processes





A snapshot in time of neural network activity in one well. Each box represents the electrical activity of neurons on 1 electrode in the array.

International Efforts on DNT NAMs

OXFORD
SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(1), 2019, 45–57

doi: 10.1093/toxsci/kfy211
Advance Access Publication Date: November 23, 2018
Forum

FORUM

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing

Magdalini Sachana,*,[1] Anna Bal-Price,† Kevin M. Crofton,‡ Susanne H. Bennekou,§ Timothy J. Shafer,¶ Mamta Behl,‖ and Andrea Terron‖

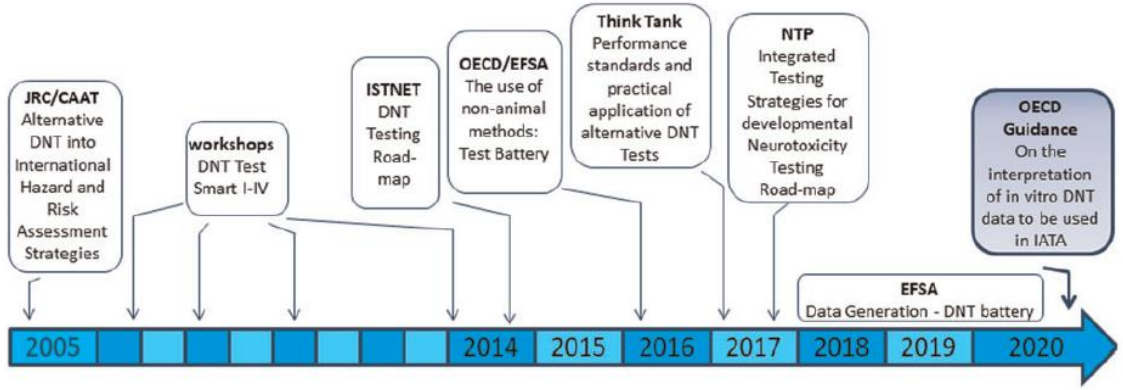
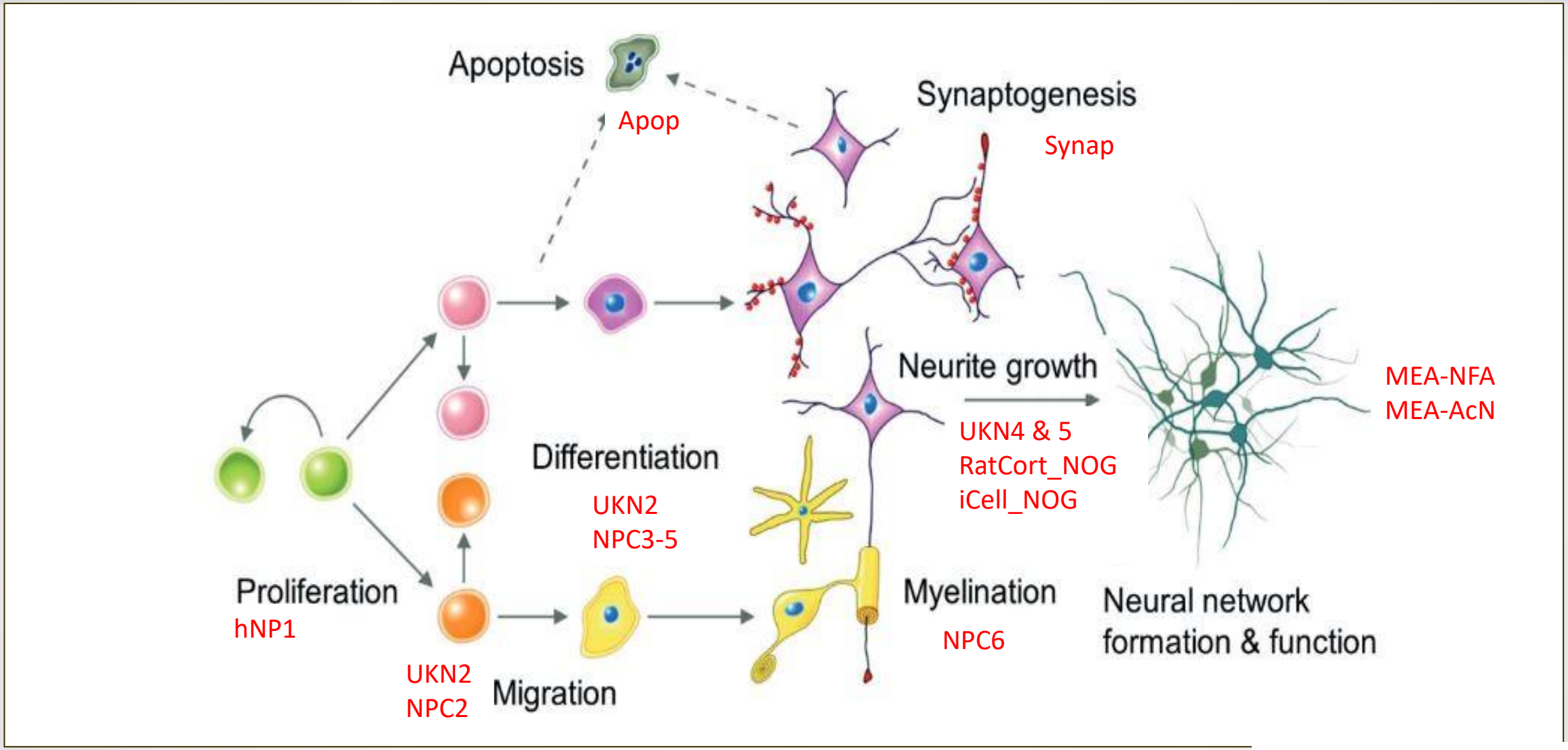


Figure 1. Timeline of efforts to develop and implement new alternative methods for developmental neurotoxicity.

Table 2. Proposed Assays for Evaluation As an *In Vitro* DNT Battery

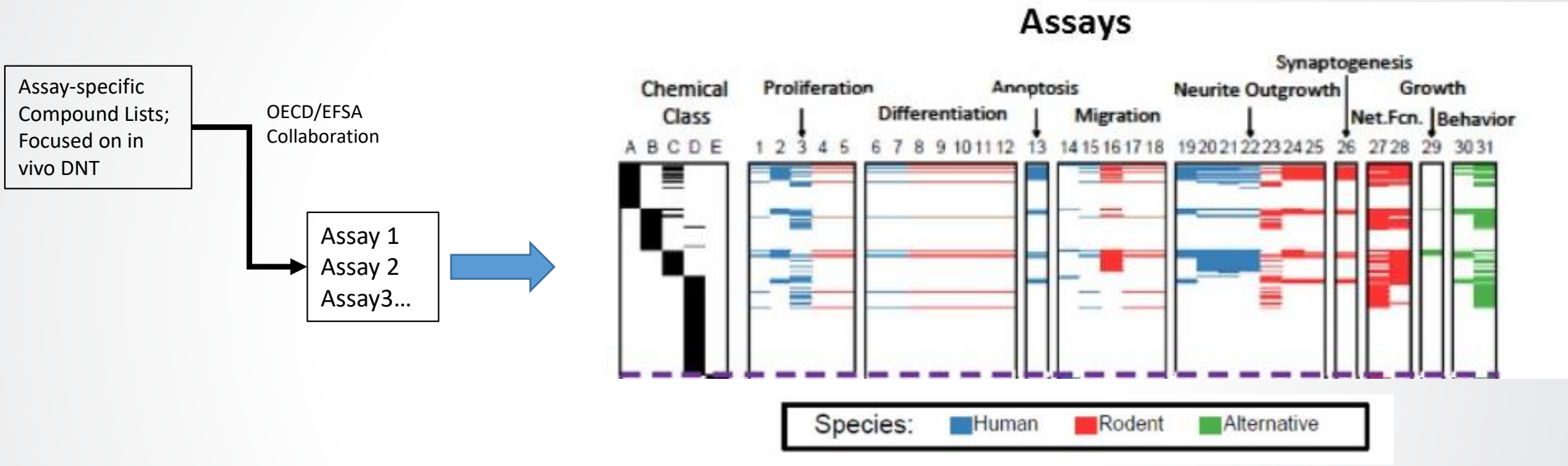
Process	Assays	References
Proliferation	hNP1	Harrill *et al.* (2018)
	NPC1	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN1	Balmer *et al.* (2012)
Apoptosis	hNP1	Harrill *et al.* (2018)
Migration	NPC2	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN2	Nyffeler *et al.* (2017)
Neuron differentiation	NPC3	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Oligodendrocyte differentiation & maturation	NPC5/6	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Neurite outgrowth	iCell gluta hN2	Harrill *et al.* (2018)
	UKN 4 & 5	Krug *et al.* (2013)
	NPC4	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Synaptogenesis	Rat primary synaptogenesis	Harrill *et al.* (2018)
Network formation	MEA-NFA	Brown *et al.* (2016) and Frank *et al.* (2018)

DNT NAMs Provide Good Coverage of Neurodevelopmental Processes



Aschner et al., 2016

OECD/EFSA-EPA Collaboration



Development of a Guidance Document for the use of DNT alternative assays in Integrated Approaches for Testing and Assessment (IATAs)

- Guidance for incorporation of in vitro assays into IATAs
- Case Studies
- Draft Guidance document expected mid 2021

Use of DNT NAMs at EPA

I. **Screening Level information**
   - APCRA, TSCA, PFAS

II. **Understanding species differences**
   - Data from DNT NAMs provided to OPP to help understand rodent-human differences in response to chemicals since the battery has both rodent and human assays

III. **Structure-activity relationships**
   - OPP requested data from selected assays on a set of structurally similar compounds
     - A DNT Guideline study existed for one compound (“compound X”)
     - Assays were selected based on the of activity of compound X in Guideline Study.
     - Structurally similar compounds were tested in vitro
     - OPP will use the data from the in vitro screens in WOE approach to deciding whether or not to request DNT guideline studies on the other compounds (Decisions are in progress).

IV. **Weight of Evidence approaches**
   - Organophosphates

Organophosphates and DNT

Organophosphate insecticides are currently regulated based on inhibition of acetylcholinesterase (AChE):

Primary Questions:

1) Does the DNT battery indicate that this may not be health protective?
2) Can data from the DNT battery contribute to a WOE approach for OPs?

Organophosphates and DNT

Study Design:

Test 27 Organophosphate insecticides in the EPA DNT assays

8 Parent/oxon pairs

Concentration-response up to 100 µM

Pipeline results through TCPL to generate AC_{50} values

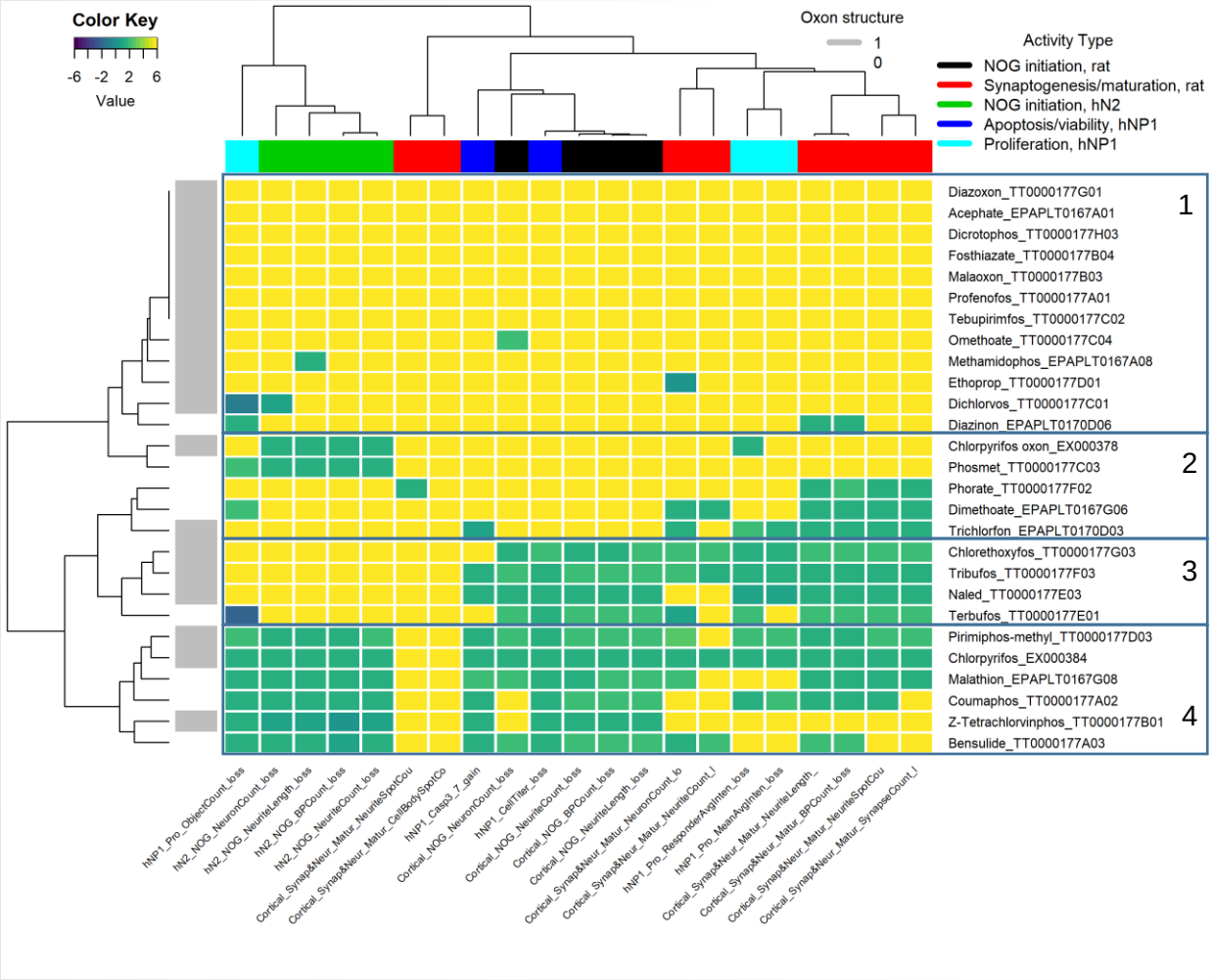
Use HTTK to convert AC_{50} values to AED_{50} values

Compare to BMD/BMDL10 values based on AChE inhibition

Assays:

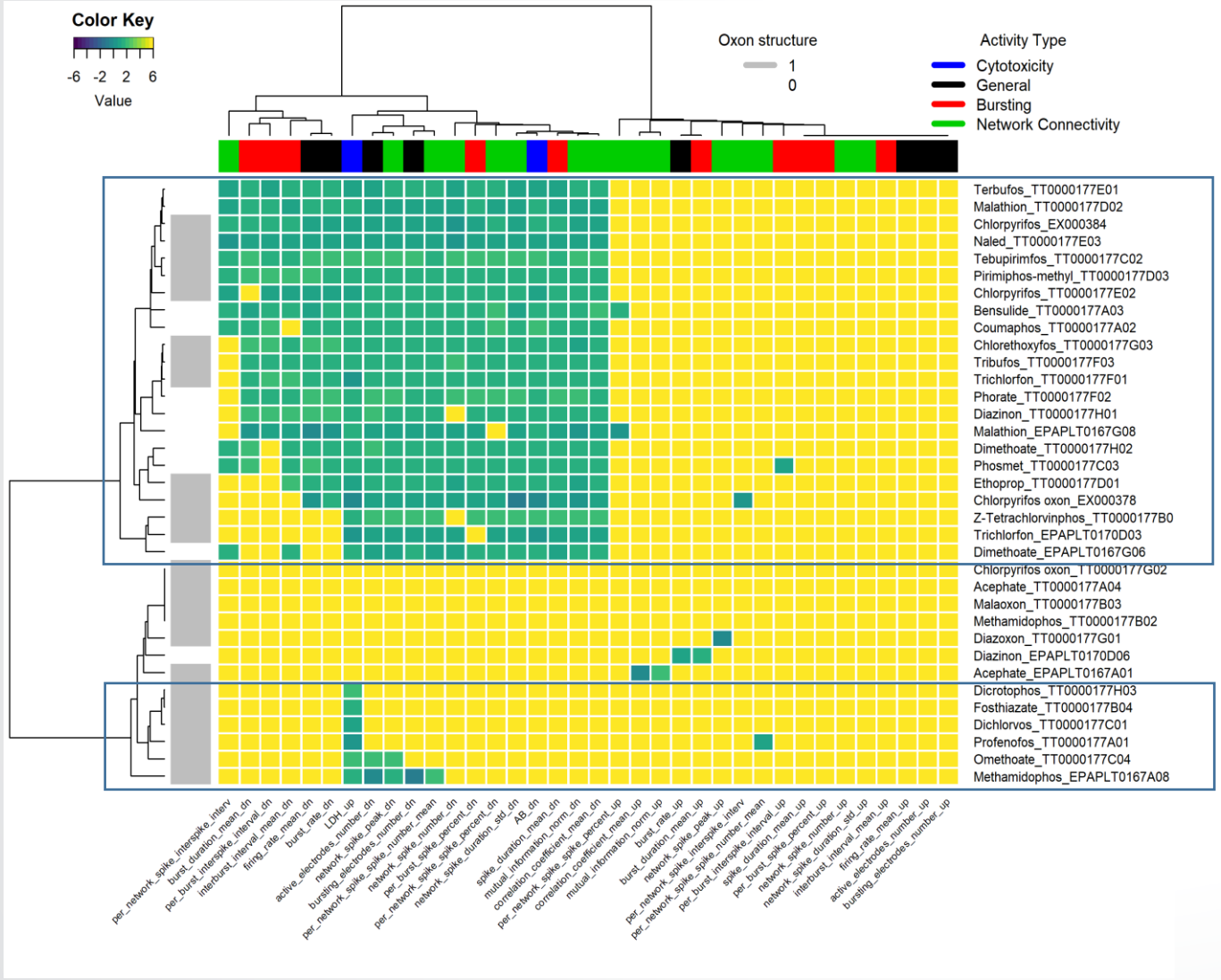
Proliferation	-	human neuroprogenitors (hNP1)
Apoptosis	-	human neuroprogenitors (hNP1)
Neurite initiation	-	human neurons (hN2)
Neurite initiation	-	rat primary neural culture
Neurite maturation	-	rat primary neural culture
Synaptogenesis	-	rat primary neural culture
Network formation (MEA)	-	rat primary neural culture
Behavior/Anatomy	-	zebrafish (data analysis pending)

OPs demonstrate differential responses in the HCI assays.



