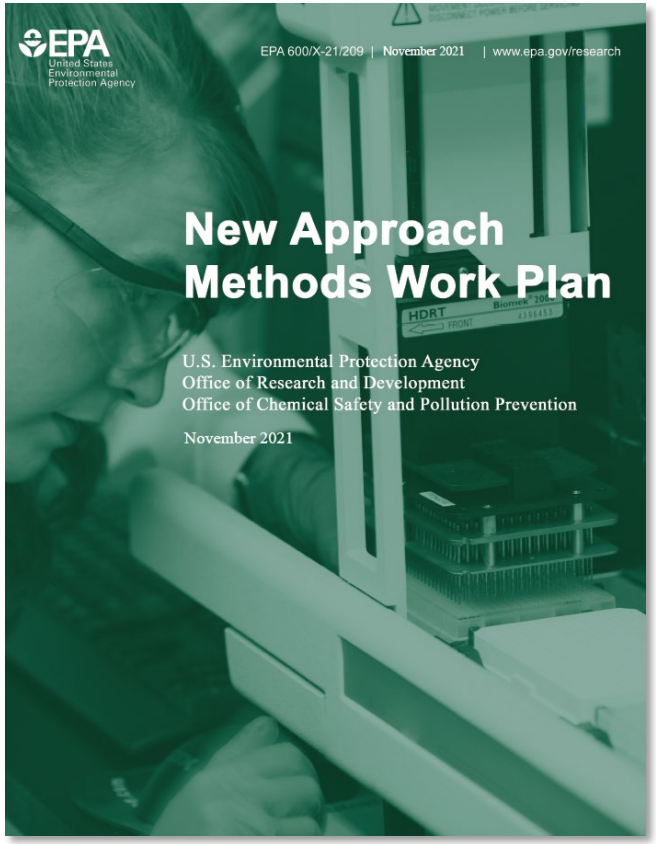
NAMs Work Plan Progress and Day 1 Overview

Fourth EPA NAMs Conference

November 5 - 6, 2024

Rusty Thomas
Director
Center for Computational Toxicology and Exposure

NAMs Work Plan Identified Objectives, Strategies and Deliverables for Applying NAMs



- Five objectives for reducing animal testing and research while ensuring that Agency decisions remain fully protective of human health and the environment

- Updated NAM Work Plan released in December 2021
  - Expansion of the species covered in the work plan to include all vertebrate animals to be consistent with TSCA.
  - Modified deliverable timelines that reflect the expansion of covered species and incorporate feedback received over the preceding years.
  - New case studies for building confidence and demonstrating application of NAMs.
  - A pilot study to develop NAMs training courses and materials.

Status of NAMs Work Plan Deliverables

Milestones/Deliverables	Projected Dates
Evaluate Regulatory Flexibility for Accommodating the Use of NAMs	
EPA report on a review of existing statutes, programmatic regulations, policies, and guidance that relate to vertebrate animal testing and the implementation and use of appropriate NAMs for regulatory purposes.	2022 ✓
Develop Baselines and Metrics for Assessing Progress	
Progress and summary metrics on reducing vertebrate animal testing requests and use.	Annually starting in Q4 2022 ✓

Status of NAMs Work Plan Deliverables

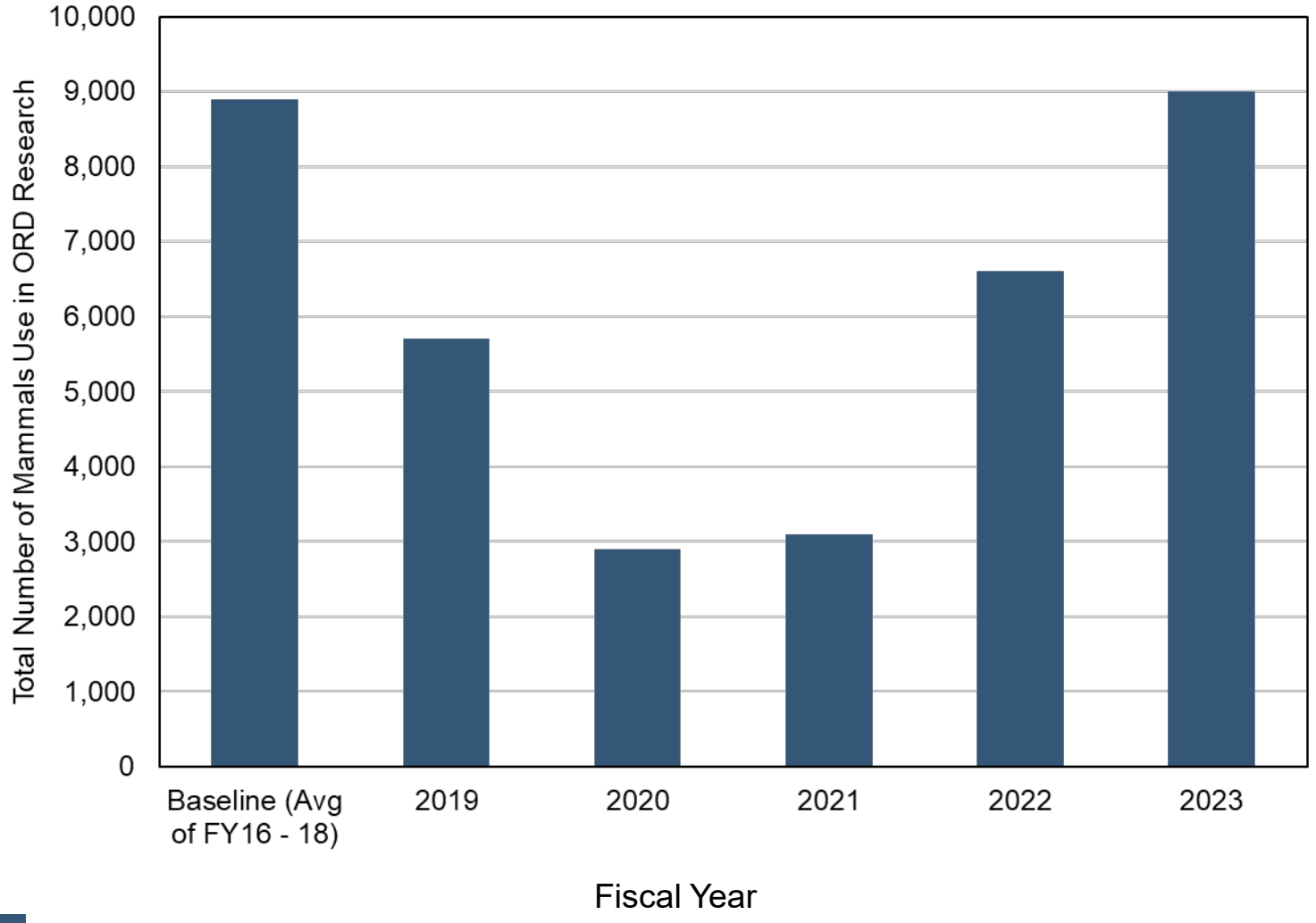
Milestones/Deliverables	Projected Dates
Establish Scientific Confidence in NAMs and Demonstrate Application to Regulatory Decisions	
U.S. National Academies of Sciences, Engineering, and Medicine study that evaluates the variability and relevance of existing mammalian toxicity tests and reviews frameworks for validation and establishing scientific confidence in testing methods.	2023 ✓
A scientific confidence framework to evaluate the quality, reliability, and relevance of NAMs.	Q4 2024
An initial set of reporting templates which may be used by EPA and stakeholders that capture the range of specific NAMs used for Agency decisions.	Q4 2024
Case studies for evaluating application to risk assessment and demonstrating protection of human health and the environment.	Ongoing ✓

Status of NAMs Work Plan Deliverables

Milestones/Deliverables	Projected Dates
Develop NAMs to Address Scientific Challenges and Fill Important Information Gaps	
EPA Strategic Research Action Plans outlining research products to develop and apply NAMs.	Q1 2023 ✓
Encourage development of NAMs through mechanisms such as the STAR program and facilitate partnerships with organizations focused on establishing scientific confidence in alternative methods.	Ongoing ✓
Engage and Communicate with Stakeholders	
EPA website to house information about NAM efforts and progress being upon release of the work plan.	2020 ✓
Public webinars and, where appropriate, peer-review on deliverables from this work plan.	Ongoing ✓
Complete NAMs pilot training program in the fourth quarter (Q4) of 2023 and provide regular scientific exchanges and progress updates through Agency sponsored and partner organized events.	Q4 2023 and Ongoing ✓

FY19 – FY23 Animal Use Metrics for ORD

Milestone/Deliverable: Progress and summary metrics on reducing vertebrate animal testing requests and use. (FY22+).

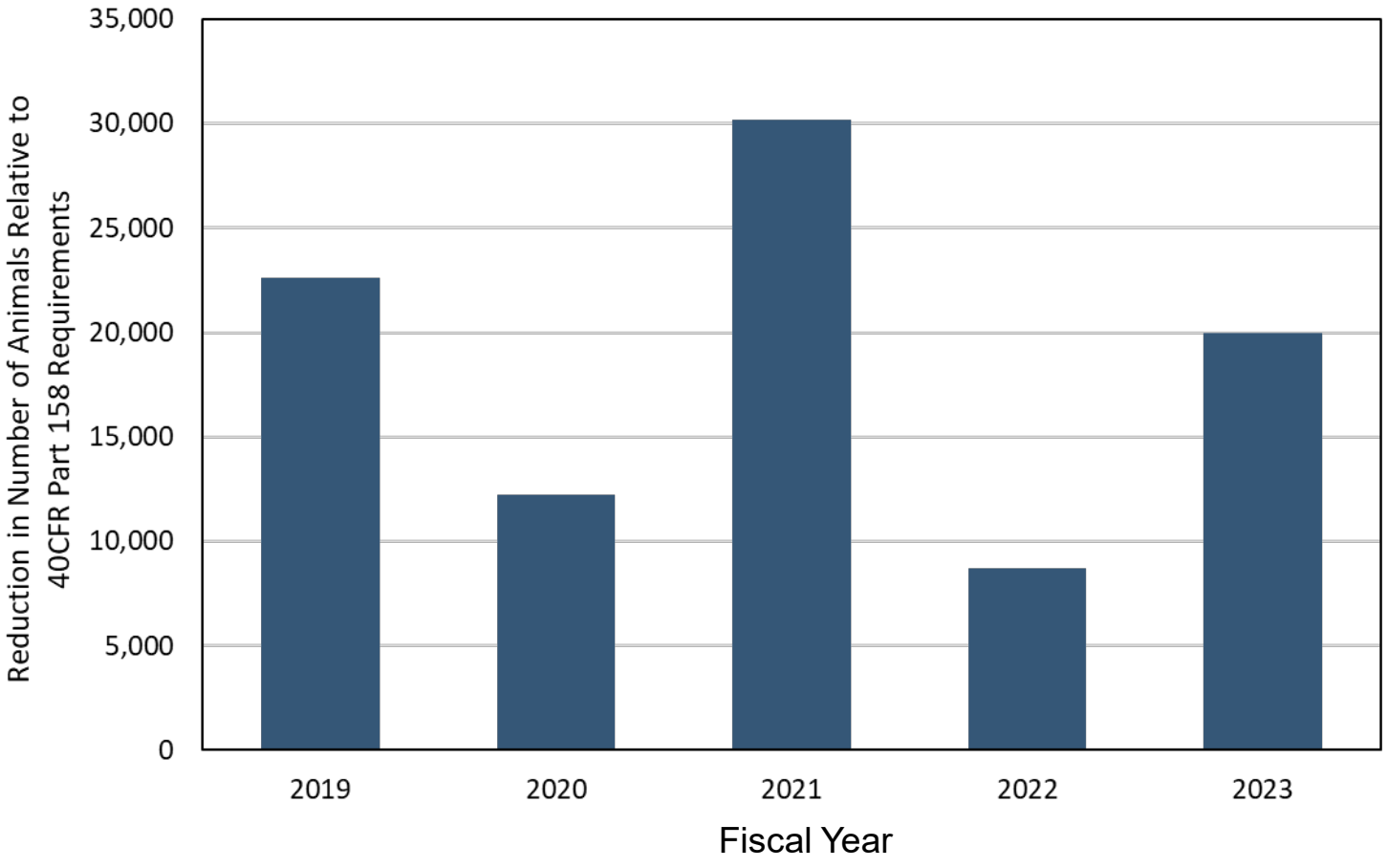


- The numbers in FY19 – 23 include those mammals used in contract research activities.
- Baseline numbers (FY16 – 18) do not include mammals used in contract research activities due to a lack of tracking at that time.
- The numbers in FY19 – 21 are likely reduced due to impacts of the ORD reorganization/lab remodeling and pandemic (FY20 – 21).
- A system for estimating vertebrate animal use in research (includes fish and amphibians in addition to mammals) has been established.
- A baseline for vertebrate animal use will be established after data has been collected over multiple years.

FY19 – FY23 Animal Reduction Metrics for OPP

Develop Baselines and Metrics

Milestone/Deliverable: Progress and summary metrics on reducing vertebrate animal testing requests and use. (FY22+).

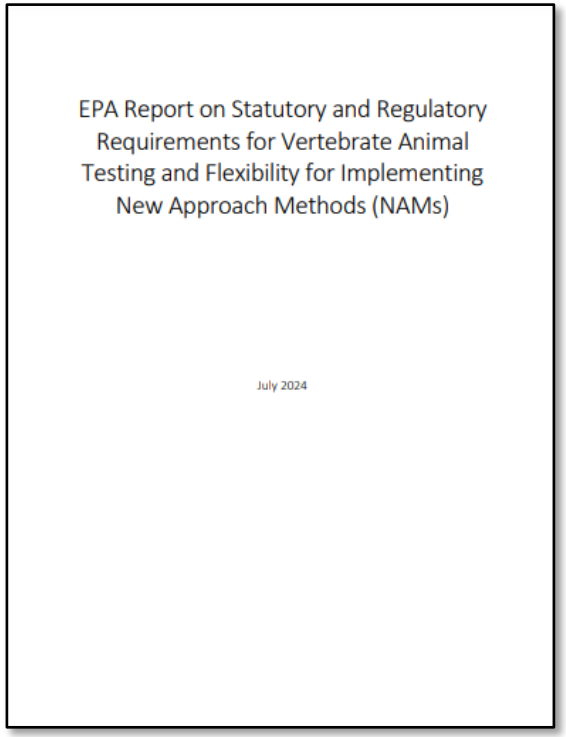


- The reduction in the number of animals were due to Hazard and Science Policy Council (HASPOC), Chemistry and Acute Toxicology Science Advisory Council (CATSAC), and Acute Dermal waivers granted under the updated waiver policies (2016/2020).
- Beginning in 2023, OPP expanded tracking of data waivers granted for the acute “6-pack”.
- The total number waivers granted from FY19 – 23 were:
  - HASPOC - 235
  - CATSAC - 62
  - Acute Dermal - 229
- The number of NAM-related endpoint data submissions from FY19 – 23 were:
  - Eye Irritation - 81
  - Skin Irritation - 57
  - Skin Sensitization - 25

Review of Statutes and Regulations for Flexibility in Incorporating NAMs

Evaluate Regulatory Flexibility

Milestone/Deliverable: EPA report on a review of existing statutes, programmatic regulations, policies, and guidance that relate to vertebrate animal testing and the implementation and use of appropriate NAMs for regulatory purposes.


EPA Report on Statutory and Regulatory Requirements for Vertebrate Animal Testing and Flexibility for Implementing New Approach Methods (NAMs)

July 2024

https://www.epa.gov/system/files/documents/2024-09/epa-regulatory-review-report_final_508_0.pdf

- Topics covered in report:
  - Overview of main environmental statutes and language on testing requirements.
  - Regulatory requirements for vertebrate animal testing
  - Research to support regulatory use of NAMs
  - Current use of NAMs in decision making
  - Barriers to implementation and use of NAMs
- Statutes are written broadly in most cases and do not generally preclude the use of scientific information or data from NAMs.
- Some regulations require a minimum set of vertebrate animal testing for decision-making.
- Program offices will need to identify the regulations that require revisions to incorporate data from NAMs when feasible and scientifically justified.

EPA STAR Grants

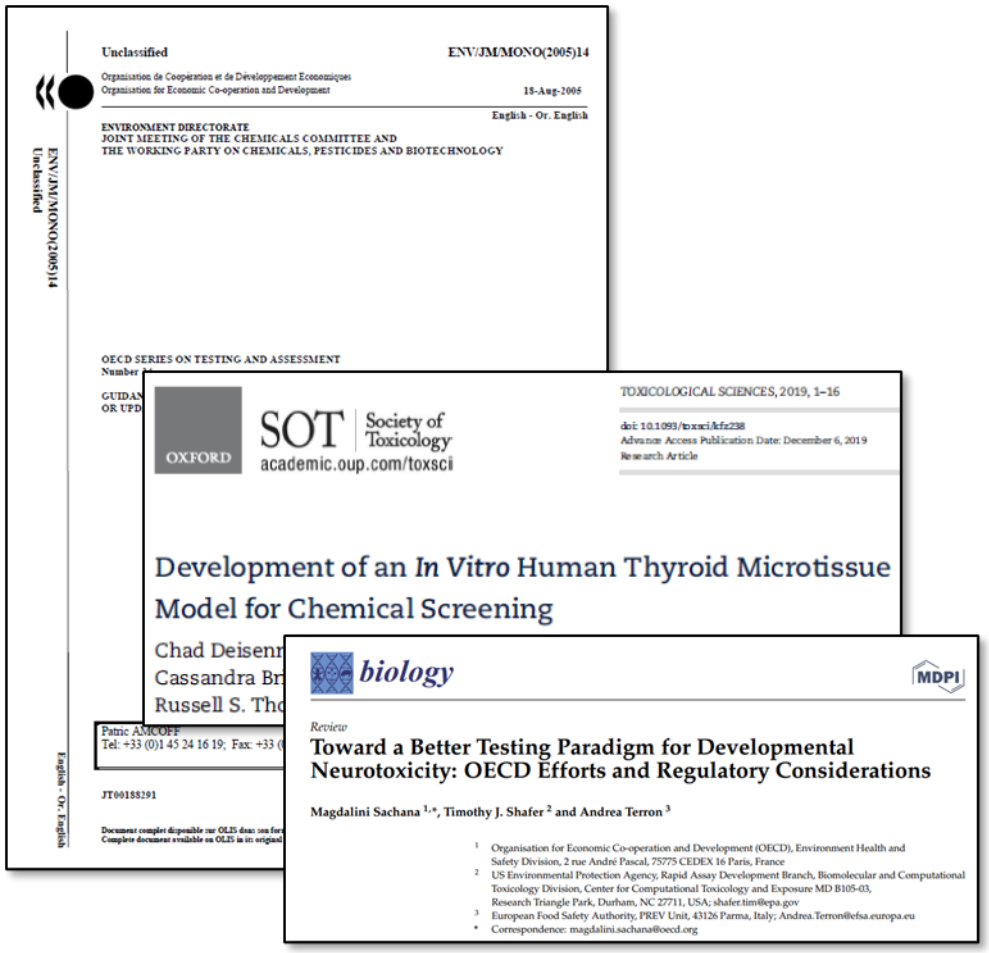
Milestone/Deliverable: Encourage development of NAMs through mechanisms such as the STAR program and facilitate partnerships with organizations focused on establishing scientific confidence in alternative methods. (Ongoing).

https://www.epa.gov/research-grants/star

- EPA STAR grants on *Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Testing (2019-22/25)*
  - Awarded ~$4.2 million to 5 universities
  - Vanderbilt University, University of California Riverside, Louisiana State University, Oregon State University, Johns Hopkins University
- EPA STAR grants on *Advancing Toxicokinetics for Efficient and Robust Chemical Evaluations (2020 – 2023/2025)*
  - Awarded ~$4 million to 5 institutions
  - Purdue University, Woods Hole Oceanographic Institution, Vanderbilt University, Texas A&M, and University of Nevada Reno
- EPA STAR grants on *Development of Innovative Approaches to Assess the Toxicity of Chemical Mixtures (2022-25)*
  - Awarded ~ $7.7 to 11 institutions
  - www.epa.gov/research-grants/development-innovative-approaches-assess-toxicity-chemical-mixtures-research-grants
- EPA STAR grants on *Advancing Sustainable Chemistry. (Awardees Coming Soon)*

Partnerships with External Organizations Focused on Scientific Confidence

Milestone/Deliverable: Encourage development of NAMs through mechanisms such as the STAR program and facilitate partnerships with organizations focused on establishing scientific confidence in alternative methods. (Ongoing).



- Partnering with 4 external organizations on an inter-laboratory prevalidation study of a human thyroid microtissue assay.
- Partnering with 5 external organizations on the development and validation of 17 assays for developmental neurotoxicity.
- Co-leading OECD activity to update Guidance Document 34.
- Collaborating with NICEATM to catalog characteristics of OECD Test Guideline validation studies.
- Participating in the NIH Complement-ARIE program.
- Co-authored ICCVAM report on the validation, qualification, and regulatory acceptance on NAMs in 2024.

Case Studies Evaluating Application of NAMs

Establish Scientific Confidence and Demonstrate Application

Milestone/Deliverable: Case studies for evaluating application to risk assessment and demonstrating protection of human health and the environment (Ongoing).

- Co-leading APCRA case study on using bioactivity for screening level risk assessment.
- Leading APCRA case study on application of *in vitro* toxicokinetics to regulatory decisions.
- Co-leading APCRA case study on incorporating NAMs into species sensitivity distributions for ecological risk assessment.
- Leading international APCRA case study on developing quantitative structure use relationship models for predicting chemical functional use.
- Leading international APCRA case study on evaluating quantitative concordance between human and rodent toxicity data.
- Participating in OECD case study on systemic toxicity.

EPA NAM Pilot Training Program and Regular Scientific Exchanges and Progress Updates

Engage and Communicate with Stakeholders

Deliverables: Completed training pilot and used lessons learned for trainings going forward. Provide regular scientific exchanges and progress updates through Agency sponsored and partner organized events (ongoing – 2026). TSCA New Chemicals Outreach due 2026.

Progress

- Public NAMs training website serves as a resource for EPA NAMs training materials and recordings
- Available Resource Hubs & Tool User Guides (comptox.epa.gov): ChemExpo Knowledgebase, Cheminformatics Modules, CompTox Chemicals Dashboard, Generalized Read-Across, ECOTOX Knowledgebase, Sequence Alignment to Predict Across-Species Susceptibility
- Available Tool Tips Videos: CompTox Chemicals Dashboard (6), ECOTOX (6)
- NAMs Update email bulletin to share progress and updates, email NAM@epa.gov to join!

https://www.epa.gov/chemical-research/new-approach-methods-nams-training

NAMs Tools Trainings

Training	Date	Attendees
ECOTOX	May 2022	321
CompTox Chemicals Dashboard	Oct 2022	554
ECOTOX	Feb 2023	575
GenRA	May 2023	730
httk R Package	Nov 2023	521
In-Person multi-tool training	April 2024	88
AOP-Wiki	Sep 2024	298

Upcoming:

- March 2025: CompTox Tools training at Society of Toxicology conference in a Satellite Meeting
- April 2025: Interactive training on Sequence Alignment to Predict Across-Species Susceptibility (SeqAPASS) tool
- Late 2025: In-person high-throughput toxicokinetics

NAM Conference Topic Survey

- More than 40 submissions from 30 individuals/ organizations
- Many submissions included multiple topics
- **All** of the topics on the agenda are from YOU!!!
  - Validation
  - Exposure
  - Toxicokinetics/IVIVE
  - Omics
- Thank you for your input!

Overview of Day 1

Validation Update		
1:30 – 2:00 pm	ICCVAM Validation Report and NICETAM Activities	Nicole Kleinstreuer (NICEATM)
2:00 – 2:30 pm	Building Confidence in NAMs via Validation Standard Setting in a Revised OECD GD 34	Alison Harrill (EPA)
Exposure NAMs		
2:30 – 3:00 pm	Development and Application of Exposure NAMs in EPA's ExpoCast Project	Kristin Isaacs (EPA)
3:00 – 3:30 pm	Break	
3:30 – 4:00 pm	Integrating Geospatial Exposure Models with NAMs to Evaluate Health Risks from Environmental Chemicals	Kyle Messier (NIEHS)
4:00 – 4:30 pm	Leveraging Non-Targeted High-Resolution Mass Spectrometry to Reveal the Complete PFAS Fingerprint in Maryland	Sin Urban (Maryland Department of Health)
4:30 – 5:00 pm	Human and Environmental Exposure Framework for Biosolids	Carsten Prasse (Johns Hopkins University)
5:00 – 5:10 pm	Session Wrap Up	Annette Guiseppi-Elie (EPA)

Acknowledgements

NAM Work Plan Implementation Team

Christina Baghdikian
Kathie Dionisio
Annette Guiseppi-Elie
Alison Harrill
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Conference Organization

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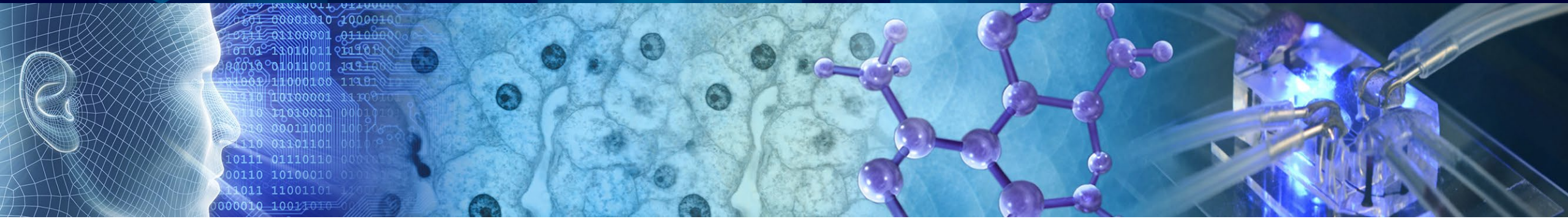
ICF

National Institute of Environmental Health Sciences
Division of Translational Toxicology

EPA NAMs Conference
5th November, 2024



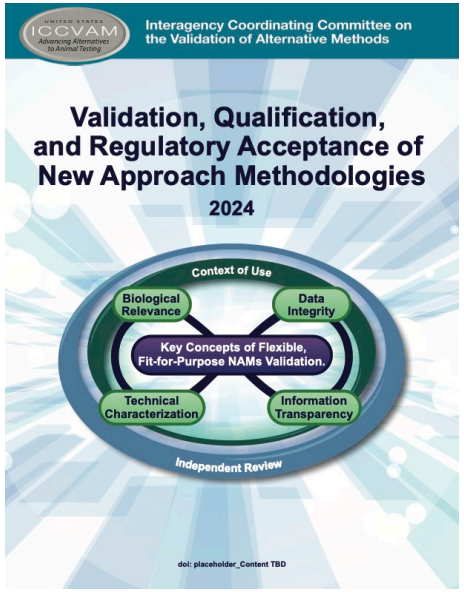
ICCVAM Validation Report and NICEATM Activities

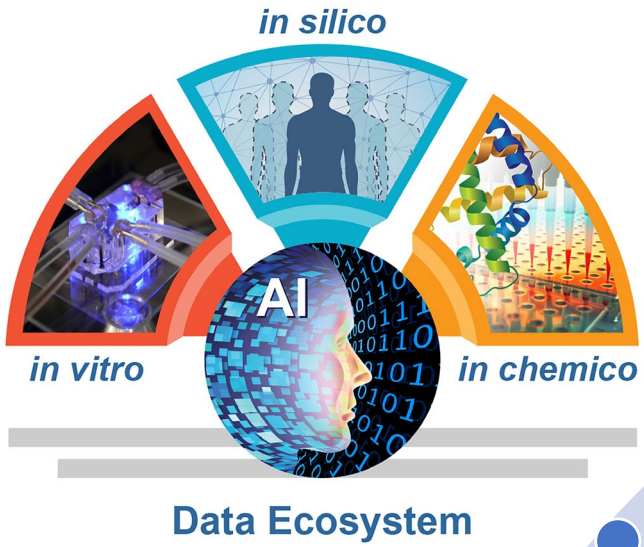
Nicole C. Kleinstreuer, PhD

Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

Executive Director, Interagency Coordinating Committee for the Validation of Alternative Methods

National Institutes of Health • U.S. Department of Health and Human Services





NIH National Institutes of Health

U.S. FOOD & DRUG ADMINISTRATION

CONSUMER PRODUCT SAFETY COMMISSION UNITED STATES

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OECD

etc.

Biomedical Research

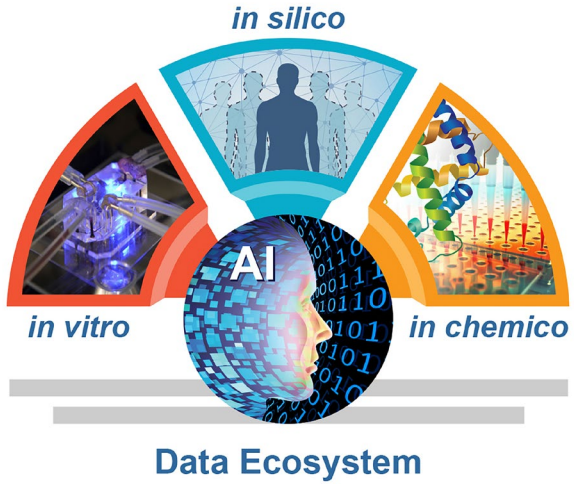
Validation/ Qualification

Adoption & Implementation

The NAMs Confidence Continuum: from *Biomedical Research* to *Validation/Qualification* to *Adoption & Implementation*

Complement-ARIE: Complement Animal Research in Experimentation

Purpose: To catalyze the development, standardization, validation and use of **human-based new approach methodologies (NAMs)** that will transform the way we do basic, translational, and clinical sciences

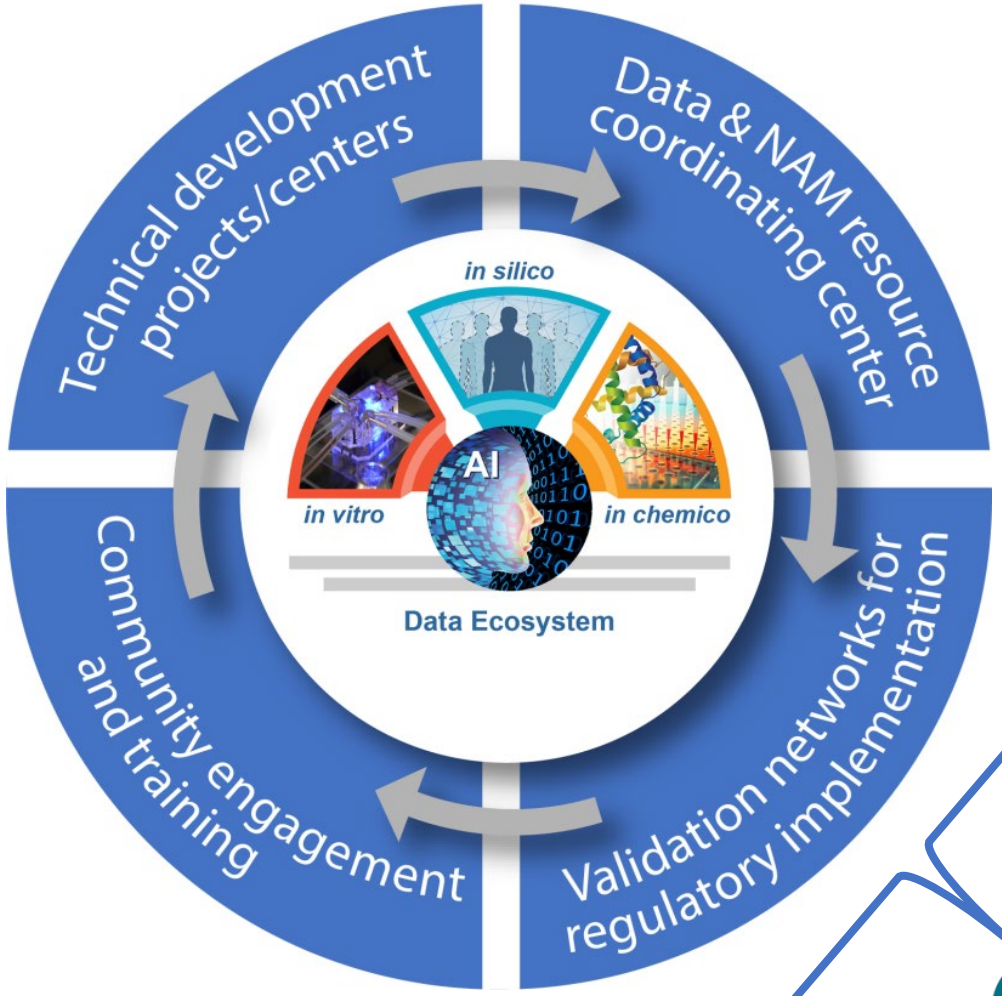


Goals:

1. Better model and **understand human health and disease** outcomes **across diverse populations**.
2. Develop NAMs that **provide insight into specific biological processes** or disease states.
3. Validate mature NAMs to **support regulatory use** and standardization.
4. Complement traditional models and **make biomedical research more efficient and effective**.

https://commonfund.nih.gov/complementarie

Complement-ARIE: Comprehensive center model



- Comprehensive centers will require embedded projects on *in vitro*, *in chemico*, and *in silico* approaches plus combinatorial approaches.
- Cores will include administrative, validation, resources, and training components.
- Phased milestone-driven projects that pilot some of the truly innovative approaches can also be transitioned for integration with the centers.

UNITED STATES ICCVAM Advancing Alternatives to Animal Testing

Key partners for validation networks include: ICCVAM, FDA, EPA, ICATM members, OECD, etc.

U.S. Validation Body: ICCVAM Authorization Act of 2000

PUBLIC LAW 106–545 (42 U.S.C. 285/-3)

"To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness."

ICCVAM Co-chairs

Suzy Fitzpatrick
FDA/CFSAN

Natalia Vinas
DoD

- Consumer Product Safety Commission
- Department of Agriculture
- Department of the Interior
- Department of Transportation
- Environmental Protection Agency
- Food and Drug Administration
- Occupational Safety and Health Administration
- National Institute for Occupational Safety and Health
- Agency for Toxic Substances and Disease Registry
- National Cancer Institute

- National Inst of Environmental Health Sciences
- National Library of Medicine
- National Institutes of Health
- Department of Defense
- Department of Energy
- National Institute of Standards and Technology (since 2017)
- Dept of Veterans Affairs Office of Research and Development (since 2020)
- National Center for Advancing Translational Sciences (since 2024)

Nicole Kleinstreuer
Executive Director, ICCVAM
Director, NICEATM

More information: https://ntp.niehs.nih.gov/go/iccvam

What is NICEATM?

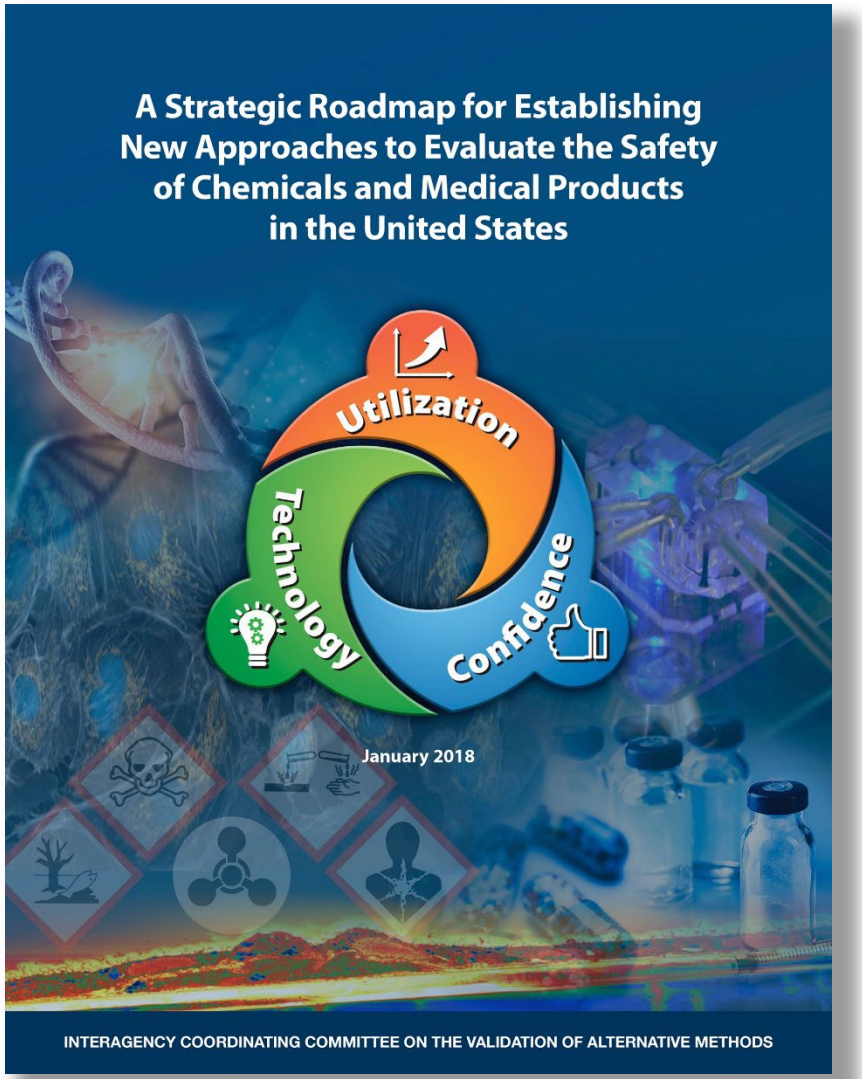
- NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- Office within the Division of Translational Toxicology (DTT), National Institute of Environmental Health Sciences (NIEHS)
- Provides scientific leadership and operational support for ICCVAM and ICCVAM workgroup activities
  - NAMs development, evaluation, validation, and implementation
  - Data compilation and review
  - Computational tools development
  - ICCVAM meeting and teleconference support
- Advised by Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

More information: https://ntp.niehs.nih.gov/go/niceatm

Existing ICCVAM Workgroups and Expert Groups

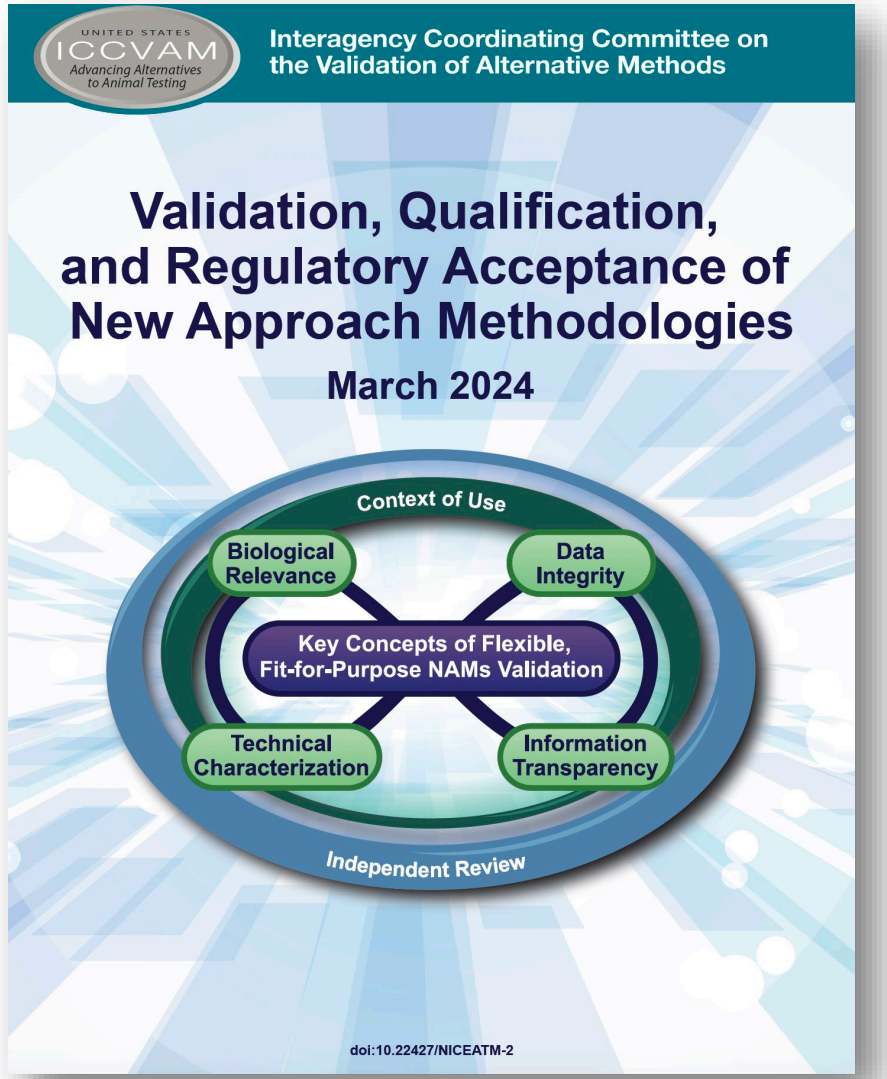
- Acute Toxicity
- Consideration of Alternative Methods
- Ecotoxicology
- In Vitro to In Vivo Extrapolation
- PFAS NAMs
- Read Across
- Validation
- Developmental and Reproductive Toxicology
- Developmental Immunotoxicity
- FAIR Data standards
- Metrics
- Microphysiological Systems
- Nanomaterials
- Ocular and Dermal Irritation
- Skin Sensitization

Interagency Coordinating Committee on the Validation of Alternative Methods



https://ntp.niehs.nih.gov/go/natl-strategy

- **Connect end users with the developers of alternative methods**
- **Establish new validation approaches that are more flexible and efficient**
- **Ensure adoption and use of new methods by both regulators and industry**



https://ntp.niehs.nih.gov/go/ICCVAM-submit

Method Developers Forum (MDF)

- A proactive effort to highlight and implement the recommendations detailed within the VWG report and provide an opportunity for NAMs developers to interact with stakeholders around regulatory issues.
- Anticipate holding approximately 2-3 MDFs per year.
- Each iteration will focus on a specific endpoint/toxicity.
  - First MDF focused on carcinogenicity (August 21-22, 2024).
  - ICCVAM agencies and industry stakeholders summarize their information needs for carcinogenicity and potential contexts of use for NAMs.
  - Developers demonstrate how their methods address the topic of interest and consider the key concepts from the VWG report in a webinar.
  - 10 submissions were reviewed and approved for presentation
- Future topics include cardiovascular toxicity, developmental toxicity, reproductive toxicity, neurotoxicity, systemic toxicity, specific target organ toxicity (e.g., liver).

https://ntp.niehs.nih.gov/go/developers-forums

MDF Process

1. Federal and industry stakeholders will record presentations that summarize their information needs and decision frameworks for the endpoint/toxicity of interest
2. Recordings will be posted on the NICEATM website.
3. A call for method developer presentations will go out in relevant media platforms.
4. Participating method developers will be asked to view the stakeholder recordings and will be provided with a basic set of questions that correspond to the key concepts in the VWG report to address in their presentations.
5. The Steering Committee will review submissions and select those to be included on the agenda for the MDF main event.
6. The MDF main event (virtual) will feature brief presentations from selected method developers that address NAMs for endpoint/toxicity of interest and will allow time for discussion.

https://ntp.niehs.nih.gov/go/developers-forums

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Ongoing NICEATM and ICCVAM Projects

- Integrated Chemical Environment
- OPERA (QSAR/QSPR)
- Computational Chemistry
- Quantitative IVIVE
- Reference data curation
- Variability of in vivo data
- Acute Systemic Toxicity
- Dermal absorption
- Eye and skin irritation
- Skin sensitization
- Ecotoxicology
- Carcinogenesis
- Cardiovascular Toxicity
- Developmental Toxicity
- DNT Testing Battery
- Zebrafish models
- Animal-free affinity reagents
- Microphysiological Systems
- Evolving Process of Validation

Integrated Chemical Environment

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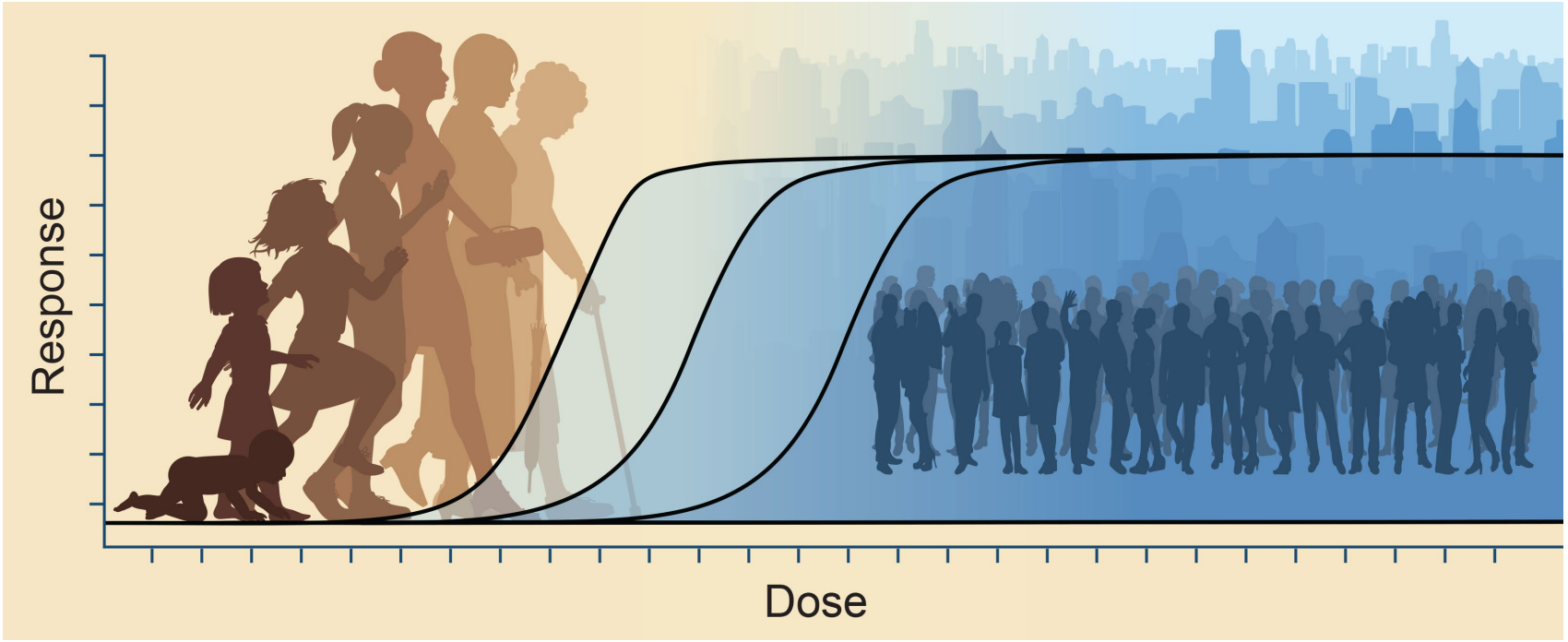
Area of Focus: NAMs to Address PopVarS

Using New Approach Methodologies to Address Variability and Susceptibility Across Populations



https://ntp.niehs.nih.gov/go/popvar

Workshop report in prep to be submitted to Human Genomics shortly

Human Genomics

SPRINGER NATURE GROUP
SDG Programme
supporting the Sustainable Development Goals

Human Genomics Call for Papers

New Approach Methodologies to Address Population Variability and Susceptibility in Human Risk Assessment

Guest Editors: Helena Hogberg, PhD; **Nicole Kleinstreuer**, PhD; **Kim To**, PhD

Submission Status: Open

Read more about the collection
https://www.biomedcentral.com/collections/NAMAPVS

OPERA v2.9 Models

Physchem properties		Chemicals	Version
BP	Boiling Point	7860	2.9
HL	Henry's Law Constant	2233	2.9
LogP	Octanol-water Partition Coefficient	18154	2.9
MP	Melting Point	22554	2.9
VP	Vapor Pressure	6764	2.9
WS	Water Solubility	9943	2.9
pKa	Acid Dissociation Constant	6503	2.6
KOA	Octanol/Air Partition Coefficient	270	2.6

Environmental fate		Chemicals	Version
AOH	Atmospheric Hydroxylation Rate	692	2.6
BCF	Bioconcentration Factor	626	2.6
BioHL	Biodegradation Half-life	150	2.6
RB	Ready Biodegradability	1603	2.6
KM	Fish Biotransformation Half-life	541	2.6
KOC	Soil Adsorption Coefficient	728	2.6

ADME properties		Chemicals	Version
FUB	Fraction unbound	3229	2.8
Clint	Intrinsic clearance	1346	2.8
CACO2	Caco-2 permeability	4601	2.8

Toxicity endpoints		Chemicals	Version
ER	Estrogen Receptor Activity	32464	2.6
AR	Androgen Receptor Activity	47673	2.6
AcuteTox	Acute Oral Systemic Toxicity	50660	2.6

Future models	
Inhalation	Acute Inhalation Systemic Toxicity
SixPack	Acute Toxicity Six-Pack Endpoints
UGT	Glucuronidation: substrate selectivity
SULT	Sulfation: substrate selectivity

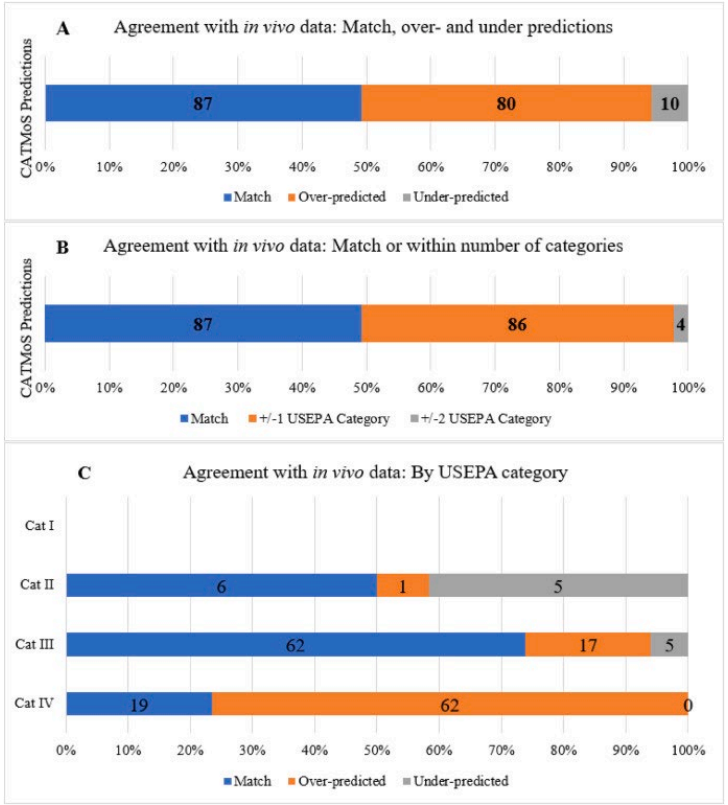
https://github.com/NIEHS/OPERA

Application of CaTMOS: Pesticide Risk Assessment

Case Study with EPA Environmental Fate and Effects Division

- Comparative analysis of 177 pesticides with LD_{50} data between CaTMOS and EPA database

Toxicity Category based on CATMoS Prediction	Number of predictions	Toxicity Category based on Empirical *In Vivo* Test Data			
		I	II	III	IV
I (<50 mg/kg)	2	-	1	1	-
II (50-500 mg/kg)	25	-	**6**	16	3
III (>500-5,000 mg/kg)	126	-	5	**62**	59
IV (>5,000 mg/kg)	24	-	-	5	**19**
III and IV combined	150	-	5	145	

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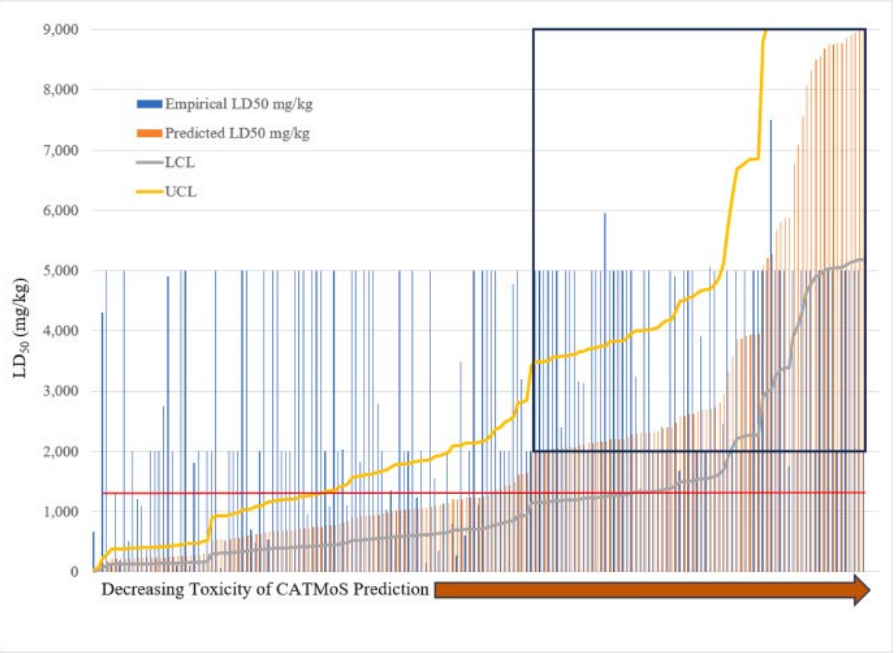
journal homepage: www.elsevier.com/locate/yrtph
ELSEVIER

Evaluation of *in silico* model predictions for mammalian acute oral toxicity and regulatory application in pesticide hazard and risk assessment

Patricia L. Bishop [a,*], Kamel Mansouri [b], William P. Eckel [c], Michael B. Lowit [c], David Allen [d,1], Amy Blankinship [c], Anna B. Lowit [e], D. Ethan Harwood [c], Tamara Johnson [c], Nicole C. Kleinstreuer [b]



Bishop et al., Reg. Tox. Pharm., 2024 https://doi.org/10.1016/j.yrtph.2024.105614

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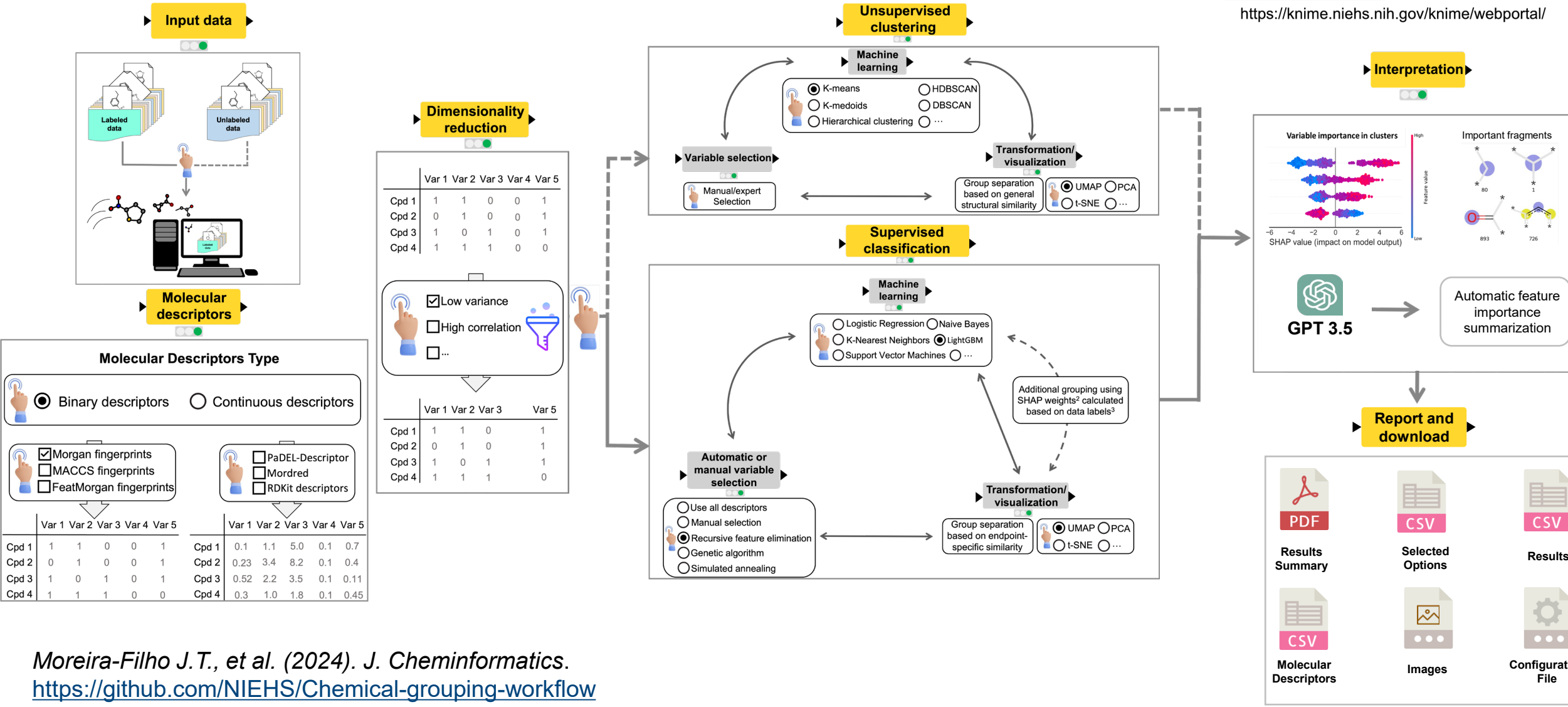
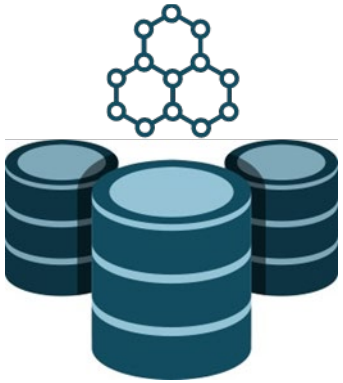
Chemical Grouping Workflow

Open for Innovation KNIME KNIME Server
https://knime.niehs.nih.gov/knime/webportal/

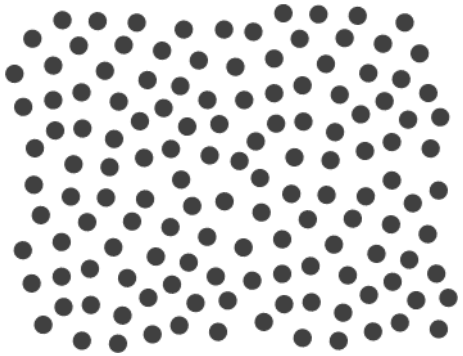


Moreira-Filho J.T., et al. (2024). J. Cheminformatics.
https://github.com/NIEHS/Chemical-grouping-workflow

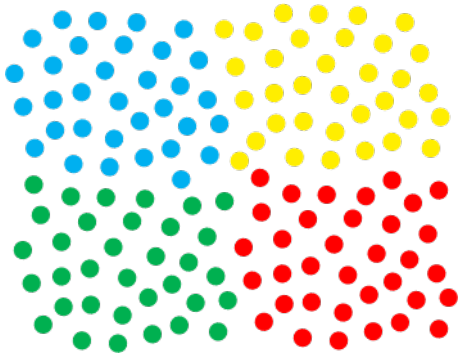
Application – DTT HTS initiative



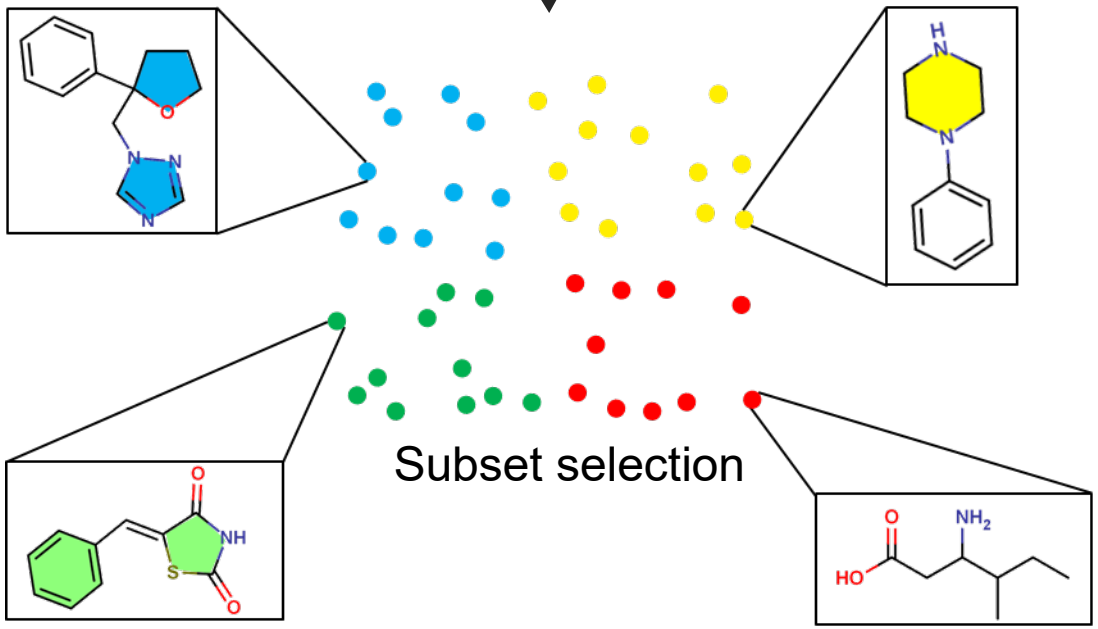
Chemical database

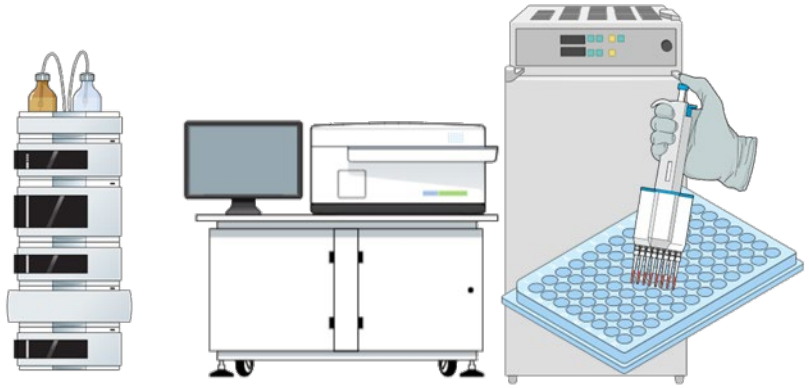


Chemical space



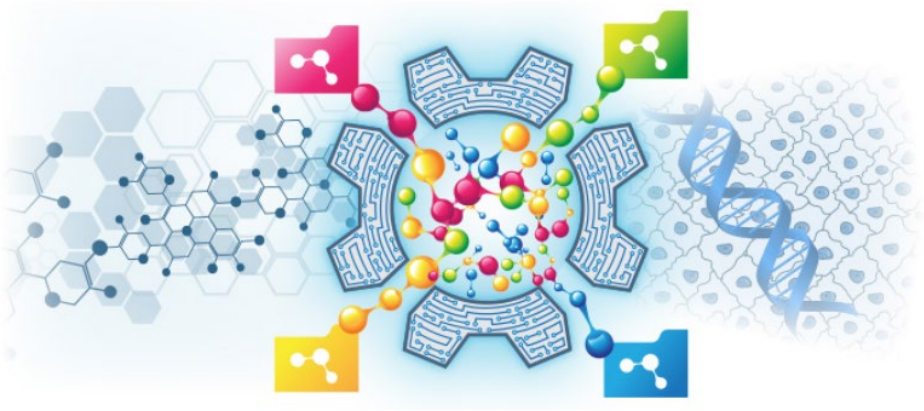
Cluster compounds





Experimental testing

Clustering and Classification Workshop



https://www.niehs.nih.gov/news/events/pastmtg/2022/nams2022/index.cfm

Convened international experts to discuss methods, their applications to guide toxicology research and inform hazard and risk assessment.

Accomplishments:

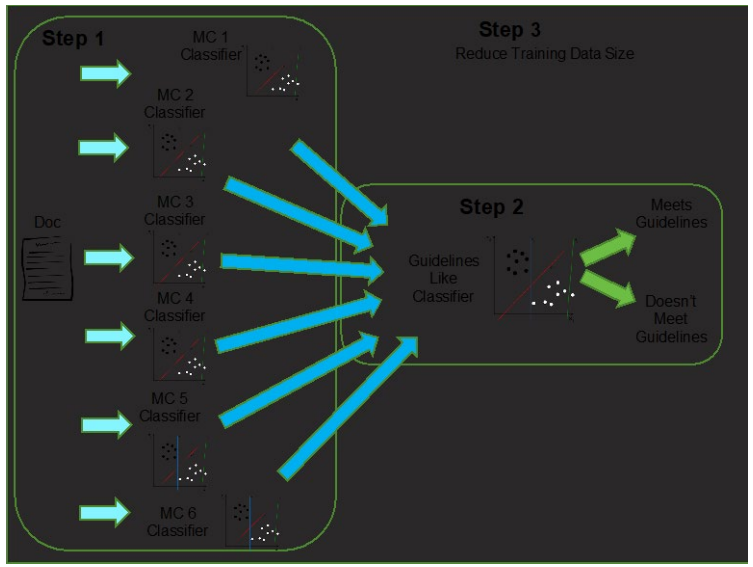
- Defined the concept similarity for supervised and unsupervised approaches
- Introduced different approaches, corrected some misconceptions
- Involved both NAM developers and users
- Established a consortium and a community for increasing communication and collaboration across sectors
- *Ongoing and future:* develop and share new ideas/concepts (best practices & innovation)

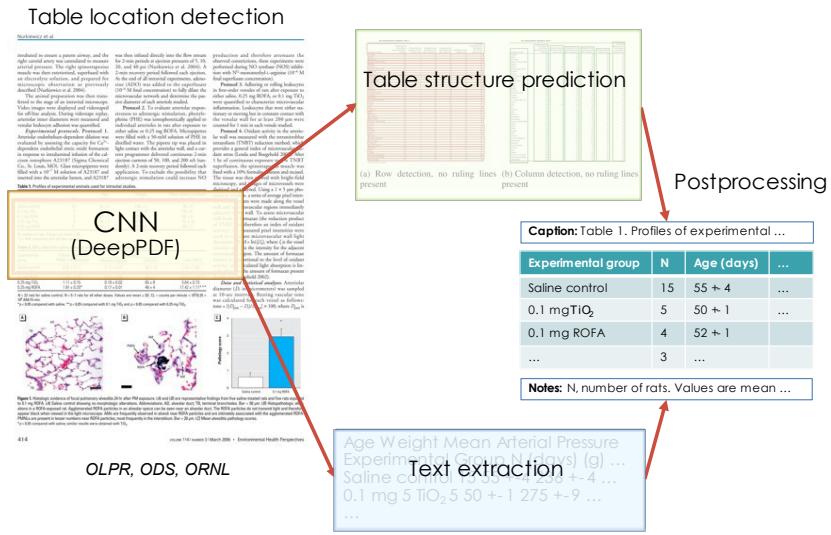
Mansouri K., et al. (2024). Env Health Persp

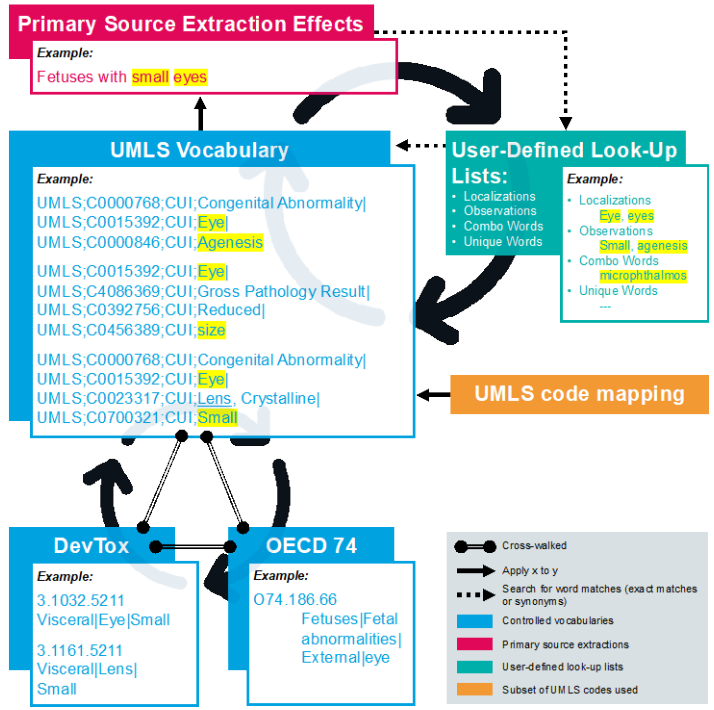
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Machine Automating Study Data Curation

Identification → Extraction → Annotation







- Important for leveraging high-quality studies in the published literature
- Applications in systematic review of chemical effects
- Establishing reference datasets for validating new methods

Foster et al. 2024 Env Health Persp

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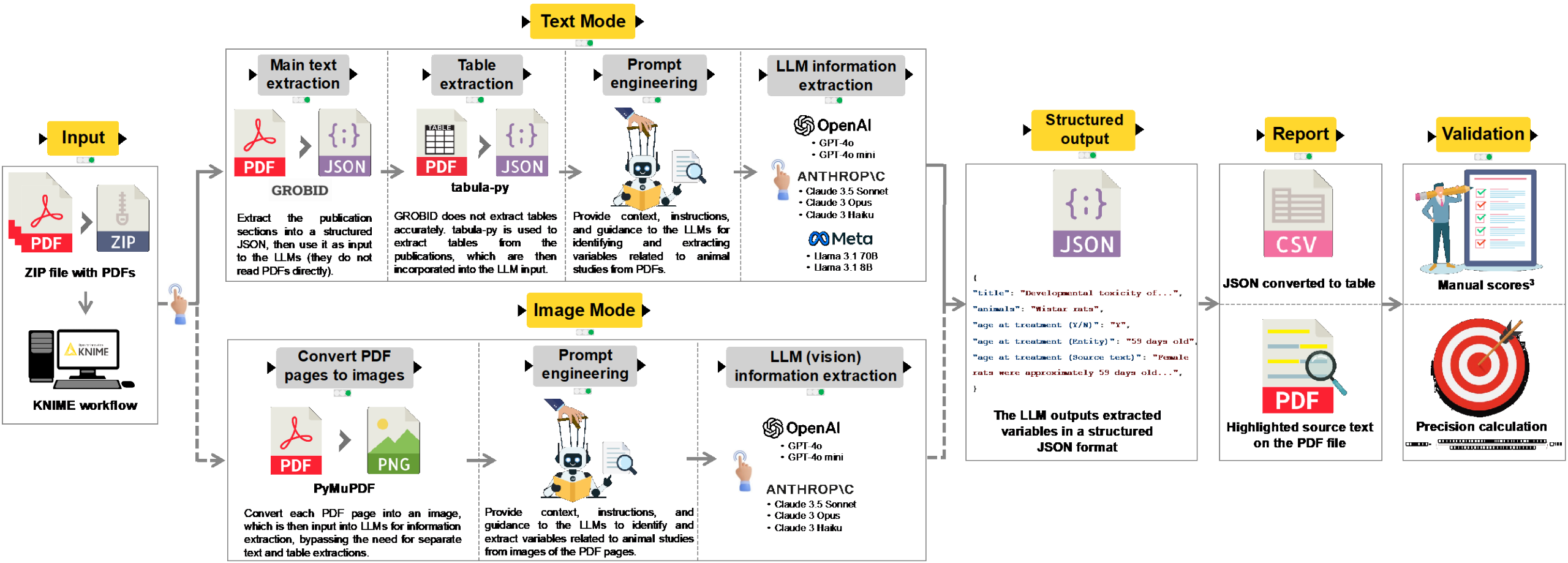
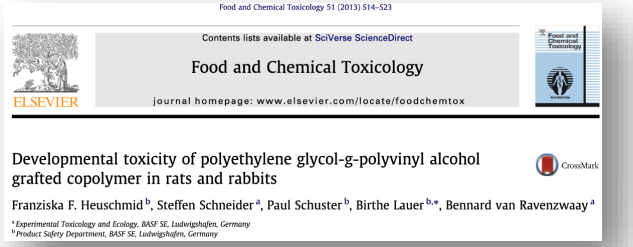
Extraction workflow



Validation - precision


Food and Chemical Toxicology 51 (2013) 514–523

Contents lists available at SciVerse ScienceDirect

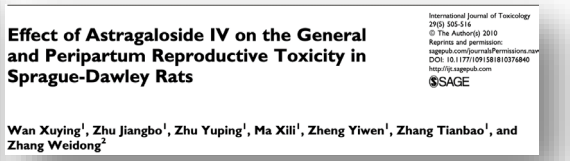
Food and Chemical Toxicology

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

Developmental toxicity of polyethylene glycol-g-polyvinyl alcohol grafted copolymer in rats and rabbits

Franziska F. Heuschmid [b], Steffen Schneider [a], Paul Schuster [b], Birthe Lauer [b,*], Bennard van Ravenzwaay [a]

[a] Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany
[b] Product Safety Department, BASF SE, Ludwigshafen, Germany


Effect of Astragaloside IV on the General and Peripartum Reproductive Toxicity in Sprague-Dawley Rats

Wan Xuying[1], Zhu Jiangbo[1], Zhu Yuping[1], Ma Xili[1], Zheng Yiwen[1], Zhang Tianbao[1], and Zhang Weidong[2]


Environmental Science and Pollution Research (2019) 26:5990–5999
https://doi.org/10.1007/s11356-018-4011-x

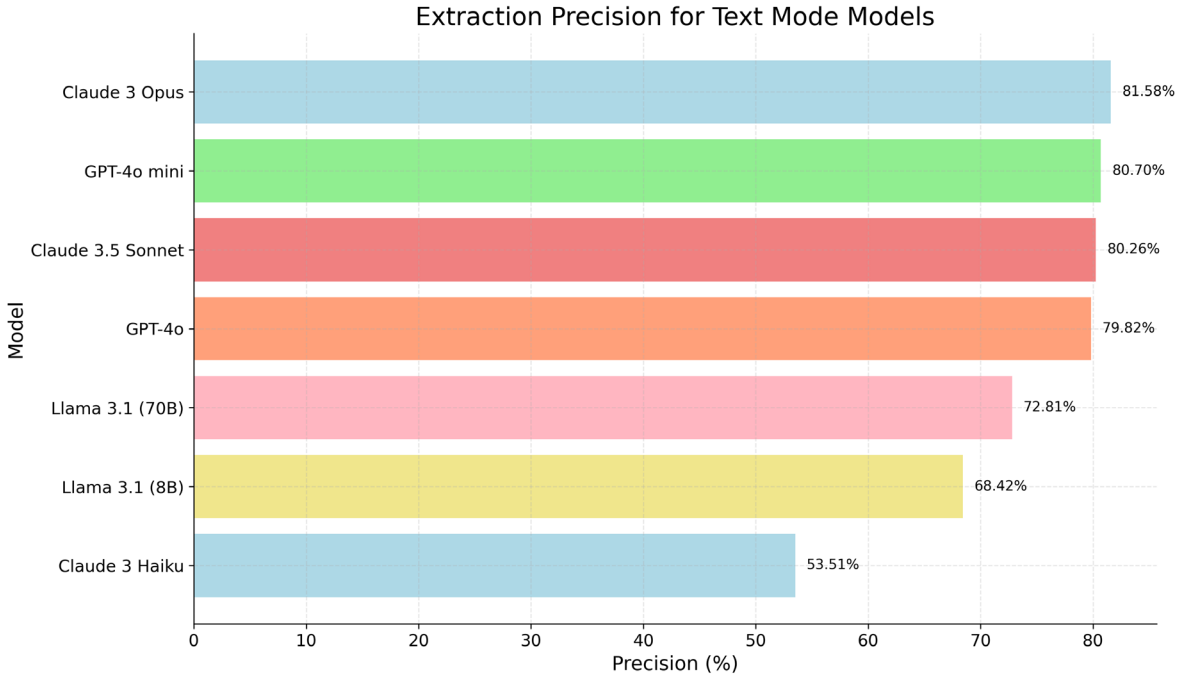
RESEARCH ARTICLE

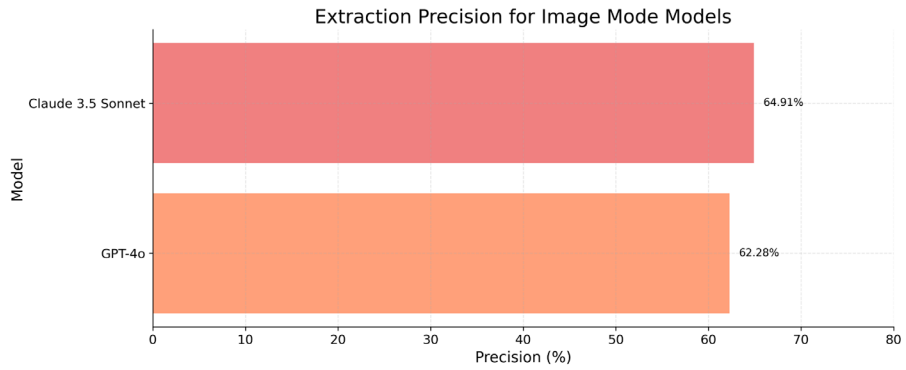
Effect of grape seed extract on maternal toxicity and in utero development in mice treated with zearalenone