- Cluster 1: negative or with effects in 1-3 endpoints.
- Cluster 2: effects on 5 or more assay endpoints
- Cluster 3: OP samples with effects on all HCI assay activity types except for NOG initiation in hN2 cells and synaptogenesis in cortical cells
- Cluster 4: widespread effects across activity types

Most OPs decreased MEA NFA activity



- Top active cluster of OPs contains oxon and non-oxon structures.
- These OPs, like the assay performance controls and many other compounds, appear to generally decrease all activity types and most assay endpoints.
- Bottom cluster with minimal actives appears somewhat driven by cytotoxicity in the LDH assay.
- Negative- 0 assay endpoints altered
- Equivocal- 1-3 assay endpoints altered
- Positive- >3 assay endpoints altered

Overall, there was agreement between the HCI and MEA_NFA assays

DTXSID	Chemical	MEA NFA			HCI			
		Neg	Equiv	Pos	1	2	3	4
DTXSID8023846	Acephate	X	X		X			
DTXSID9032329	Bensulide			X				X
DTXSID2032344	Chlorethoxyfos			X			X	
DTXSID4020458	Chlorpyrifos			X,X				X
DTXSID1038666	Chlorpyrifos oxon	X		X		X		
DTXSID2020347	Coumaphos			X				X
DTXSID9020407	Diazinon		X	X		X		
DTXSID5037523	Diazoxon		X		X			
DTXSID5020449	Dichlorvos		X		X			
DTXSID9023914	Dicrotophos		X		X			
DTXSID7020479	Dimethoate			X		X		
DTXSID4032611	Ethoprop			X	X			
DTXSID0034930	Fosthiazate		X		X			
DTXSID9020790	Malaoxon	X			X			
DTXSID4020791	Malathion			X				X
DTXSID6024177	Methamidophos	X	X			X		
DTXSID1024209	Naled			X			X	
DTXSID4037580	Omethoate		X		X			

DTXSID	Chemical	Neg	Equiv	Pos	1	2	3	4
DTXSID4032459	Phorate			X		X		
DTXSID5024261	Phosmet			X		X		
DTXSID0024266	Pirimiphos-methyl			X				X
DTXSID3032464	Profenofos		X		X			
DTXSID1032482	Tebupirimfos			X	X			
DTXSID2022254	Terbufos			X			X	
DTXSID1024174	Tribufos			X			X	
DTXSID0021389	Trichlorfon			X		X		
DTXSID1032648	Z-Tetrachlorvinphos			X				X

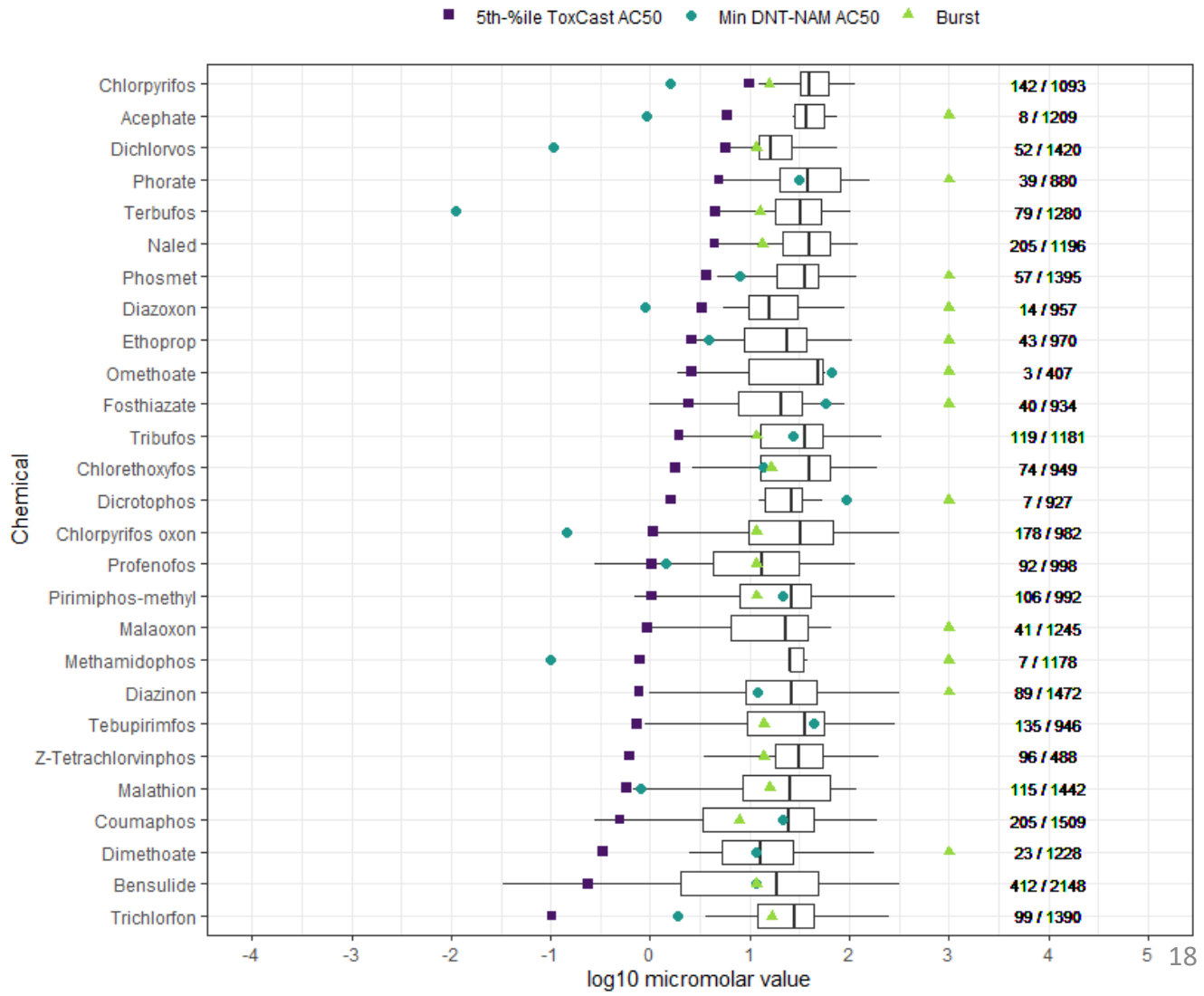
- *Equiv or Pos in MEA NFA and negative in HCI*: Acephate, diazoxon, dichlorvos, dicrotophos, fosthiazate, malaoxon, omethoate, profenofos
- *Positive in MEA NFA and negative in HCI*: Ethoprop
- *Positive in HCI and negative in MEA NFA:* OP chemical (methamidophos) was neg/equiv in the MEA NFA
- **If activity is observed in the HCI assays, it is likely that the OP chemical will also be active in the MEA NFA.**

For some OPs, DNT-NAM AC_{50} < bioactivity estimate from the rest of ToxCast.

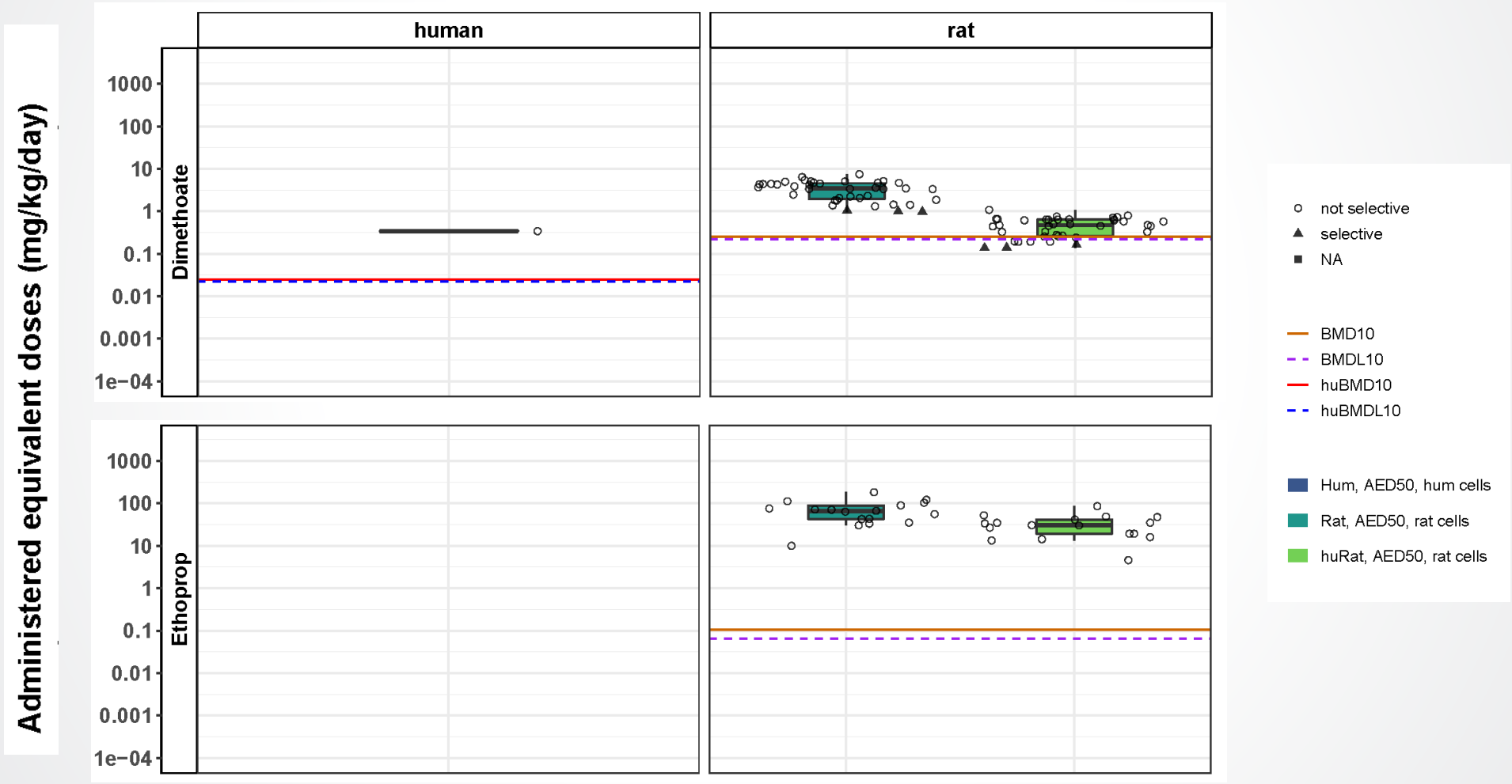
DNT-NAM battery may provide a more potent estimate of bioactivity for substances with minimum DNT-NAM AC50 < 5th percentile of filtered ToxCast AC50 values:

- Chlorpyrifos and chlorpyrifos oxon
- Acephate
- Dichlorvos
- Terbufos
- Diazoxon
- Methamidophos

Suggests that the DNT-NAM battery, in covering some new biology not previously in ToxCast, may yield bioactivity threshold concentrations lower than what is already available for some neuroactive substances in ToxCast.



AED50 to BMD/BMDL10 comparisons



Summary of the AED50 to BMD/BMDL comparison

	Chemicals with AED50 values >>> BMD/BMDL comparator	Chemicals with lowest AED50 within 1 log10 order of magnitude of BMD/BMDL comparator	Chemicals with lowest AED50 approaching BMD/BMDL comparator	Missing in vitro data for comparison
Rat/HuRat	Coumaphos, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate	acephate, bensulide, chlorpyrifos, chlorpyrifos oxon, diazinon, dimethoate, malathion, methamidophos, and phorate	dimethoate and methamidophos (lower quartile of huRat AED_{50} values dichlorvos (huRat AED_{50}; only one positive rat assay endpoint) overlaps with the BMDL10 value, and it was not based on selective bioactivity in the DNT-NAM battery. malathion (huRat AED_{50} (selective) for also approach the BMD/BMDL10 values.	Malaoxon (negative in all assays)
Human	bensulide, chlorpyrifos, chlorpyrifos oxon, coumaphos, diazinon, dimethoate, malathion, methamidophos, phosmet, pirimiphos-methyl, tribufos, and trichlorfon		dichlorvos, only two AED_{50} values are available for comparison, and these values are centered around the BMD10/10 and BMDL10/10 values. terbufos, only 3 human AED_{50} values are available for comparison, and the lowest one of these values approaches the BMD10/10 value.	**Negative in all assays with human cells:** Acephate, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate, phorate, profenofos, and tebupirimfos Malaoxon was negative in all assays.

AEDs from DNT NAMS are more sensitive than LOAELs for other compounds

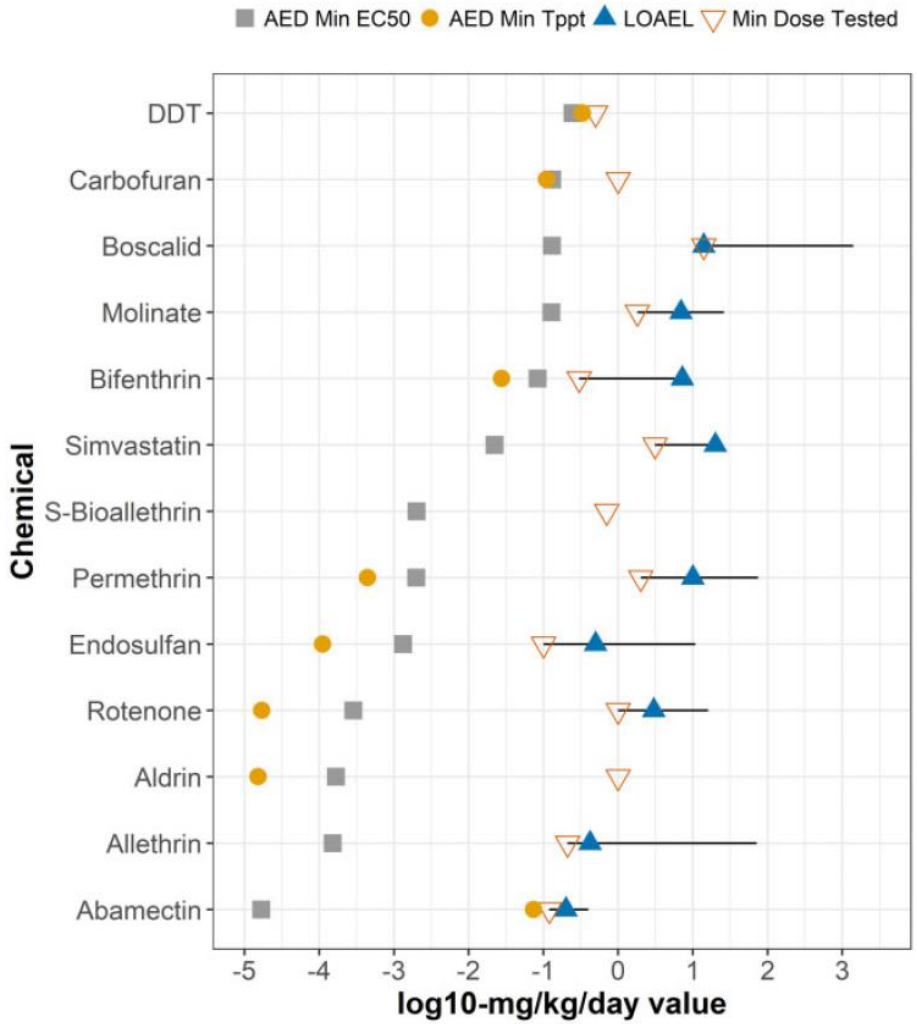
OXFORD
SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 169(2), 2019, 436–455

doi: 10.1093/toxsci/kfz052
Advance Access Publication Date: February 28, 2019
Research Article

Evaluation of Chemical Effects on Network Formation in Cortical Neurons Grown on Microelectrode Arrays

Timothy J. Shafer,*,1 Jasmine P. Brown,*,2 Brittany Lynch,†
Sylmarie Davila-Montero,‡ Kathleen Wallace,* and Katie Paul Friedman§



Even though AEDs were not more sensitive than BMDLs for OPs, DNT NAMs can still be sensitive indicators of potential disruption of nervous system development

Overall conclusion

The development of a DNT-NAM battery for assessing potential DNT-related effects:

- Provides an opportunity to overcome some of the challenges with the *in vivo* DNT guideline study
- Evaluates critical processes underlying neurodevelopment
- Incorporating human relevant information.
- Represents a significant advancement toward developing a DNT-NAM battery for DNT evaluation.
- Is currently being utilized for a variety of regulatory decision-making processes at EPA

Future Directions

I. Continue to Improve Current Assays
    I. Scale up to higher throughput
    II. Increase # compounds tested
II. Contribute to Development of AOPs (CSS 4.2.4)
III. Incorporate Next Generation Technologies
IV. Incorporate 3D Models

Collaborators

EPA

- Theresa Freudenrich
- Kathleen Wallace
- Jasmine Brown
- Chris Frank
- Stephanie Padilla
- Josh Harrill
- Megan Culbreth
- Bill Mundy (retired)
- Kevin Crofton (retired)
- Katie Paul-Friedman
- Richard Judson
- Anna Lowit (OPP)
- Monique Perron (OPP)
- Liz Mendez (OPP)
- Sarah Dobreniecki (OPP)