Nouf J. Althali [1] · Aziza M. Hassan [2,3] · Mosaad A. Abdel-Wahhab [4]

- ❖ Extracted if the following variables are present (Y/N), the entity, and the source text:

- Title
- Animal
- Age at treatment
- Body weight at treatment
- Number of animals per treatment group
- Route of administration
- Dose
- Daily dosing
- Dose day
- Animal checks during treatment
- Body weights during treatment
- Food consumption during treatment
- Sacrifice
- Maternal body weight at sacrifice
- Fetal body weight at sacrifice, individual
- Fetal body weight at sacrifice, combined
- Uterine weight
- Organ weights
- Pregnancy status
- Number of Live fetuses
- Number of Dead fetuses
- Fetal sex
- Number of Implantation sites
- Number of Corpora lutea
- Number of Resorptions
- Placental evaluation
- Fetal exam, any type
- Fetal external examination
- Fetal visceral examination
- Fetal skeletal examination





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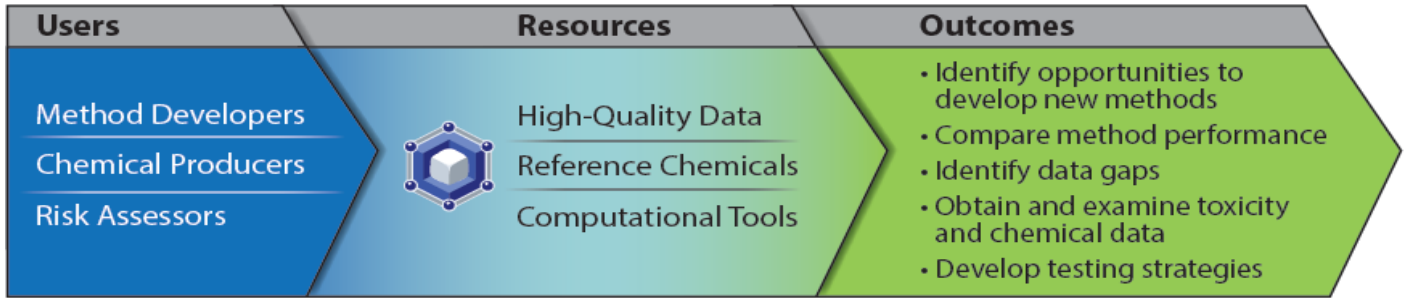
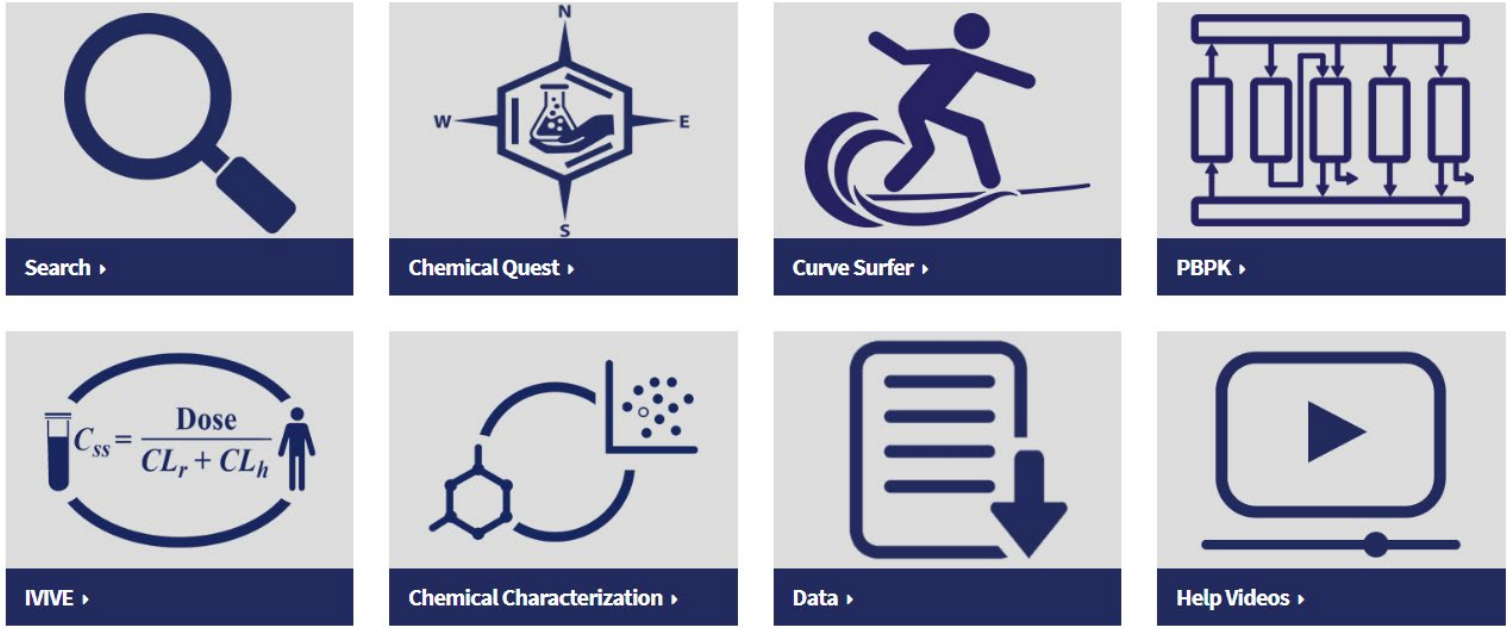
Division of Translational Toxicology

ICE: The Integrated Chemical Environment



Integrated Chemical Environment



Findable Accessible Interoperable Reusable

https://ice.ntp.niehs.nih.gov/

Bell et al. 2017 EHP
Bell et al. 2020 Tox In Vitro
Abedini et al. 2021 Comp Tox
Daniel et al. 2022 Front Toxicol

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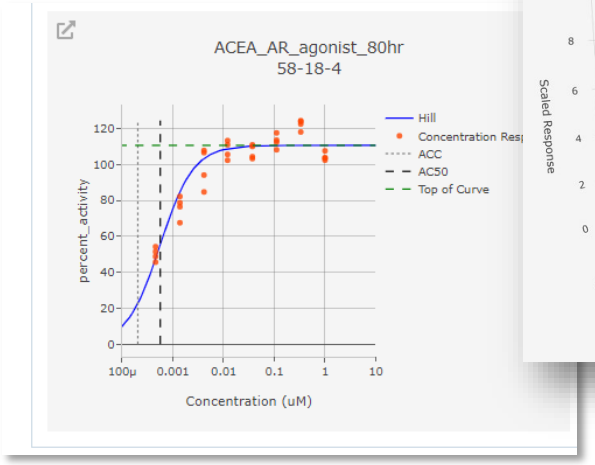
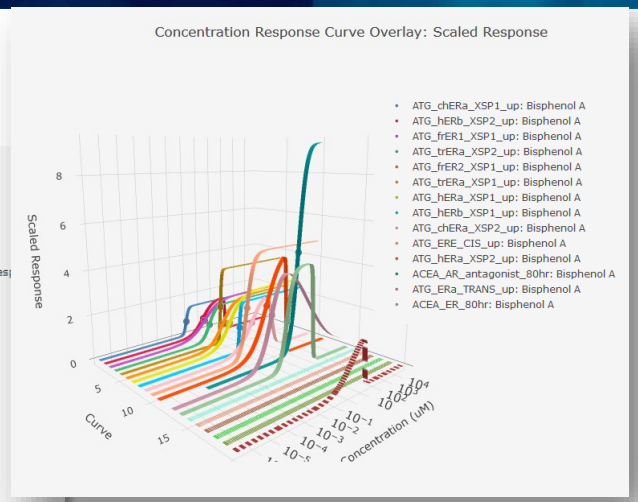
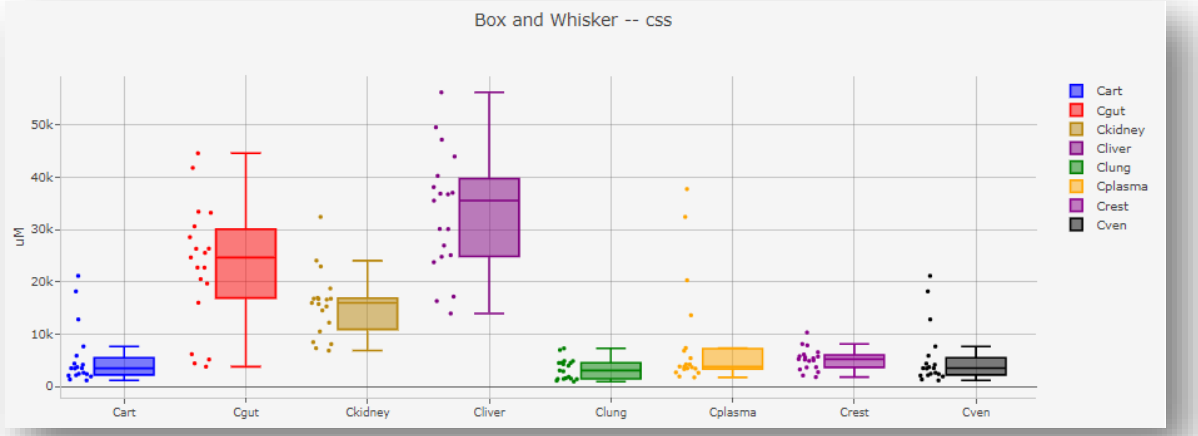
Division of Translational Toxicology

ICE Tools: Examples

HTS Data Exploration

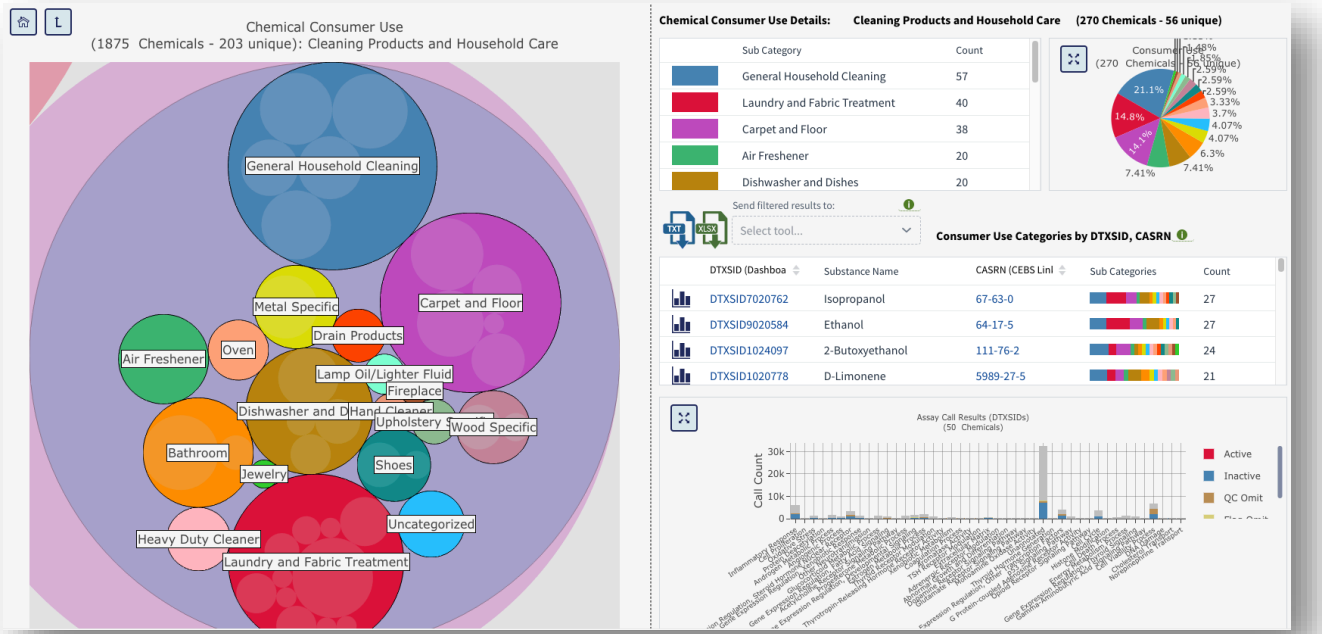






Predicting Chemical Exposure: Body Tissues, Consumer Products

Chemical Similarity



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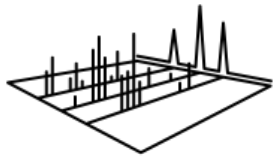
Risk Characterization of Triazole Fungicides using Human Biomonitoring and Mechanistic Data

Sampling

- Rural area, n = 140
  - Farrmers, n = 88
  - Women residents, n = 52
- Urban area, n = 50
  - Men, n = 25
  - Women, n = 25

Biomarkers



Urinary Triazoles

Internal Dose Indicator

Oxidative Stress Markers

Plasma Bile Acids

Liver Enzymes

Steroid Hormones

Cytome assay – Genotoxicity

Risk Calculations

HQ Calculation at the highest quantified value:

EDI = 6.31 µg/kg-bw/day

HQ = 2.1
Farmers

EDI = 8.77 µg/kg-bw/day

HQ = 2.9
Rural Women Residents

Marciano, et al. Regul Toxicol Pharm. 2024

Costa, et al. Chem Biol Interact. 2023

Unifal MG
Universidade Federal de Alfenas

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Association of *in vitro* molecular targets and human biomarker alterations

Integrated Chemical Environment v.4.0.2

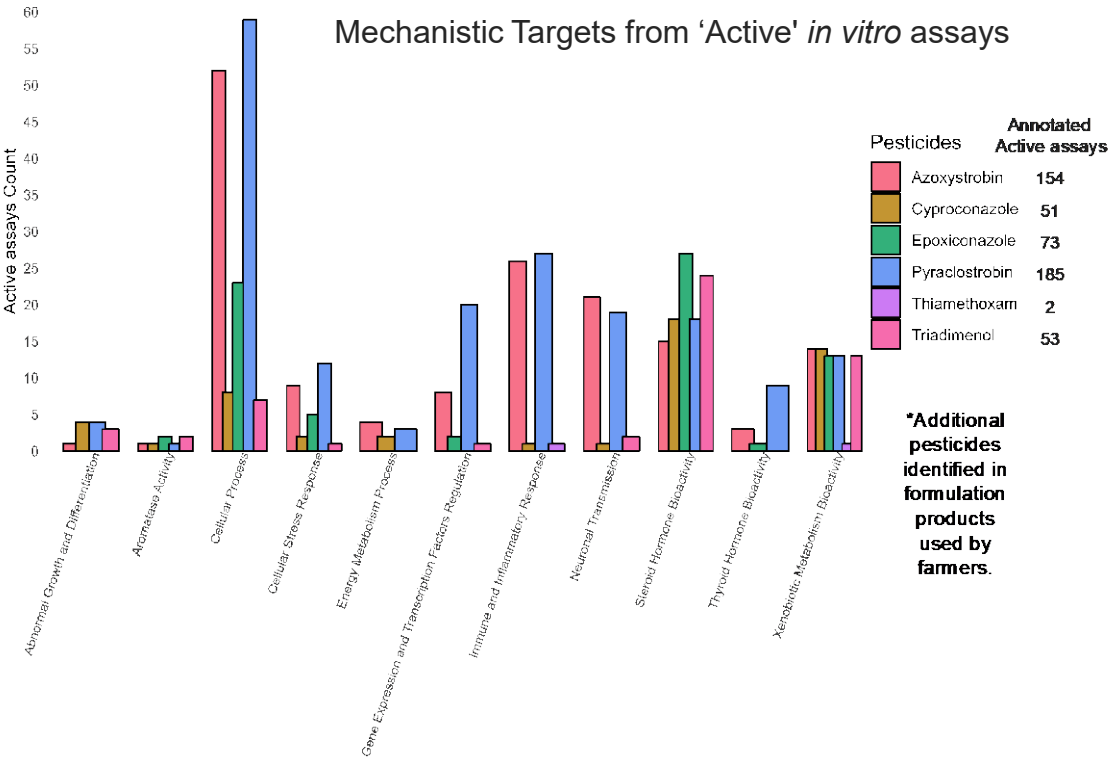
Concentration-Response Curves

- cHTS data from Tox21/ToxCast

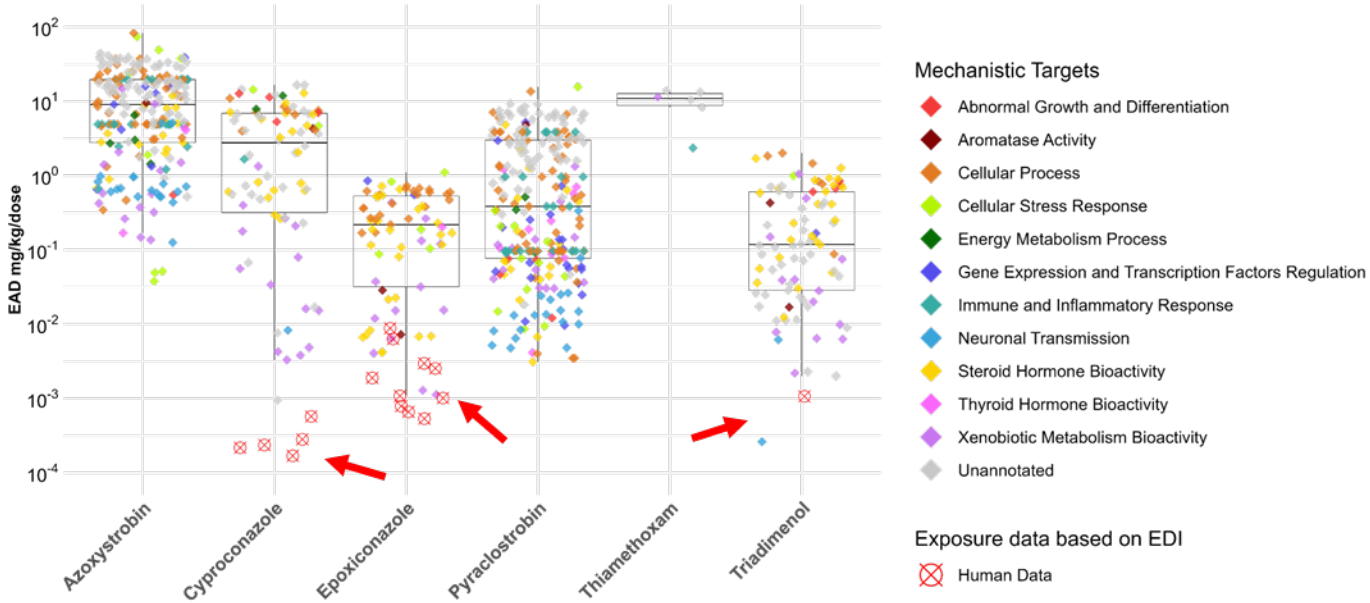
In Vitro to *In Vivo* Extrapolation

- Calculate equivalent doses from cHTS data
- Comparison with human exposure

Mechanistic Targets from 'Active' *in vitro* assays



Solve_oral_pbtk model



Marciano et al. 2024 STOTEN

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Integrated Approach to Testing and Assessment (IATA) DNT Case Study for Prioritization

Guidance document to inform on the DNT IVB, its usage and interpretation

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2023)13

Unclassified — English - Or. English
10 July 2023

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 4 July 2023

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

Series on Testing and Assessment
No. 377

IATA case studies to exemplify different regulatory needs

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)26

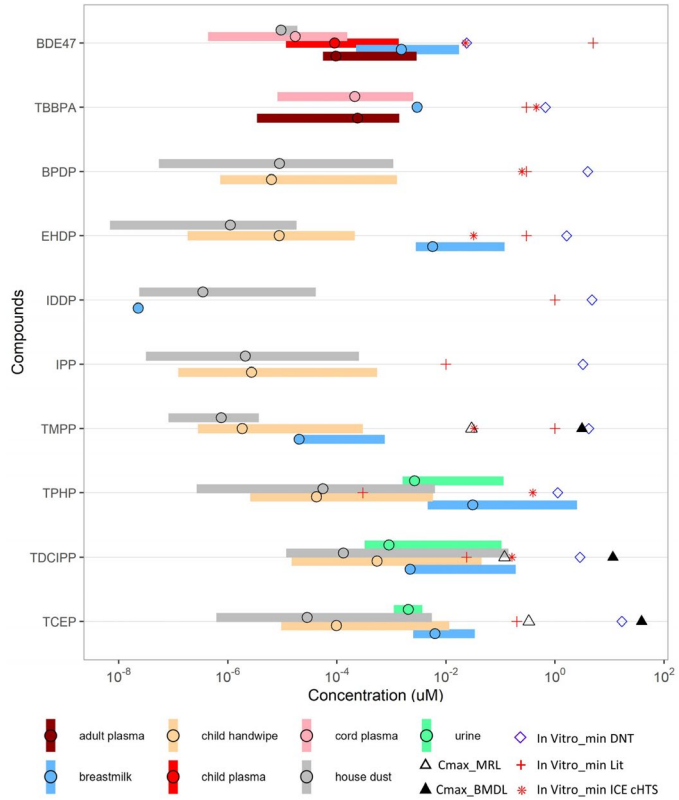
Unclassified — English - Or. English
1 September 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case study on the use of Integrated Approaches for Testing and Assessment for DNT to prioritize a class of Organophosphorus flame retardants

Series on Testing and Assessment
No. 364

Updated IATA case study for prioritization



Kreutz A et al. (2024)
Toxics 12(6):437

Integrated Chemical Environment

Advisory Committee to the NIH Director:
NAMS WORKING GROUP CHARGE

Catalyze the Development and Use of NAMs

- Identify the types of alternative methods and assess their general strengths and weaknesses for studying human biology, circuits, systems, and disease states

- Characterize the types of research, condition, or disease for which NAMs are most applicable or beneficial

- Articulate high-priority areas for NIH investment in the **use and development with human applicability** to:
  - Advance progress into understanding specific biological processes or states
  - Augment the tools and capabilities for biomedical research to complement and/or potentially replace traditional models

Implementing the ACD NAMs WG recommendations

RECOMMENDATIONS TO CATALYZE THE DEVELOPMENT AND USE OF NAMs	
Recommendation 1.	Prioritize the development and use of combinatorial NAMs.
Recommendation 2.	Establish resources, infrastructure, and collaborations to promote the use of interoperable, reliable, and well curated/high quality datasets produced from research using NAMs.
Recommendation 3.	Promote effective dissemination and interconnection of NAMs technologies.
Recommendation 4.	Invest in comprehensive training to bolster continuous advances in NAMs development and use.
Recommendation 5.	Facilitate multidisciplinary teams with expertise across technologies and the lifecycle of NAMs development and use.
Recommendation 6.	Promote social responsibility in both the creation and deployment of NAMs across the research lifecycle.
Recommendation 7.	Support and maintain coordinated infrastructure to catalyze effective and responsible NAM development and use.

New interactive database of validated/qualified NAMs

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Acknowledgments

The NICEATM Group

UNITED STATES ICCVAM
Advancing Alternatives to Animal Testing

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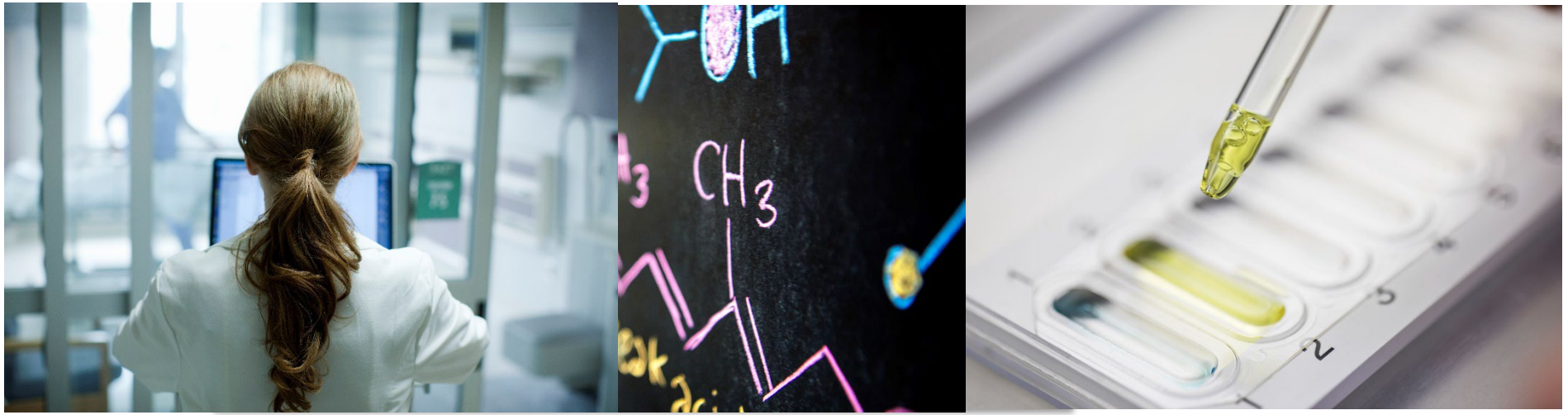
Integrated Chemical Environment

Now Available:
2022 – 2023 ICCVAM Biennial Report

Building Confidence in NAMs via Validation Standard Setting in a Revised OECD GD 34

Alison Harrill, PhD

Associate Director for Toxicology, Center for Computational Toxicology and Exposure



The views expressed in this presentation are those of the presenter and do not represent the views or policies of the U.S. EPA

OECD Guidance Document 34

Unclassified

ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

18-Aug-2005

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2005)14
Unclassified

OECD SERIES ON TESTING AND ASSESSMENT
Number 34

GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

- Expert group working to update
- Pre-dates development of many in vitro NAM and in silico approaches
- Pre-dates the institution of the Defined Approach
- Best practices have changed somewhat

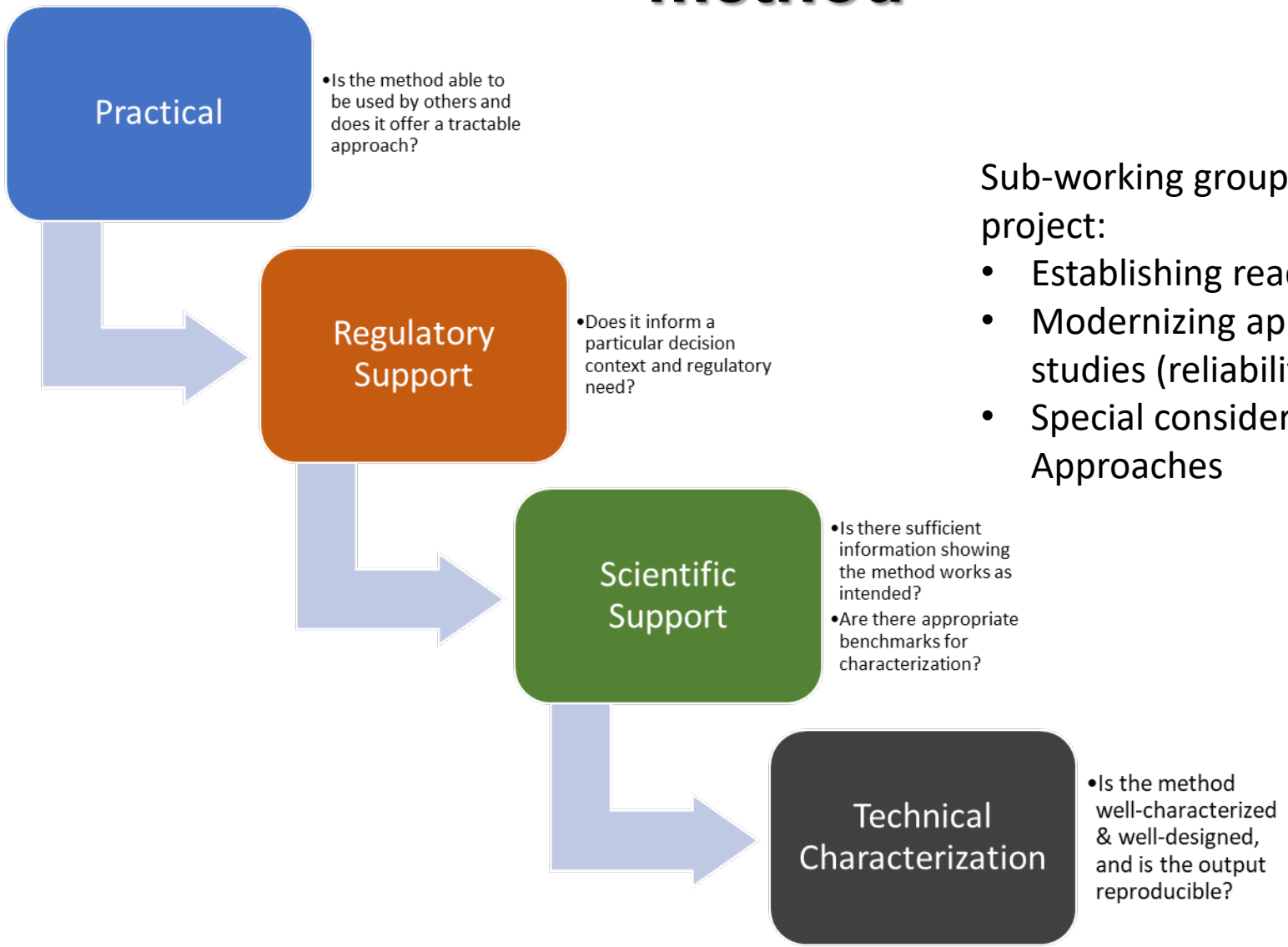
Definitions and Recommendations from Existing GD34 Guidance

- OECD definition of "validation" as a *"process based on scientifically sound principles (5)(6) by which the reliability and relevance of a particular test, approach, method, or process are established for a specific purpose."*
- Reliability is defined as *"the extent of reproducibility of results from a test within and among laboratories over time, when performed using the same standardised protocol."*
- The relevance of a test method describes *"the relationship between the test and the effect in the target species and whether the test method is meaningful and useful for a defined purpose, with the limitations identified."*
- Other recommendations…
  - "*the validation process should be flexible and adaptable*",
  - performance must be "*demonstrated using a series of reference chemicals*", and
  - "*evaluated in relation to existing relevant toxicity data*."

Mutual Acceptance of Data (MAD) system dictates data to be accepted from OECD TGs by all participating countries.

The foundation of the MAD system is the approximately 150 OECD Test Guidelines (methods).

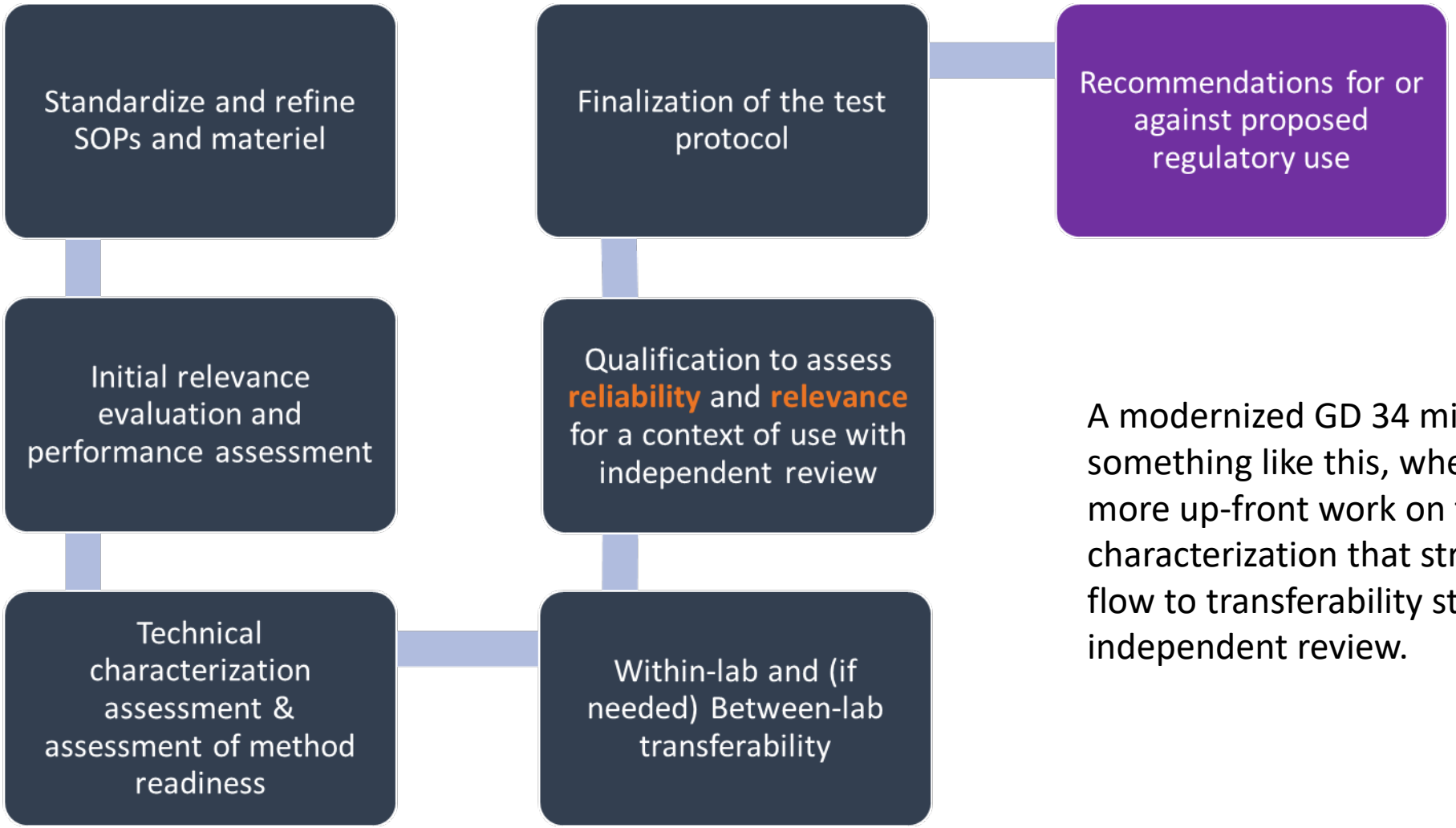
Building international scientific support for a new method



Sub-working groups of the GD 34 revision project:

- Establishing readiness criteria
- Modernizing approaches to transferability studies (reliability)
- Special considerations for Defined Approaches

Envisioning a more streamlined GD 34 process

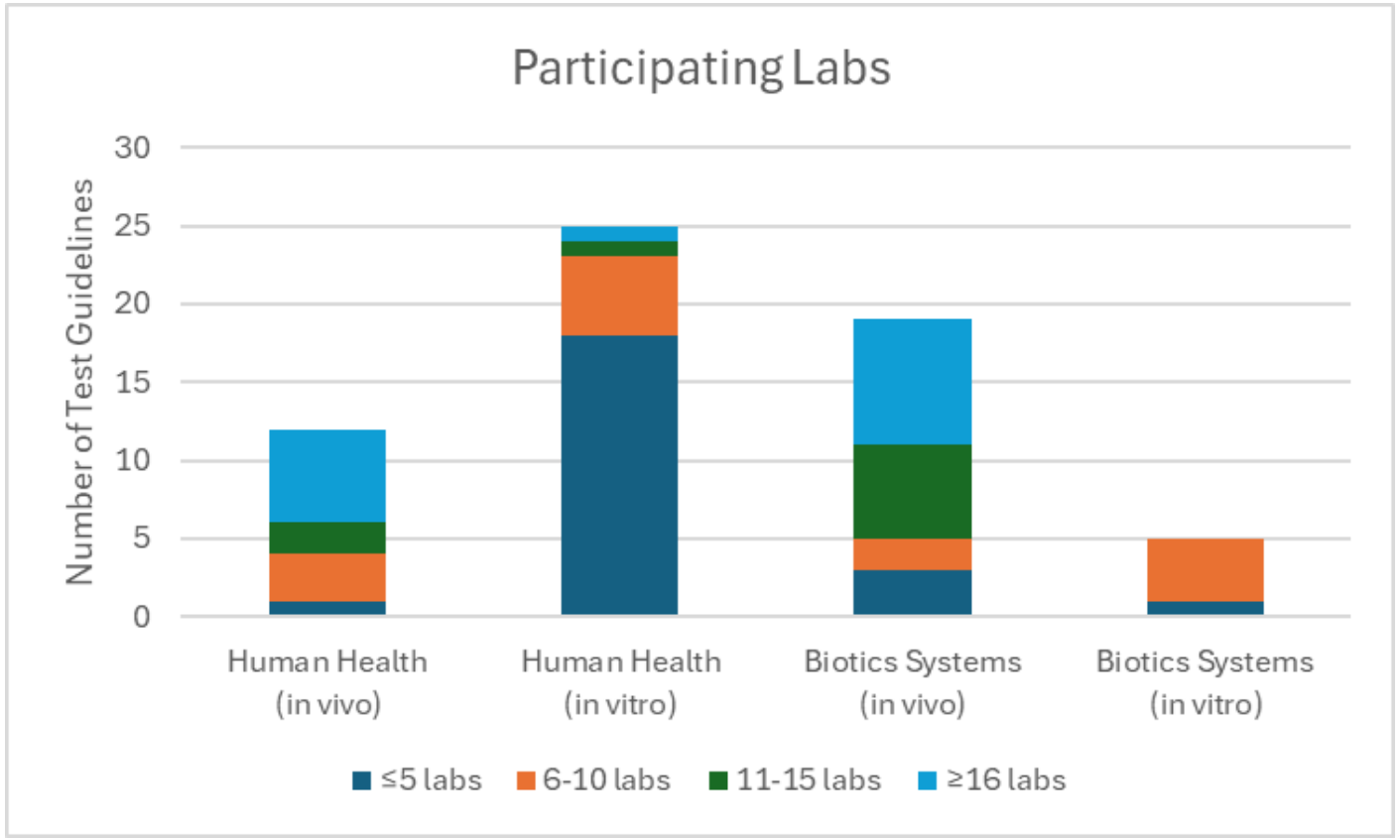


A modernized GD 34 might look something like this, where there is more up-front work on technical characterization that streamlines flow to transferability studies and independent review.

What do validation efforts typically entail for OECD Test Guidelines?

- Transferability studies are typically performed across multiple labs to assess the variability of point estimates of the readout across labs (how reproducible is the result?)
- While all of the OECD Test Guidelines have been *standardized* into a formal method, a subset of these have been formally 'validated' using between-lab transferability studies
- To assess assay reliability in a validation study, performance is frequently assessed in both *quantitative terms* (variability around a point estimate) AND *qualitative terms* (how well the assay predicts the endpoint)
- To validate an assay, lists of reference chemicals with association (or lack thereof) to the measured effect are used to assess assay performance and reproducibility (reliability)

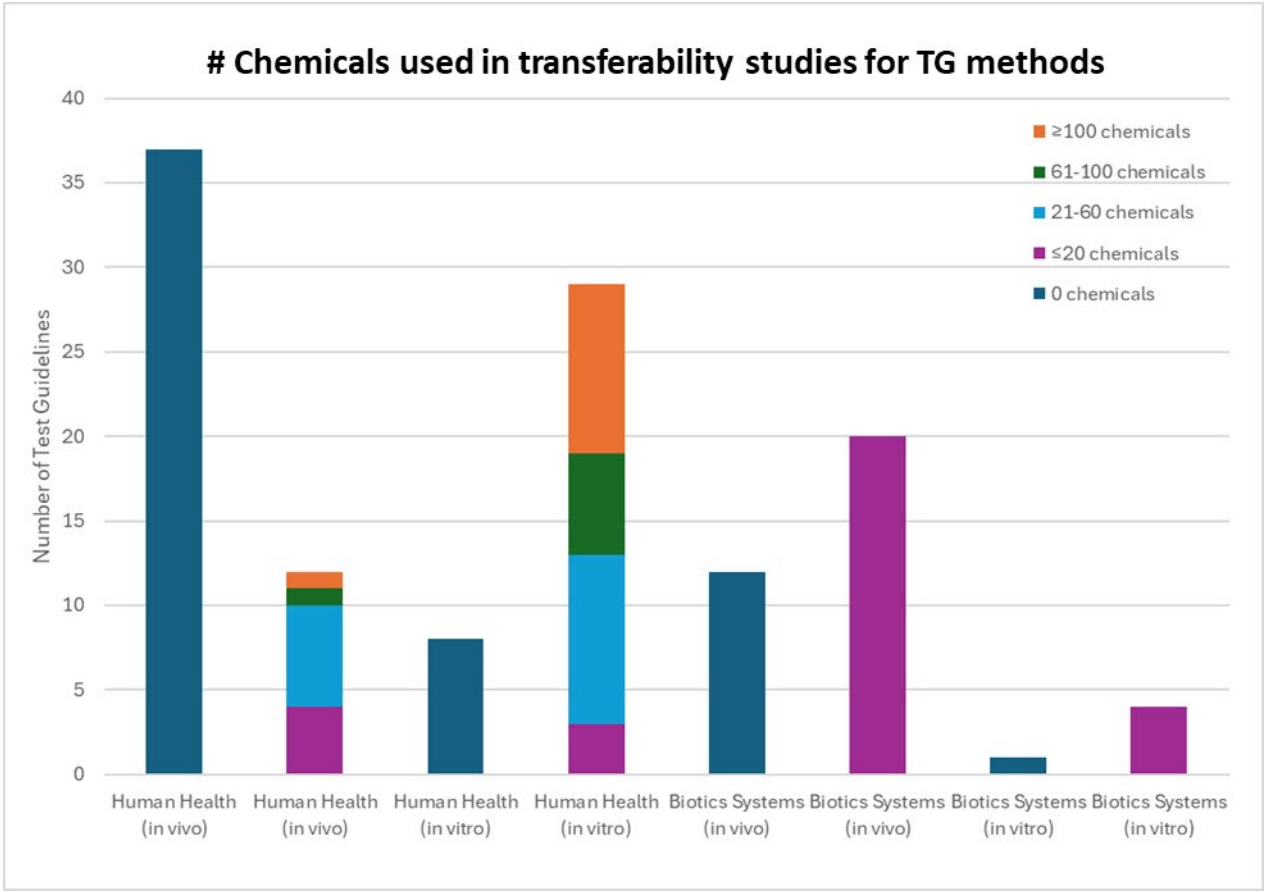
Numbers of labs involved in transferability and validation studies that led to OECD TGs



- Not all OECD records of transferability studies had accessible data on the numbers of participating labs
- Participating lab numbers were based on the particular phase of validation that had greatest number of participating labs

Not all OECD TGs have undergone transferability studies to assess method reliability

Test Guideline Type	Total TGs	Total with Transferability	% with Transferability Studies
Human Health (in vivo)	49	12	24%
Human Health (in vitro)	36	29	81%
Biotics Systems (in vivo)	34	21	62%
Biotics Systems (in vitro)	6	5	83%



Expectations for the size of reference chemical sets for NAMs exceeds those of *in vivo* TGs

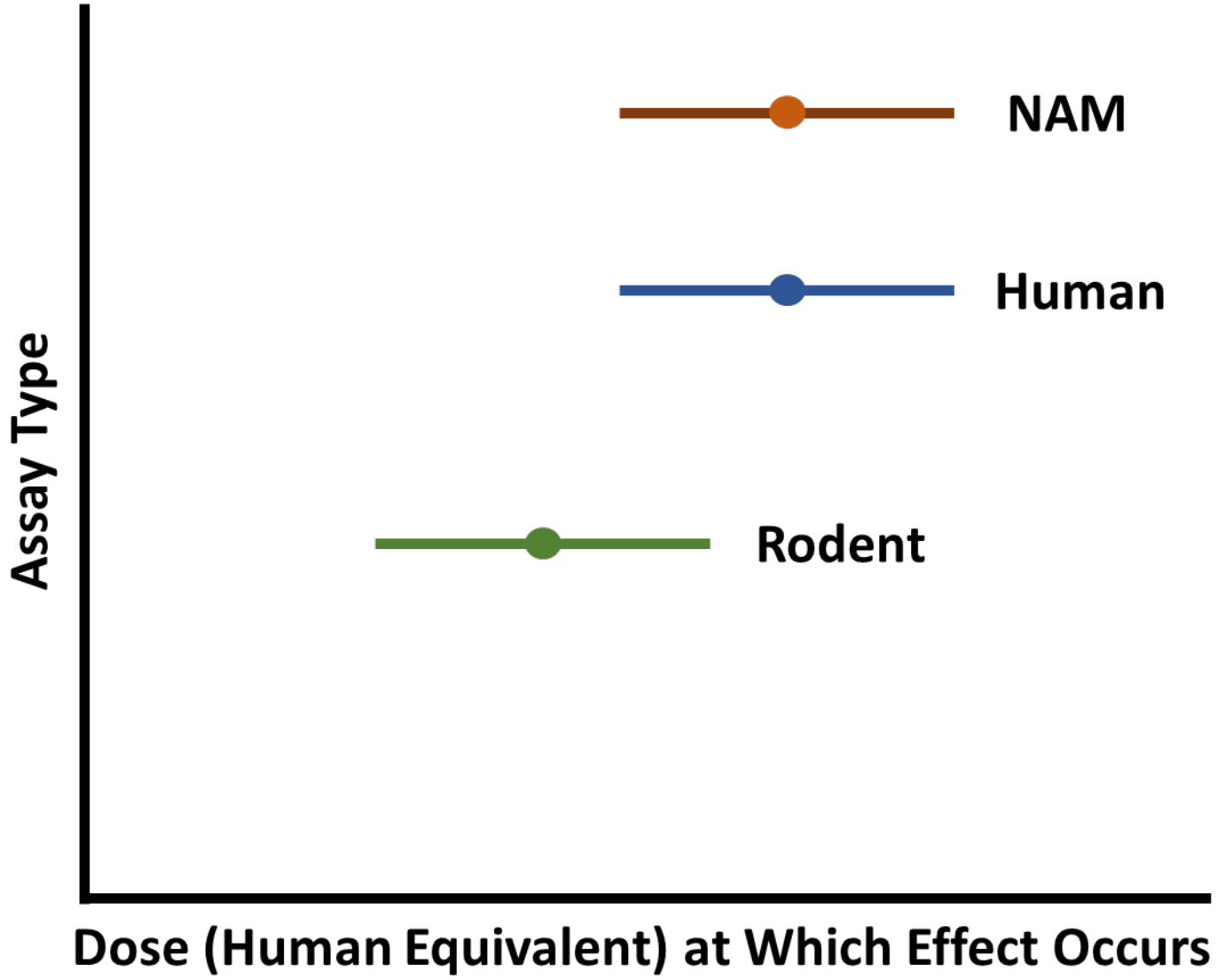
- Data were extracted from 125 TGs covering vertebrate and invertebrate species and associated in vitro alternatives
- *In vitro* methods for both human health and biotic systems had a larger proportion of TGs that had been formally validated (81 and 83%) and had larger numbers of chemicals associated with the validation studies overall
  - 55% of validated *in vitro* human health TGs > 61 chemicals, 34% had greater than 100 chemicals
  - 16% of human health *in vivo* studies with >60 chemicals, only 8% had greater than 100 chemicals
  - Majority of all biotic systems validation studies had 20 or fewer chemicals (1 in vitro with 61-100 chemicals)
- *In vitro* test methods overall have larger chemicals sets for transferability and greater numbers of validation studies, although *in vivo* human health appeared to have larger numbers of participating labs
- One reason for the increase in numbers of test chemicals being used is that assessing reliability and relevance may be challenging, and doing so requires appropriate benchmarks.

Data are preliminary and analysis is ongoing

EPA efforts may inform scientific support elements that relate to reliability and relevance

- In what contexts do we need to require inter-laboratory validation versus ensuring the method is standardized?
- How does the human relevance of the new approach compare to the "gold standard" or traditional test (if a comparator assay exists)?
- Is the assay result reliable when compared to repeated studies using traditional approaches?
- How do we contextualize a result from a new method, particularly if the result has more uncertainty than the traditional approach?

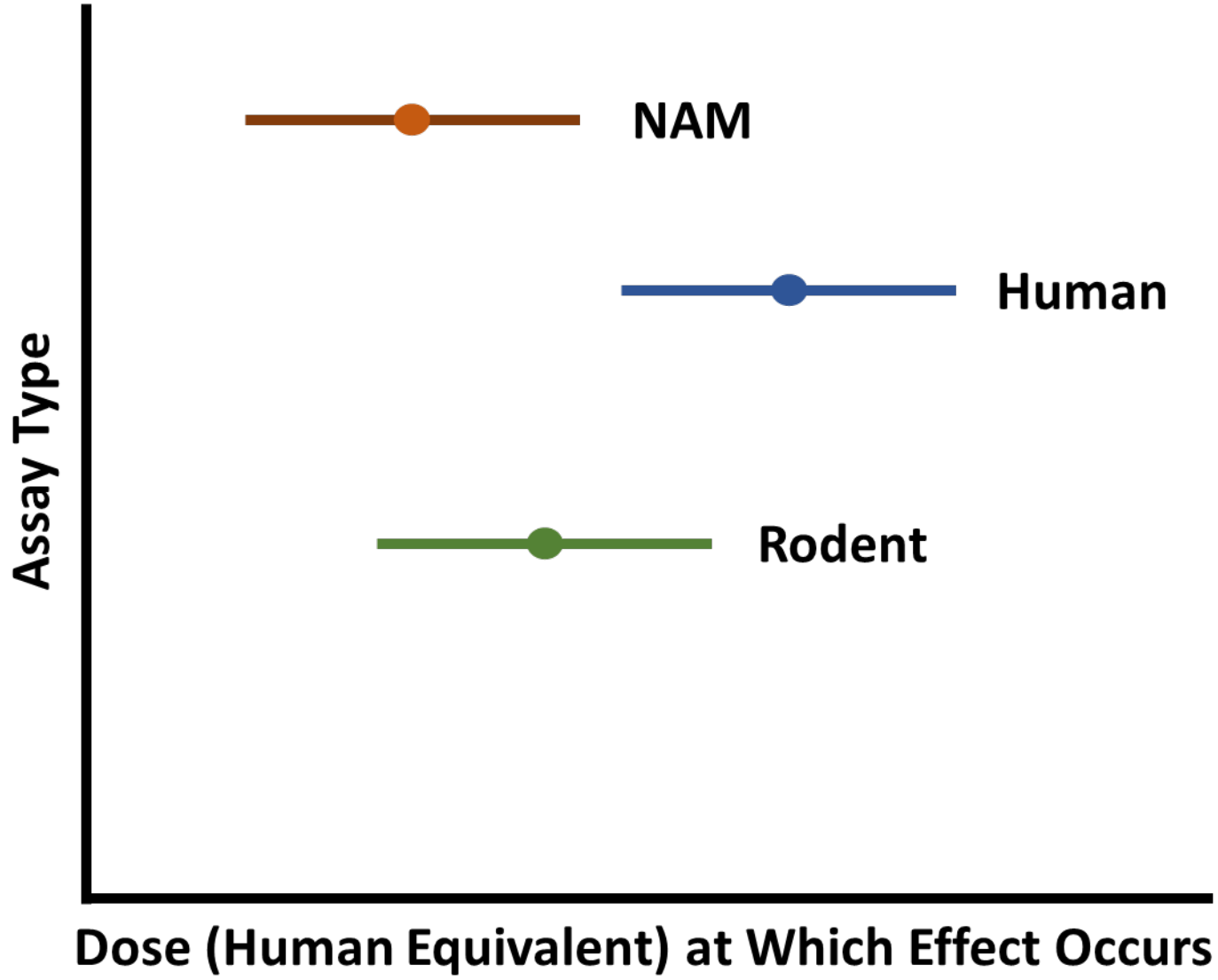
Relevance: Cross-species concordance



Some expect that a human cell-based NAM will be more predictive of human responses than an animal-based NAM due to species differences.

If prediction is highly concordant, then the point estimate of the effect should overlap the human in-life point estimate.

Relevance: Cross-species concordance



However, because NAMs are often MOA-based, the sensitivity may be greater, which means that the dose-effect linkage is **less concordant** with human, but potentially may be **more protective** of human responses.

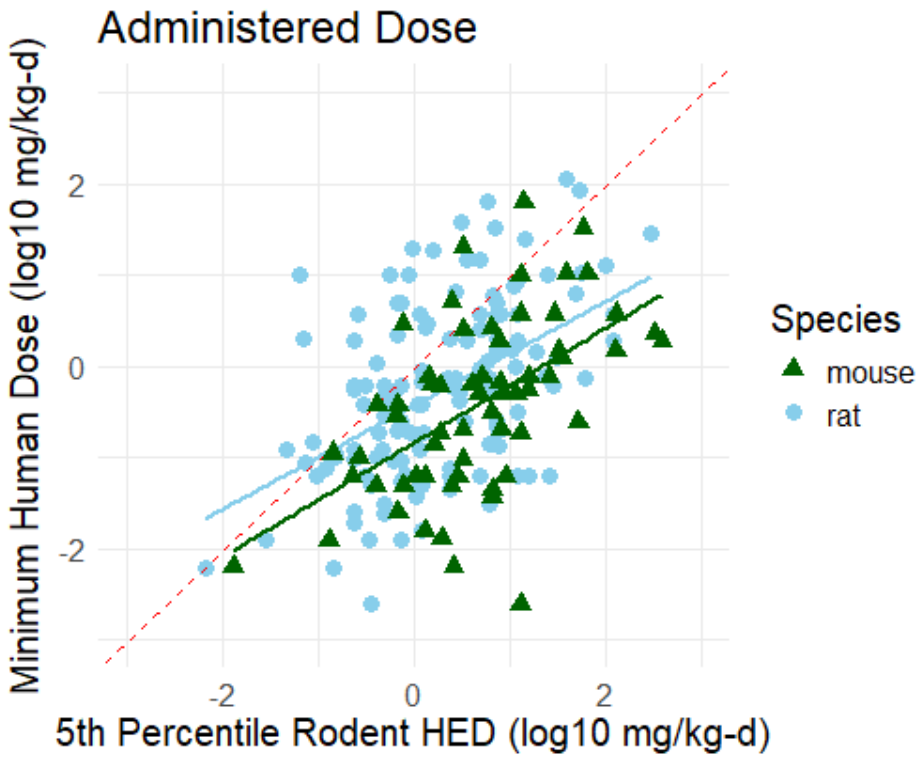
Most frameworks for confidence building require predictions to be "as good or better" than current models.

A good starting place would be to assess concordance between doses and adverse effects that occur in both humans and animal models for the same chemicals.

Benchmarks for assessing relevance in validation studies

- We can leverage pharmaceutical data to assess benchmarks for
  - *Quantitative* concordance – dose matching across species
  - *Qualitative* concordance – hazard matching across species
- Pharmaceutical data have the benefit of providing both human (clinical) and rodent (non-clinical) dose-effect linkage data

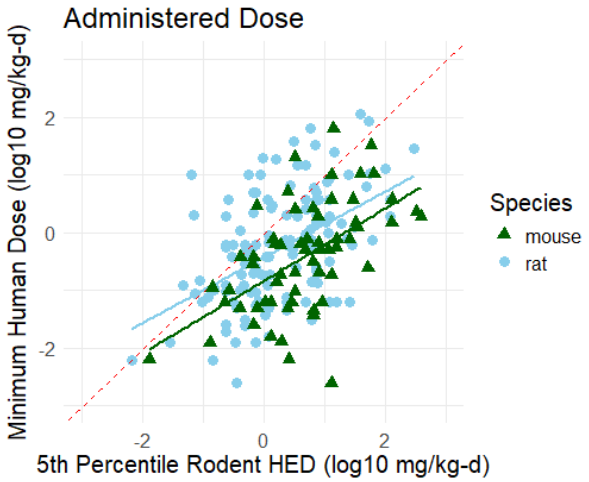
Benchmarks for human relevance – dose concordance in pharmaceuticals

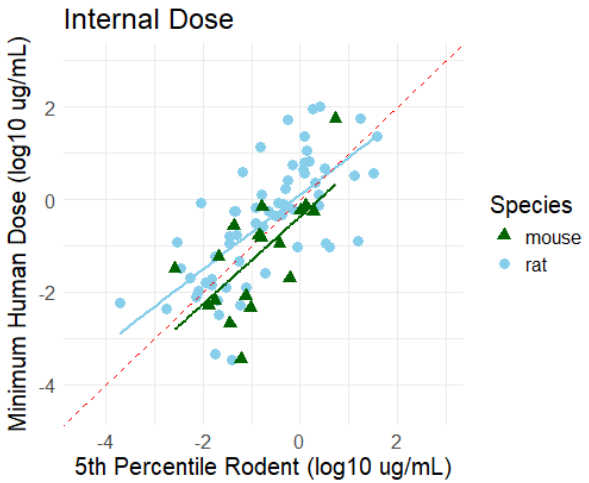


- Data derived from extracting relevant information from new drug applications (NDA) to the FDA
- Rodent doses adjusted to human equivalent dose
- Multiple studies submitted on single compounds – 5th percentile of the distribution of POD values for each species used as a conservative lower bound estimate

- Human administered dose associated with adverse event (AE) is not strongly concordant with mouse or rat, with mouse potentially more protective than rat

Benchmarks for human relevance – dose concordance in pharmaceuticals





- Data derived from extracting relevant information from new drug applications (NDA) to the FDA
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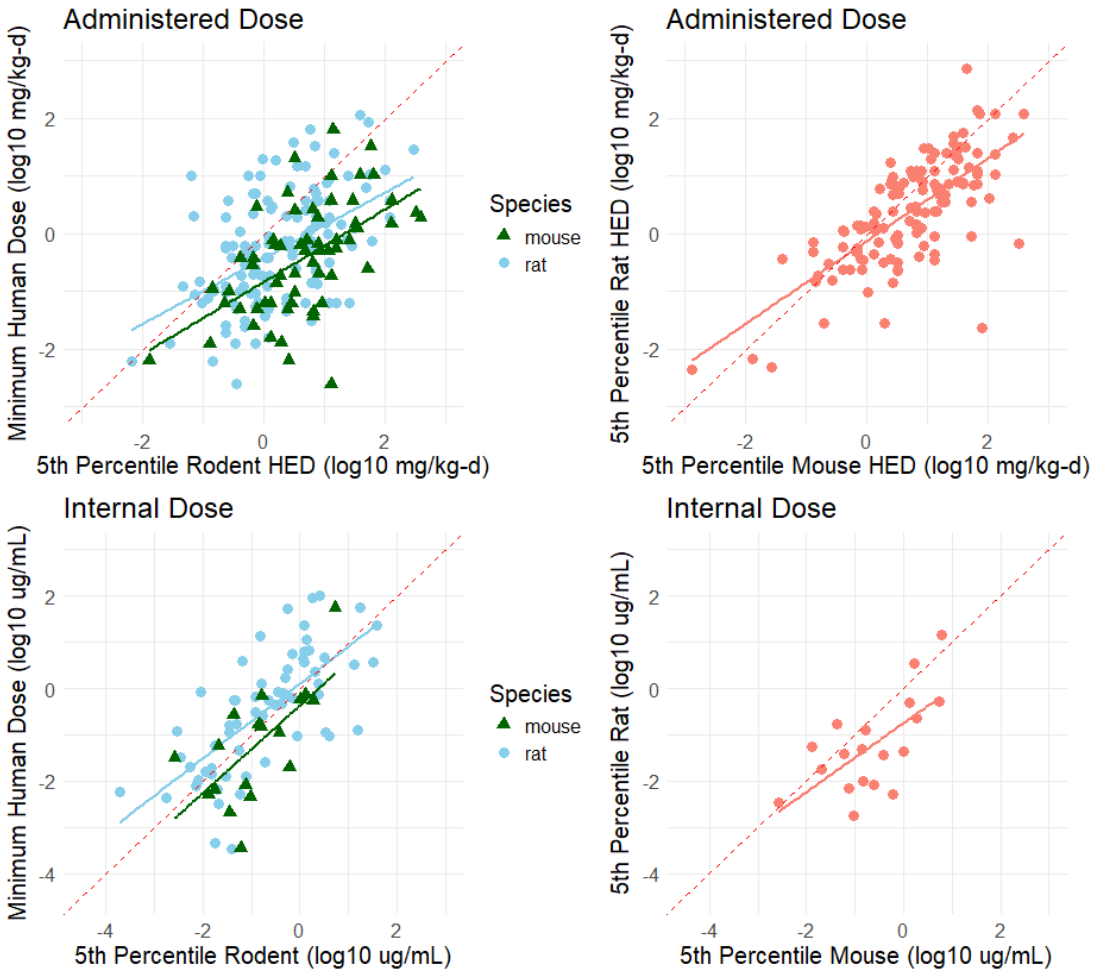
- Human administered dose associated with adverse event (AE) is not strongly concordant with mouse or rat, with mouse potentially more protective than rat
- For drugs where we have internal dose (PK) information – internal dose for human associated with AE is more concordant with rodent

Benchmarks for human relevance – dose concordance in pharmaceuticals



- Rat tends to be more concordant with mouse than either rodent species is with human
- ToxCast assay-> human is similar to human->rodent
- Provides a starting point for assessing where dose concordance should fall for NAM->human concordance

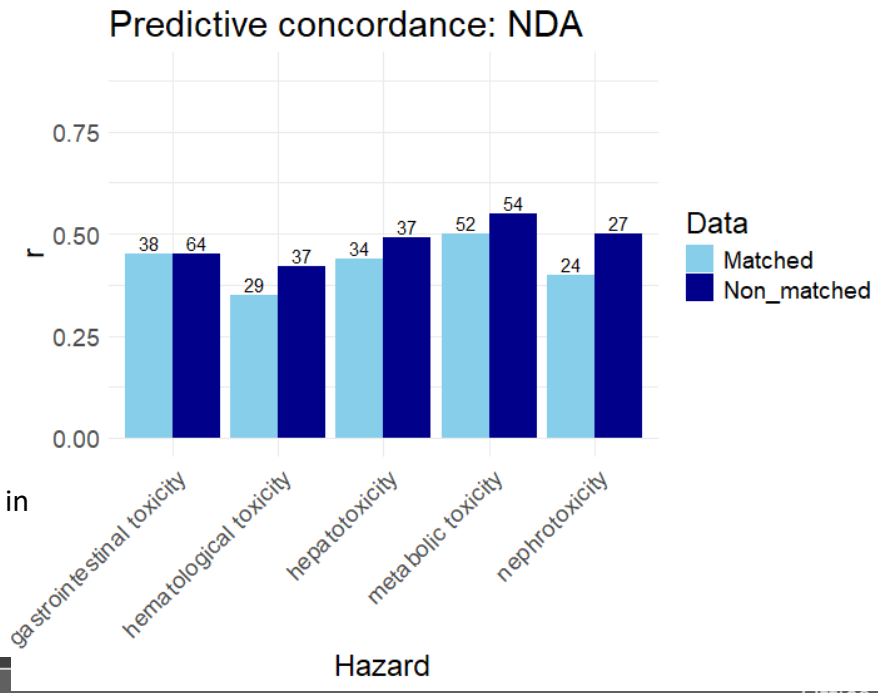
		Correlation statistics				Regression statistics	
	N	r	MAD	Mean bias	RMSD	R^2	RMSE
Comparison with rodent human equivalent dose effect levels							
Human – Rat	134	0.49	0.85	-0.52	1.1	0.24	0.84
Human – Mouse	61	0.56	1.13	-1.1	1.4	0.31	0.79
Rat – Mouse	133	0.72	0.57	-0.35	0.71	0.52	0.6
Comparison with internal dose adjusted effect levels							
Human – Rat	64	0.7	0.79	0.26	0.97	0.49	0.91
Human – Mouse	18	0.67	0.75	-0.31	0.94	0.44	0.89
Rat – Mouse	19	0.65	0.8	-0.57	0.98	0.43	0.75

Benchmarks for human relevance – qualitative concordance for particular adverse events

- Nonclinical and clinical trial data have been analyzed from the perspective of *qualitative* concordance (presence/absence of effects)
- Monticello *et al.* 2016
  - Low PPV (~30%) but high NPV (~86%)

Current nonclinical testing paradigm enables safe entry to First-In-Human clinical trials: The IQ consortium nonclinical to clinical translational database

Thomas M. Monticello[a,*], Thomas W. Jones[b], Donna M. Dambach[c], David M. Potter[d], Michael W. Bolt[e], Maggie Liu[f], Douglas A. Keller[g], Timothy K. Hart[h], Vivek J. Kadambi[i]

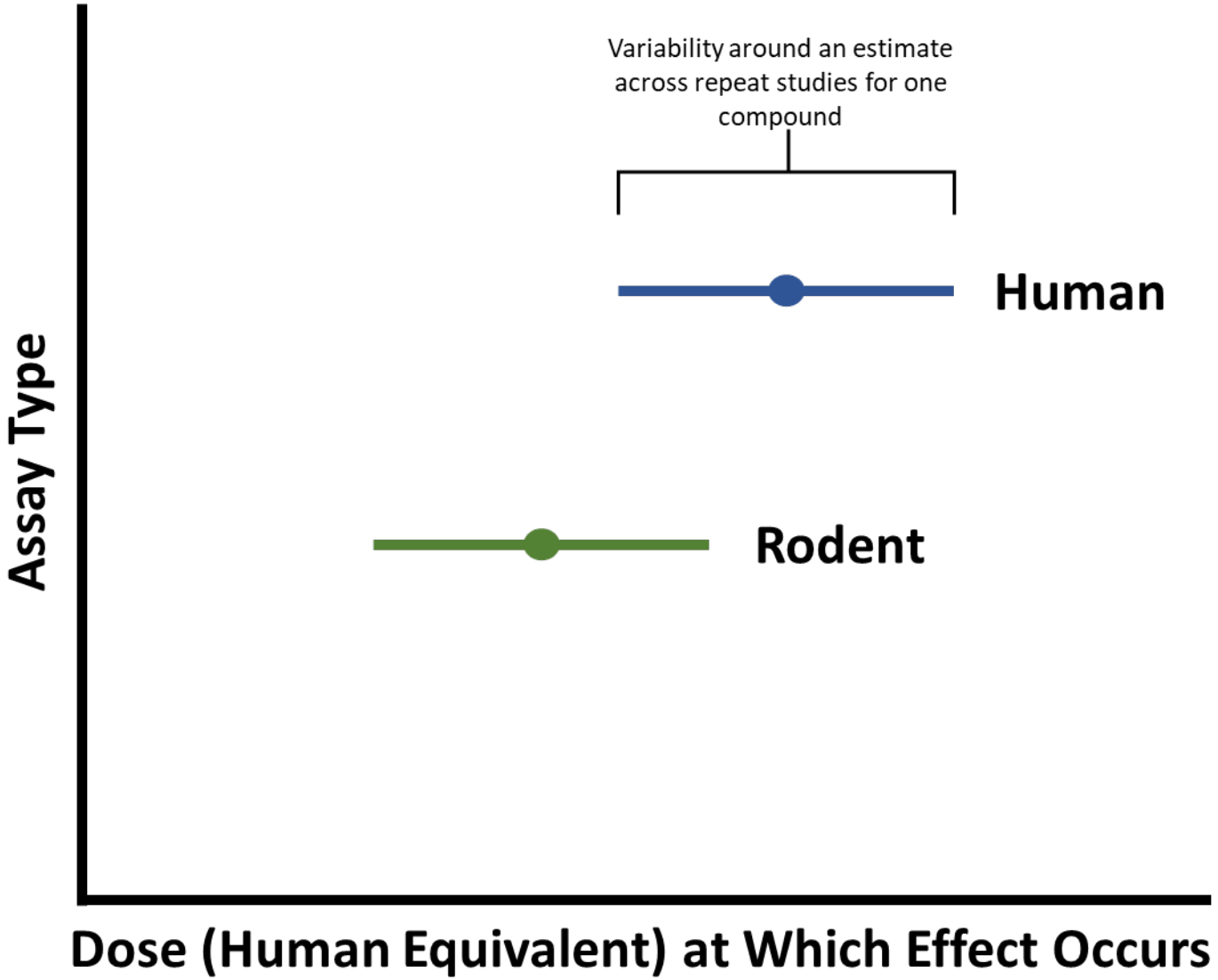


Bar labels = # of drug compounds in analysis

There is a low concordance between rodent and human both when the effects are matched (hepatic-hepatic) and when the effects are unmatched (hepatic-any other adverse effect).

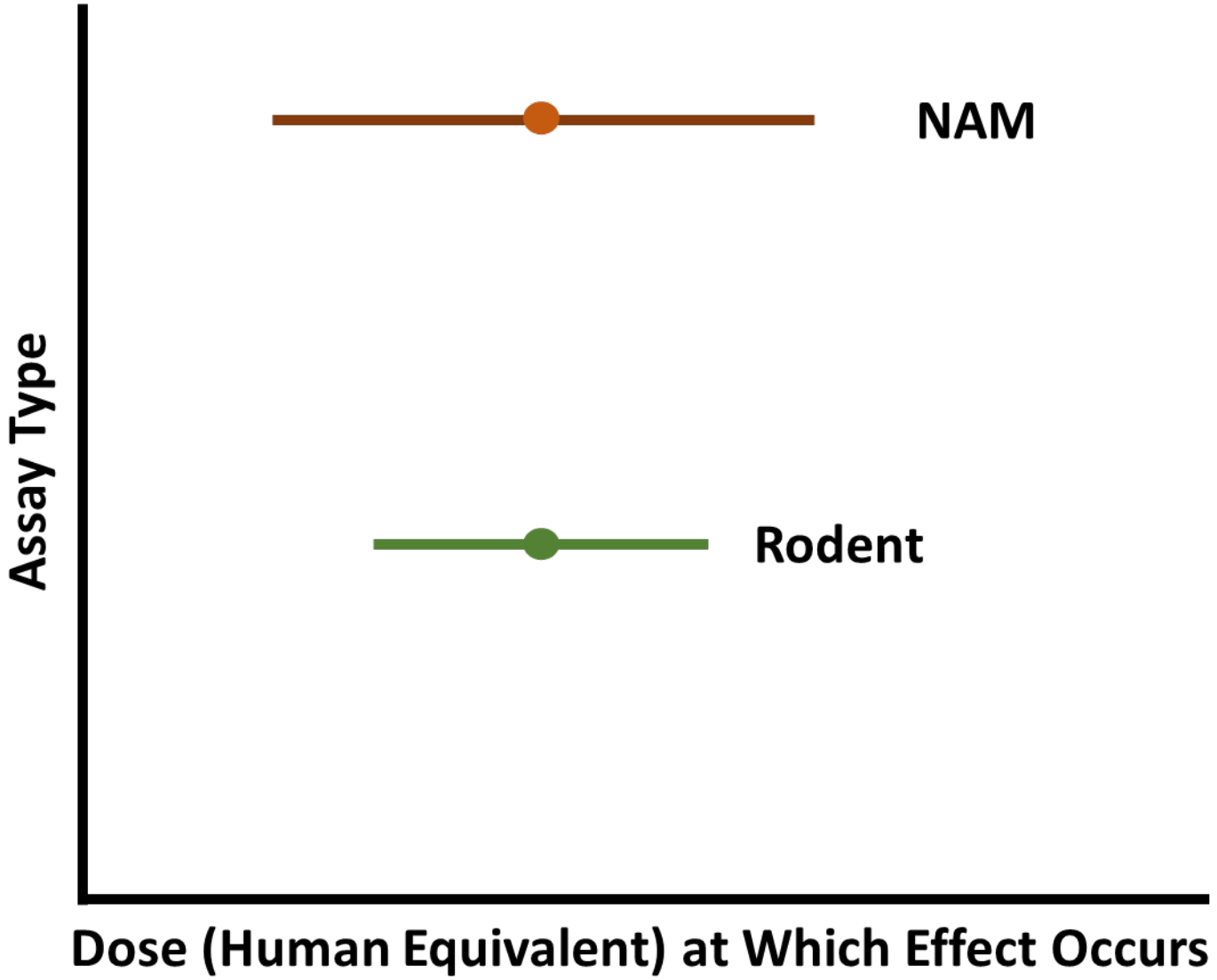
We can use these concordance estimates as potential benchmarks when assessing expectations for *in vitro* systems to predict effects in the human population.

Reliability: What is an acceptable amount of variability around the assay estimate across studies?



For environmental chemicals, we typically do not have repeated studies on chemicals in human populations.

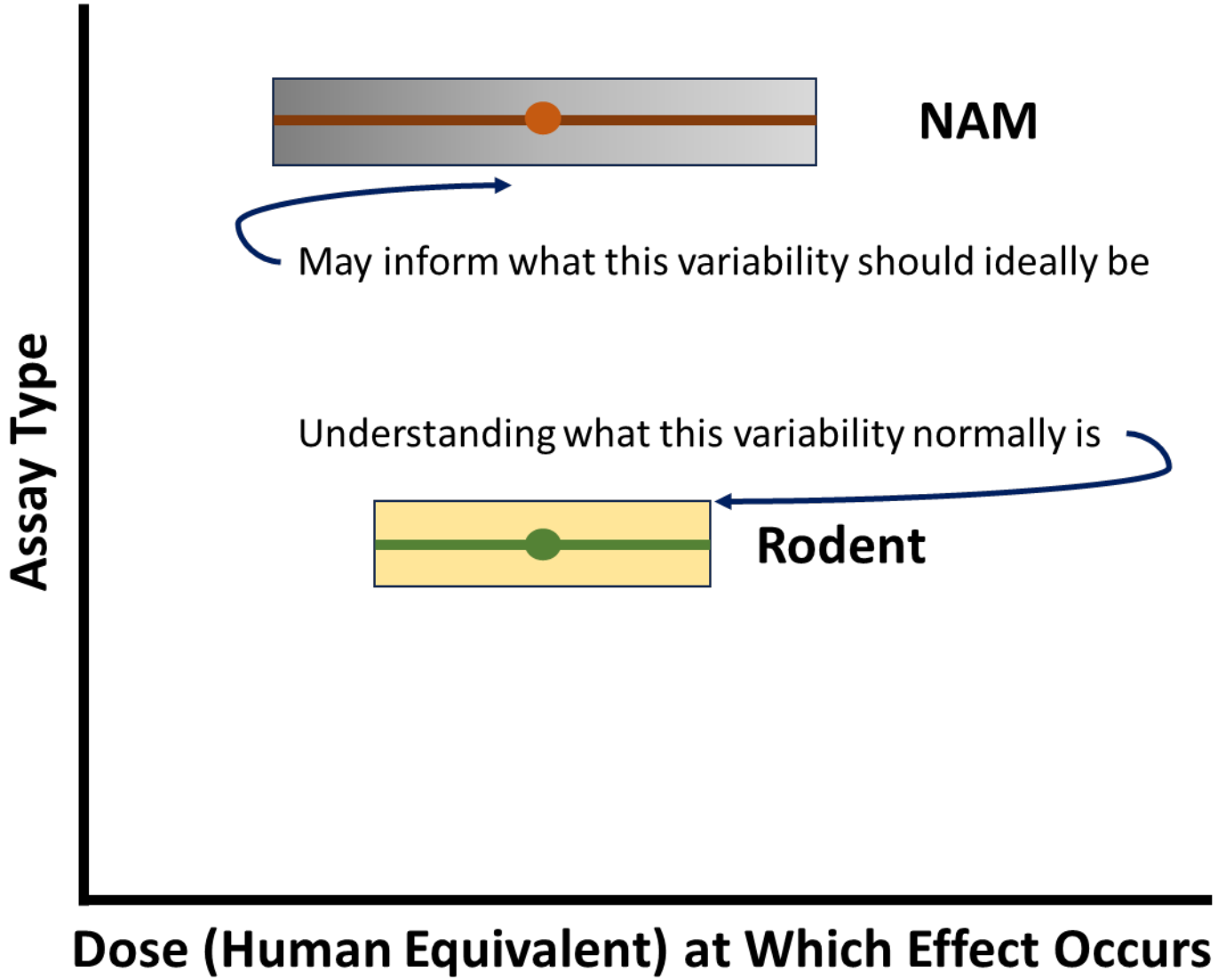
Reliability: Benchmarks for the amount of variability around the assay estimate



We may be able to estimate variability around point estimates from repeat rodent studies as an appropriate benchmark for NAMs.