University of Konstanz

- Marcel Leist
- Johanna Nyffeler

Düsseldorf

- Ellen Fritsche

Support:

- EPA CSS Research Program
- EPA Pathway Innovation Projects

United States Environmental Protection Agency

ExpoCast
exposure forecasting

Implementing a Workflow for Exposure Screening of Drinking Water Contaminants of Concern

Kristin Isaacs

US EPA CSS-HERA Board of Scientific Counselors
Chemical Safety Subcommittee Meeting

February 2-5, 2021

Background

- The US Environmental Protection Agency's Center for Computational Toxicology and Exposure (CCTE) and the Minnesota Department of Health (MDH) are collaborating to use new chemical data generated from scientific approaches such as read-across, QSAR, high-throughput toxicology screening, and computational modeling of exposure and toxicokinetics to prioritize chemicals for further evaluation and inform risk assessment
- CCTE and MDH finalized a formal Cooperative Research and Development Agreement (CRADA) in 2019
  - CRADA has a goal of addressing up to five MDH chemical evaluation activities

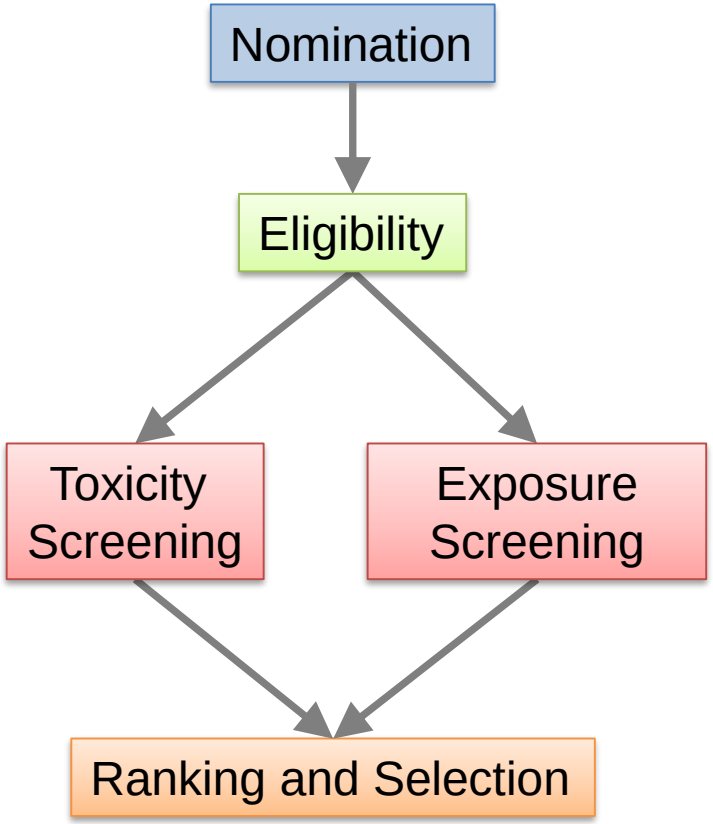
Problem: MDH CEC Initiative

- Through its **Contaminants of Emerging Concern** (CEC) initiative, the Minnesota Department of Health (MDH) collaborates with partners and the public to identify contaminants of interest in drinking water

- Substances that **have been released to, found in, or have the potential to enter Minnesota waters**, and:
  - Real or perceived health threat,
  - No current Minnesota human health-based guidance
  - New information that increases the level of concern

Problem: MDH CEC Initiative

MDH CEC Process



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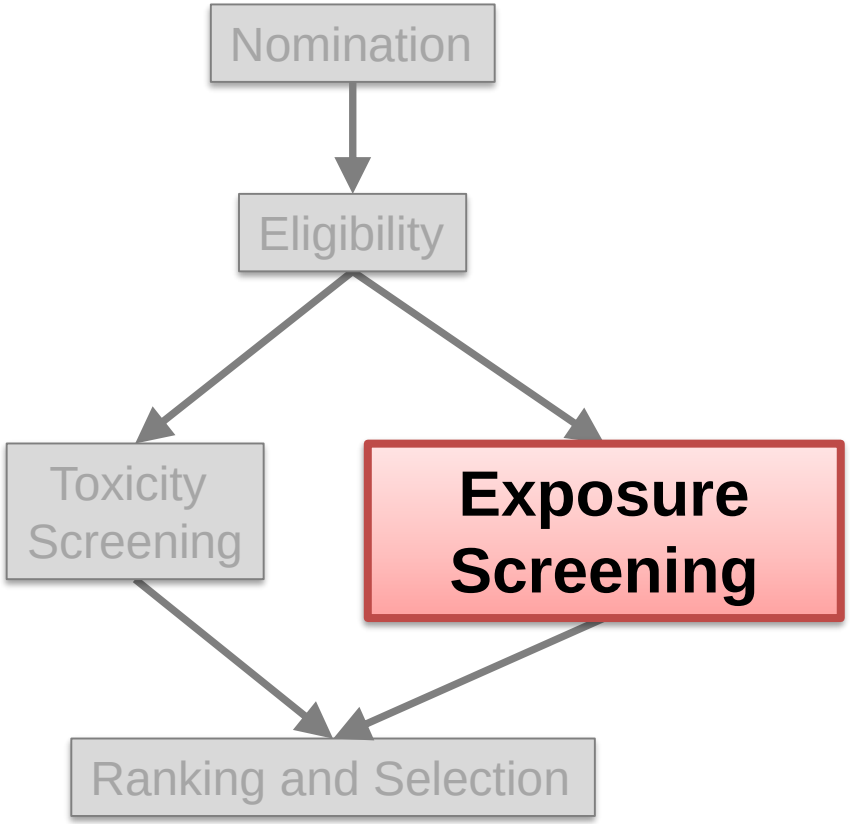
  - Real or perceived health threat,
  - No current Minnesota human health-based guidance
  - New information that increases the level of concern

- Substances selected via a nomination process, followed by:
  - **Screening-level evaluation and ranking of nominated chemicals based on exposure and toxicity potential**
  - Screening informs selection of contaminants for an in-depth toxicological review and guidance development

Problem: CEC Exposure Screening

MDH CEC Process

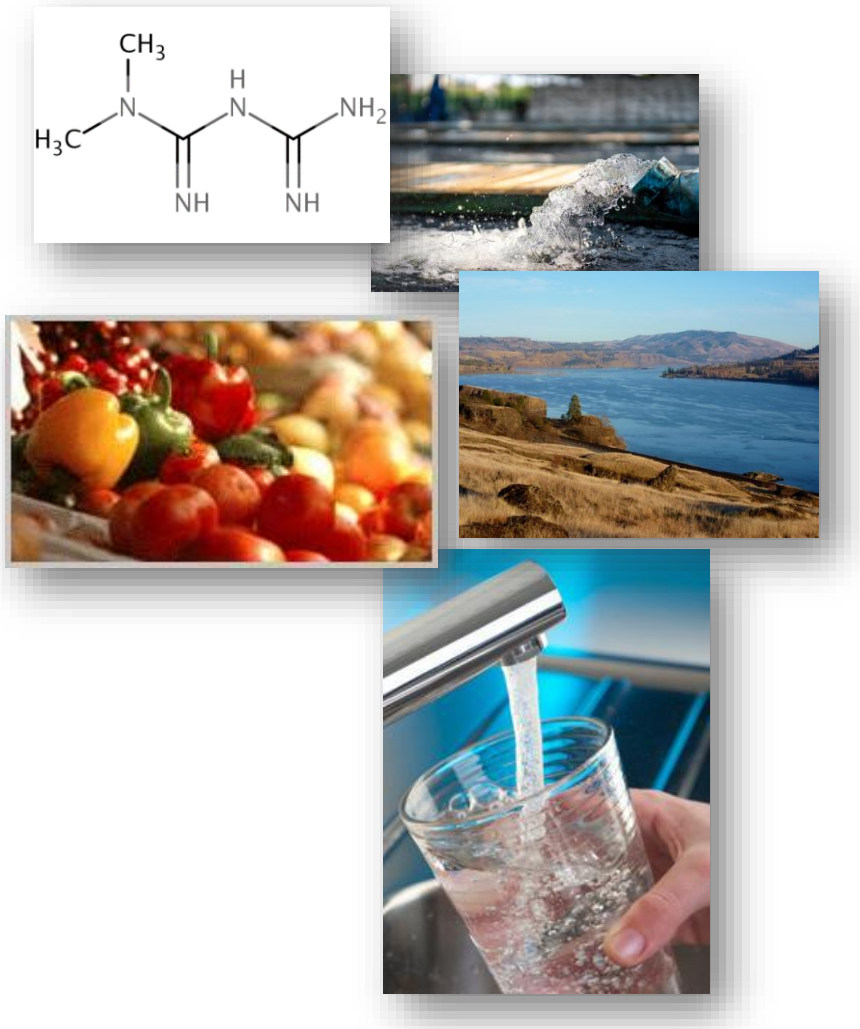


- **Exposure screening** was identified by MDH as a high-priority workflow for implementation under the CRADA
- Past approach: **manual** exposure screening by MDH staff
  - Data identification is time-consuming process (multiple days to a week for 1 chemical)
    - Disparate data sources
    - Synthesis can be challenging
  - Scoring is also manual: tedious/unreproducible
  - Many chemicals are **data-poor** based on traditional approaches (for example, existing regulatory exposure assessments, traditional monitoring data)

Approach

- Establish collaboration between MDH and CCTE accelerate the **exposure screening** process
- Develop a **proof-of-concept** automated workflow for scoring chemicals and reporting results according to MDH screening criteria
- Incorporate New Approach Methodologies (NAMs) for exposure from ORD's Exposure Forecasting (ExpoCast) project
- Apply workflow to two chemical lists
  - 87 chemicals previously manually evaluated by MDH (for assessment of workflow performance)
  - 171 proof-of-concept chemicals of interest to MDH and EPA

CEC Exposure Screening Criteria

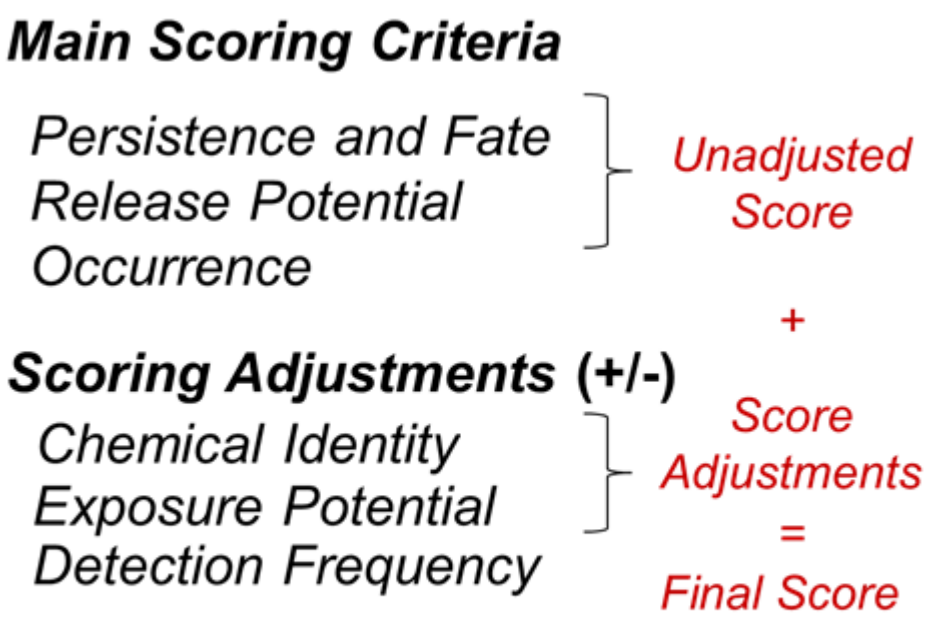


- Uses components of the US EPA's Office Water Candidate Contaminant List (CCL) methodology and incorporates the recommendations from MDH Stakeholder Task Group

- Considers data and criteria associated with multiple domains, including
  - Chemical identity and use
  - Chemical properties
  - Chemical emissions and disposal
  - Chemical occurrence in environment, drinking water, and food
  - Human exposure potential

- Incorporates MN information where possible

CEC Exposure Screening Criteria



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  - Chemical identity and use
  - Chemical properties
  - Chemical emissions and disposal
  - Chemical occurrence in environment, drinking water, and food
  - Human exposure potential
- Incorporates MN information where possible
- Evaluates and scores chemicals using algorithm developed by MDH (primary unadjusted score + score adjustments= final score)

Eight Classes of NAMs for Exposure from the ExpoCast Project

ELSEVIER

Current Opinion in Toxicology

Available online 31 July 2019

In Press, Journal Pre-proof

New Approach Methodologies for Exposure Science

John F. Wambaugh [1], Jane C. Bare [2], Courtney C. Carignan [3], Kathie L. Dionisio [4], Robin E. Dodson [5, 6], Olivier Jolliet [7], Xiaoyu Liu [8], David E. Meyer [2], Seth R. Newton [4], Katherine A. Phillips [4], Paul S. Price [4], Caroline L. Ring [9], Hyeong-Moo Shin [10], Jon R. Sobus [4], Tamara Tal [11], Elin M. Ulrich [4], Daniel A. Vallero [4], Barbara A. Wetmore [4], Kristin K. Isaacs [4]

- ***Chemical descriptors*** that provide information on chemicals in an exposure context (e.g., how chemicals are used)
- ***Machine-learning approaches*** that use these descriptors to fill gaps in existing data
- ***High-throughput exposure models*** that address various pathways
- ***High-throughput measurements*** that fill gaps in monitoring data
- High-throughput approaches that measure or predict chemical ***toxicokinetics***
- New ***evaluation frameworks*** that integrate models and monitoring to provide consensus exposure predictions

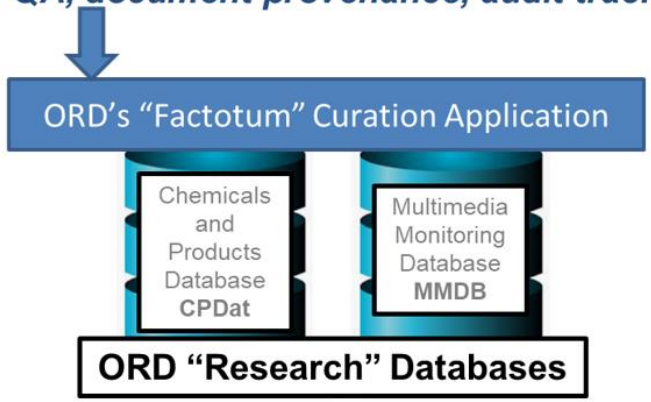
All these pieces together provide can accelerate high-throughput risk-based chemical prioritization

Workflow Design and Implementation

Data Curation

MN-specific documents and other source documents extracted and curated into ORD's research databases via the Factotum curation application.

QA, document provenance, audit tracking



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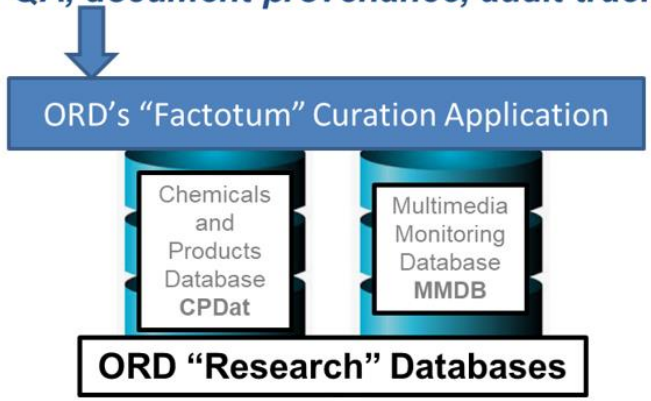

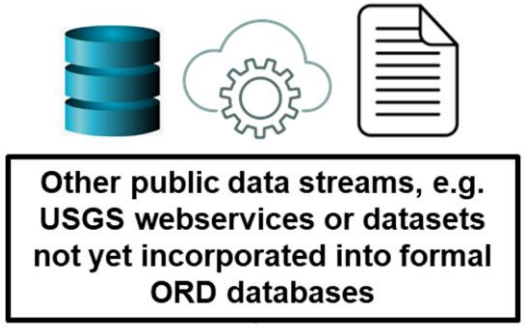

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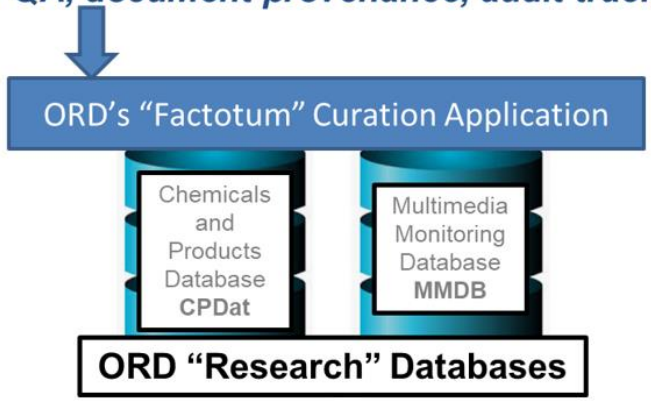