These benchmarks would inform reliability estimates from derived from between-lab reproducibility studies.

Reliability: Benchmarks for the amount of variability around the assay estimate



What is an expected level of variance around a point estimate?

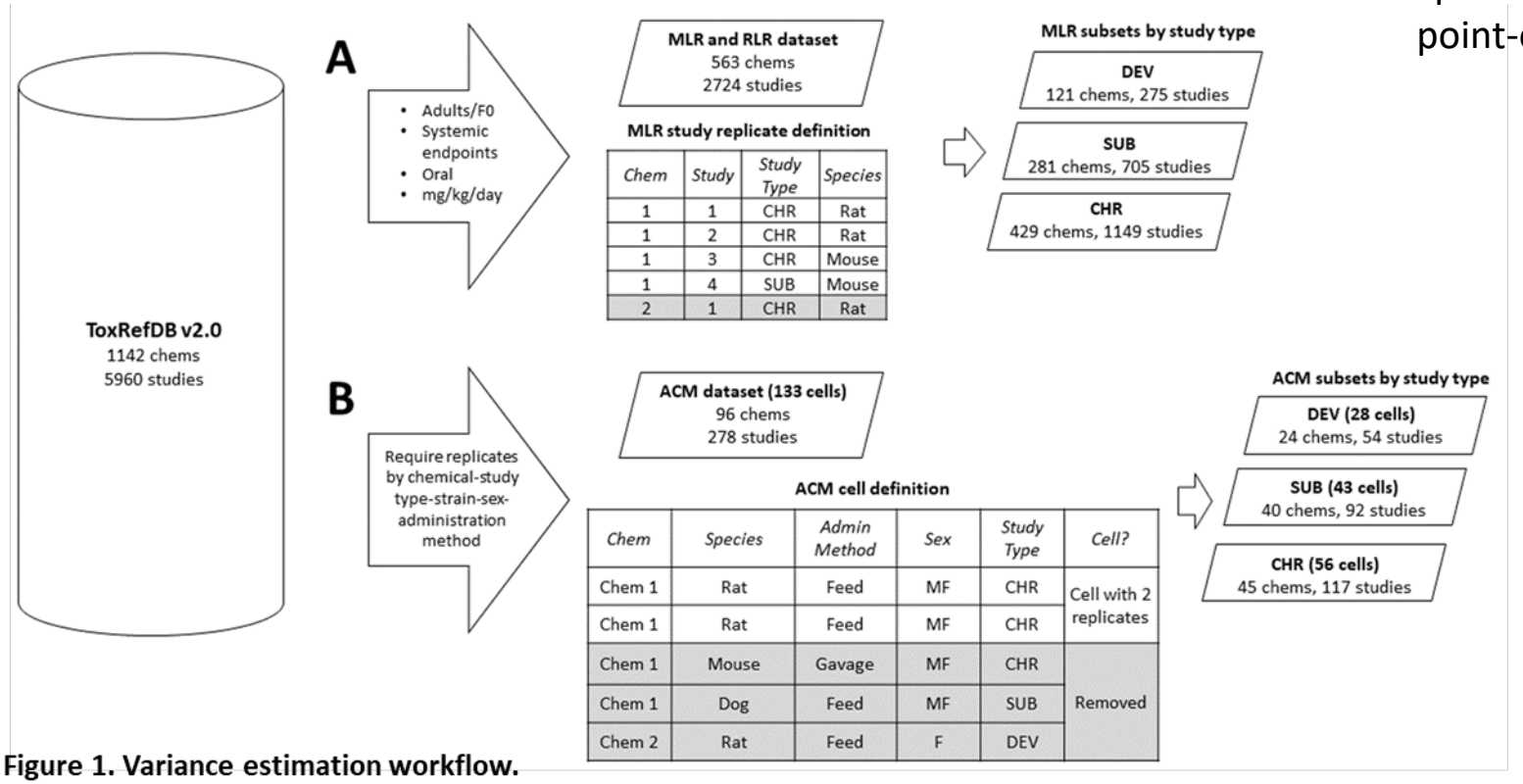
A potential benchmark is to examine variability around a POD using estimates from multiple animal studies on a chemical as a measure of reliability.

To do this, we can leverage ToxValDB, EPA's largest repository of published in-life study data.

Reliability: Benchmarks for variability around the POD in repeated studies

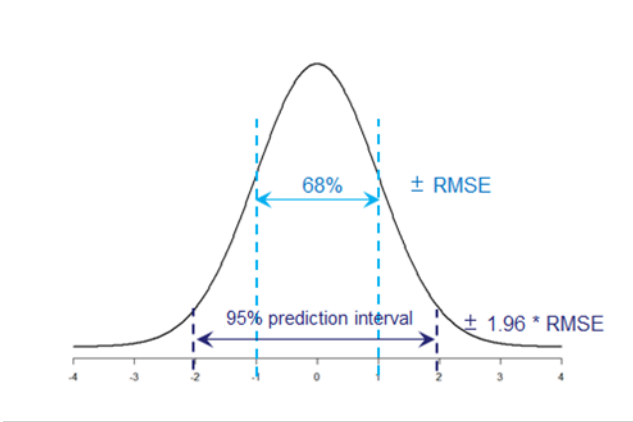
28 different statistical models to approximate total variance, unexplained variance, and the spread of the residuals from statistical models of study-level points-of-departure in adult animals.

The variance, as approximated by RMSE, is **0.45-0.56 log_{10}-mg/kg-bw/day**. This helps us estimate a minimum prediction interval for a new estimation of study-level point-of-departure and to set a benchmark for NAMs to predict these values.



Chem	Study	Study Type	Species
1	1	CHR	Rat
1	2	CHR	Rat
1	3	CHR	Mouse
1	4	SUB	Mouse
2	1	CHR	Rat

Chem	Species	Admin Method	Sex	Study Type	Cell?
Chem 1	Rat	Feed	MF	CHR	Cell with 2 replicates
Chem 1	Rat	Feed	MF	CHR	
Chem 1	Mouse	Gavage	MF	CHR	Removed
Chem 1	Dog	Feed	MF	SUB	
Chem 2	Rat	Feed	F	DEV	



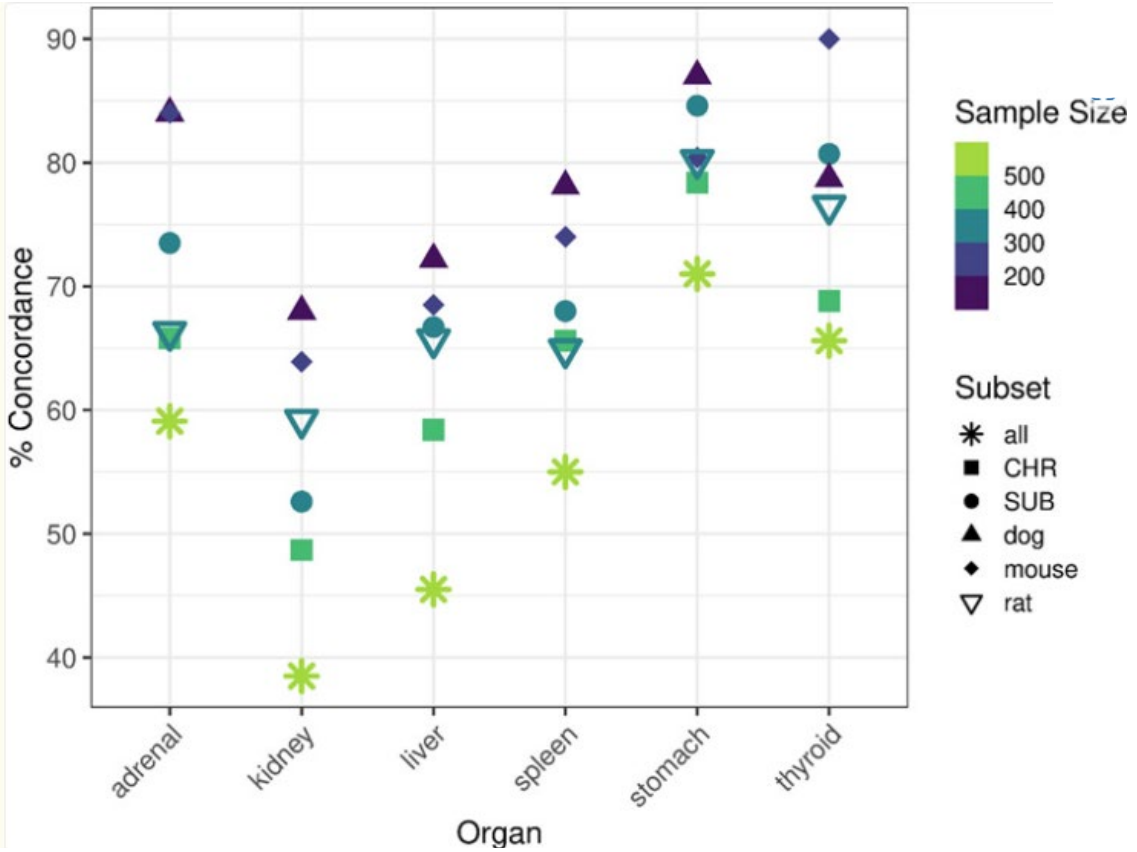
Pham *et al., Comp Toxicol., 2020*

Figure 1. Variance estimation workflow.

CHR = chronic; DEV = developmental (adults only); SUB = subchronic; cells are defined by the factor of all categorical variables; MF = males and females; F = females; MLR = multilinear regression; POD = point of departure; RLR = robust linear regression; ACM = augmented cell means.

Reproducibility of organ-level effects in repeat dose animal studies

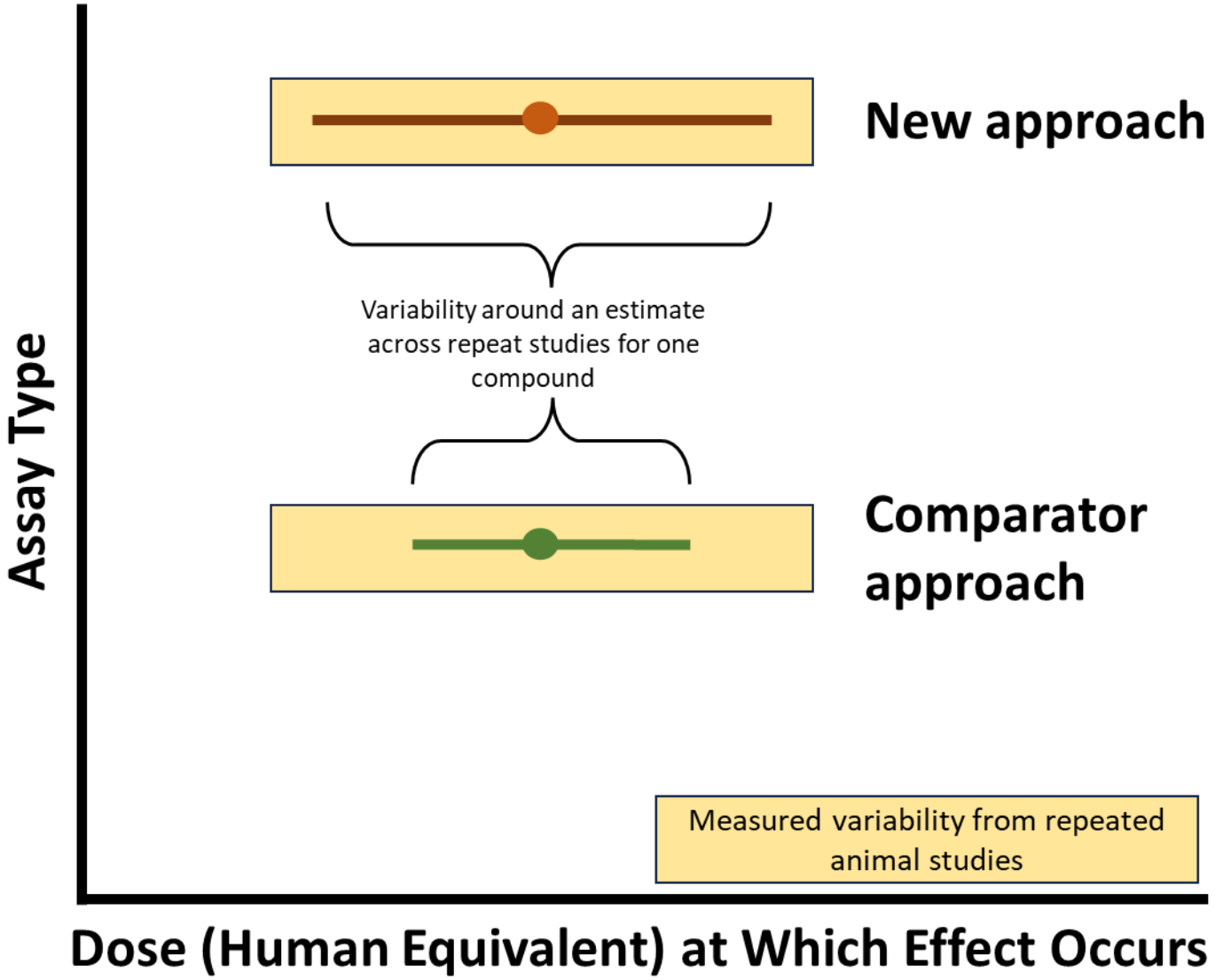
Qualitative reproducibility of organ-level effect observations in repeat dose studies of adult animals



- Data for 538 chemicals across 2284 studies
- Study replicates considered at the chemical level
- Liver and kidney were associated with the greatest number of studies with positive reporting, yet had the lowest concordance of findings
- Can be used as potential benchmark for qualitative concordance of endpoint effects

Paul-Friedman et al. Comput Toxicol. 2023 Nov;28:1–17

Reliability: Contextualizing Variability of a New Method



If reproducibility in the new method is within the limits of historical in vivo data, but the variability of the new method is still greater than a comparator test, is there value in using the new approach?

Value of Information (VOI) can be used as a decision-making framework to contextualize trade-offs in uncertainty around the POD, costs, and time to decision between a choice of methods than can lead to informing a decision.

Value of Information: EPA-developed framework

DOI: 10.1111/risa.13931

ORIGINAL ARTICLE

A value of information framework for assessing the trade-offs associated with uncertainty, duration, and cost of chemical toxicity testing

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Abstract
A number of investigators have explored the use of value of information (VOI) analysis to evaluate alternative information collection procedures in diverse decision-making contexts. This paper presents an analytic framework for determining the value of toxicity information used in risk-based decision making. The framework is specifically designed to explore the trade-offs between cost, timeliness, and uncertainty reduction associated with different toxicity-testing methodologies. The use of the proposed framework is demonstrated by two illustrative applications which, although based on simplified assumptions, show the insights that can be obtained through the use of VOI analysis. Specifically, these results suggest that timeliness of information collection has a significant impact on estimates of the VOI of chemical toxicity tests, even in the presence of smaller reductions in uncertainty. The framework introduces the concept of the expected value of delayed sample information, as an extension to the usual expected value of sample information, to accommodate the reductions in value resulting from delayed decision making. Our analysis also suggests that lower cost and higher throughput testing also may be beneficial in terms of public health benefits by increasing the number of substances that can be evaluated within a given budget. When the relative value is expressed in terms of return-on-investment per testing strategy, the differences can be substantial.

KEYWORDS
cost of delay, return on investment, risk decision making, social cost, toxicity testing, value of information

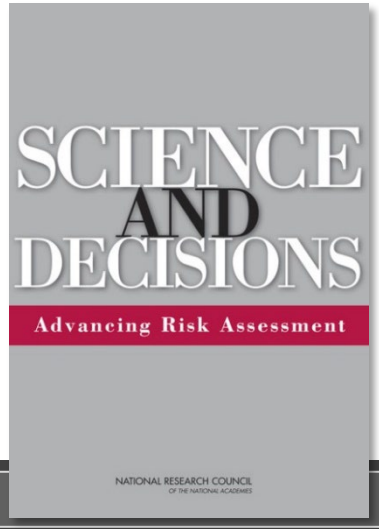
1 | INTRODUCTION

Evidence-based risk assessment has become a cornerstone of public and population health risk decision making, integrating evidence on toxicity and exposure from multiple evidence streams. When the available evidence is insufficient to allow a decision to be made with confidence, consideration can be given to gathering additional evidence to strengthen the evidence base. The present paper focuses on the use of value of information (VOI) analysis to evaluate the utility of gathering additional evidence on the toxicity of chemicals. Specifically, we present a VOI analytic framework that builds on previous methodological work in this field, explicitly incorporating the value of additional test data resulting from reductions in the uncertainty in estimates of a chemical's toxicity, the cost of delay in decision making that results

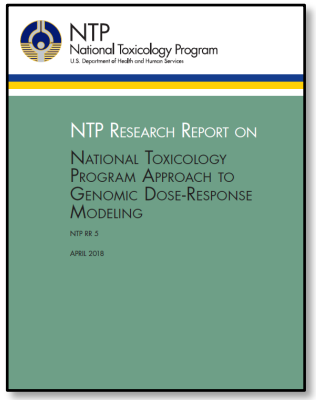
Utilize the EPA-developed VOI framework that is ground-breaking because it explicitly considers the impact of delay in decision-making.

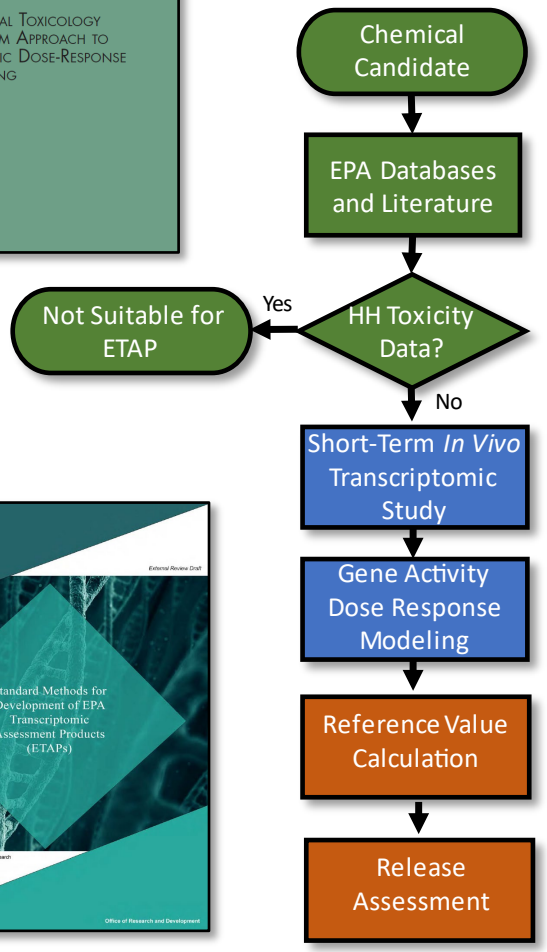
The framework takes into account:

- Amount of **uncertainty** reduced
- **Cost** of additional toxicity testing
- **Delay** in obtaining and evaluating toxicity testing data



Case Study: Value of Information associated with ETAP





The VOI analysis in this study aimed to answer the following question: ***given that additional toxicity testing data may be beneficial, which toxicity testing methodology and assessment process provides the most value?***

Case study compared chronic 2-year rodent toxicity test & assessment to shorter-duration transcriptomic study (ETAP)

Variability around the POD for ETAP was within estimates from Pham *et al.* 2020.

	Transcriptomics Study and Human Health Assessment	Traditional Toxicity Testing and Human Health Assessment
Time Required	<1 year	8 years
Quantitative uncertainty	Modestly greater	Modestly less
Costs	~$200,000	~$4 million

VOI considers socieoeconomic factors and public health benefit to assess return on investment

- **Not testing a chemical may have a cost** borne by the public in terms of healthcare costs arising from exposure to a chemical
  - Economists think in terms of annualized health costs for a variety of outcomes, in terms of healthcare costs, lost productivity, and direct non-medical costs such as education or transportation
  - Annual economic values for a variety of conditions have been estimated
    - Ex: autism spectrum disorder ($69,530/year), asthma ($36,500/yr), pervasive developmental disorders ($10,538/yr), EPA economic guidance estimates fatality at $110,000/yr, considering a value of statistical life (VSL) of $8.8 mil and an 80-year life span

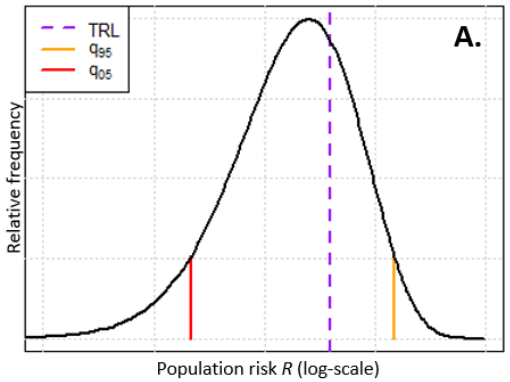
- **Delay has a cost –** Annualized healthcare costs accumulate over time if the exposure is not mitigated and are multiplicative based on the size of the affected population
  - 100,000 people exposed for 5 years prior to mitigation with a $10k annual healthcare cost (total health cost is $5 billion)
    - Mitigating exposure after 2 years saves the public $3 billion
  - For VOI, we consider a time horizon over which benefits of a particular testing strategy may be realized, economists typically use a 20-year time horizon

Additional socioeconomic cost to consider

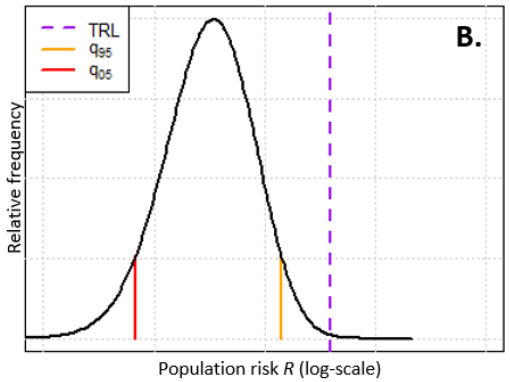
- **Another cost to be considered once a regulatory action is finalized –** cost of control
  - Variety of actions that can be taken – ex. reducing emissions, incorporating water treatment/purification modalities, excavating and moving soil, substituting one chemical in a product formulation for an alternative
  - Under REACH (2021), annualized control costs had a mean of $50.6M and a median of $5.7M

Two idealized decision makers in case study

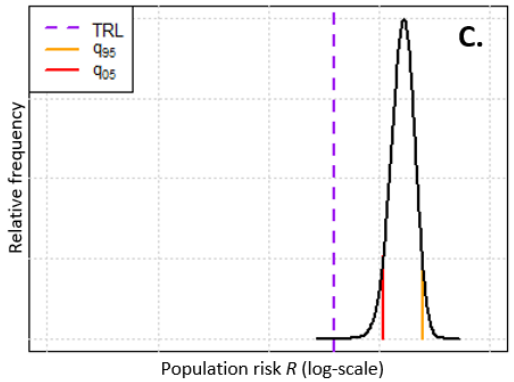
- Benefit-Risk Decision Maker (BRDM): Chooses to regulate a chemical if the reduction in health cost (or increased health benefit) outweighs the associated cost of control
- Target-Risk Decision Maker (TRDM): Chooses to regulate a chemical if the (lower quantile of) risk exceeds the pre-specified target risk level



TRDM would need additional evidence to make a decision



Target risk level is greater than the uncertainty distribution, no regulatory action required

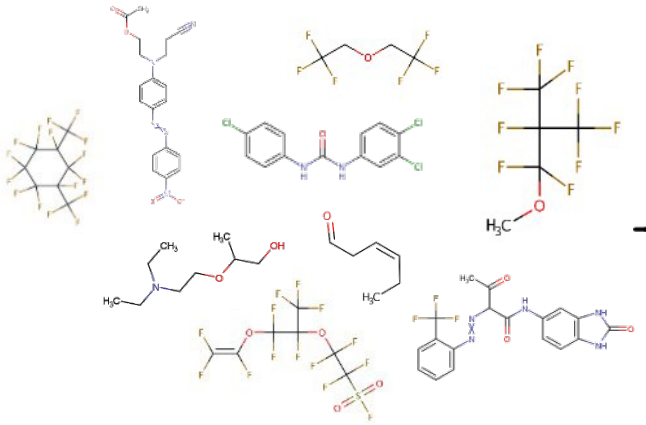


Target risk level is below the 5th percentile of uncertainty distribution, regulatory action is required

TRL: Prespecified Target Risk Level

Contextualizing reliability of two assays with VOI analysis

Diverse Range of Chemicals



Exposure Level
Population Variability in Exposure
Affected Population Size
Health Effects
Population Variability in Toxicity
Control Costs

↓

Uncertainty in Effect Level
Timeliness
Cost

↓

Regulatory Decision Context

↓

Bounded Range of VOI metrics

360 Data Driven Scenarios Examined Comparing ETAP vs Traditional HHA Process

- SHEDS-HT exposure tertiles
- Population sizes (US)
  - 33, 165, **330 million** (10, 50, **100%**)
- Time horizons
  - **20**, 40, 75 years
- Testing costs
  - THHA $1M or **$4M** (M=million)
  - ETAP **$200K** or $250K (K=thousand)
- Time from testing start to assessment finish
  - THHA 6, **8**, 14 years
  - ETAP **0.5**, 1, 2 years
- Control costs
  - $50M or **$23.1B** for 25% reduction
- Annualized health costs
  - $1K, **$10K**, $110K
- Discount rate: 3, **5**, 7%
- Uncertainty around the point-of-departure
  - SD about the mean for each assay from empirical measurements
  - Additional uncertainty added to ETAP

New approach was preferred over the traditional approach in most scenarios, despite greater uncertainty in estimates derived from repeated studies

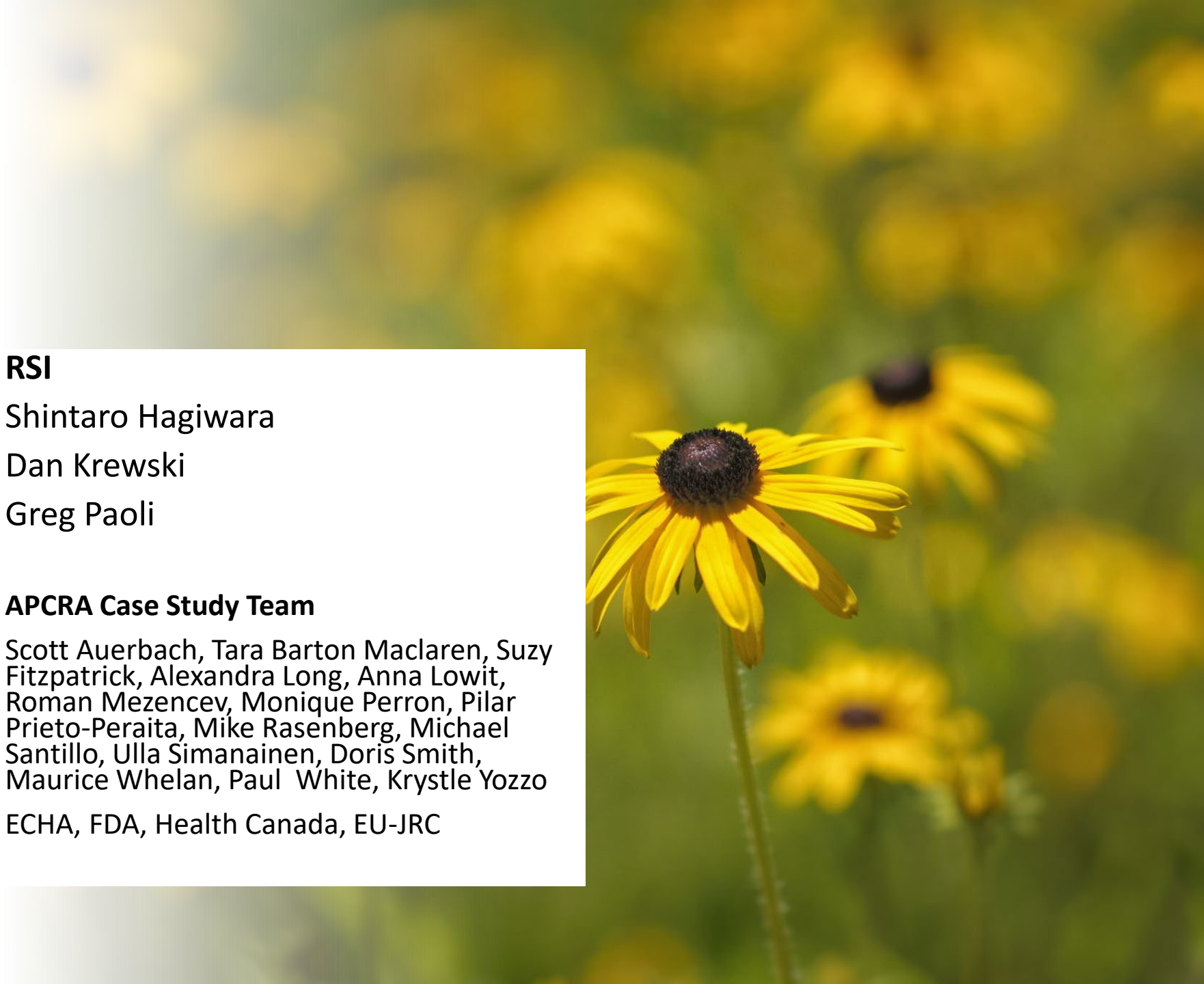
- The VOI Case study evaluated 360 scenarios
  - For each decision context, 9 baseline and 171 sensitivity scenarios

- Benefit-Risk Decision Maker (180 scenarios)
  - In 82% of scenarios, ETAP was preferred with favorable ROI & ENBS
  - 18% - no testing preferred
  - Average benefit was $44 billion for BRDM
- Target-Risk Decision Maker (180 scenarios)
  - ETAP was preferred in 89% of scenarios (ENBS) and 99% of scenarios (ROI)
  - 7.2% - no testing preferred
  - Average benefit was $81 billion for TRDM

Conclusions

- Understanding the validation efforts to date may help inform optimization of the numbers of participating labs and reference chemicals required for building confidence in a method's reliability
- Retrospective analysis of available *in vivo* datasets allows for understanding of appropriate qualitative and quantitative benchmarks for assessing relevance and reliability of NAMs
- Value of Information frameworks can assist with contextualizing relative value in socioeconomics terms of using a more uncertain versus less uncertain assay or method
- These efforts inform updates to confidence building frameworks, including the GD 34 revision

Thank you



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Scott Auerbach, Tara Barton Maclaren, Suzy Fitzpatrick, Alexandra Long, Anna Lowit, Roman Mezencev, Monique Perron, Pilar Prieto-Peraita, Mike Rasenberg, Michael Santillo, Ulla Simanainen, Doris Smith, Maurice Whelan, Paul White, Krystle Yozzo

ECHA, FDA, Health Canada, EU-JRC

United States Environmental Protection Agency

Development and Application of Exposure New Approach Methodologies in EPA's ExpoCast Project

Kristin Isaacs

Center for Computational Toxicology and Exposure
Office of Research and Development
United States Environmental Protection Agency

4th US EPA Conference on the State of Science on Development and Use of NAMs for Chemical Safety Testing
November 5, 2024

United States Environmental Protection Agency

Disclaimer

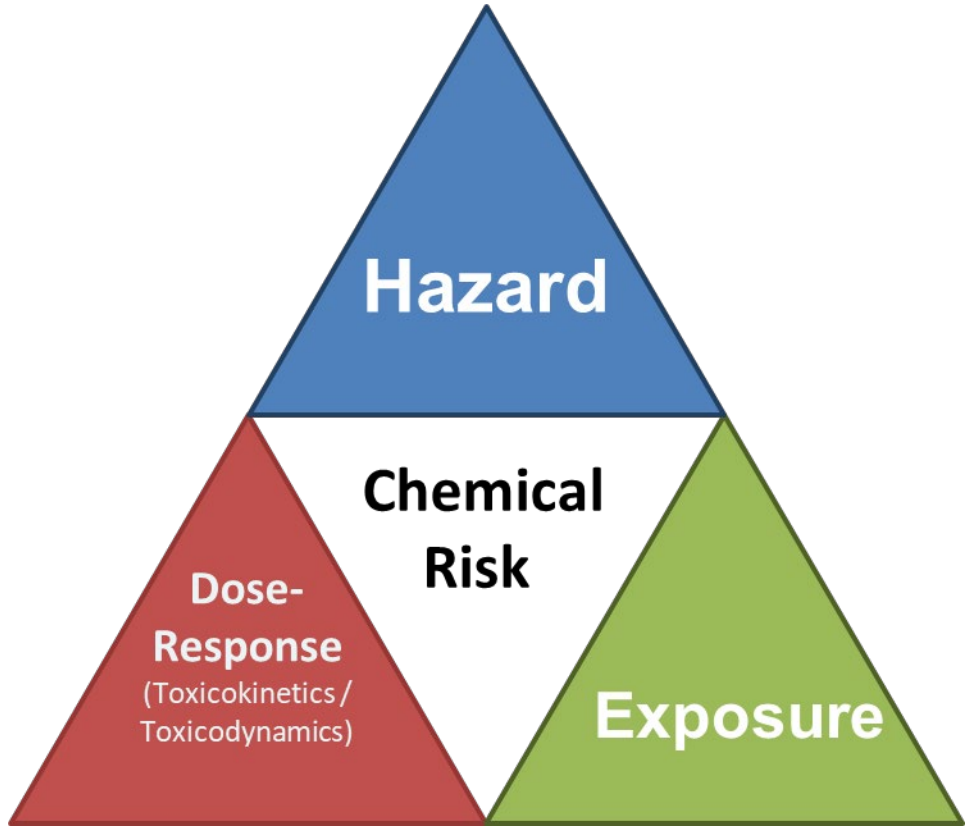
The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

NAMs and Chemical Risk

USING 21ST CENTURY SCIENCE TO IMPROVE RISK-RELATED EVALUATIONS

NASEM (2017)

"Recent advances in high throughput toxicity assessment, notably the **ToxCast and Tox21** programs… and in high throughput computational exposure assessment [**ExpoCast**] have enabled first-tier risk-based rankings of chemicals on the basis of **margins of exposure**"



Material from John Wambaugh

NAMs and Chemical Risk

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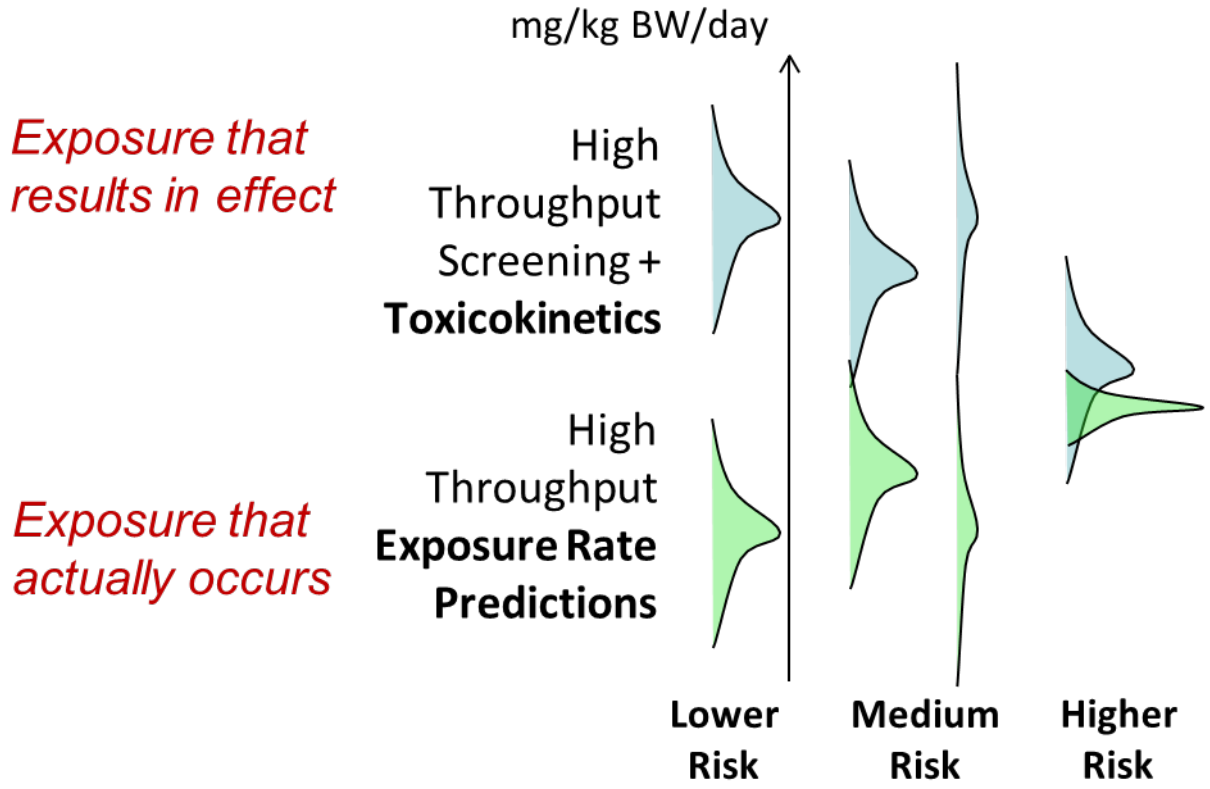
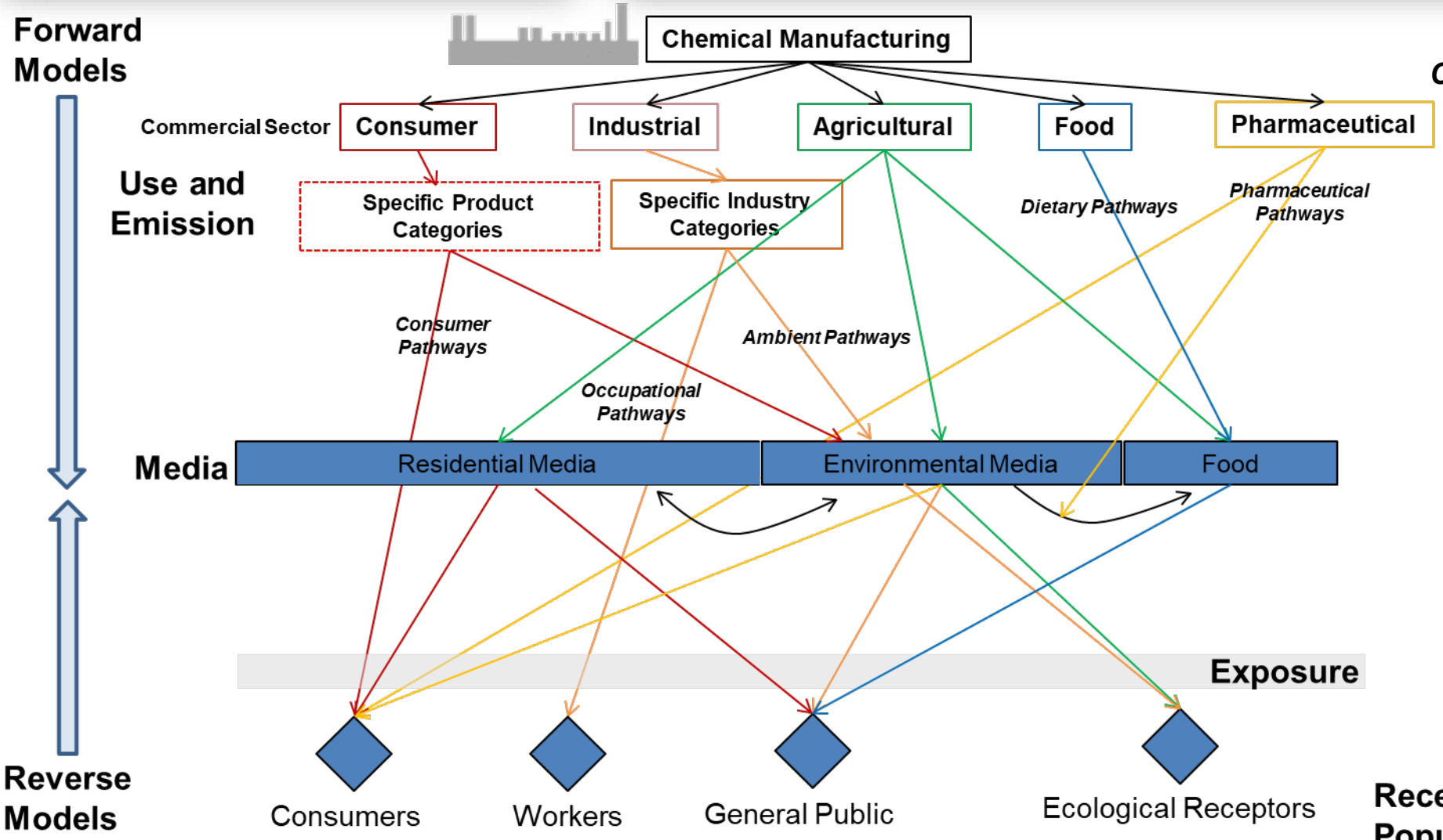


New approach methodologies (NAMs) enable risk assessors to more rapidly address public health challenges and chemical regulation

Material from John Wambaugh

Exposure Pathways from Source to Receptor

Exposure Pathways from Source to Receptor



Critical Exposure-Relevant Domains

- ***Chemical use and emission.*** Provides critical information for identifying chemical sources, exposure pathways, and relevant models for a given chemical.
- ***Media occurrence, environmental surveillance, and biomonitoring***. Provides exposure data for evaluating predictive models.
- ***Toxicokinetics***. Provides real-world exposure context to *in vitro* high-throughput screening data and biological receptor monitoring information.