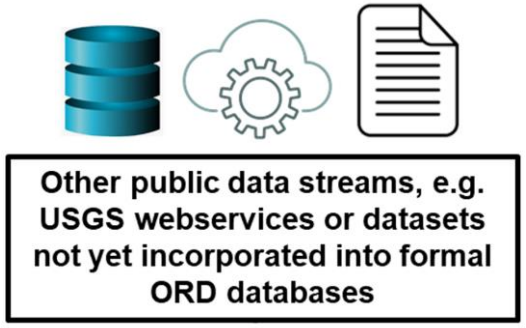

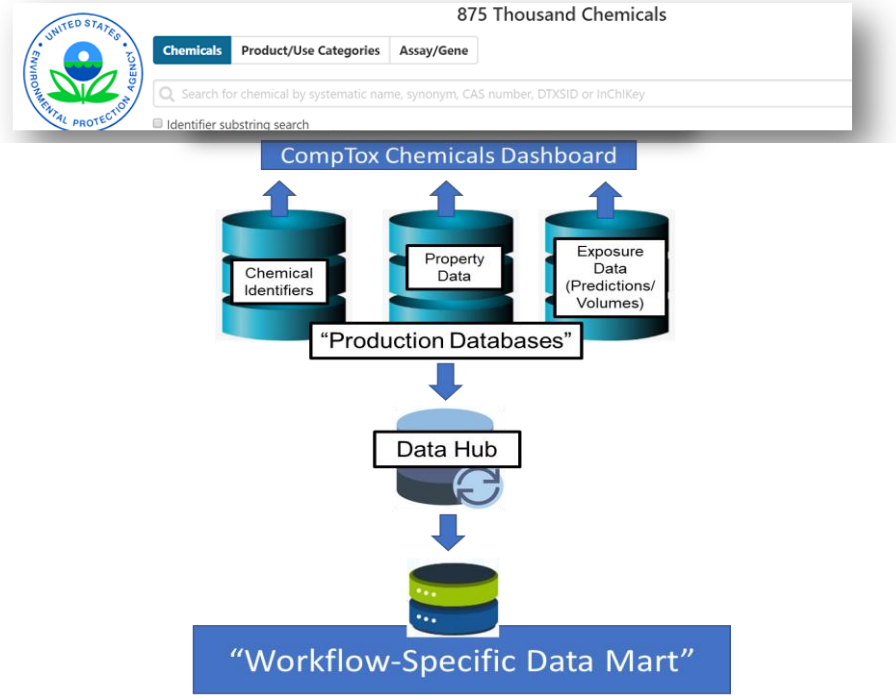

Workflow Design and Implementation

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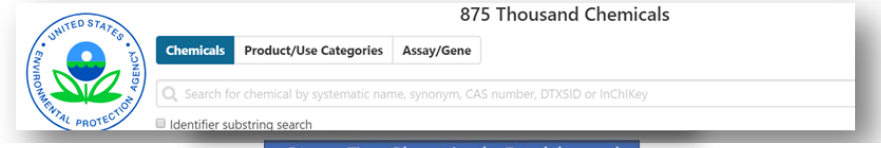
ORD's "Factotum" Curation Application

Chemicals and Products Database **CPDat**

Multimedia Monitoring Database **MMDB**

ORD "Research" Databases

Other public data streams, e.g. USGS webservices or datasets not yet incorporated into formal ORD databases



CompTox Chemicals Dashboard

Chemical Identifiers

Property Data

Exposure Data (Predictions/ Volumes)

"Production Databases"

Data Hub

"Workflow-Specific Data Mart"

- Data retrieval and caching

Workflow Design and Implementation

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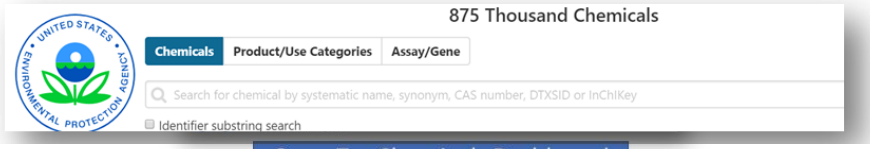

CompTox Chemicals Dashboard

Chemical Identifiers

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Exposure Data (Predictions/ Volumes)

"Production Databases"

Data Hub

"Workflow-Specific Data Mart"

- Data retrieval and caching
- Chemical scoring
- Summary report and data table generation

Main Scoring Criteria

Persistence and Fate
Release Potential
Occurrence
→ *Unadjusted Score*

\+

Scoring Adjustments **(+/-)**

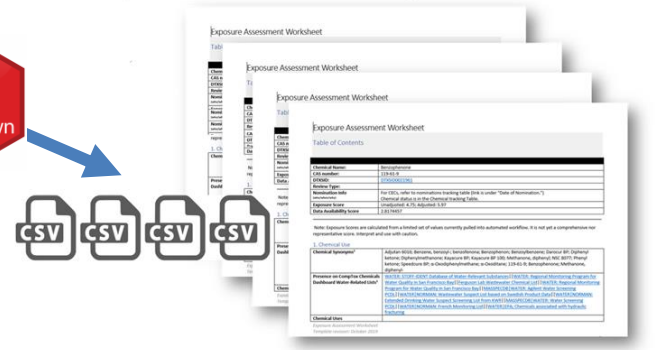
*Chemical Identity**
Exposure Potential
Detection Frequency
→ *Score Adjustments*

=

Final Score

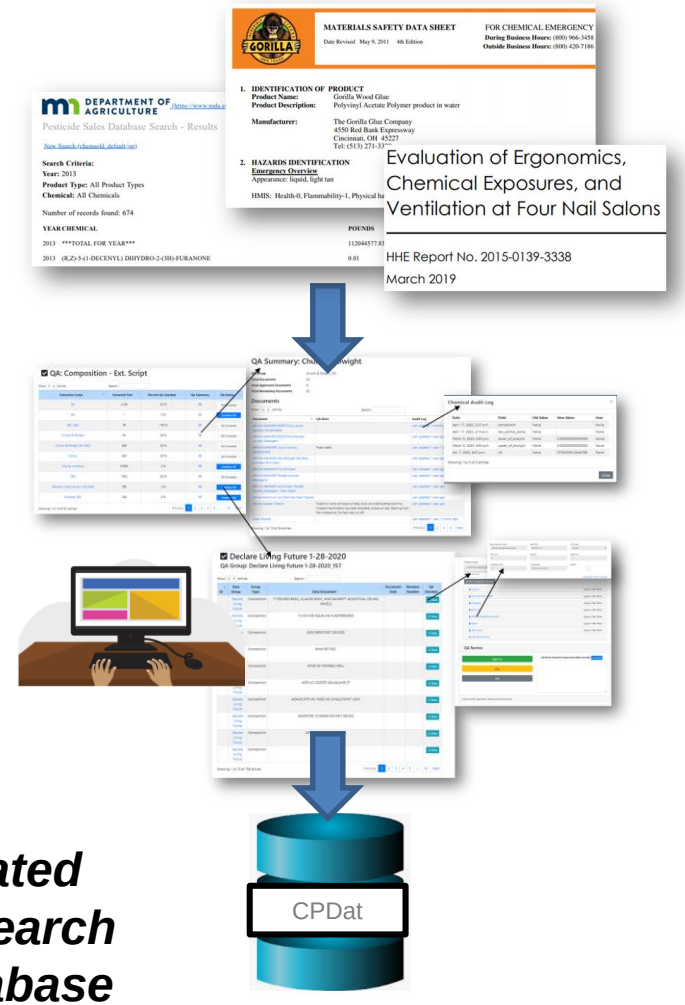
rmarkdown

Automated Reporting and Data Generation for In-Depth Assessment

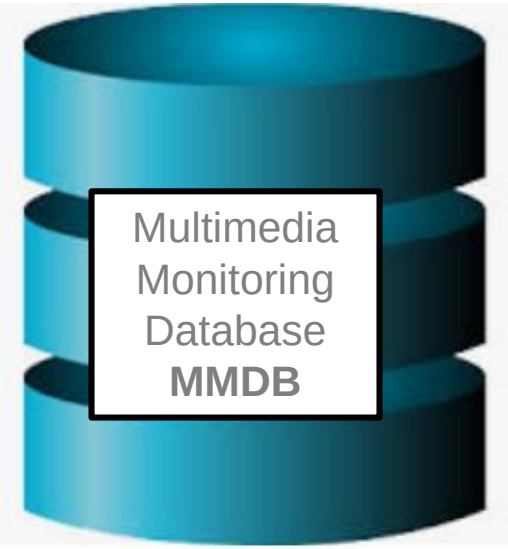


Curation of Chemical Use Descriptors with Factotum

- We are using informatics approaches to obtain and curate chemical use descriptor information
- Public data sources: reports, consumer product ingredient data, etc.
- Utilizing standard curation/QA procedures and tools
- Currently supports EPA's Chemical and Products Database
- Integrates with ORD's chemical curation workflows
- Allowed us to curate many MN-specific documents for use in the workflow



Multimedia Monitoring Database (MMDB)



- ORD research database of measurements from over 20 public data sources
  - Includes data from several EPA programs, California state monitoring programs, the FDA, the Comparative Toxicogenomics Database, the EU's Information Platform for Chemical Monitoring Data (IPCHEM), the National Health and Nutrition Examination Survey (NHANES), the USDA, the International Council for the Exploration of the Sea (ICES), and the International Council of Chemical Associations' Long-Range Research Initiative (ICCA-LRI)
  - Harmonized to **chemical identifier and media** (e.g., drinking water, surface water, human blood or urine, soil, food, and ecological species).
- Developed in collaboration with OPPT
- Contains over **250 million** individual data records covering over **3200** unique chemicals
- Basis for future QSAR-like models for occurrence in different media
- Manuscript for submittal for peer-reviewed publication in internal EPA clearance

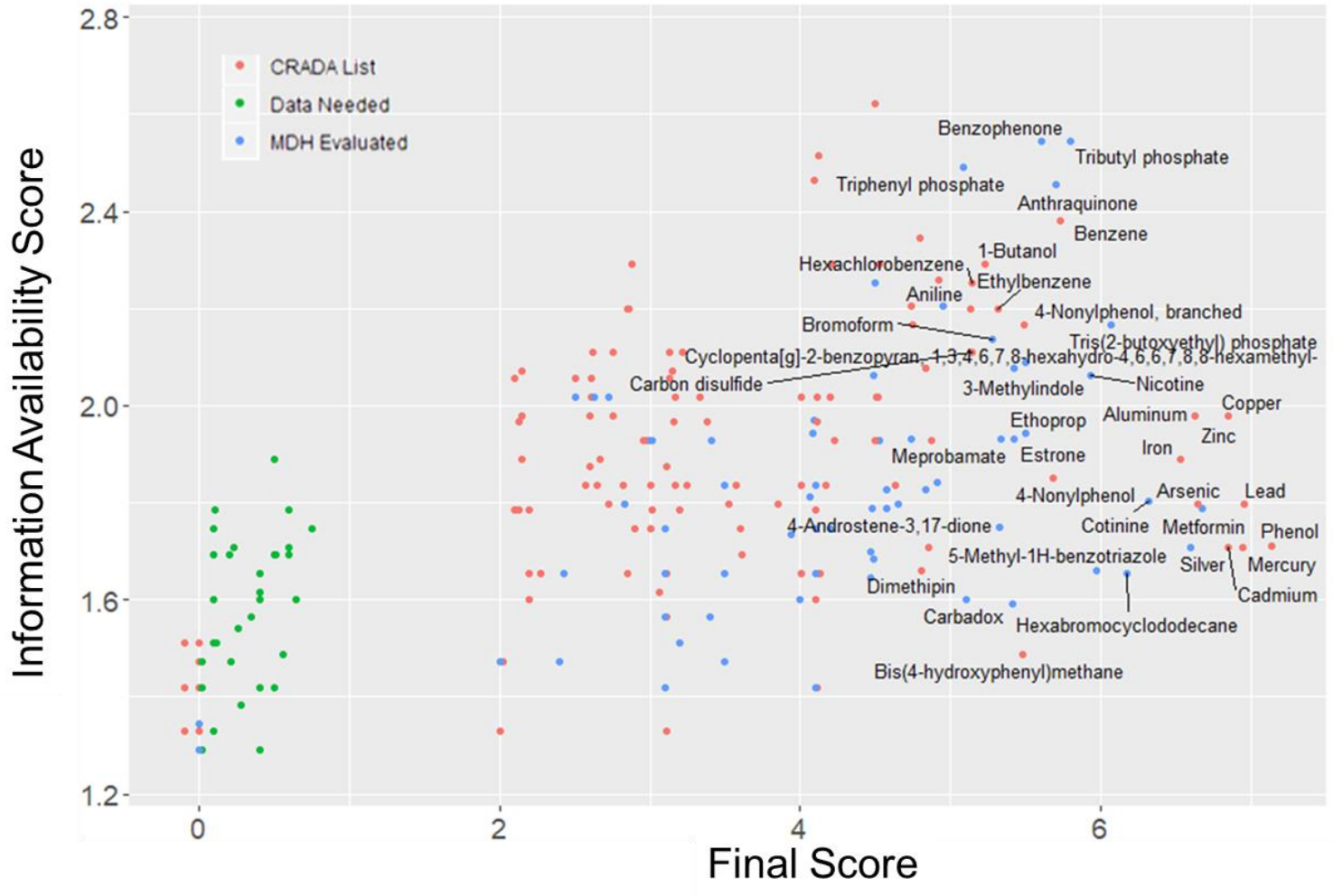
Data Source Summary

* Incorporate Exposure NAM data

Chemical Identity and Use	
Chemical Identifiers and Synonyms	EPA-ORD's CompTox Chemicals Dashboard/Underlying Databases
Uses	EPA-ORD's Chemicals and Products Database[1] (CPDat)
Uses	EPA's Chemical Data Reporting (CDR) Consumer, Commercial, Industrial uses
National Production Volume	EPA-ORD's CompTox Chemicals Dashboard (Underlying data)
Uses	EPA Safer Chemical Ingredients List
Chemical Properties	
Measured Properties	EPA-ORD's CompTox Chemicals Dashboard/Underlying Databases
Predicted Properties	EPA-ORD's CompTox Chemicals Dashboard (OPERA QSAR Models[4])
Predicted Wastewater Treatment Removal	EPA's Estimation Program Interface Suite (EPI-Suite)
Transformation Products	EPA-ORD's CompTox Chemicals Dashboard/Underlying Databases
Chemical Emissions and Disposal	
Pesticide Releases	National Agricultural Statistic Service
Chemical Releases	EPA's Toxics Release Inventory
Down-the-Drain Releases	EPA's SHEDS-HT model
Chemical Occurrence in Environment, Drinking Water, and Food	
Occurrence in Environmental Media, Including Drinking and Surface Water	EPA-ORD Multimedia Monitoring Database (MMDB)
Occurrence in US Water	US Geological Survey (USGS) Water Quality Portal data, via its application programming interface (API)
Occurrence in MN Water	Custom Database developed by USGS for MDH
Occurrence in MN Water	MN-specific reports, curated into EPA's chemical databases
Occurrence in Food	US Department of Agriculture (USDA) Pesticide Data Program
Occurrence in Food	US Food and Drug Administration (FDA) Substances Added to Food Database
Occurrence in Food	US Food and Drug Administration (FDA) Indirect Food Additives Database
Human Exposure	
Intake Exposures Inferred from Biomonitoring Data	EPA-ORD's CompTox Chemicals Dashboard/Underlying Databases
Biomonitoring Data	EPA-ORD Multimedia Modeling Database (MMDB)
Consumer Exposure Predictions	EPA-ORD's SHEDS-HT Model
General Population Exposures	EPA-ORD's Systematic Empirical Evaluation of Models (SEEM) Consensus Predictions
Presence on Biomonitoring Lists	Biomonitoring California

Results

- The automated workflow was applied to the 258 chemicals (87 evaluated by MDH previously, 171 on the current proof-of-concept list)
- Also defined an “Information Availability Score”
- All data collection, scoring, and report/table writing were completed in approximately 18 hours



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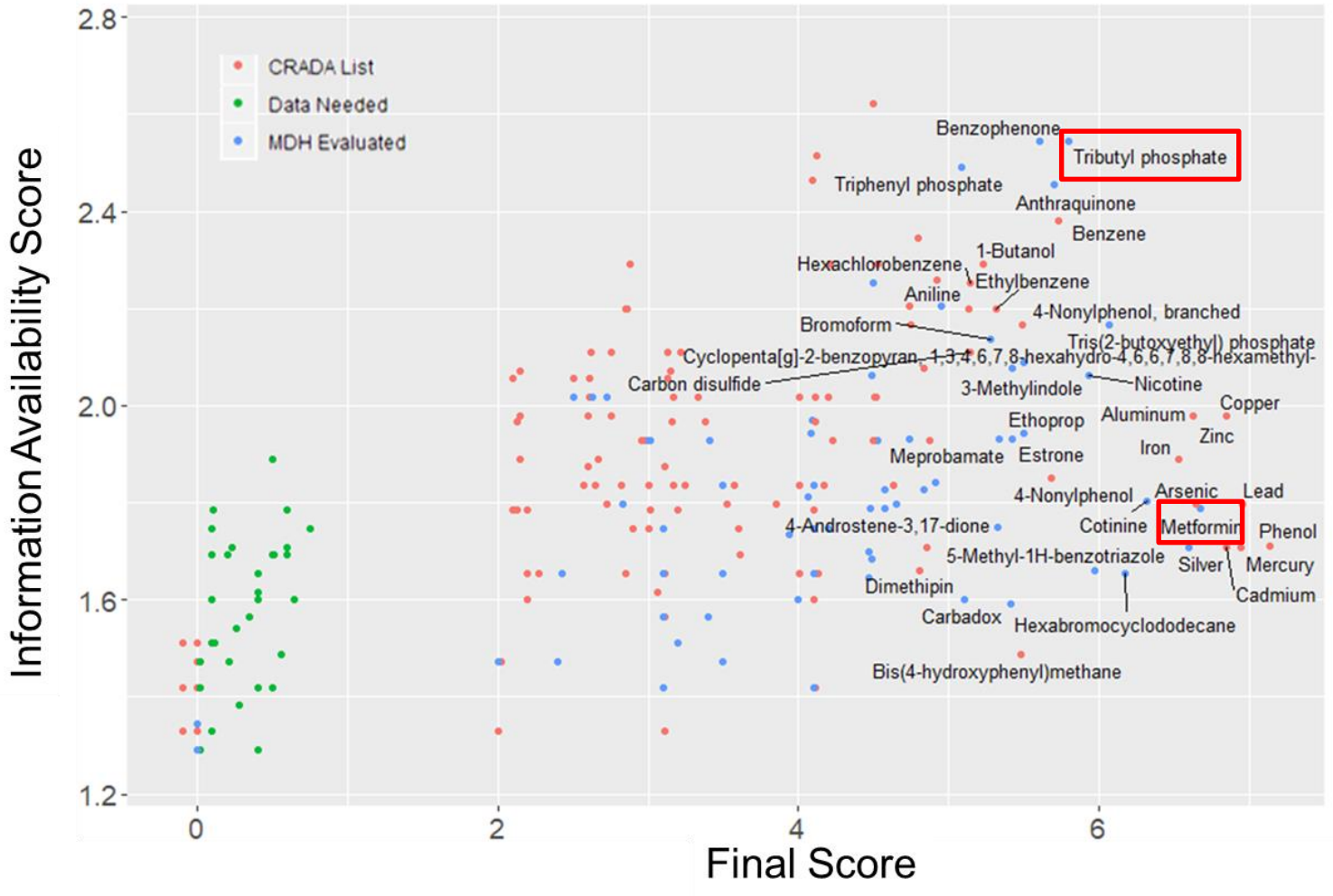

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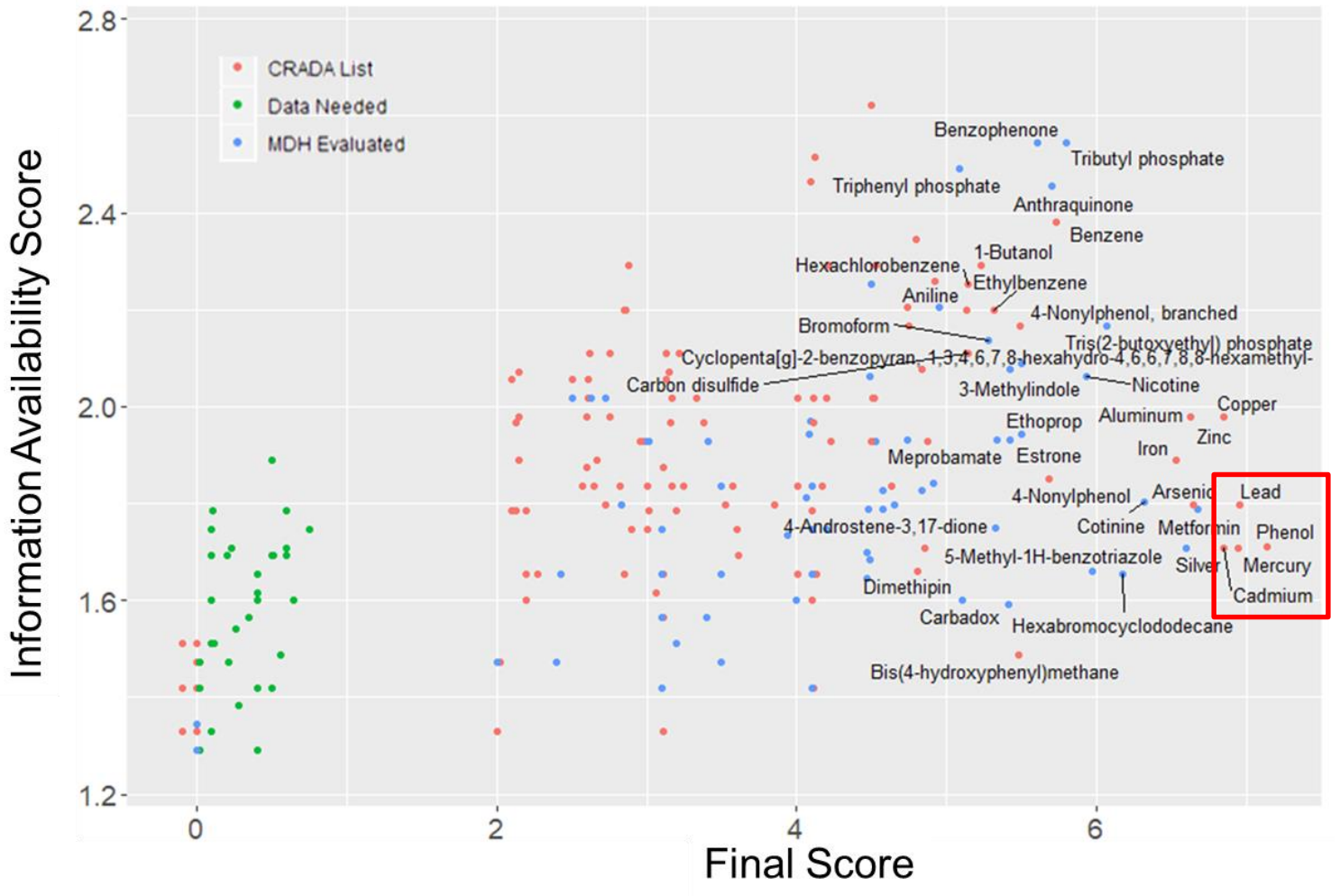

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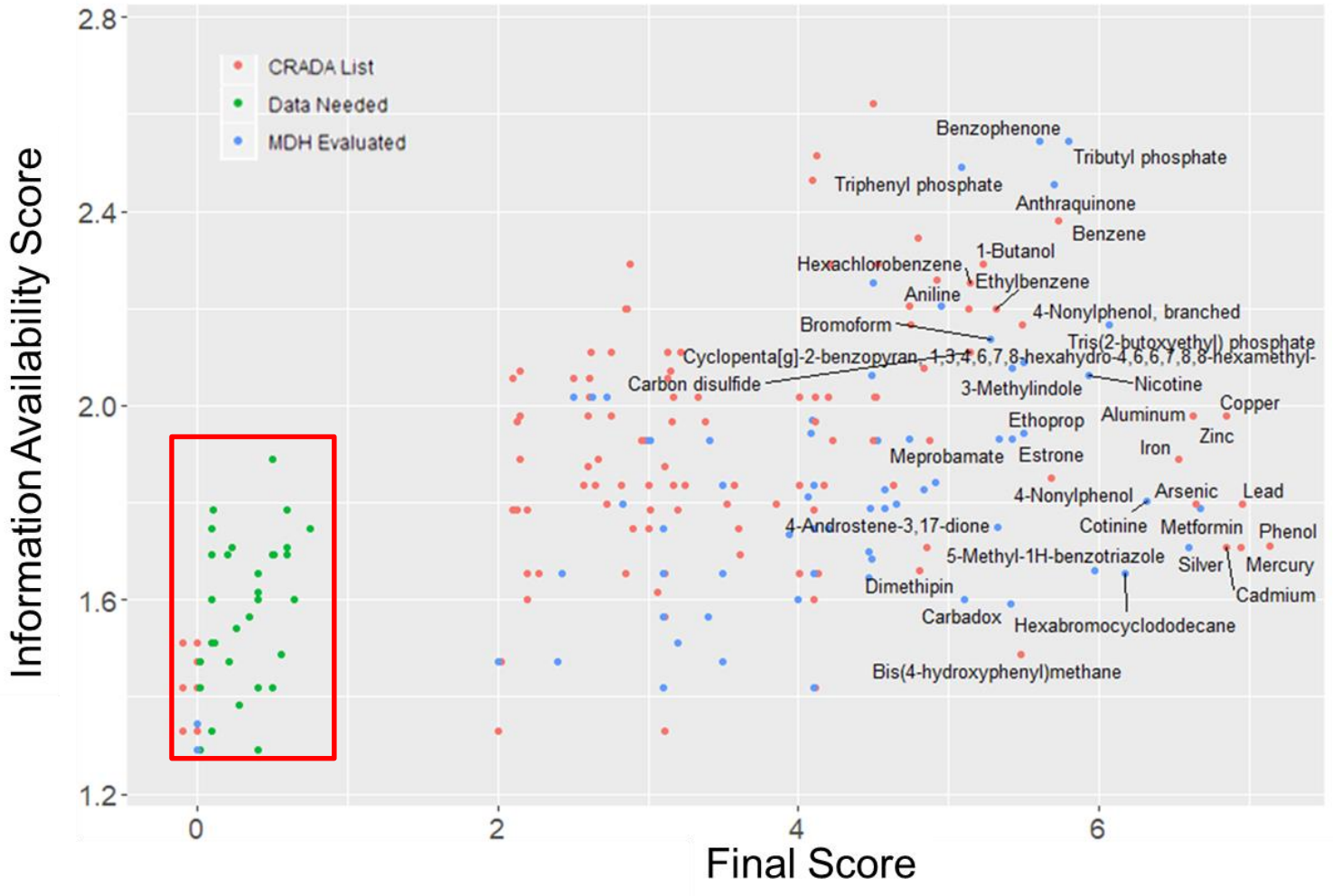
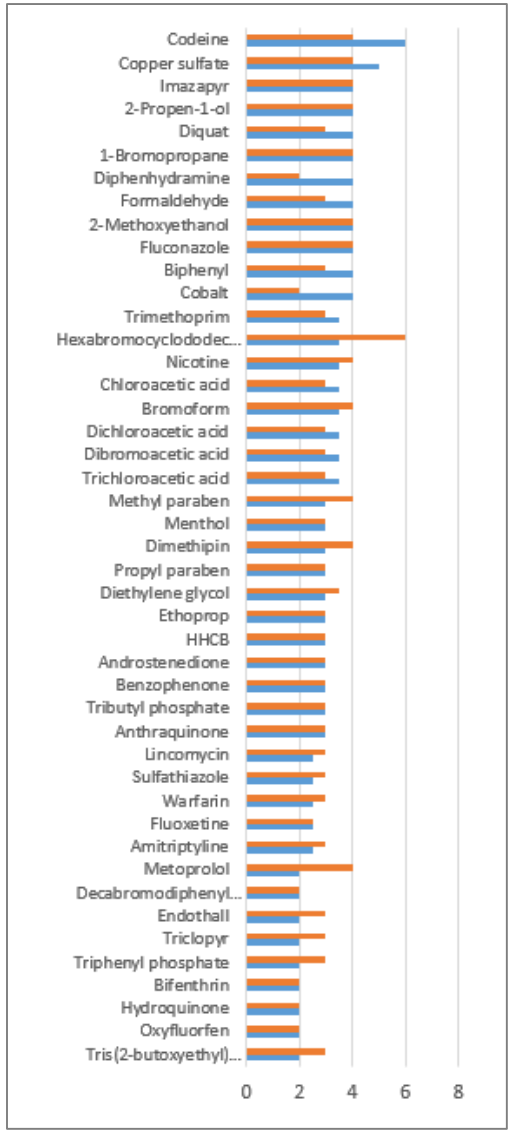
  - Identified several other chemicals that have not undergone explicit exposure screening process by MDH but have been identified as priority to evaluate via assessments outside the CEC initiative
- There were 82 chemicals that did not have enough data for main unadjusted scores to be calculated
  - 36 had positive exposure scoring adjustment (might be priority for additional data collection/curation)



Initial Evaluation of Automated Workflow and Manual Results

- Excellent agreement between scores in **Persistence and Fate** and **Occurrence** domains



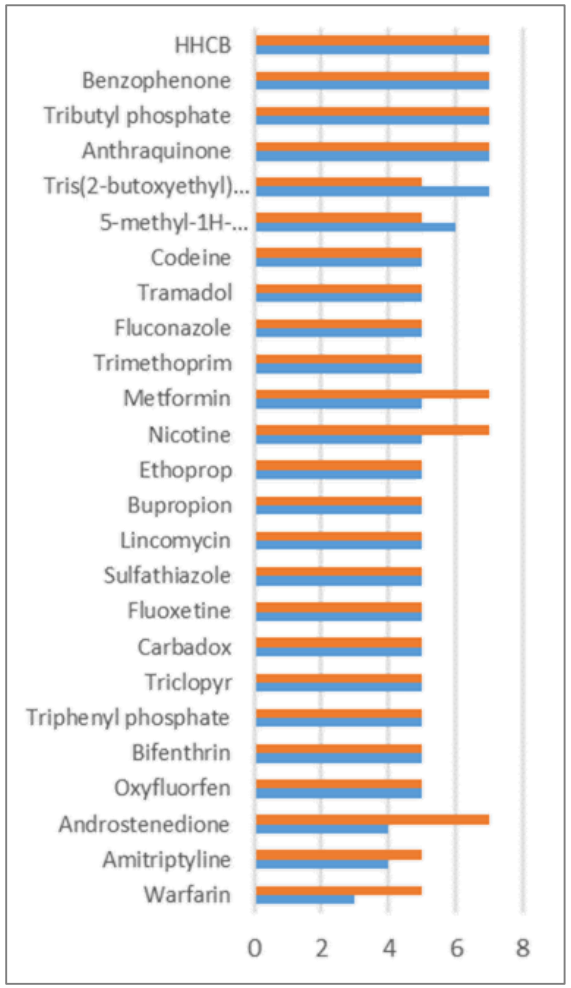
Persistence and Fate

Manual Score

Workflow Score

Initial Evaluation of Automated Workflow and Manual Results

- Excellent agreement between scores in **Persistence and Fate** and **Occurrence** domains



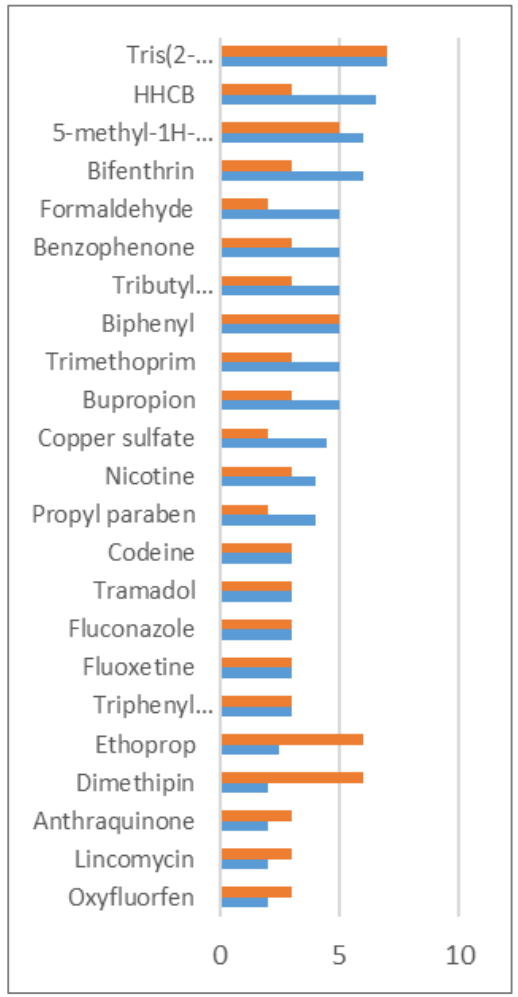
Occurrence

Manual Score

Workflow Score

Initial Evaluation of Automated Workflow and Manual Results

- Excellent agreement between scores in **Persistence and Fate** and **Occurrence** domains
- Somewhat poorer alignment in the **Release Potential** domain



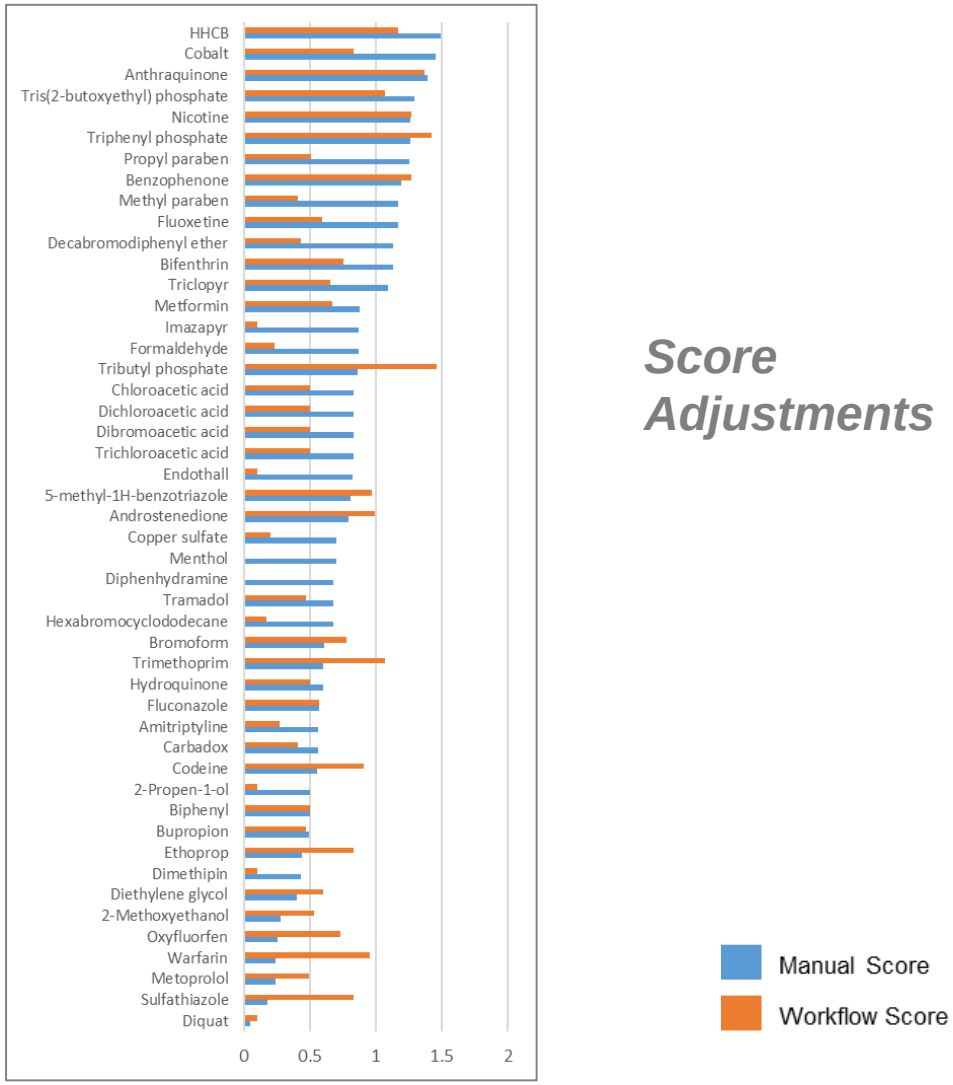
Release Potential

Manual Score

Workflow Score

Initial Evaluation of Automated Workflow and Manual Results

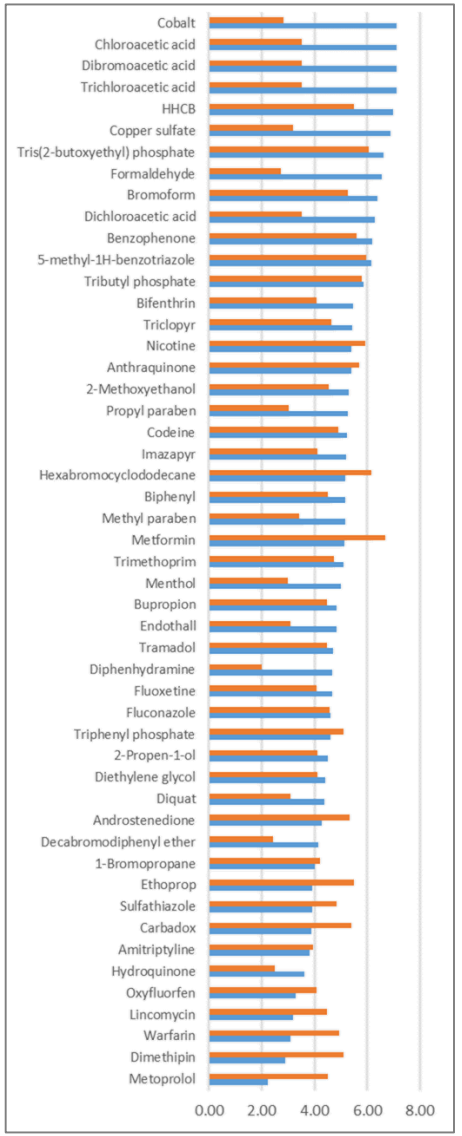
- Excellent agreement between scores in **Persistence and Fate** and **Occurrence** domains
- Somewhat poorer alignment in the **Release Potential** domain
- Poor agreement in score adjustments (i.e., detection frequency, human exposure potential)
  - Difference in estimates of detection frequencies in MMDB and MN sources
  - New exposure information from ExpoCast