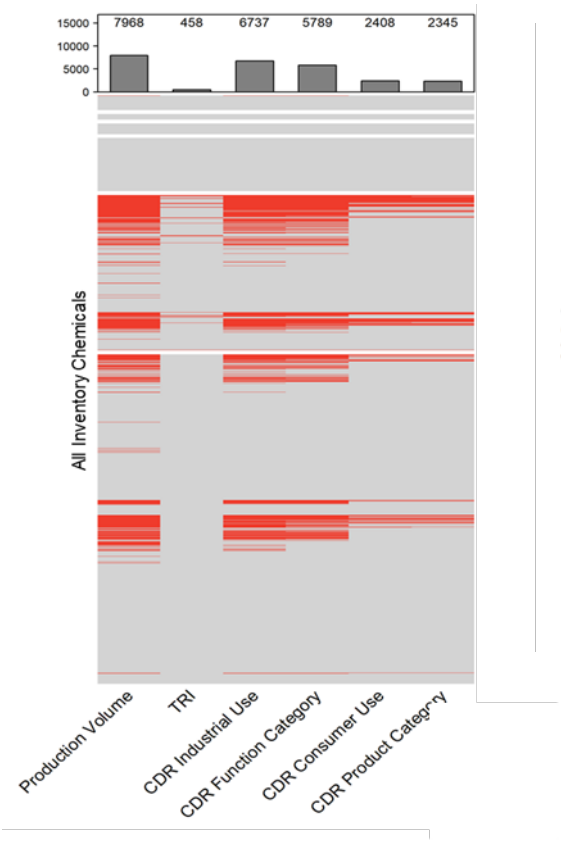
Traditional Exposure Data are Scarce

Accelerating the Pace of Chemical Risk Assessment

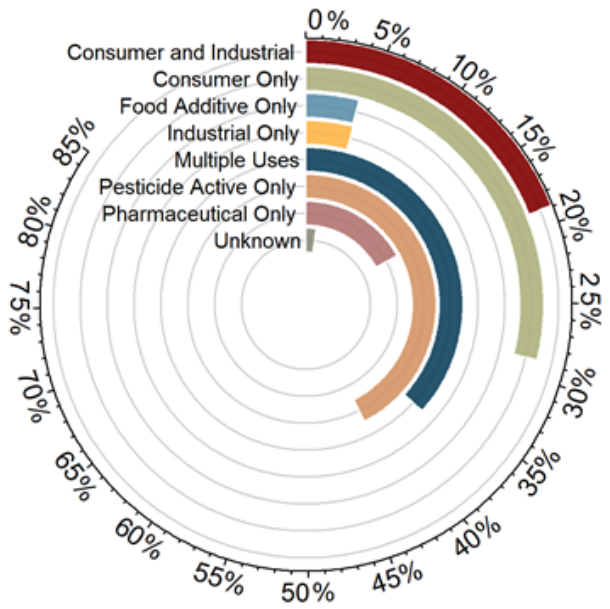
APCRA

- Examined combined list of 38,715 chemicals from government regulatory inventories

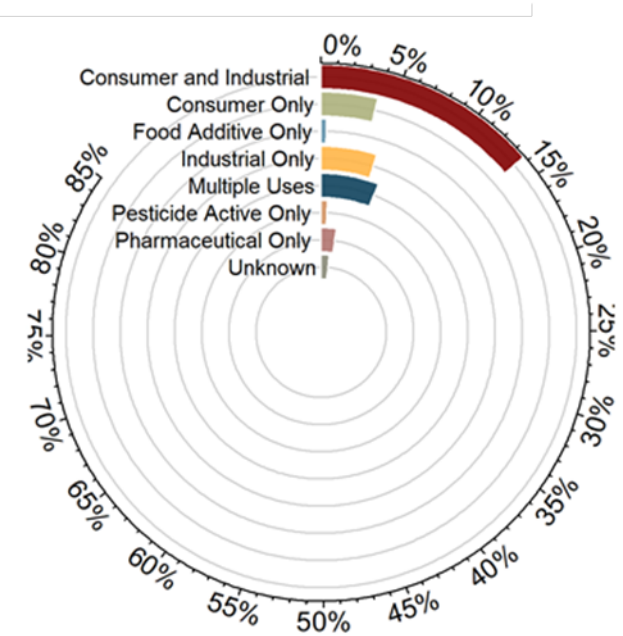
Available Use and Emission Data



Percent of Commercial Sector with Traditional Monitoring Data



Percent of Commercial Sector with Exposure Estimates



Isaacs et al., J Exp. Sci. Env. Epidem. (2022)

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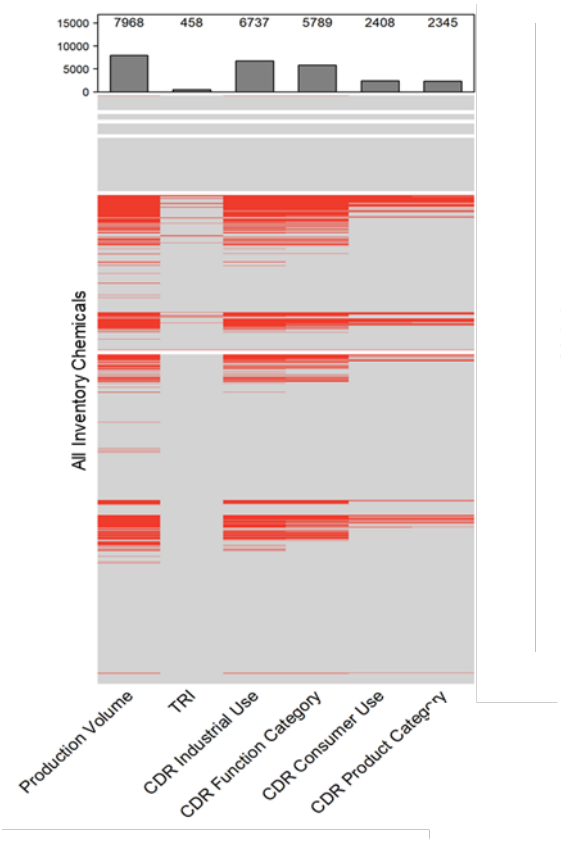

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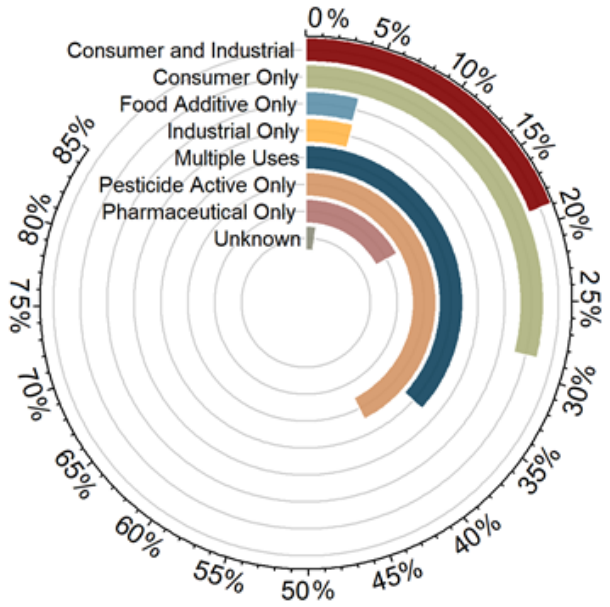

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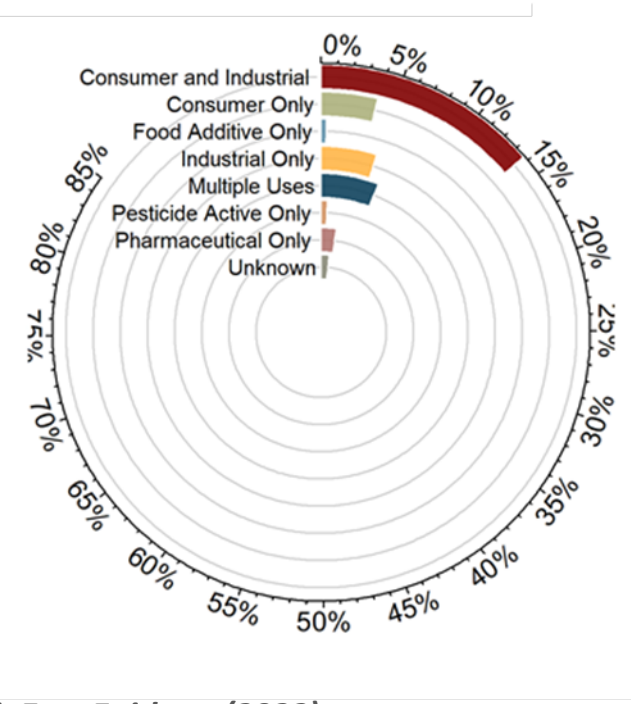


Isaacs et al., J Exp. Sci. Env. Epidem. (2022)

*The ExpoCast project and its collaborators are working to fill gaps in exposure data for 1000s of chemicals using high-throughput **new approach methods (NAMs)** for exposure*

New Approach Methods and Exposure

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]

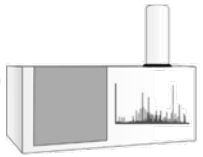
Defined 7 classes of Exposure NAMs oriented toward high-throughput application: suitable for dealing with the thousands of chemicals in commerce with limited sources of chemical exposure information

Exposure NAMs in the ExpoCast Paradigm

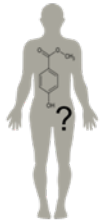
Chemical descriptors that provide information on chemicals in an exposure context (e.g., how chemicals are used or released)

Collect
relevant data

High-throughput measurements to fill gaps in monitoring data

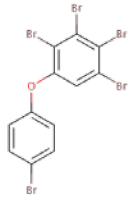


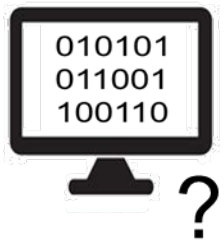
High-throughput toxicokinetics approaches for measuring and predicting chemical fate *in vivo*



Machine-learning approaches that use these descriptors to fill gaps in existing data

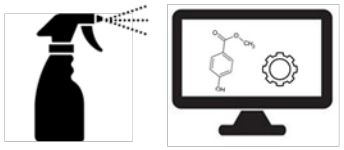
Extrapolate
to fill data gaps



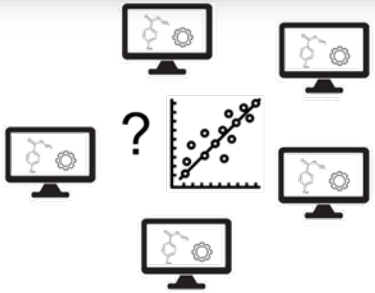


High-throughput exposure models for various exposure pathways

Predict
exposures for specific pathways



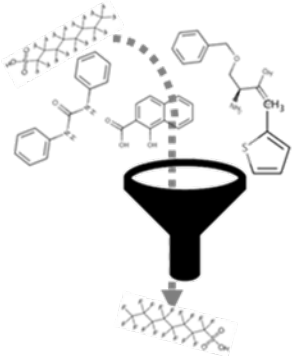
Forecast
exposures for 1000s of chemicals using consensus approaches



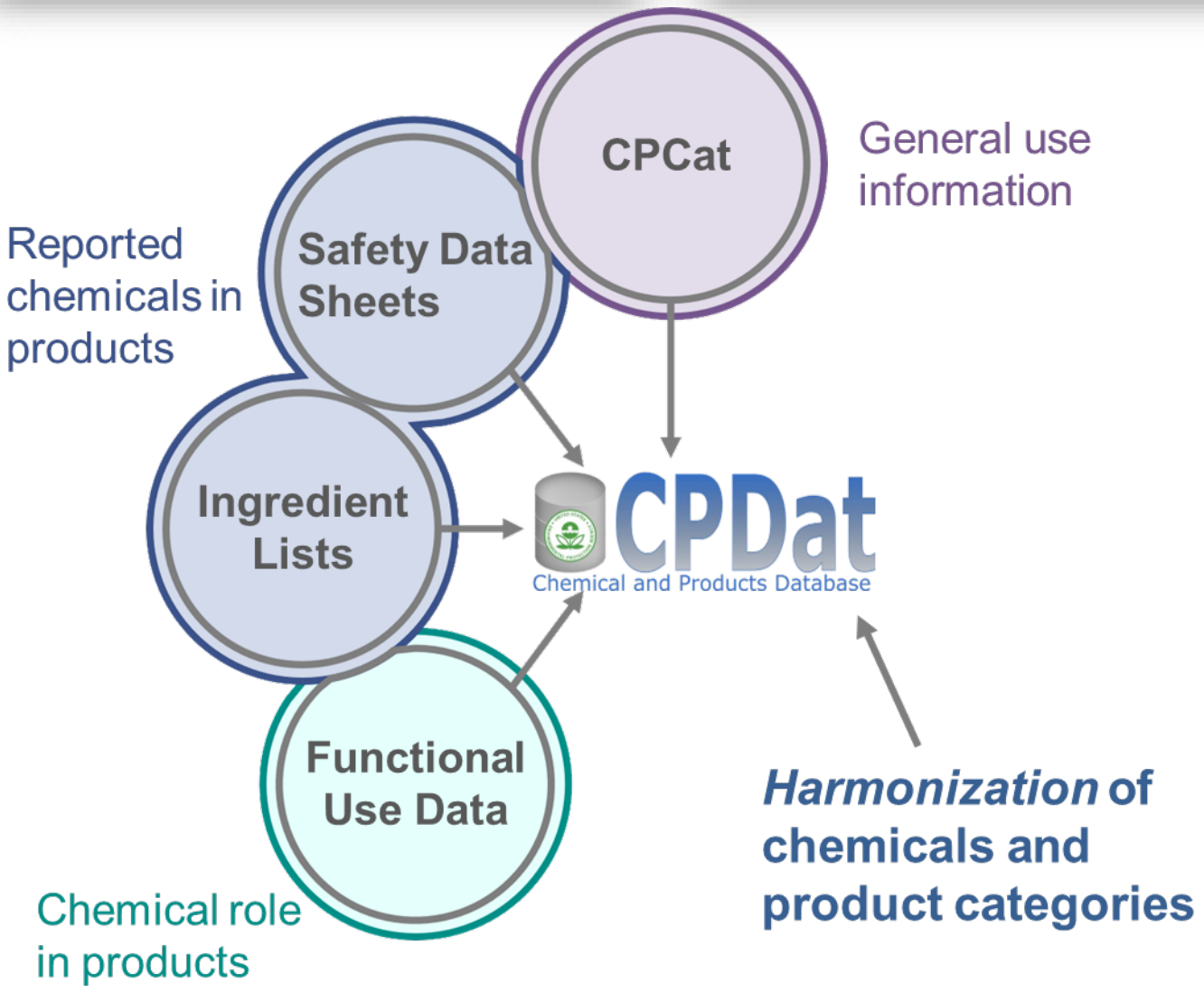
Statistical frameworks for integrating models for multiple pathways with exposure data to provide calibrated exposure predictions

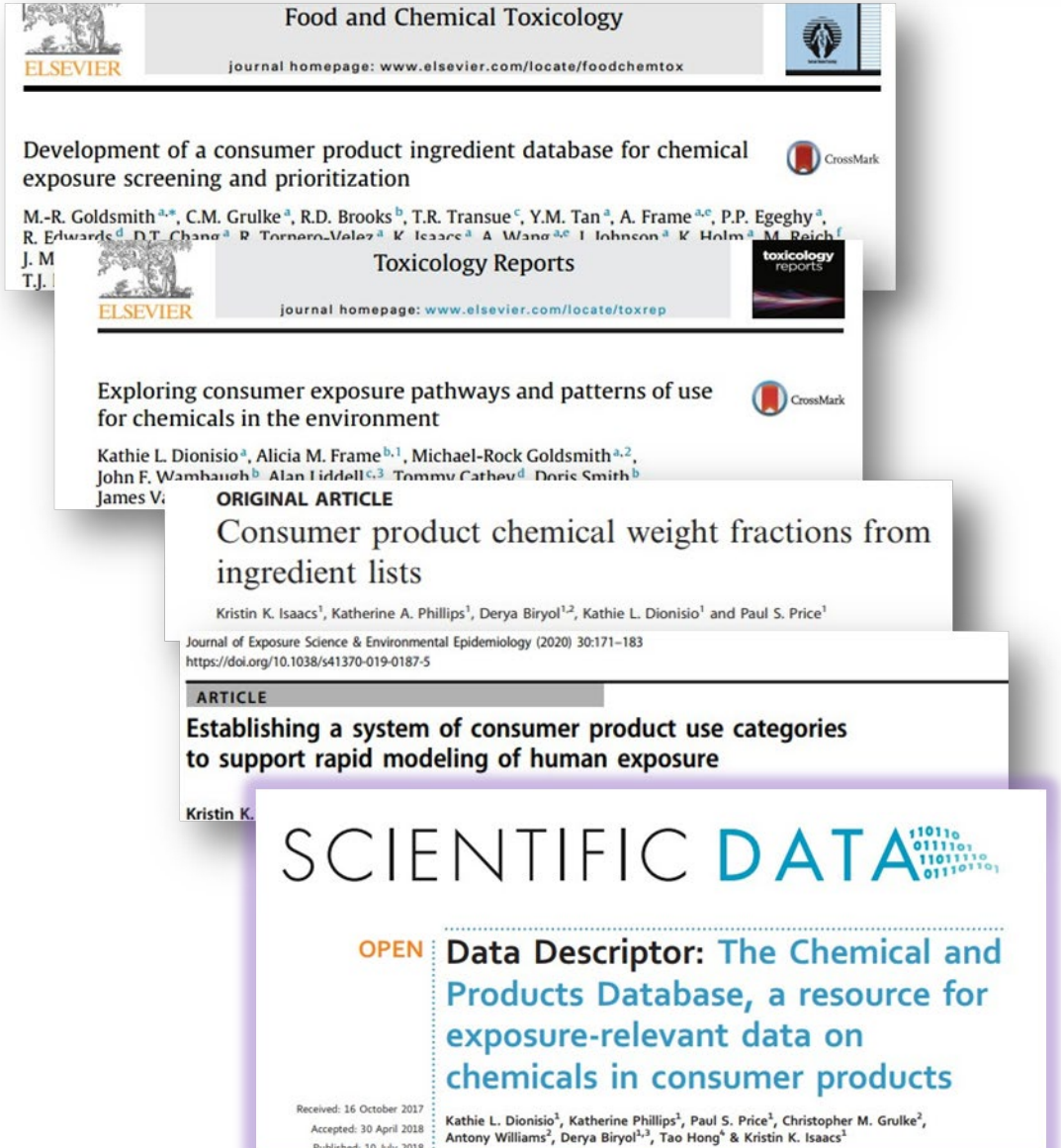
Integration of hazard and exposure NAMs for high-throughput ***chemical prioritization***

Prioritize
chemicals for further evaluation



Curation of Chemical Descriptor NAMs





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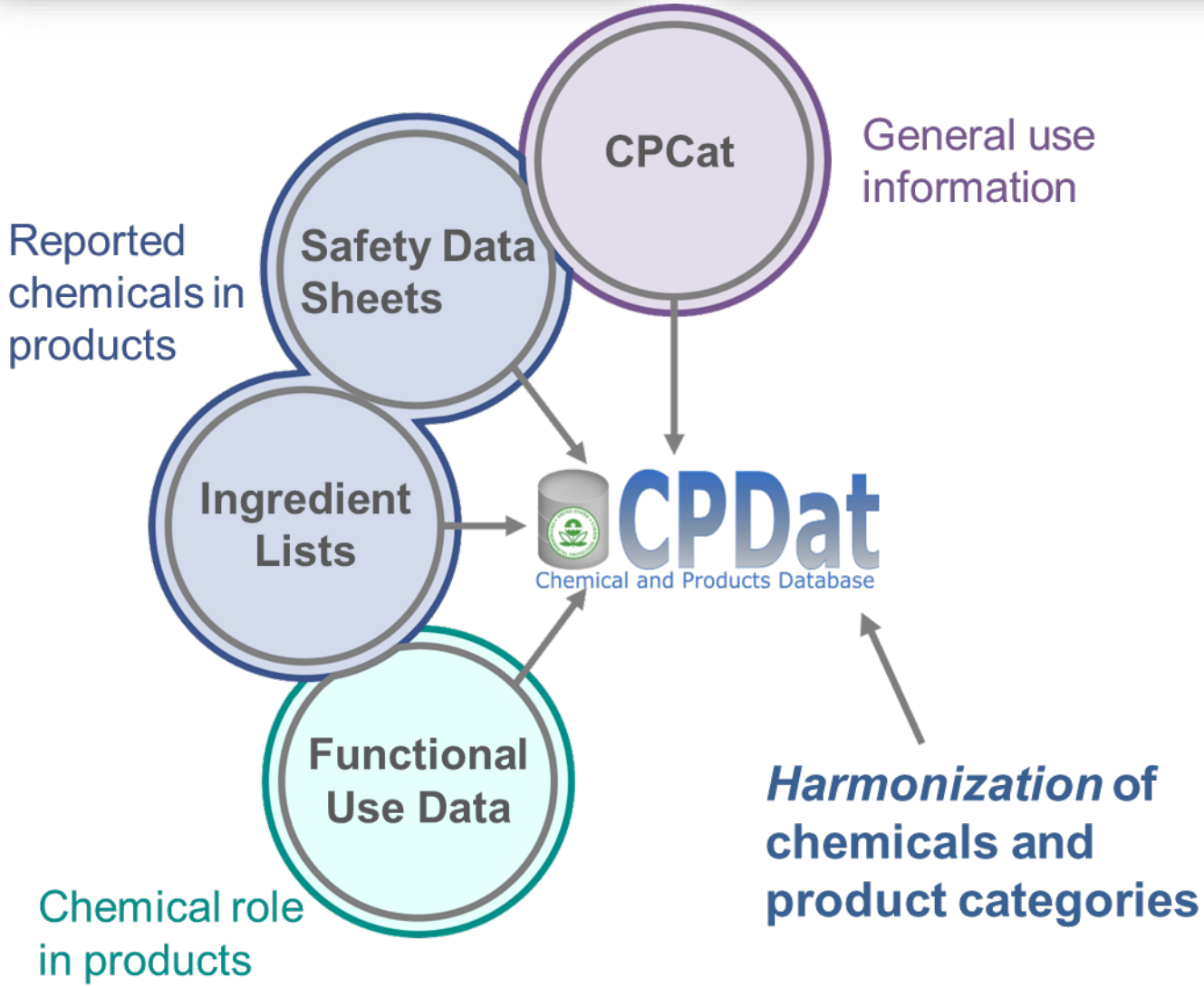
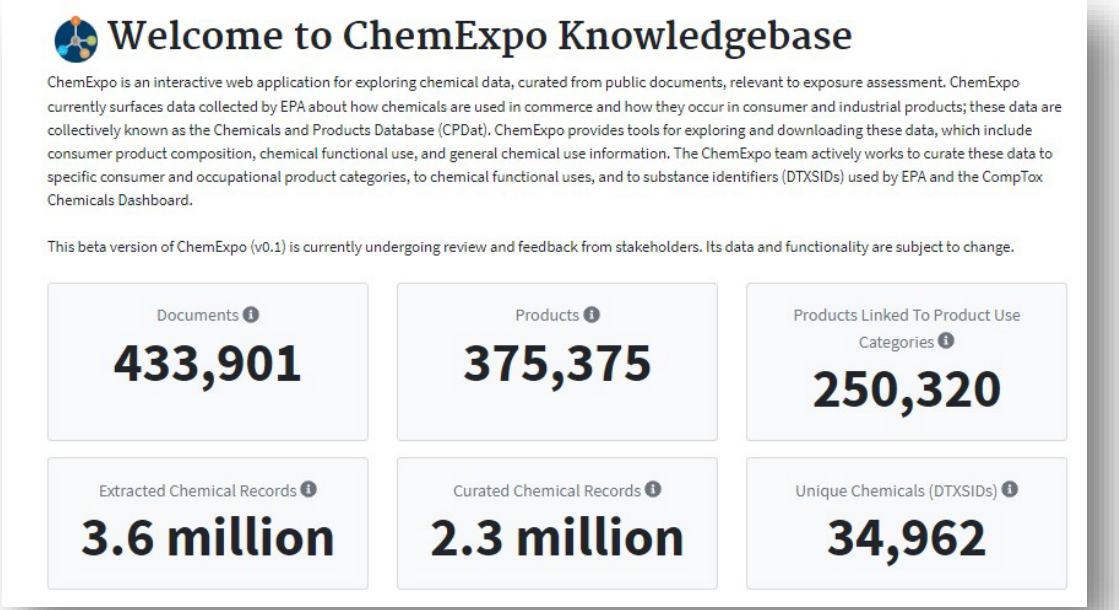
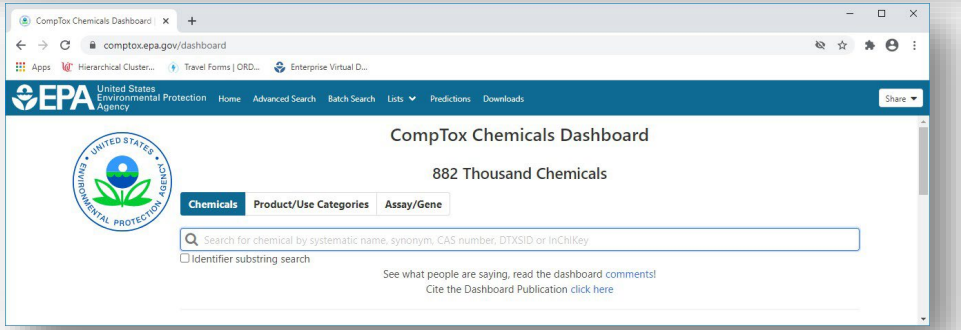


https://comptox.epa.gov/chemexpo/

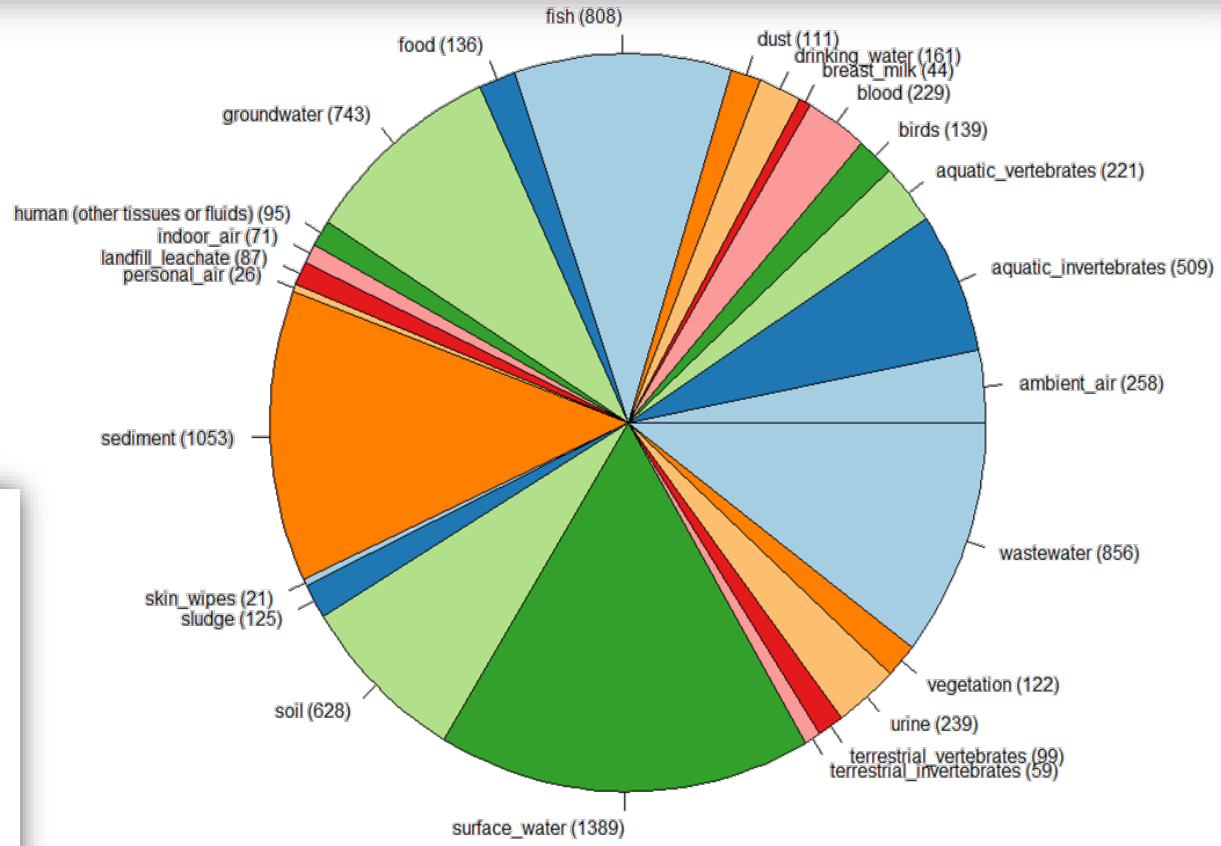




https://comptox.epa.gov/dashboard/

Curation of Chemical Descriptor NAMs

- Other curation efforts have focused on media occurrence of chemicals
- 63 million+ chemical records from 20 sources mapped to harmonized chemical identifiers and ~30 media categories
- 3271 unique chemicals



Data Descriptor | Open Access | Published: 16 June 2022

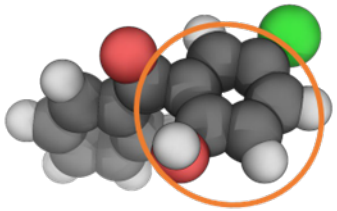
A harmonized chemical monitoring database for support of exposure assessments

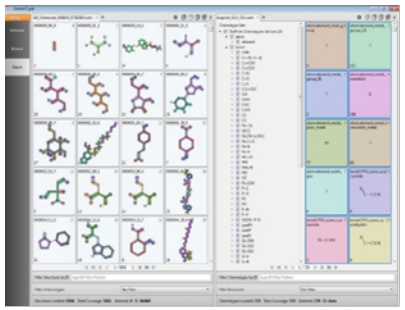
Kristin K. Isaacs, Jonathan T. Wall, Ashley R. Williams, Kevin A. Hobbie, Jon R. Sobus, Elin Ulrich, David Lyons, Kathie L. Dionisio, Antony J. Williams, Christopher Grulke, Caroline A. Foster, Josiah McCoy & Charles Bevington

Scientific Data **9**, Article number: 314 (2022) | Cite this article

MMDB
Multimedia Monitoring Database

Machine Learning NAMs



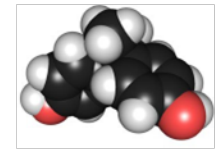


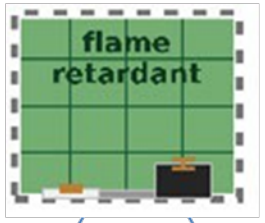
CPDat Chemical and Products Database

Training Sets of Chemical Descriptor NAMS

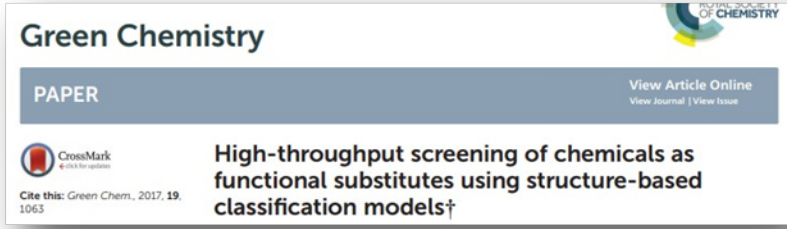
Machine Learning Based Classification Models

Prediction of Potential Alternatives from Chemical Libraries





YES NO



Phillips et al., Green Chemistry 2017

High-throughput Measurement NAMs

- "Targeted" Analysis:
  - We know exactly what were looking for
  - 10s – 100s of chemicals
- "Non-Targeted" Analysis (NTA):
  - We have no preconceived lists
  - 1,000s – 10,000s of chemical
- NTA research is being performed both in-house by EPA investigators and via contract

- Key focus on developing **reproducible and defensible** NTA methods and results
- Ultimate goal is to develop tools, databases, and workflows for rapid analysis of any sample for chemicals of interest, i.e. ***exposure forensics***

Material from Jon Sobus

High-throughput Measurement NAMs

Source and Release

Pilot: 20 Consumer Product Categories

Phillips et al., Env. Sci. Tech. 2018

Recycled Consumer Materials

Lowe et al., Env. Sci. Tech. 2018

Consumer Product Emissions from Different Substrates

Watson et al., Env. Sci. Tech. 2024 (in press)

Media Occurrence

Residential Air

Residential Dust

Rager *et al., Env. Int.*, 2016

Drinking Water

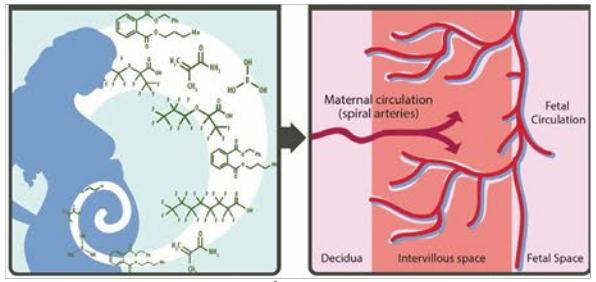
Newton et al., Env. Pollut., 2019

Exposure

Pooled Human Blood

Phillips et al., 2023

Human Placenta

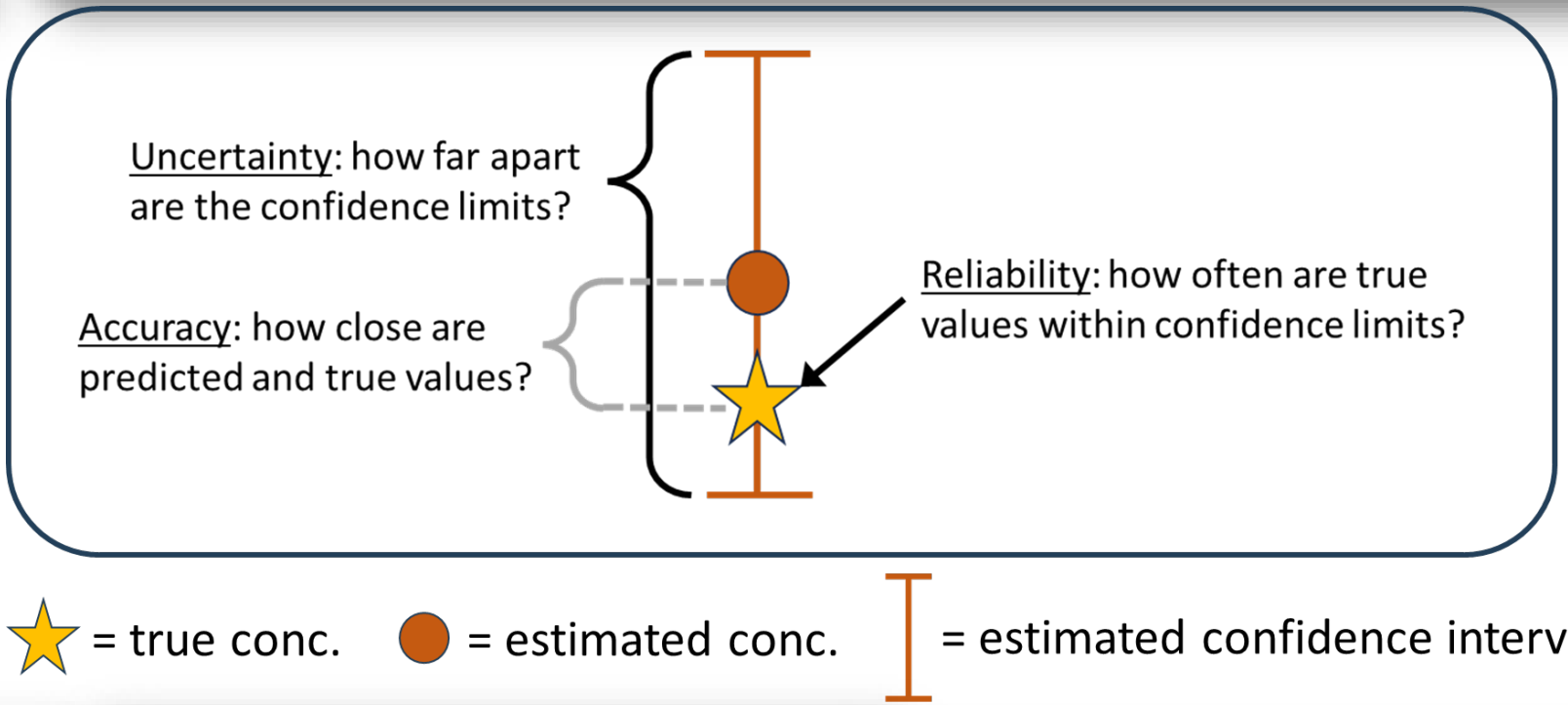


Rager *et al., Repro. Tox.* , 2020
Chao *et al., Env. Int.*, 2022

Current Focus and Challenge: How can we **quantify** concentrations of chemicals in media using NTA?