Score Adjustments

Initial Evaluation of Automated Workflow and Manual Results

- Excellent agreement between scores in **Persistence and Fate** and **Occurrence** domains
- Somewhat poorer alignment in the **Release Potential** domain
- Poor agreement in score adjustments (i.e., detection frequency, human exposure potential)
  - Difference in estimates of detection frequencies in MMDB and MN sources
  - New exposure information from ExpoCast
- Reflected in final scores



Final Score

Manual Score

Workflow Score

Next Steps

- Continue evaluations
  - Closer look at differences across the data domains
  - Are there priority data sources to be added?
- Incorporation of additional data streams into workflow
  - Integration into workflow of MN-specific water measurement database
  - Additional exposure NAMs, including machine-learning models for media occurrence built using the MMDB monitoring descriptors
- ORD toxicologists are working with MN to gather hazard data (including data from NAMs) for data-poor nominated CECs and those identified as having high exposure potential

Impact

- This workflow allows MDH health scientists to accelerate and expand exposure screening evaluations, freeing resources to complete the more complex aspects of exposure assessment
- Large libraries of chemicals relevant to MDH can be rapidly screened for *a priori* identification of new potential nominees (something that has never been feasible)
- The implemented workflow has formed a basis for exposure screening under another MDH regulatory program, the **Toxic Free Kids** initiative (implementation now underway, MDH concurrently developing screening algorithm in collaboration with ORD)
- ORD has had initial conversation with Office of Water to discuss potential use of a similar automated workflow approach for future CCL phases

CRADA Team
(Exposure Forecasting)

EPA-ORD

Kathie Dionisio
Jill Franzosa
Kristin Isaacs
Jason Lambert
Monica Linnenbrink
Katie Paul-Friedman
Amar Singh
Jonathan Taylor Wall
Antony Williams

MDH

Christopher Greene
Helen Goeden
David Bell
Sarah Johnson
James Jacobus

ExpoCast Project (Exposure Forecasting)

CCTE

Linda Adams
Miyuki Breen*
Alex Chao*
Dan Dawson*
Mike Devito
Kathie Dionisio
Christopher Ecklund
Marina Evans
Peter Egeghy
Michael-Rock Goldsmith
Chris Grulke
Mike Hughes
Kristin Isaacs
Richard Judson
Jen Korol-Bexell*
Anna Kreutz*
Charles Lowe*
Seth Newton
Katherine Phillips
Paul Price
Tom Purucker
Ann Richard
Caroline Ring
Marci Smeltz*
Jon Sobus
Risa Sayre*
Mark Sfeir*
Mark Strynar
Zach Stanfield*
Rusty Thomas
Mike Tornero-Velez
Elin Ulrich
Dan Vallero
John Wambaugh
Barbara Wetmore
Antony Williams

CEMM

Xiaoyu Liu

CPHEA

Jane Ellen Simmons

CESER

David Meyer
Gerardo Ruiz-Mercado
Wes Ingwersen

***Trainees**

Collaborators

Arnot Research and Consulting
Jon Arnot
Johnny Westgate
Institut National de l'Environnement et des Risques (INERIS)
Frederic Bois
Integrated Laboratory Systems
Kamel Mansouri
National Toxicology Program
Steve Ferguson
Nisha Sipes
Ramboll
Harvey Clewell
ScitoVation
Chantel Nicolas
Silent Spring Institute
Robin Dodson
Southwest Research Institute
Alice Yau
Kristin Favela
Summit Toxicology
Lesa Aylward
Technical University of Denmark
Peter Fantke
Tox Strategies
Miyoung Yoon
Unilever
Beate Nicol
Cecilie Rendal
Ian Sorrell
United States Air Force
Heather Pangburn
Matt Linakis
University of California, Davis
Deborah Bennett
University of Michigan
Olivier Jolliet
University of Texas, Arlington
Hyeong-Moo Shin

Application of NAMs and AOPs to Surface Water Surveillance and Monitoring in the Great Lakes (EPA Region 5) and a Western River (EPA Region 8)

Daniel L. Villeneuve, US EPA, Office of Research and Development, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division

Problem/Need

- Regions, states, tribes, and communities are monitoring an ever-growing list of contaminants in water and other environmental matrices.
- Established water quality standards / guidelines are lacking for many of the chemicals detected.
  - Uncertainty about whether the chemicals detected are likely to be harmful at the concentrations detected
- Need to focus limited resources available for monitoring, research, and/or source reduction on the substances most likely to cause adverse effects.
- Even with extensive contaminant monitoring, undetected compounds and mixtures leave uncertainty about whether assessments based on individual chemicals are sufficiently protective.

Role for NAMs

- In the absence of traditional animal toxicity data, NAMs can provide a provisional, protective (?), benchmark to support risk-based prioritization

- When traditional animal toxicity data are limited (scope of endpoints or taxa), NAMs can protect against mode of action-based toxicities that may be overlooked in traditional guideline studies or QSARs.

- NAMs can be used to directly test complex mixtures, providing bioactivity data that account for unknowns and cumulative/integrated effects.

EPA Region 5
Great Lakes Restoration Initiative – Emerging Contaminants

Focus Area 1: Toxic Substances and Areas of Concern

Goal 5: The health and integrity of wildlife populations and habitat are protected from adverse chemical and biological effects associated with the presence of toxic substances in the Great Lake Basin.

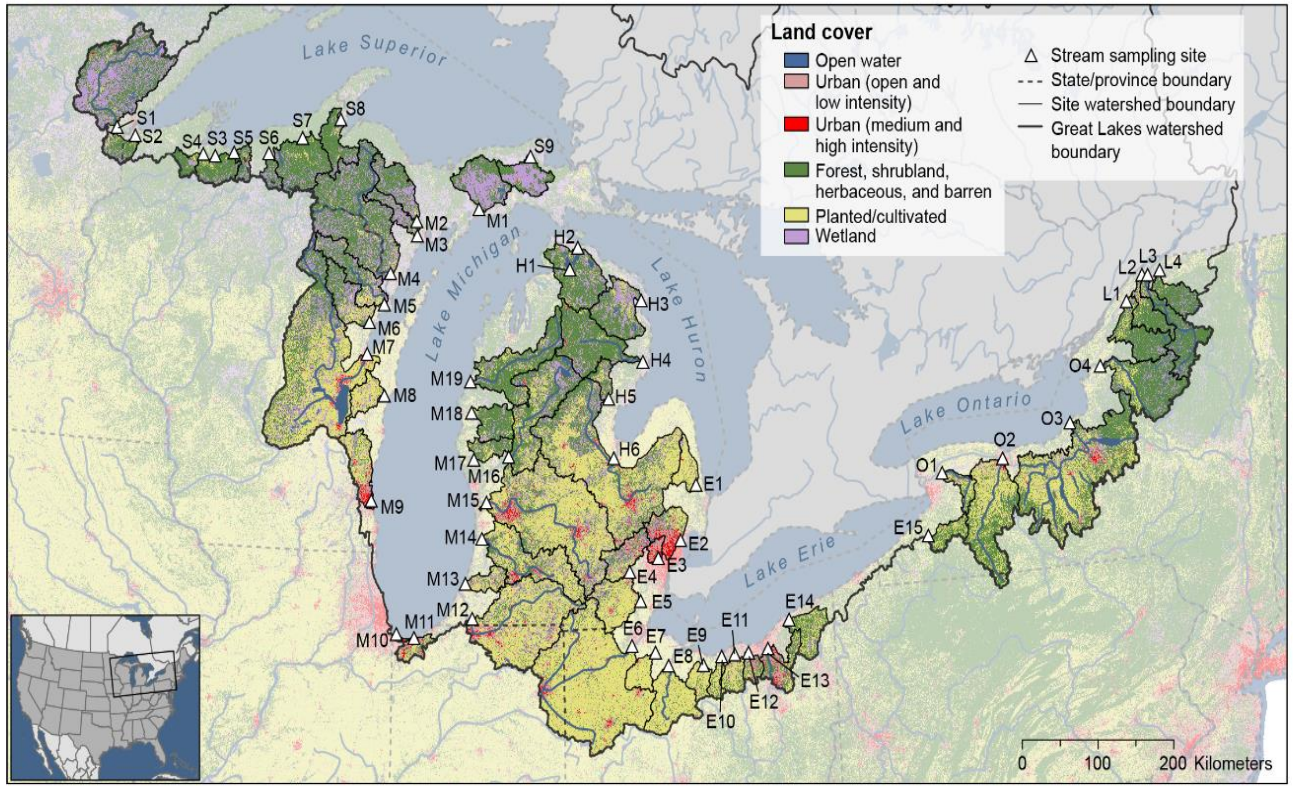
- Identify significant sources and impacts of **new toxics** to the Great Lakes ecosystem ….., in order to devise and implement effective control strategies.

Focus Area 1: Toxic Substances and Areas of Concern

Increase knowledge about contaminants in Great Lakes fish and wildlife

- Identify **emerging contaminants** and assess impacts on Great Lakes fish and wildlife

Chemical monitoring



709 water samples collected 2010-2013

57 Great Lakes tributaries

38 sites sampled 1-2 times

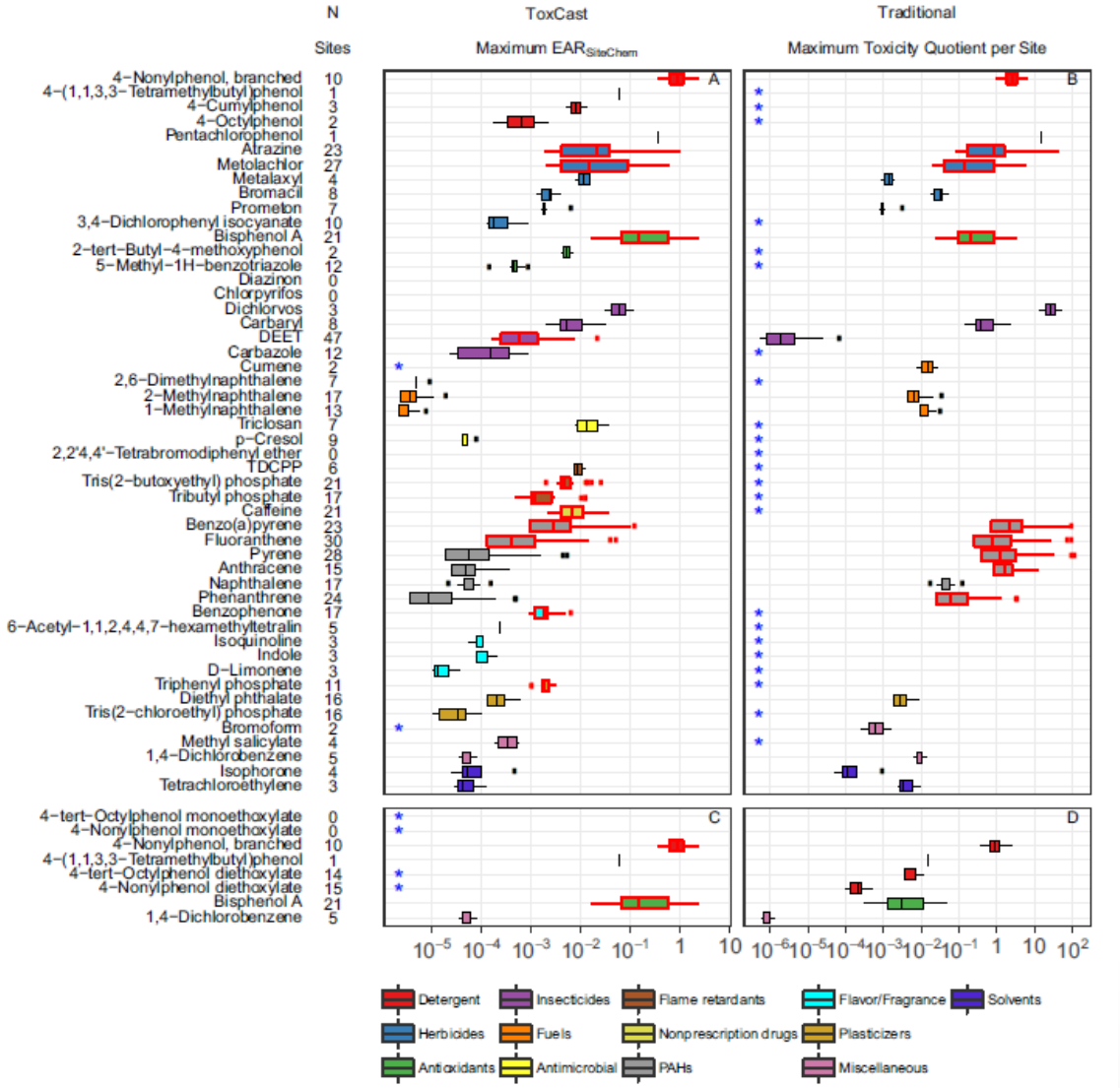
19 sites sampled 7-64 times

Analyzed for 67 organic contaminants

- Water quality benchmarks (27/67 = 40%)
- In vivo toxicity data (34/67 = 51%)
- ToxCast data (54/67 = 81%)

Which chemicals are of concern?
Where are we most likely to see impacts?
What kinds of effects might we expect to see?

Which chemicals?



$$EAR = \frac{Measured\ Concentration\ (\mu M)}{Activity\ Concentration\ at\ Cut{-}off\ (ACC;\ \mu M)}$$

$$TQ = \frac{Measured\ Concentration\ (mg/L)}{In\ vivo\ effect\ concentration\ (\frac{mg}{L})}$$

1. 4 nonylphenol
2. bisphenol A
3. Metolachlor
4. Atrazine
5. DEET
6. Caffeine
7. tris(2 butoxyethyl) phosphate
8. tributyl phosphate
9. triphenyl phosphate
10. benzo(a)pyrene
11. Fluoranthene
12. benzophenone.

Which sites?