Towards Quantitative NTA (qNTA)

Methods are being developed for selecting appropriate calibration surrogates and characterizing qNTA performance

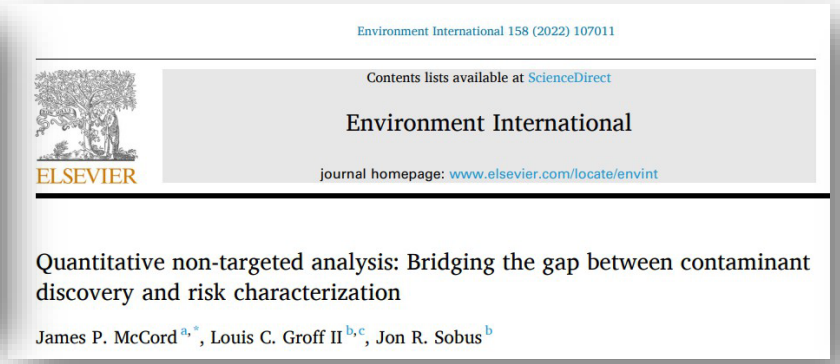



Analytical and Bioanalytical Chemistry (2022) 414:4919–4933
https://doi.org/10.1007/s00216-022-04118-z

RESEARCH PAPER

Uncertainty estimation strategies for quantitative non-targeted analysis

Louis C. Groff II[1,2] · Jarod N. Grossman[2,3] · Anneli Kruve[4] · Jeffrey M. Minucci[1] · Charles N. Lowe[1] · James P. McCord[1] · Dustin F. Kapraun[1] · Katherine A. Phillips[1] · S. Thomas Purucker[1] · Alex Chao[1] · Caroline L. Ring[1] · Antony J. Williams[1] · Jon R. Sobus[1]


Environment International 158 (2022) 107011

Contents lists available at ScienceDirect

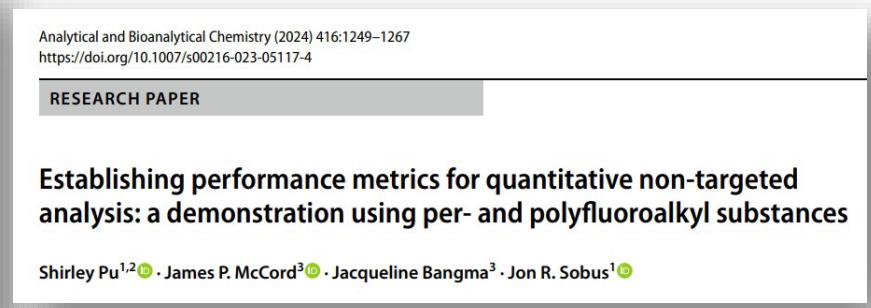
Environment International

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

ELSEVIER

Quantitative non-targeted analysis: Bridging the gap between contaminant discovery and risk characterization

James P. McCord[a,*], Louis C. Groff II[b,c], Jon R. Sobus[b]


Analytical and Bioanalytical Chemistry (2024) 416:1249–1267
https://doi.org/10.1007/s00216-023-05117-4

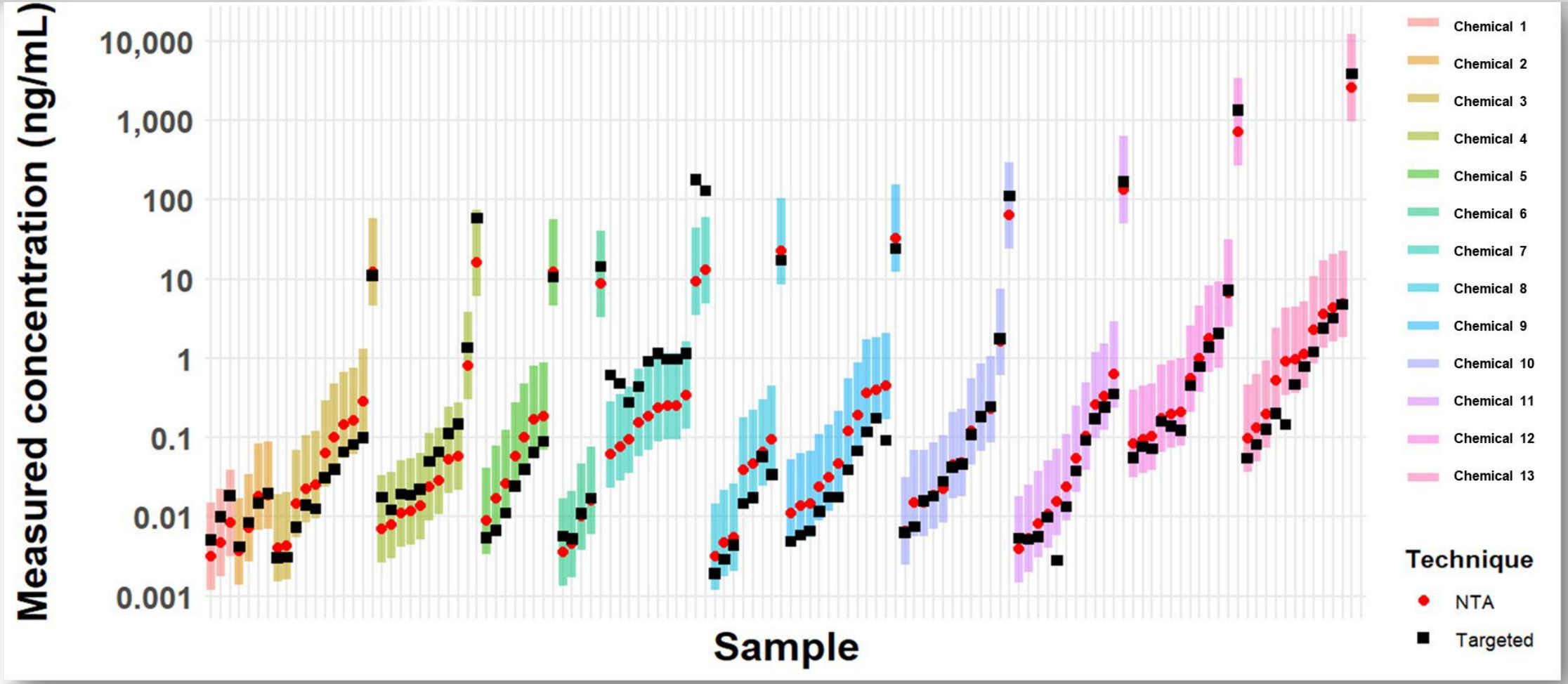
RESEARCH PAPER

Establishing performance metrics for quantitative non-targeted analysis: a demonstration using per- and polyfluoroalkyl substances

Shirley Pu[1,2] · James P. McCord[3] · Jacqueline Bangma[3] · Jon R. Sobus[1]

Material from Jon Sobus

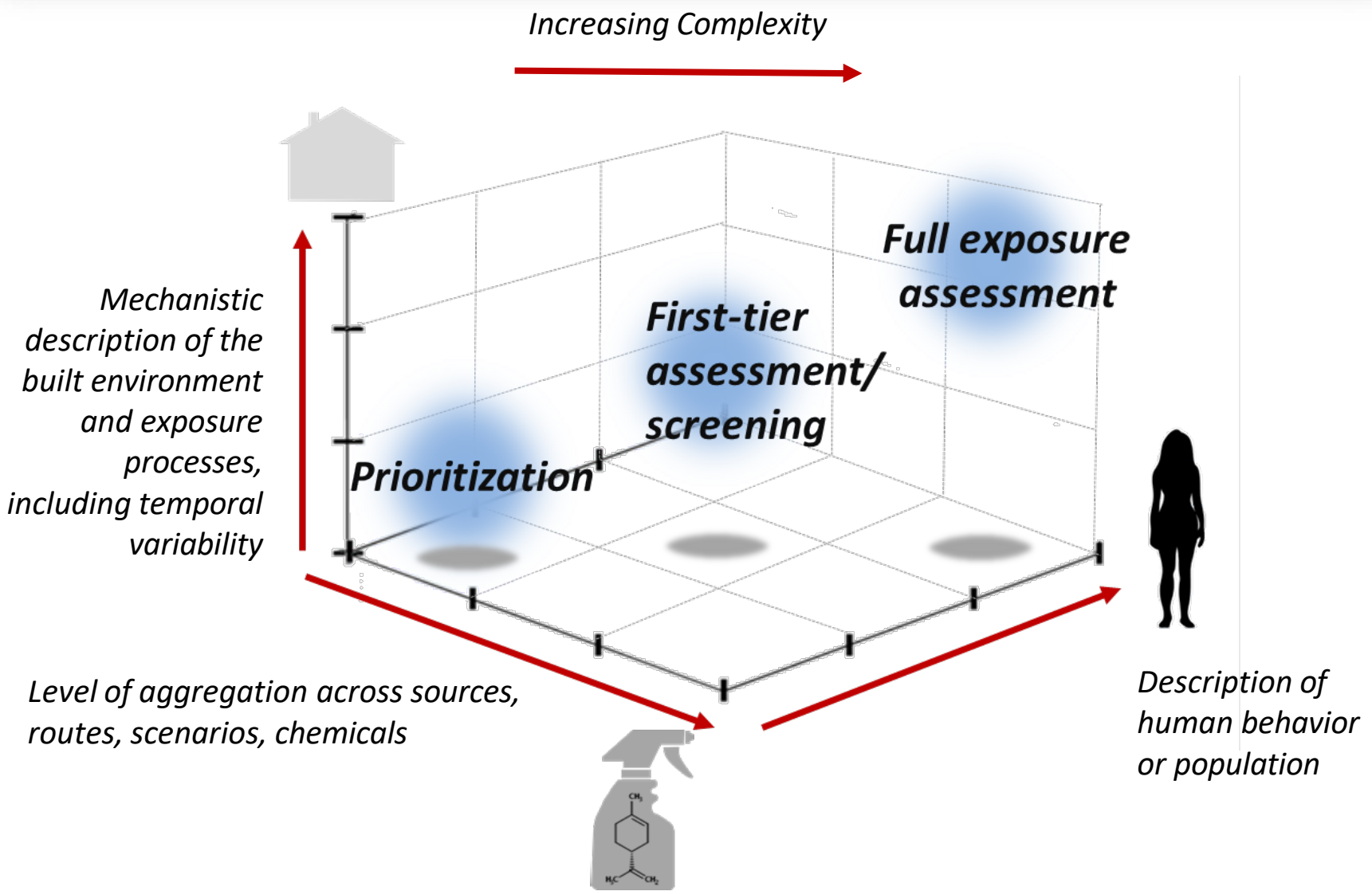
Towards Quantitative NTA (qNTA)



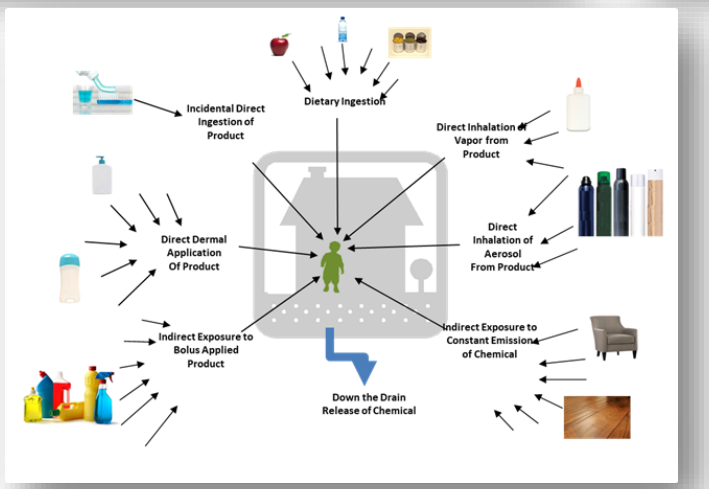
Material from Jon Sobus

High Throughput Exposure Model NAMs

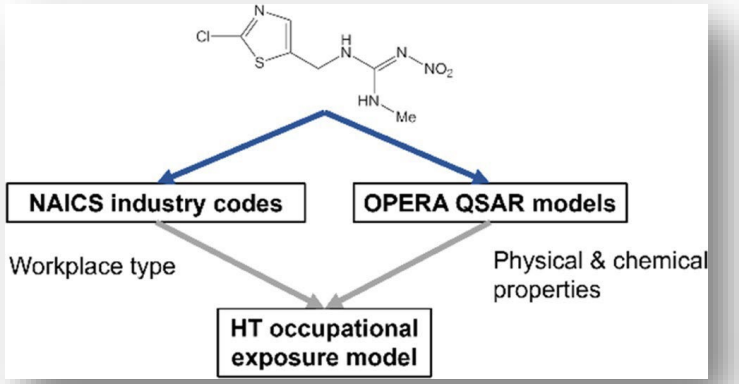
- All models vary in complexity and data needed to describe chemical exposure
- **High throughput exposure (HTE) models** can handle many chemicals with minimal descriptive information
- HTE models can provide rough but quantitative estimates of exposure



High Throughput Exposure Model NAMs

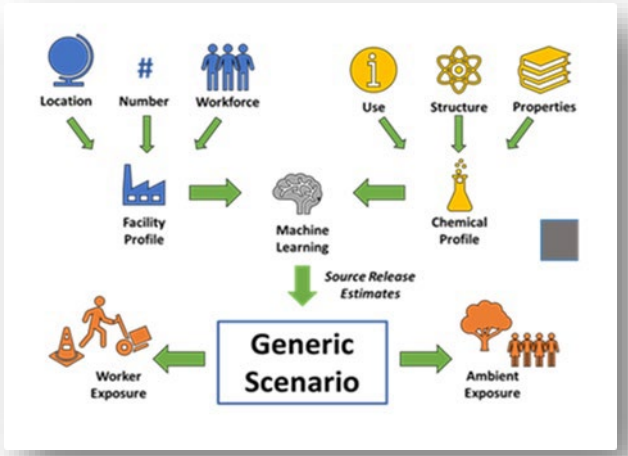


Consumer *Isaacs et al., Env. Sci. Tech. (2014)*



Occupational

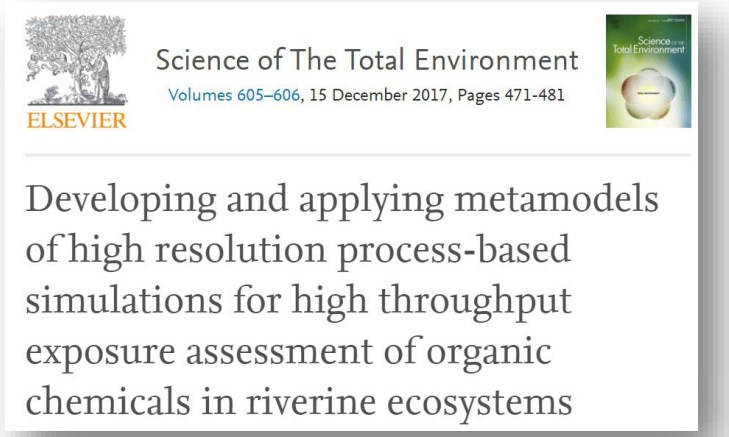
Minucci et al., Env. Int. (2023)



Ambient

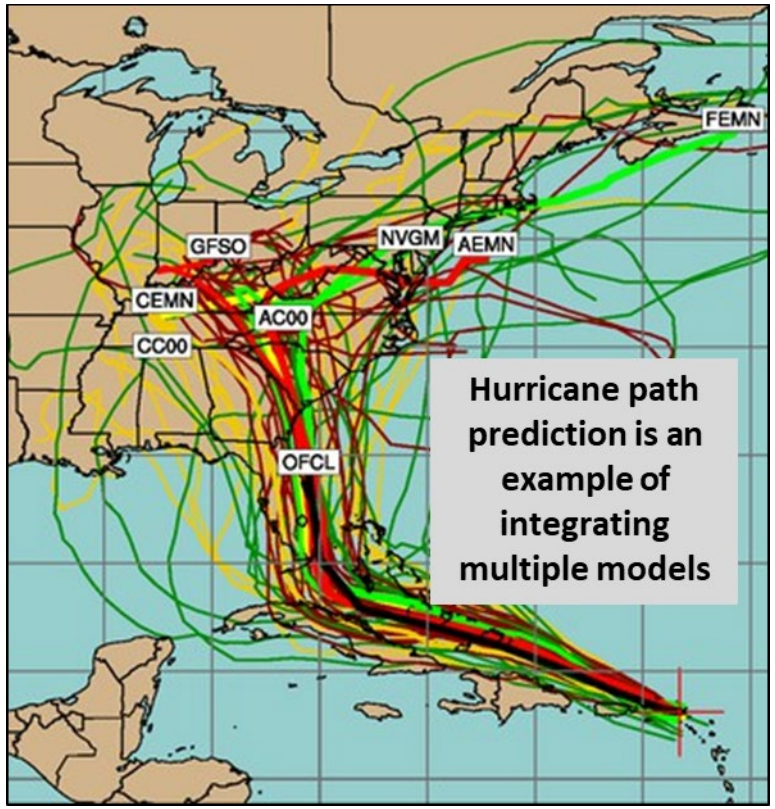
Meyer et al., ACS Sustain Chem Eng. (2019)

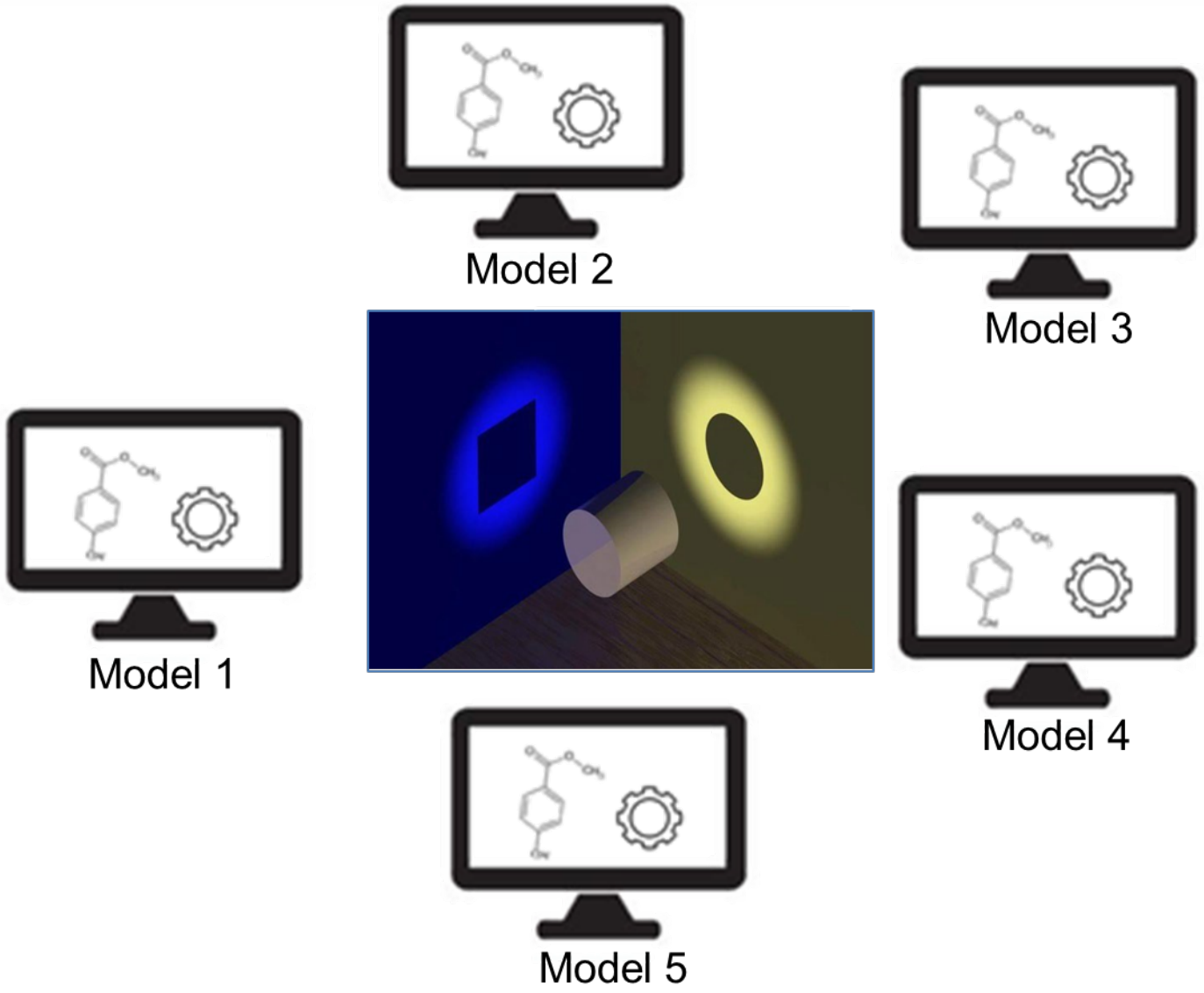
Dietary *Biryol et al., (2017)*



Ecological *Barber et al., (2017)*

Evaluation Framework NAMs and Consensus Models





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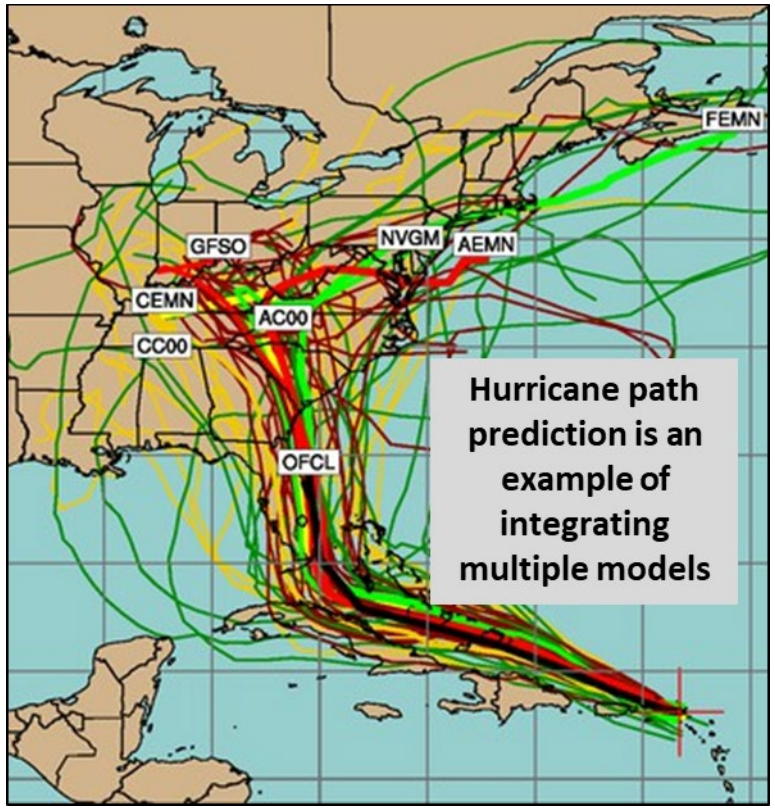
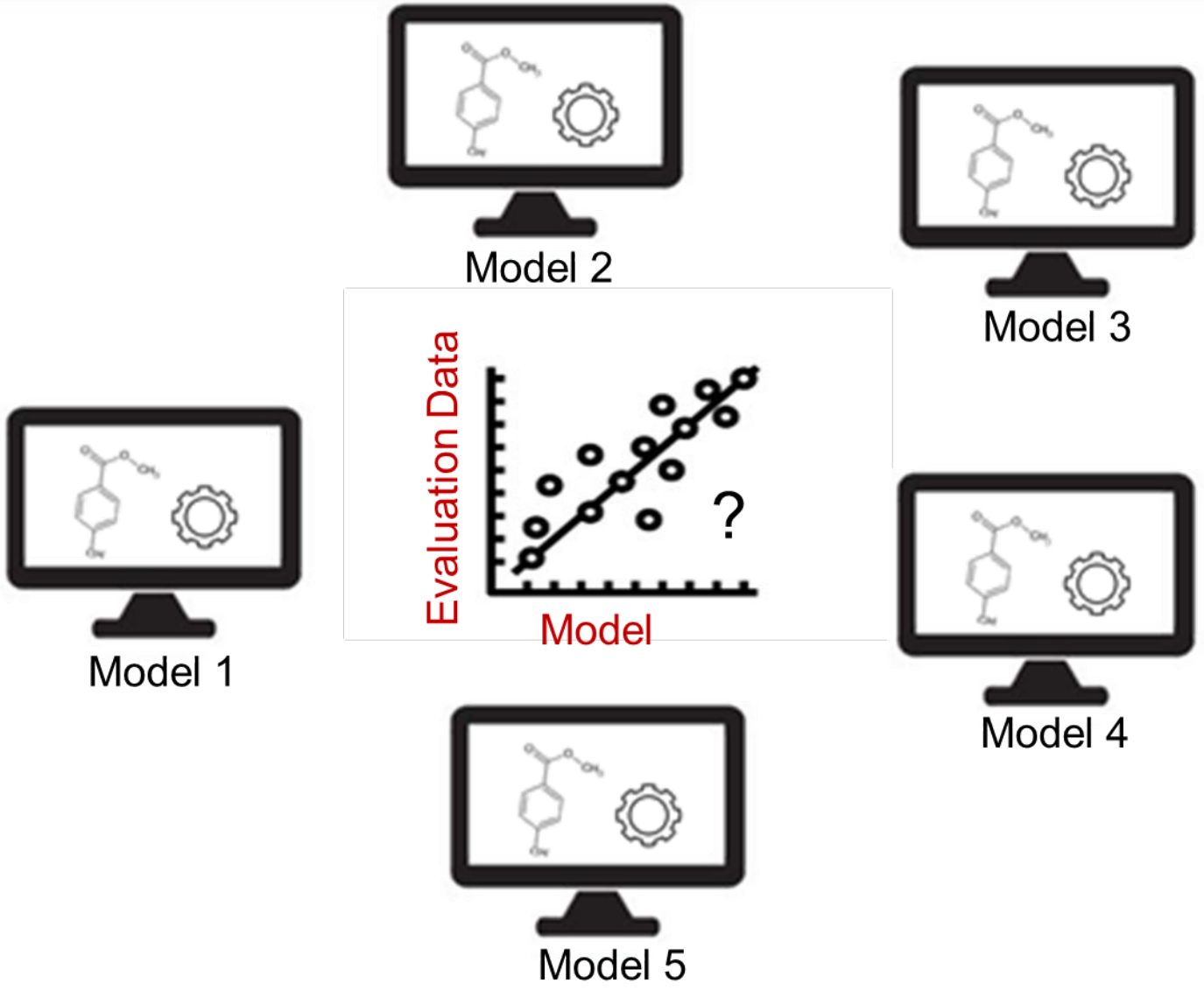
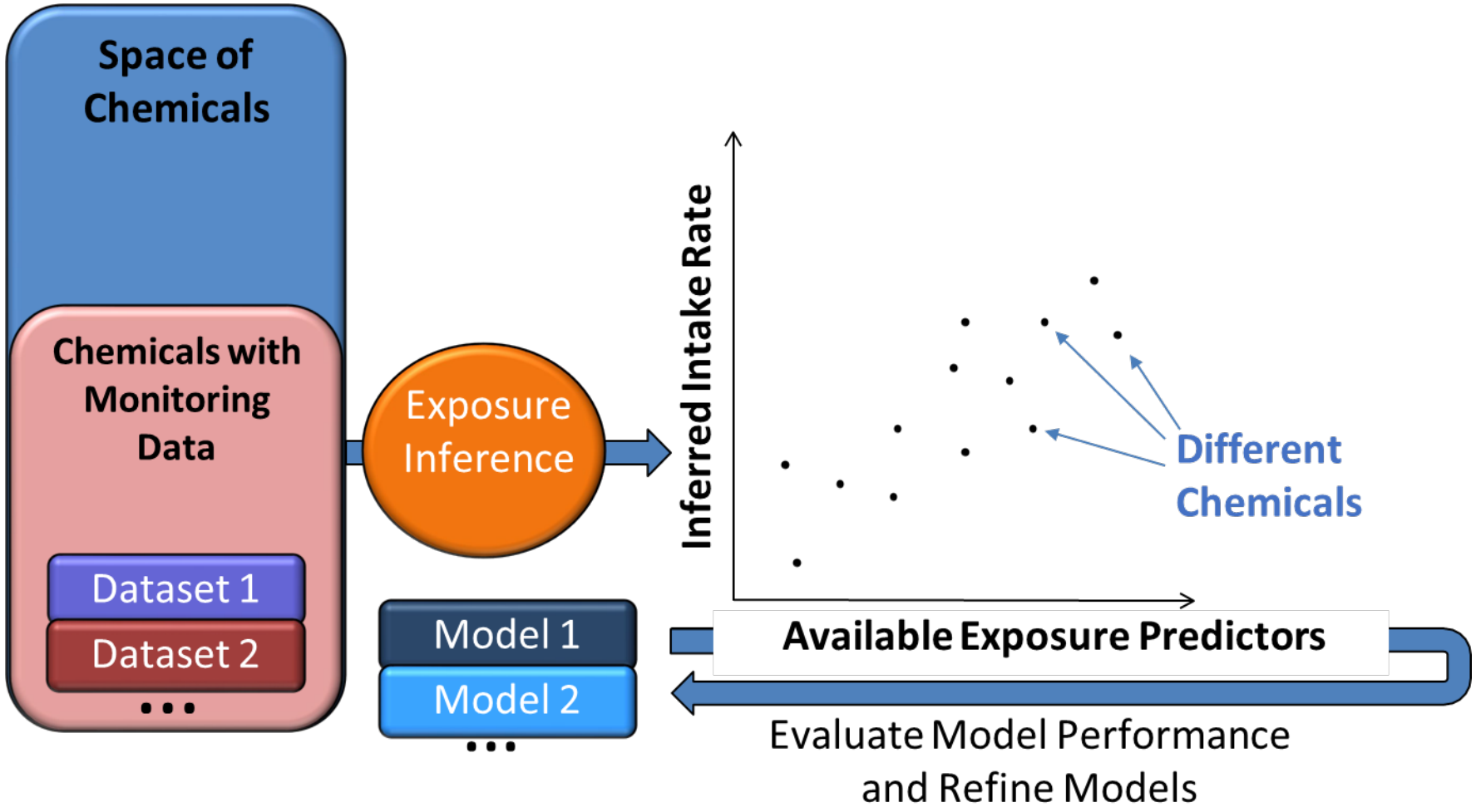




Evaluating Exposure Models with the SEEM Framework

- We use Bayesian methods to incorporate multiple models into consensus forecasts for 1000s of chemicals within the **Systematic Empirical Evaluation of Models (SEEM)** (Wambaugh et al., 2013,2014)



Wambaugh et al., Cur. Opin. Toxicol. 2019

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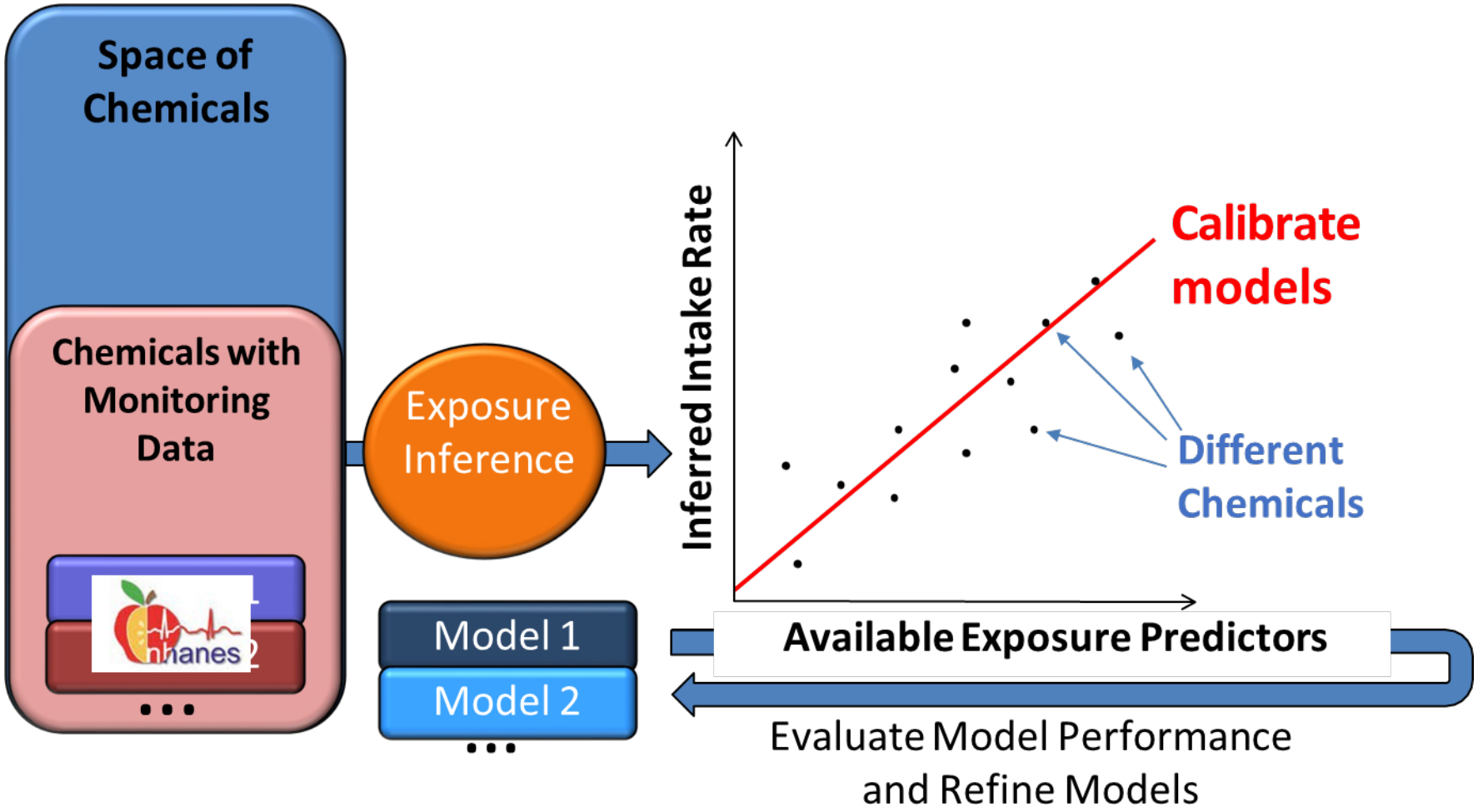

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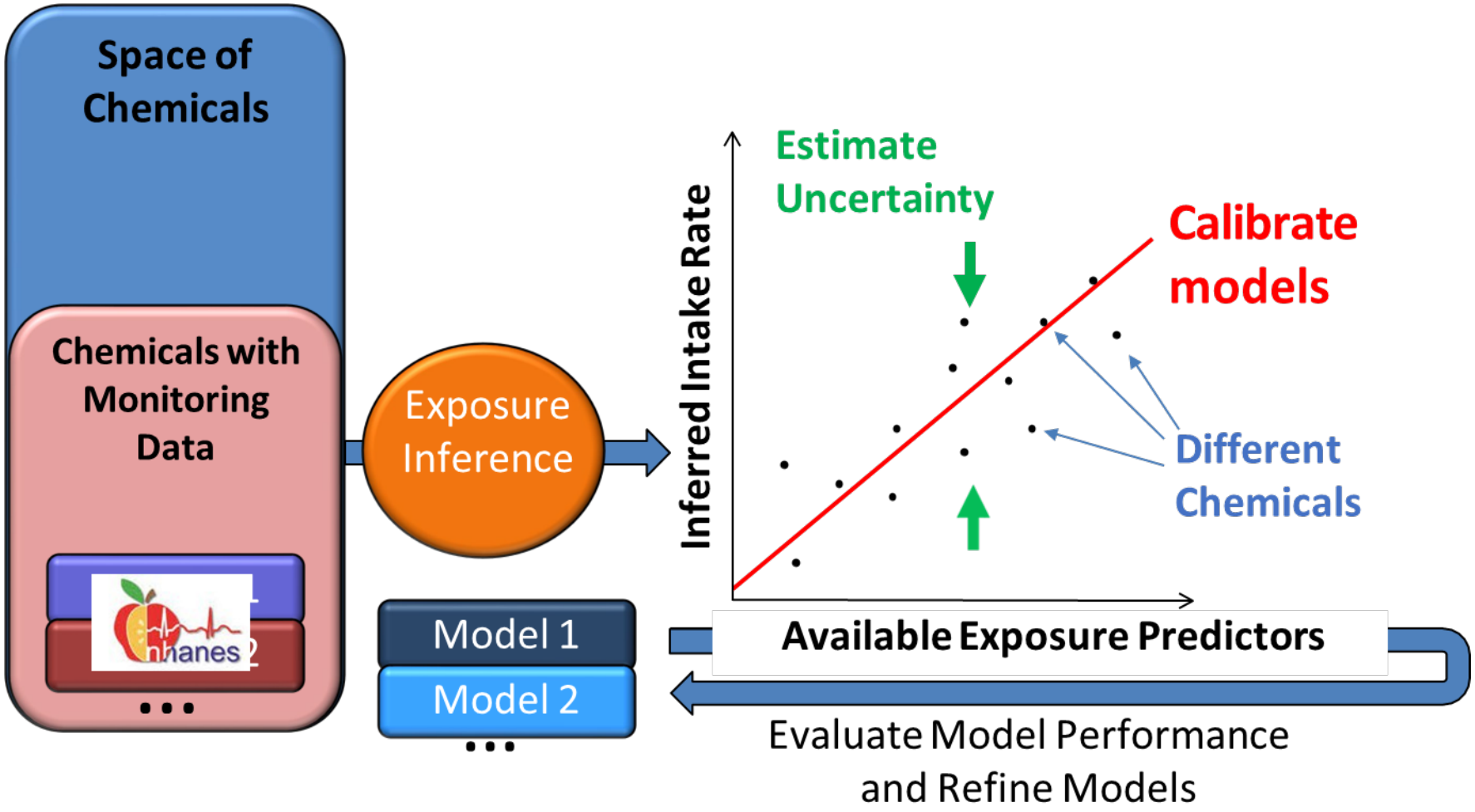