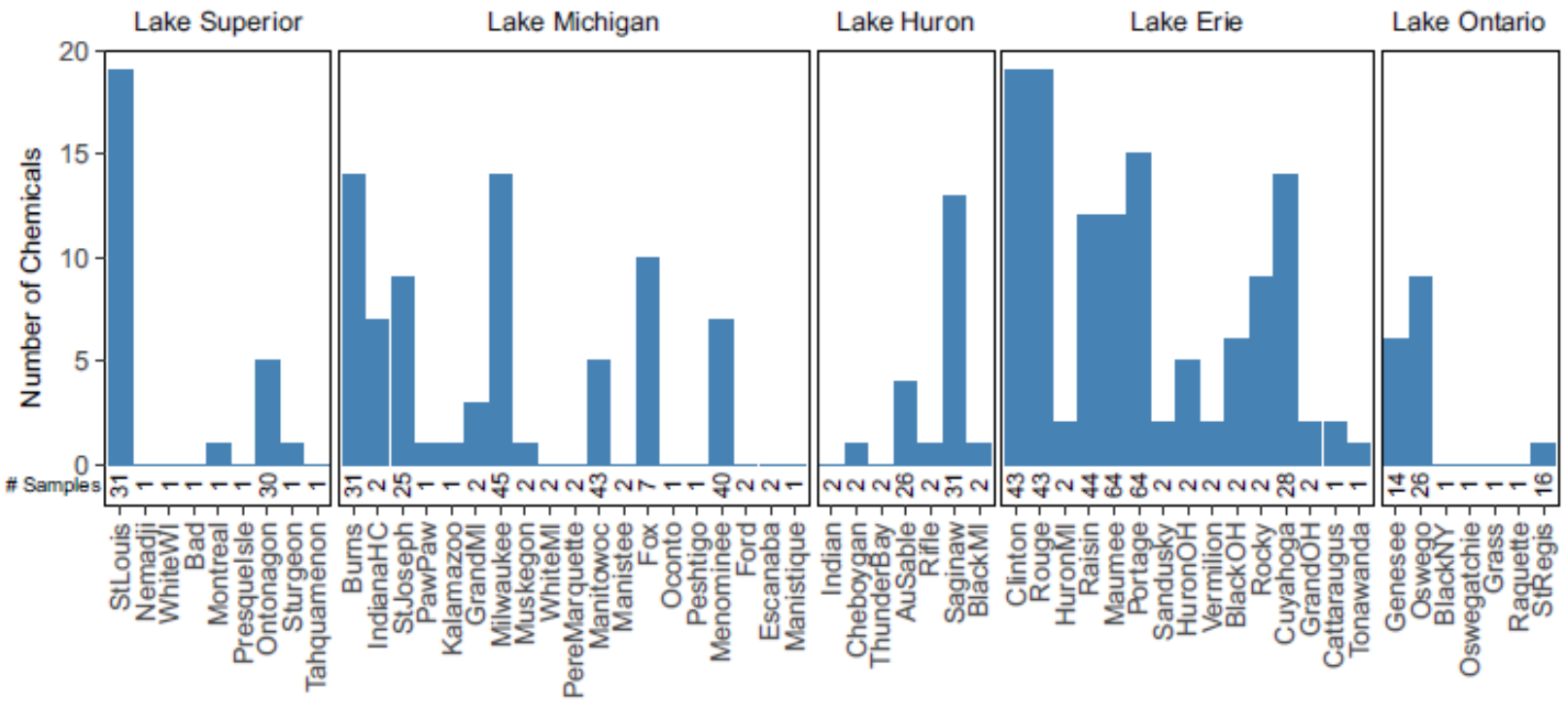
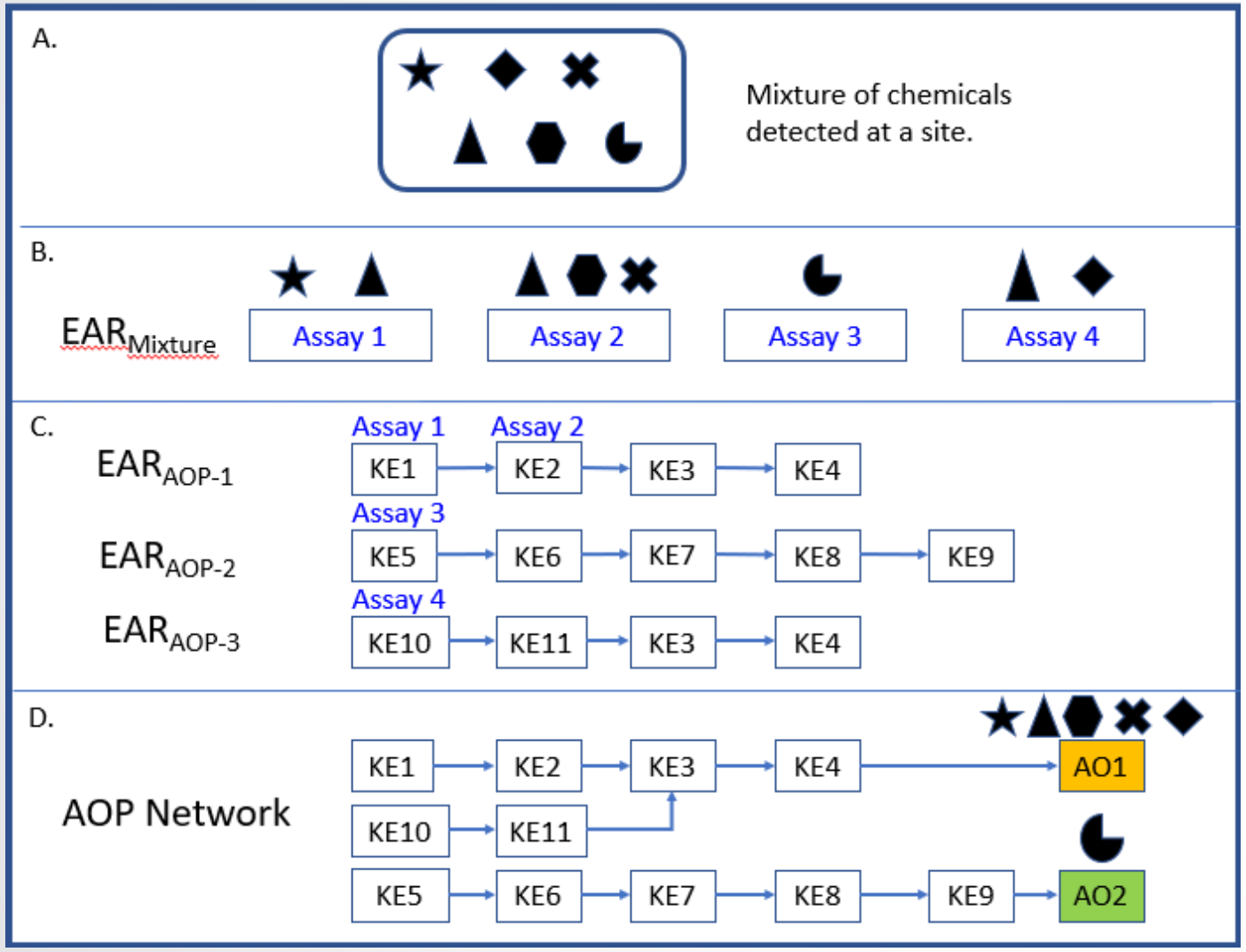


Fig. 3. Number of individual chemicals with at least one sample that resulted in a maximum exposure–activity ratio ($EAR_{SiteChem}$) > 10^{-3} for each site.

Sites link to sources and stakeholders

What effects?



Considers cumulative effects of **detected** chemicals

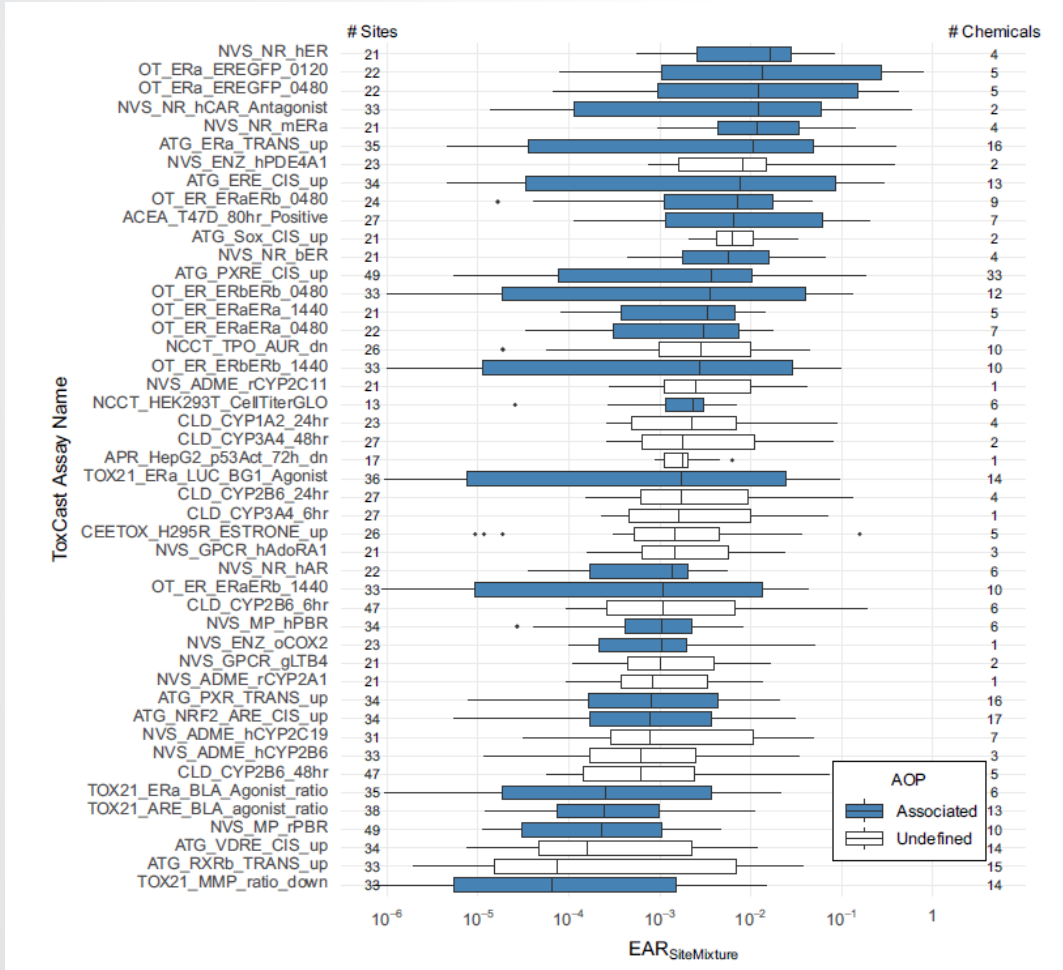
Assume additivity within each ToxCast assay/endpoint

Assay endpoints map to key events
Redundant KEs not double-counted

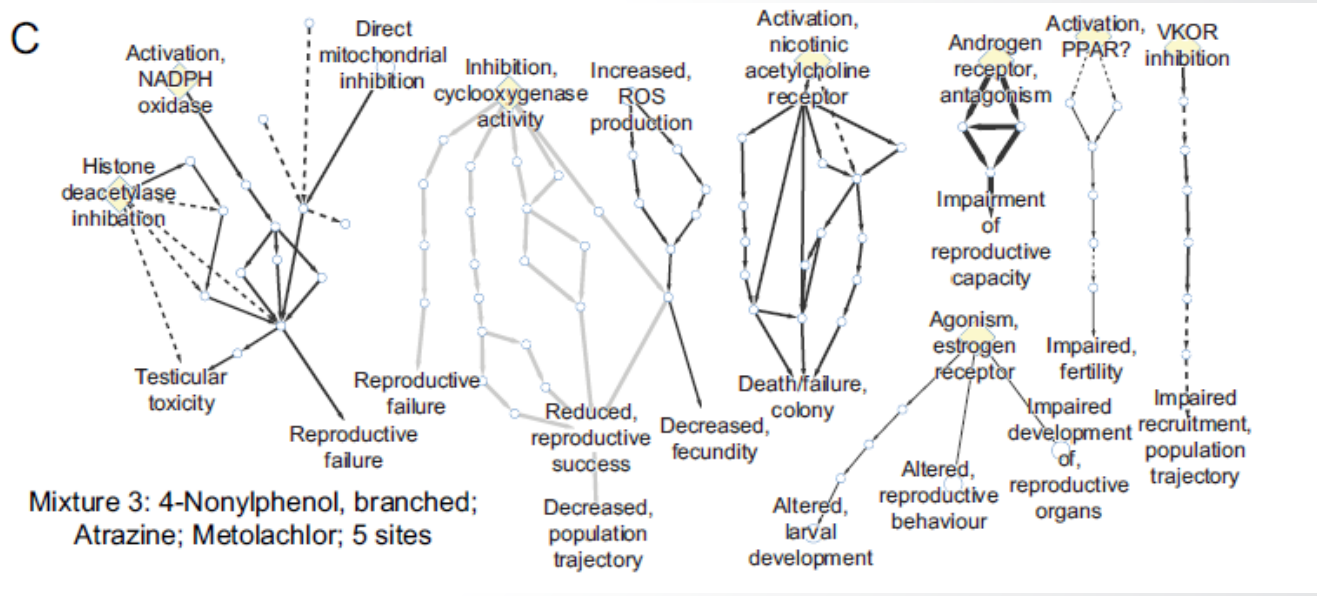
Considers cumulative impacts of multiple pathway perturbations on potential adverse outcomes.

What Effects?

Assay endpoints associated with higher EARs



Associated AOPs / AOP networks



GLRI-CECs, On-going research

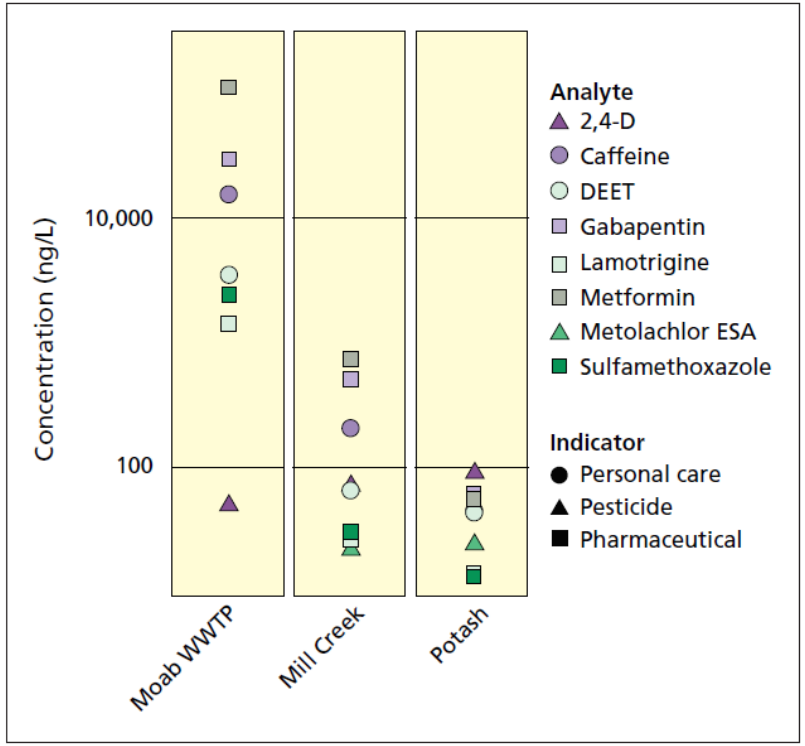
- NAMs-based prioritization being applied to other data sets
  - Fill gaps when water quality benchmarks and in vivo toxicity data are lacking or limited
  - Additional GLRI data sets
  - Other USGS monitoring studies (including drinking water)

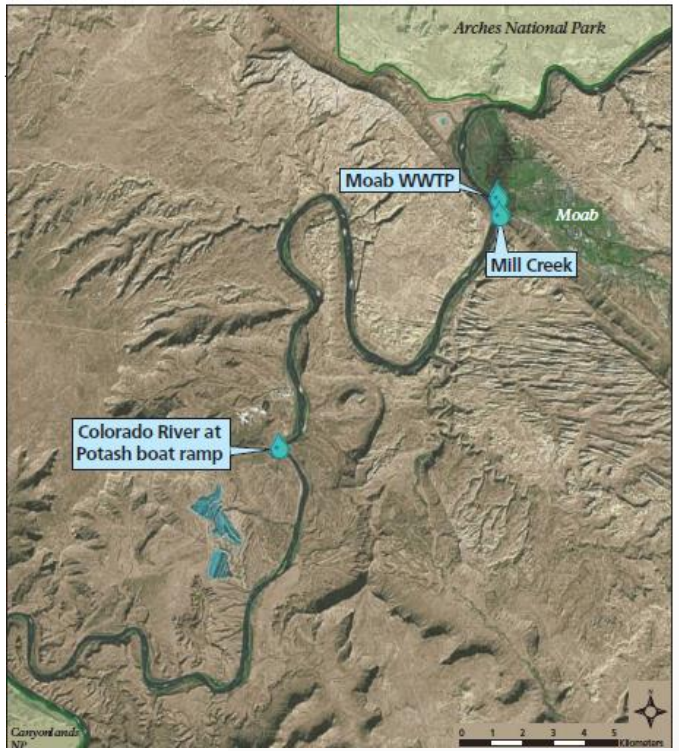
- Risk-based prioritization (incorporating NAMs) is now being applied to over 800 organic contaminants detected over 10 years of CEC monitoring
  - Includes water, sediment, passive samplers, mussels, fish
  - Help inform nomination of potential chemicals of mutual concern as defined through Annex 3 of binational Great Lakes Water Quality Agreement.

EPA Region 8
Waste-water treatment upgrade, Moab, UT

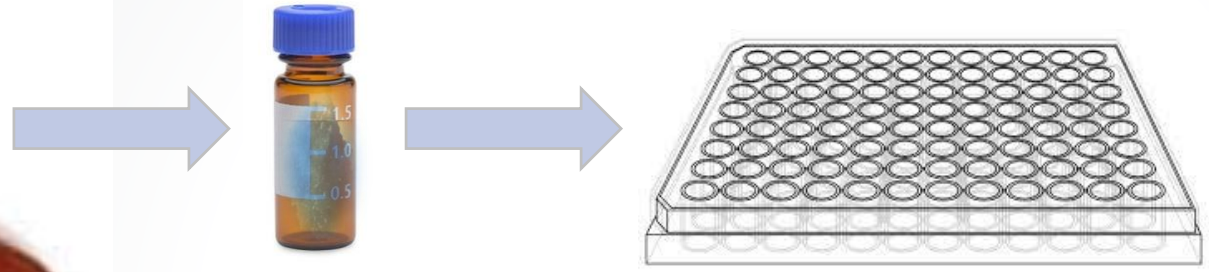
- 2013 National Park Service and USGS measured contaminants along Colorado River between Arches NP and Canyonlands NP

- Variety of pharmaceuticals, pesticides and personal care products detected
  - Greatest concentrations at the Moab WWTP discharge
  - Detectable concentrations extended > 15 km downstream





What about chemicals that weren't monitored



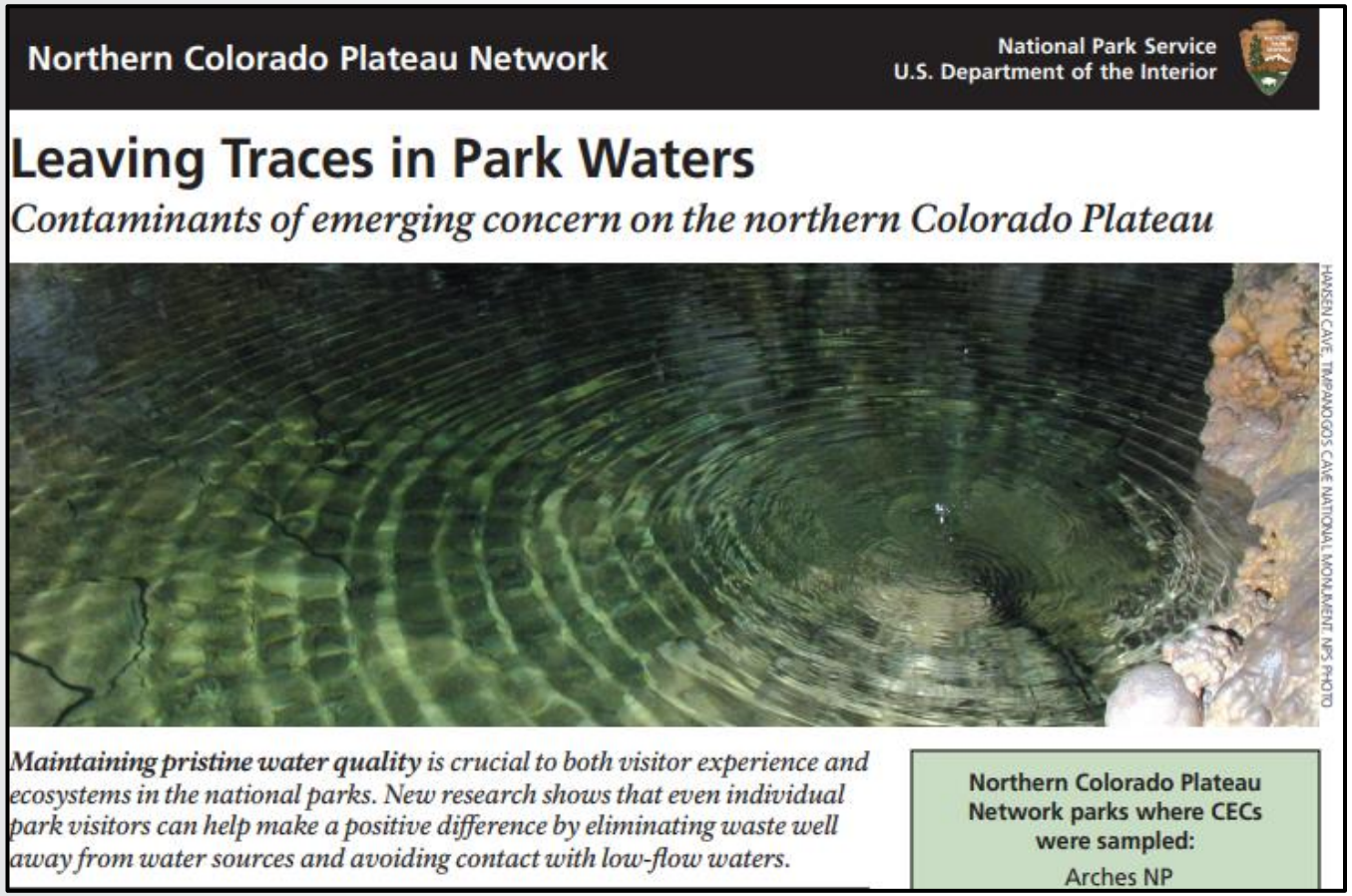
Screened samples using the Attagene trans-Factorial assay

- ToxCast assay platform
- Screens for activation of 24 different nuclear receptors

Three prominent activities were detected

- Estrogen-like (important to reproduction)
- Glucocorticoid-like (important to stress response)
- PPARγ activation (involved in regulation of body fats)

EPA Region 8
Waste-water treatment upgrade, Moab, UT



Moab UT

- 5000 year-round residents
- >1 million visitors per year

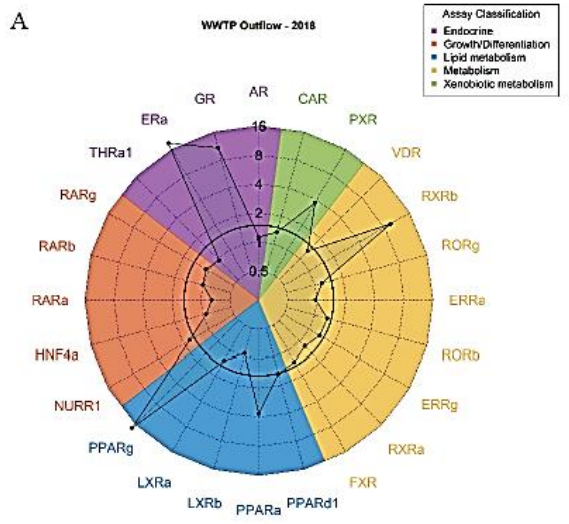
Moab WWTP

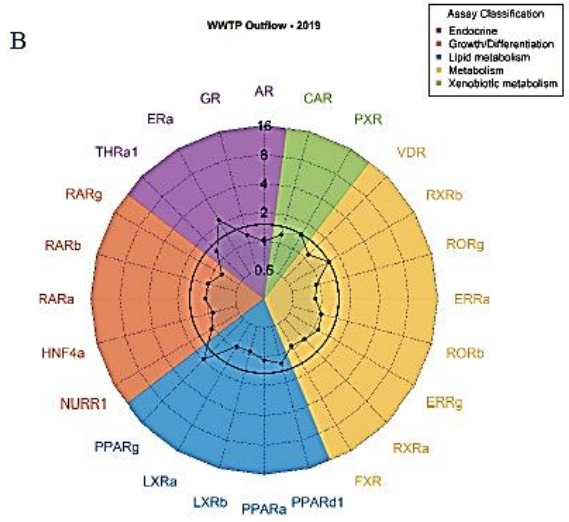
- Originally built in the 1950s
- Upgraded 1996 (trickling filter, chlorine disinfection)
  - Ammonia and nutrient violations with increasing tourism pressure and age
- 2018 new WWTP (activated sludge, UV disinfection)

- Parks and tourism are important to the local economy

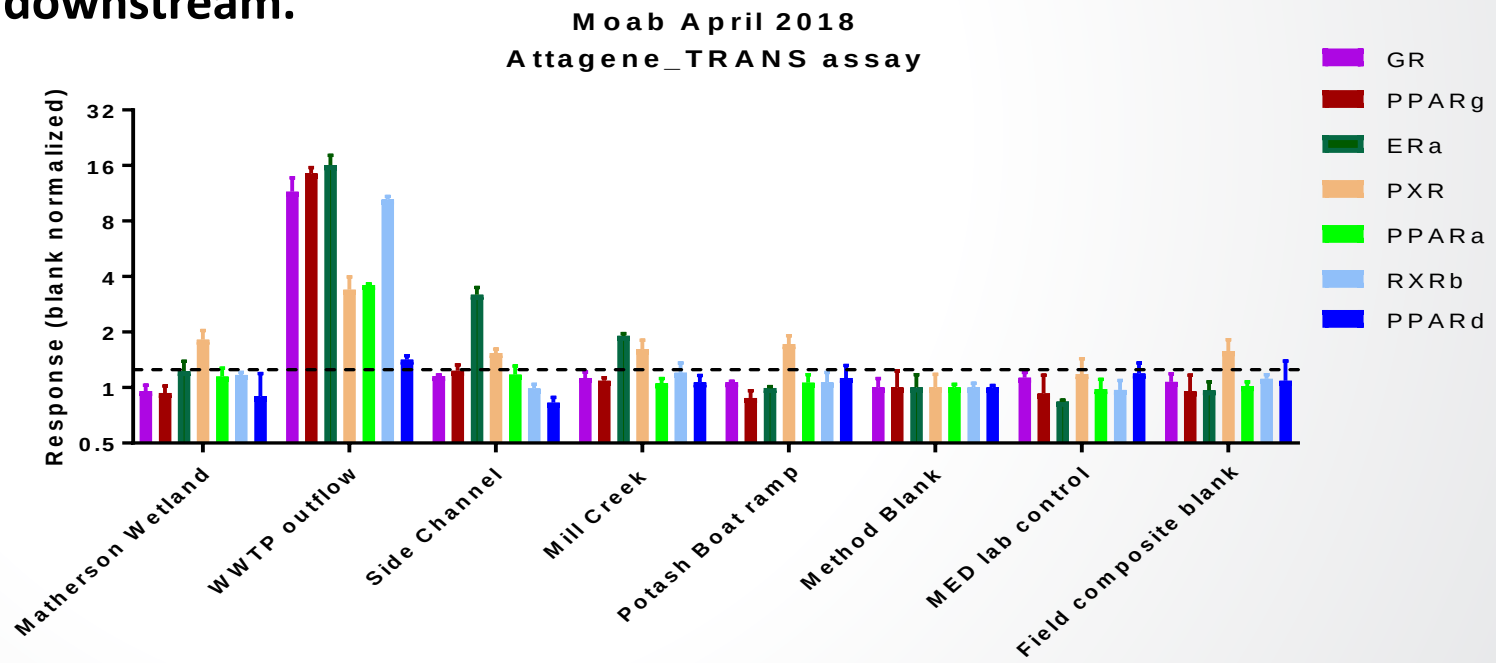
Would the treatment upgrade reduce the loading of bioactive CECs to the Colorado River?

Bioactivity Screening with Attagene



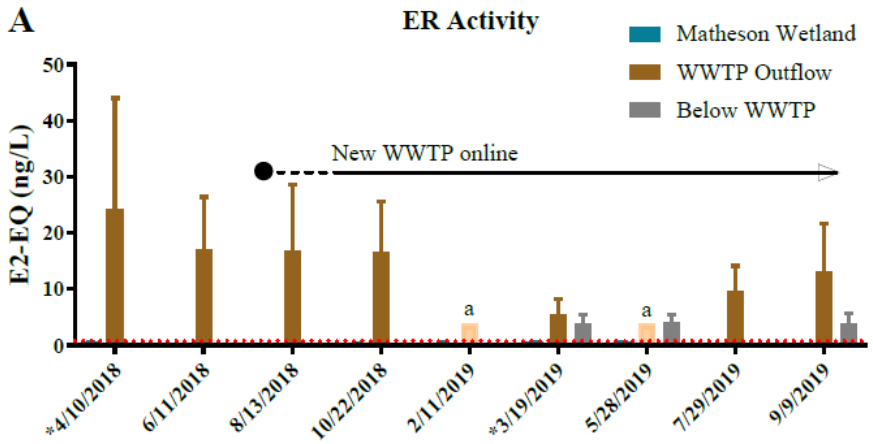


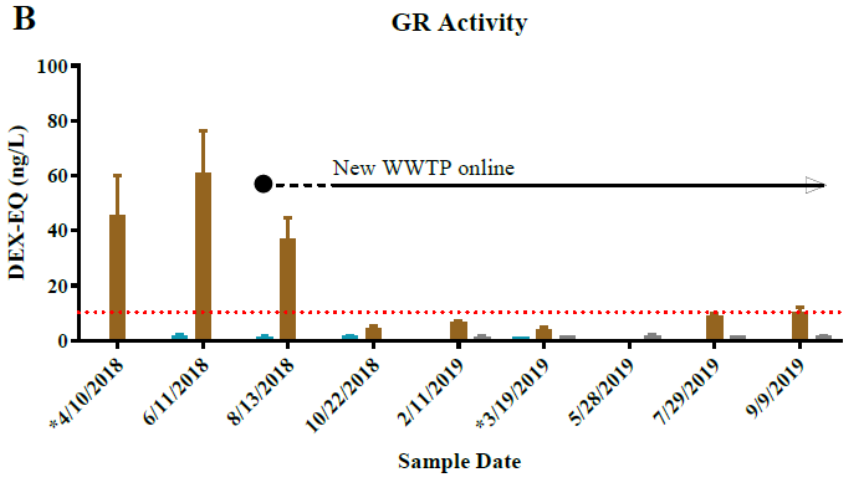
- Six sites, once per year
- Biological activities observed (ER, GR, PPARg) were consistent with pilot years.
- Activity was greatest at the WWTP outflow, diminished rapidly downstream.



- Activity in 2019 was much lower than in 2018

Targeted Bioassays





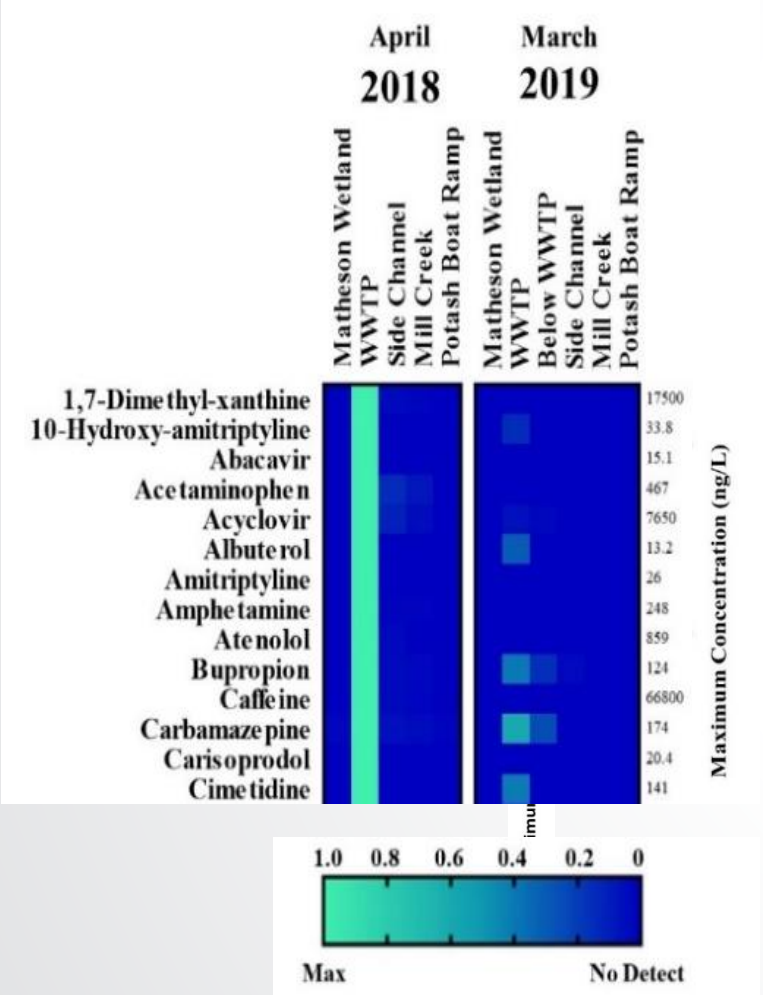
12 sites, bi-monthly, spring to fall over two years

- ER activity declined shortly after WWTP replacement
  - A little lag
  - Possibly trending back up in summer
  - Much lower immediately downstream

- PPARγ activity not detected in targeted assay
  - Slightly less sensitive

- GR activity declined immediately after WWTP replacement
  - Only detected at WWTP outflow

Chemical Monitoring



Only partial heat map shown

- **2018**
  - 62 (out of 131) chemicals detected at outflow
- **2019**
  - 36 (out of 131) chemicals detected at outflow
  - Generally lower concentrations than 2018
- Consistent with bioassay results
- Detections and concentrations quickly decrease away from WWTP
- Guanylurea increased in 2019
  - WWTP transformation product of metformin
  - Metformin below detection limits
  - Recent studies in our lab suggest very low toxicity to aquatic organisms

Good news!

Community investments in upgraded WWTP infrastructure appear to have had a positive effect on the loading of biologically active contaminants to the Colorado River.

- In vitro bioactivities (ER, GR, and PPARγ) reduced and rapidly decline downstream
- Fewer contaminants and lower concentrations
- Caged-fish survival drastically improved

- **Additional contaminant and bioactivity monitoring, if desired, can be focused in close proximity to the WWTP outflow**
  - Some on-going sample collection in 2020-2021 monitor trends in ER- and GR- activity

Conclusions

- Practical applications of NAMs and NAMs data in chemical safety assessment is not limited to prospective assessments of individual chemicals.
- NAMs data can help inform risk-based screening based on environmental monitoring, particularly where traditional toxicity benchmarks are lacking.
- NAMs can be applied to evaluate complex mixtures with both known and unknown compositions.
- NAMs applications can aid in environmental decision-making

Acknowledgements

Brett Blackwell, Kellie Fay, Gerald Ankley, Dan Villeneuve
Erin Maloney, Shibin Li, Joe Swintek,

Steve Corsi, Laura De Cicco, Austin Baldwin

Jenna Cavallin, Jon Beihoffer, Brett Blackwell, Alex Cole, Drew Ekman, Rachel Hofer, Julie Kinsey, Kristen Keteles, Daniel Villeneuve

Additional technical support and assistance was provided by Jon Doering, Correne Jenson, Kathleen Jensen, Ashley Kittelson, Shane Poole, Michael Kahl, Dustin Woodruff, Jonathon Launspach, and Terri Jicha.

Bill Battaglin, Paul Bradley, Dana Winkelman

Chris Wilkowske and colleagues at the USGS Utah Water Science Center Field Office provided laboratory space for this study.

Northern Colorado Plateau Network — National Park Service, U.S. Department of the Interior

Rebecca Weissinger

Anna Marie Forest of the State of Utah,
Nature Conservancy, Scott and Norma Matheson Wetland Preserve
Moab WWTP
City of Moab

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Great Lakes Restoration Initiative, Action Plan II. https://www.glri.us/sites/default/files/glri-action-plan-2.pdf