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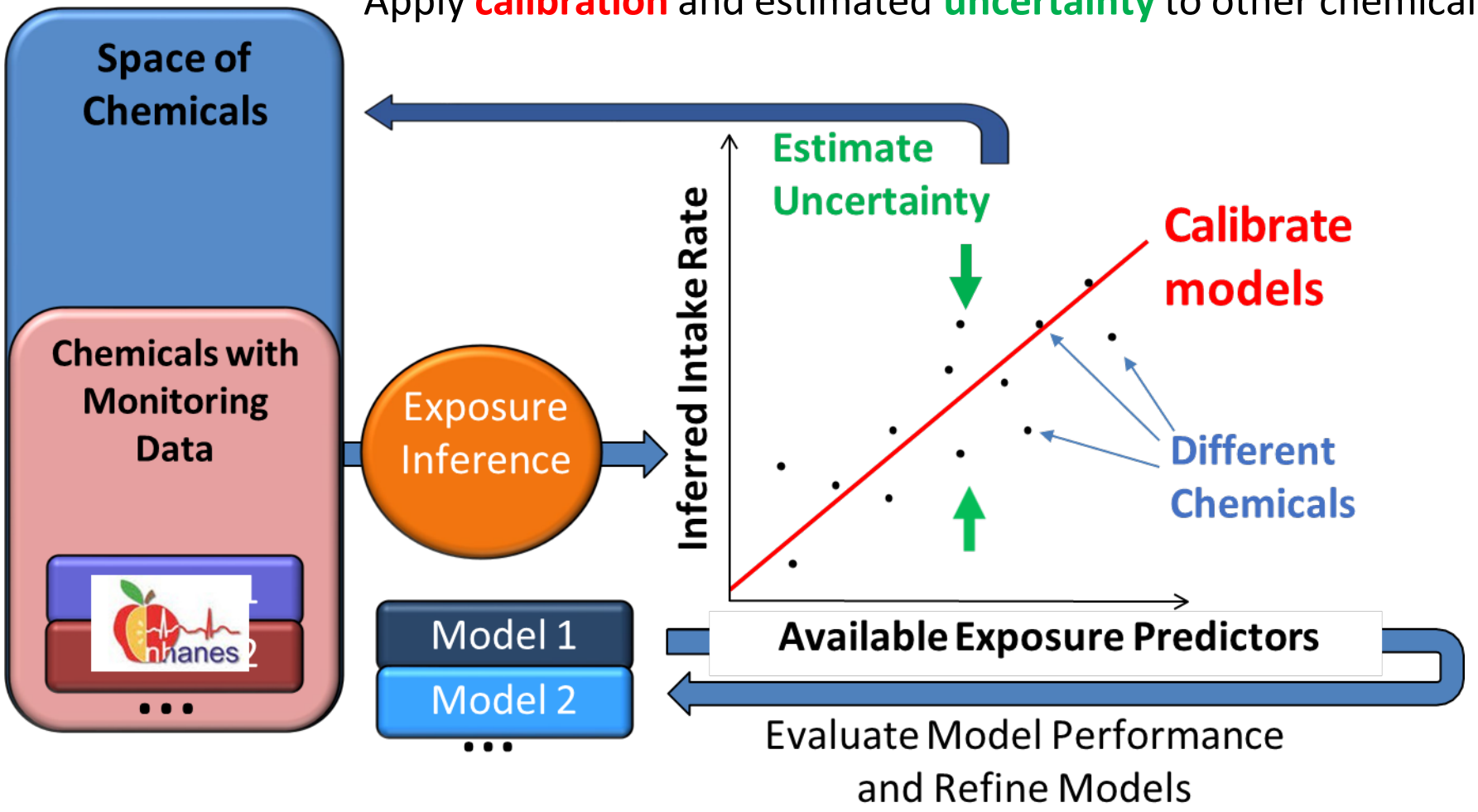
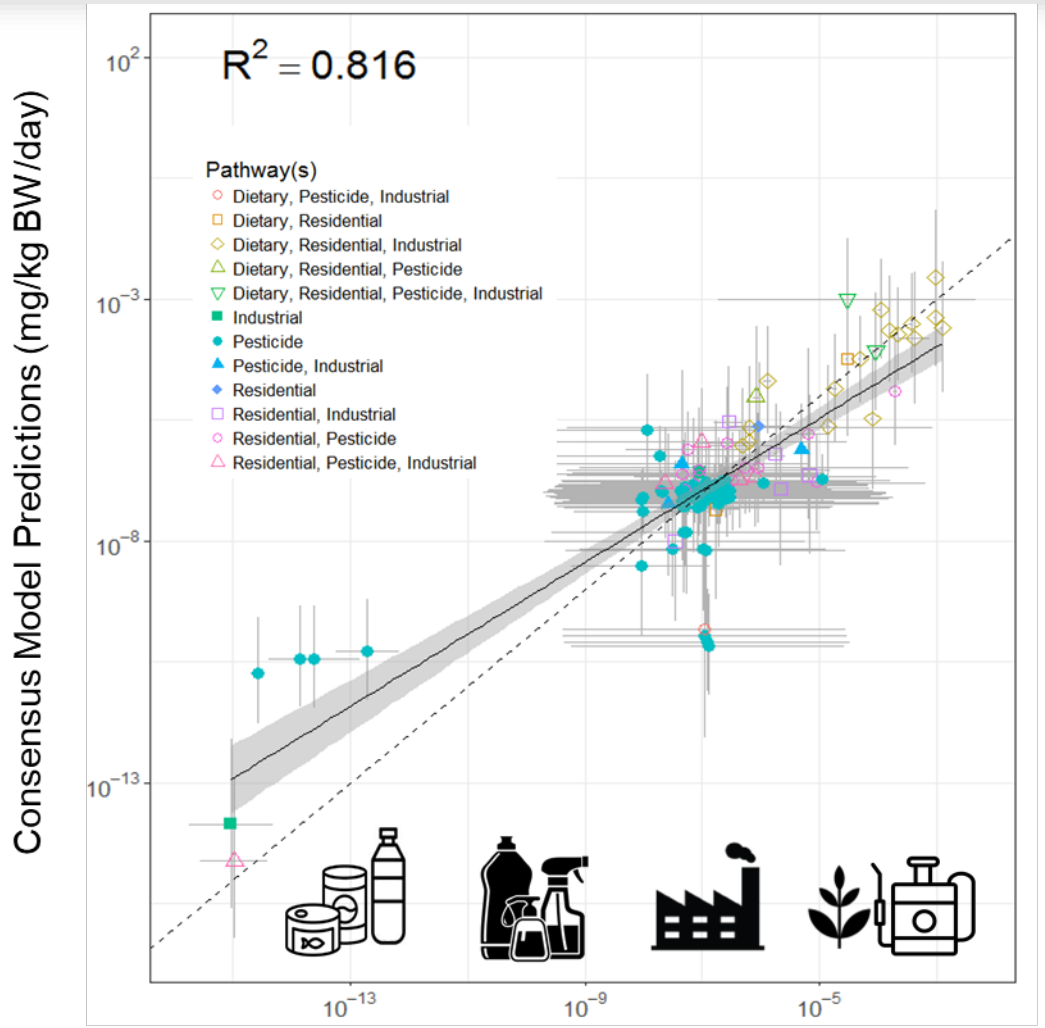
- We apply the SEEM regression to thousands of other chemicals with predictors



Wambaugh et al., Cur. Opin. Toxicol. 2019

SEEM3 Collaboration

- Third generation SEEM model incorporates 12 exposure predictors, including high-throughput exposure models from ExpoCast and its collaborators
- SEEM3 first predicts relevance of **four exposure pathways** from chemical structure using machine learning
- Predictors are weighted according to their ability to explain NHANES data



Ring et al., Env. Sci. Tech. 2019

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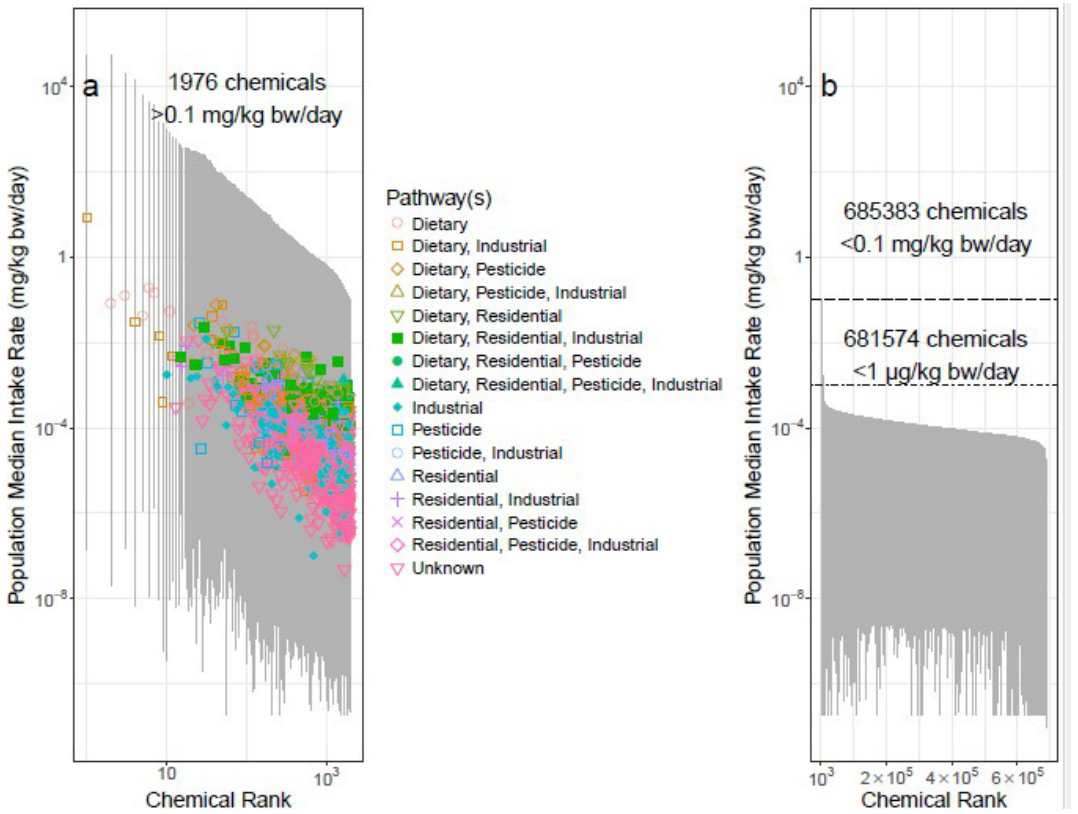

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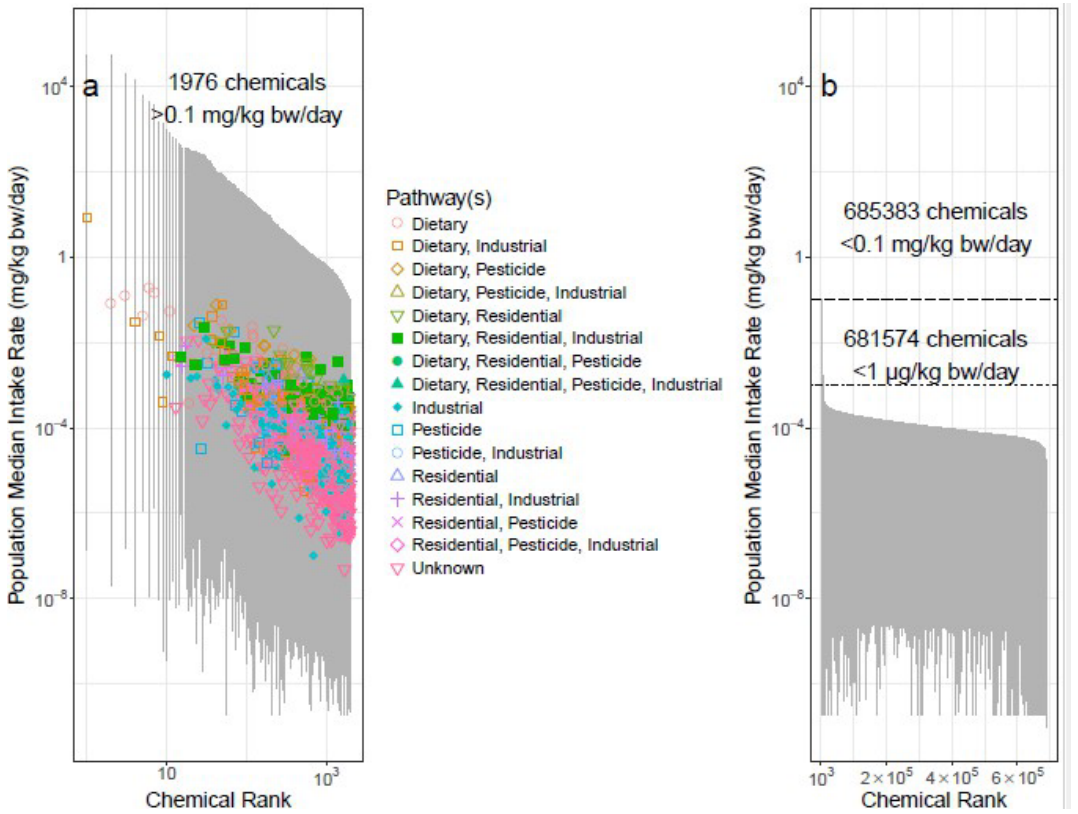
- **SEEM4: Considering cohort in evaluation data (including new NHANES data for children) and HT model predictions**

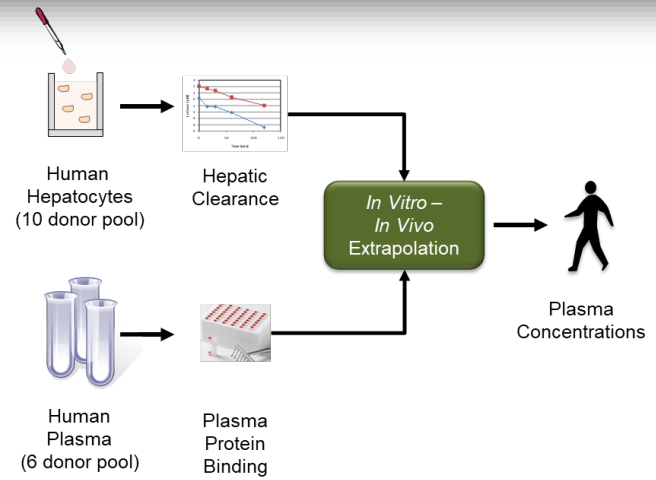


Ring et al., Env. Sci. Tech. 2019

Toxicokinetics NAMs

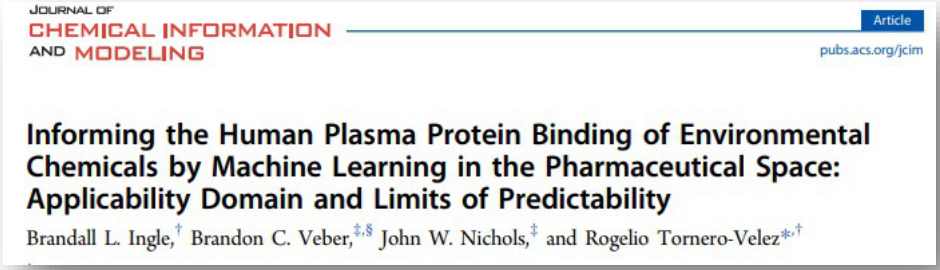
- Chemical-specific data for toxicokinetics (TK) are as sparse as they are for exposure
- High-throughput TK measurements have provided data for nearly 1000 chemicals over the past decade
- However, thousands of chemical still have no data – therefore we employ machine learning and QSAR approaches
- Data and models incorporated into open source R package, httk:

https://CRAN.R-project.org/package=httk



Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (2019) + ~300 chemicals

***In vitro* Measurements**


JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

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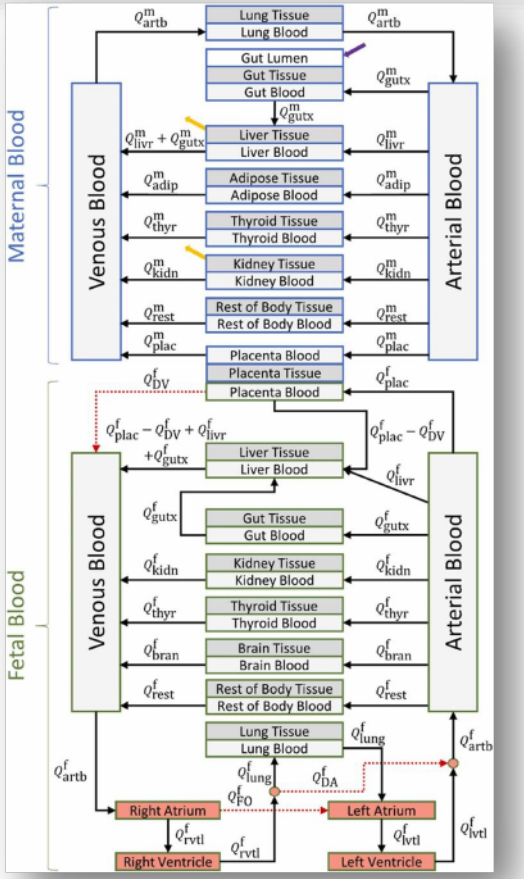
Informing the Human Plasma Protein Binding of Environmental Chemicals by Machine Learning in the Pharmaceutical Space: Applicability Domain and Limits of Predictability

Brandall L. Ingle,[†] Brandon C. Veber,[‡,§] John W. Nichols,[‡] and Rogelio Tornero-Velez*[,†]

Machine Learning Models

Fetal-Maternal PBTK Model

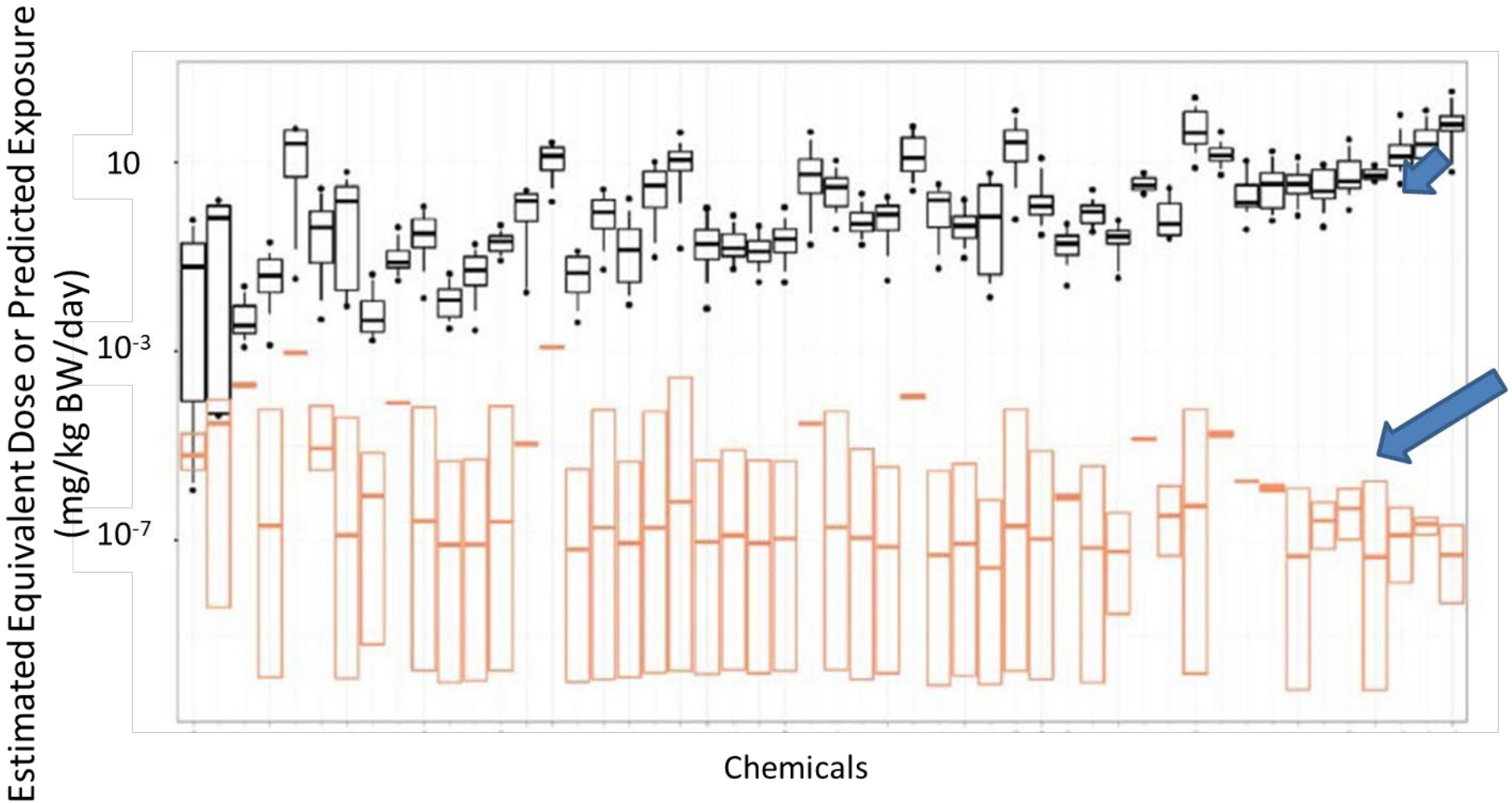


Kapraun et al., Repro Tox 2022

Generic Physiologically-Based Toxicokinetic Models

Material from John Wambaugh

Prioritization NAMs: Risk-Based Evaluation in Practice



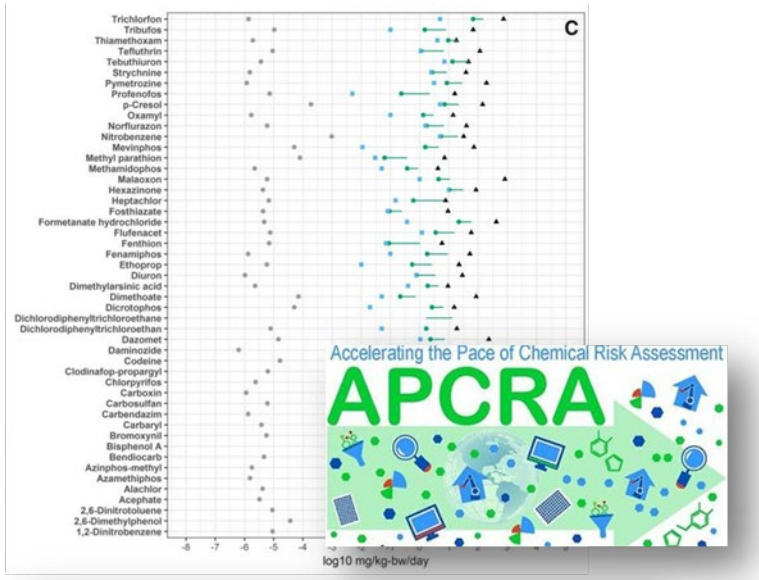
High throughput *in vitro* screening + toxicokinetics NAMs can estimate intake exposures needed to cause bioactivity

Consensus exposure rates with uncertainty (e.g., SEEM3)

Estimates of bioactivity, TK, and exposure are available for thousands of chemicals from:
https://comptox.epa.gov/dashboard/

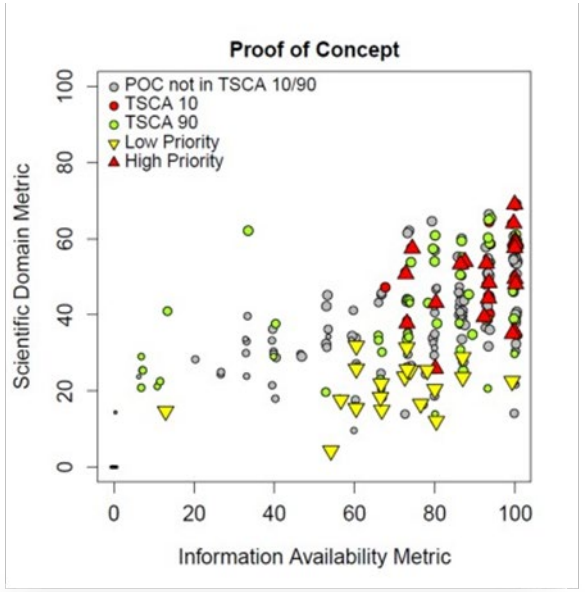
Prioritization NAMs: Risk-Based Evaluation in Practice

- ***Informing an international government-to-government initiative advancing risk evaluation***

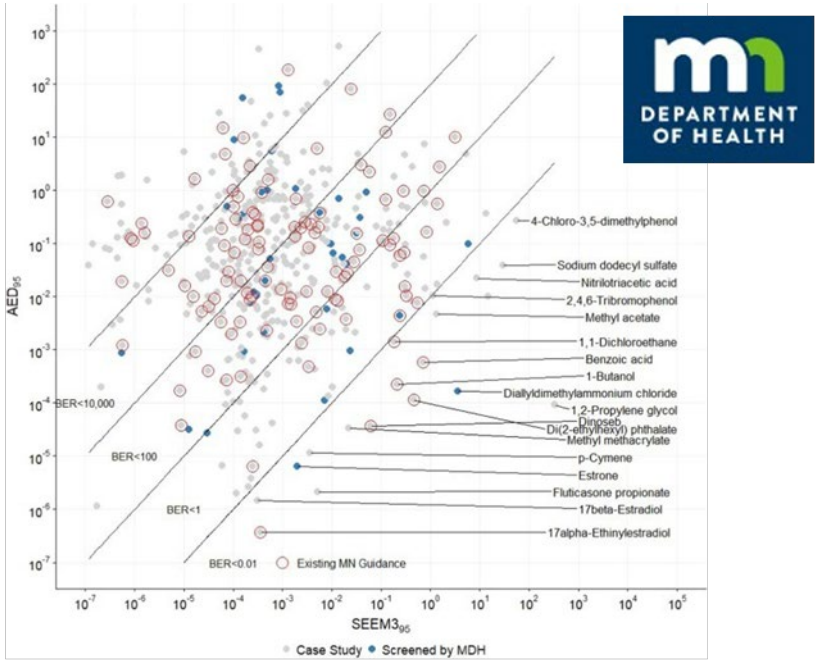


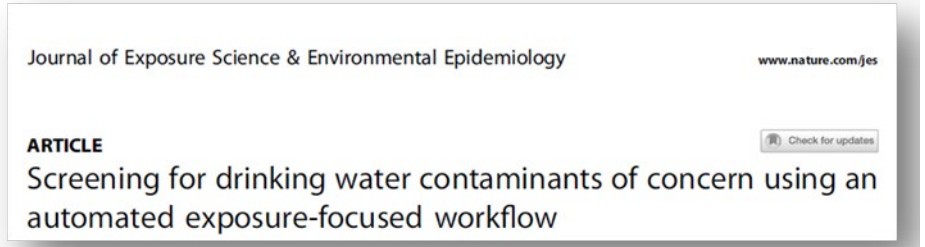
Paul-Friedman et al. (2020)

- ***Screening candidates for chemical prioritization under TSCA***



- ***Evaluating chemicals in state regulatory programs***





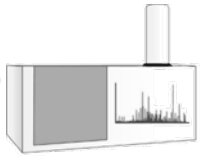
Isaacs et al., (2024)

In Review…

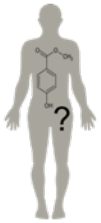
Chemical descriptors that provide information on chemicals in an exposure context (e.g., how chemicals are used or released)

Collect
relevant data

High-throughput measurements to fill gaps in monitoring data

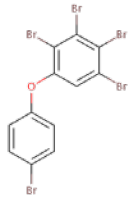


High-throughput toxicokinetics approaches for measuring and predicting chemical fate *in vivo*



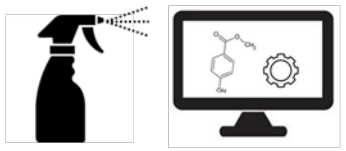
Machine-learning approaches that use these descriptors to fill gaps in existing data

Extrapolate
to fill data gaps



High-throughput exposure models for various exposure pathways

Predict
exposures for specific pathways

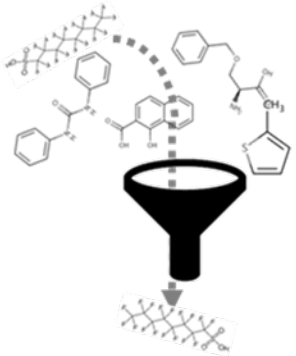


Forecast
exposures for 1000s of chemicals using consensus approaches

Statistical frameworks for integrating models for multiple pathways with exposure data to provide calibrated exposure predictions

Integration of hazard and exposure NAMs for high-throughput ***chemical prioritization***

Prioritize
chemicals for further evaluation



Summary

- Exposure and toxicokinetic data are required as critical input to risk-based prioritization and screening of chemicals.
- The ExpoCast project seeks to develop the data, tools, and evaluation approaches required to generate rapid and scientifically-defensible:
  - Exposure predictions for the full universe of existing and proposed commercial chemicals.
  - The toxicokinetic data required to relate bioactive concentrations identified in high-throughput screening to predicted real-world doses (i.e. *in vitro-in vivo extrapolation*).
- We are developing and applying computational and analytical new approach methods for exposure science and toxicokinetics that are appropriate for application to 1000s of chemicals.
- Rapid prediction of chemical exposure and bioactive doses allows prioritization based upon risk.
- We aim to expand our current approaches to individual cohorts and populations.

ExpoCast Project (Exposure Forecasting)

Center for Computational Toxicology and Exposure

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Division of Translational Toxicology

Integrating geospatial exposure models with NAMs to evaluate health risks from environmental chemicals

Kyle P Messier, PhD

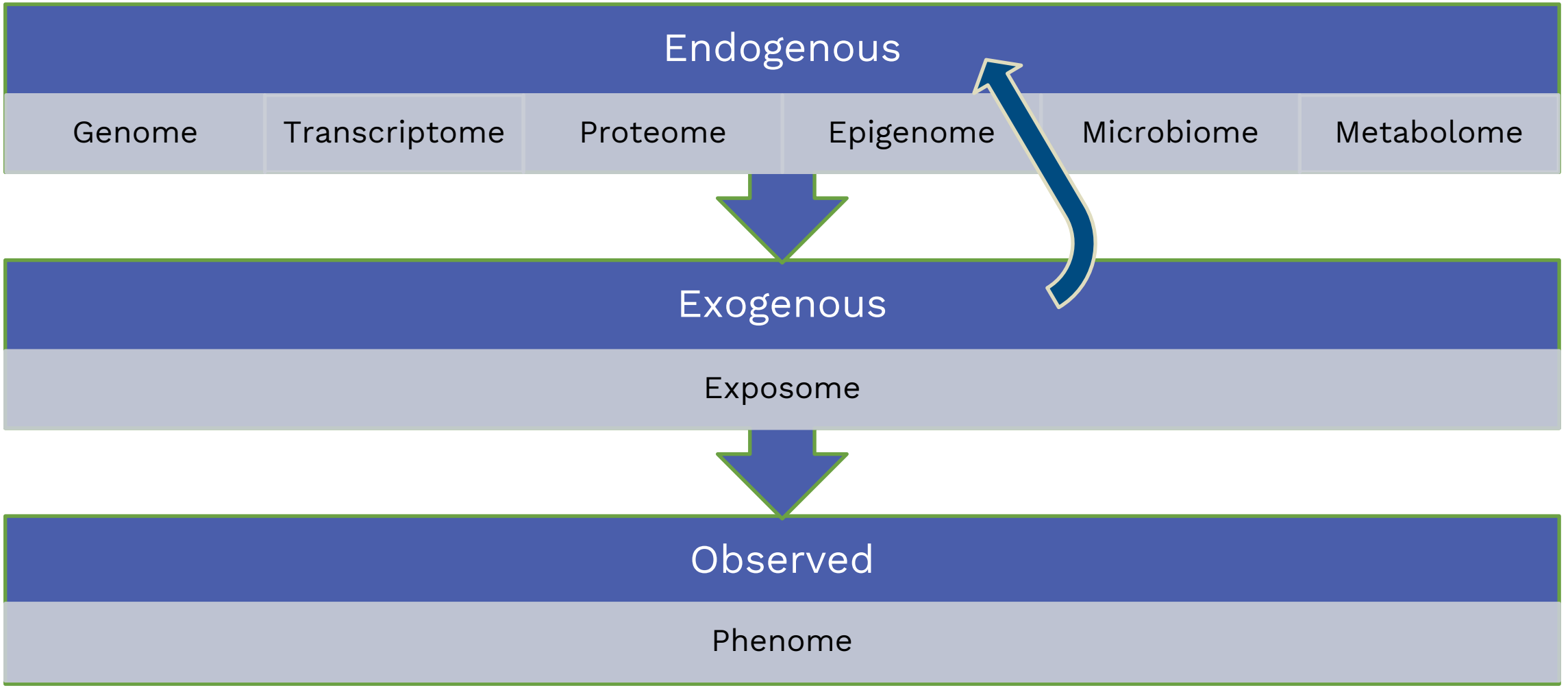
Tenure Track Investigator

Division of Translational Toxicology & Division of Intramural Research

Overview

- Exposome
- Geospatial Exposomics via integration of spatial exposure and hazard NAMs
- GeoTox Software
- Best-Practices in Software to Address Complex Challenges

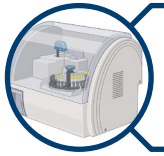
Exposomics

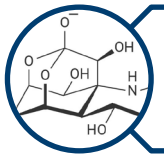

Endogenous
Genome
Transcriptome
Proteome
Epigenome
Microbiome
Metabolome
Exogenous
Exposome
Observed
Phenome

Internal Exposomics

Strengths


Non-Targeted Analysis


Novel Exposure Discovery



High Chemical Throughput

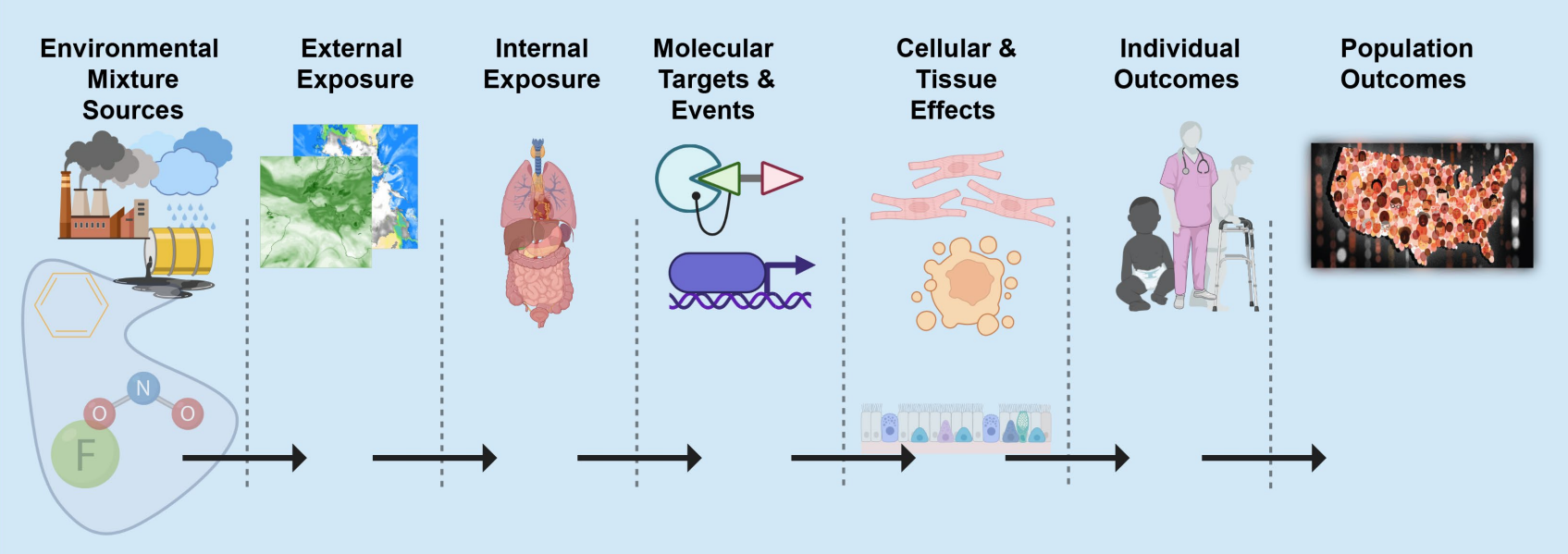

Individual Biological Samples


-Omic measurements

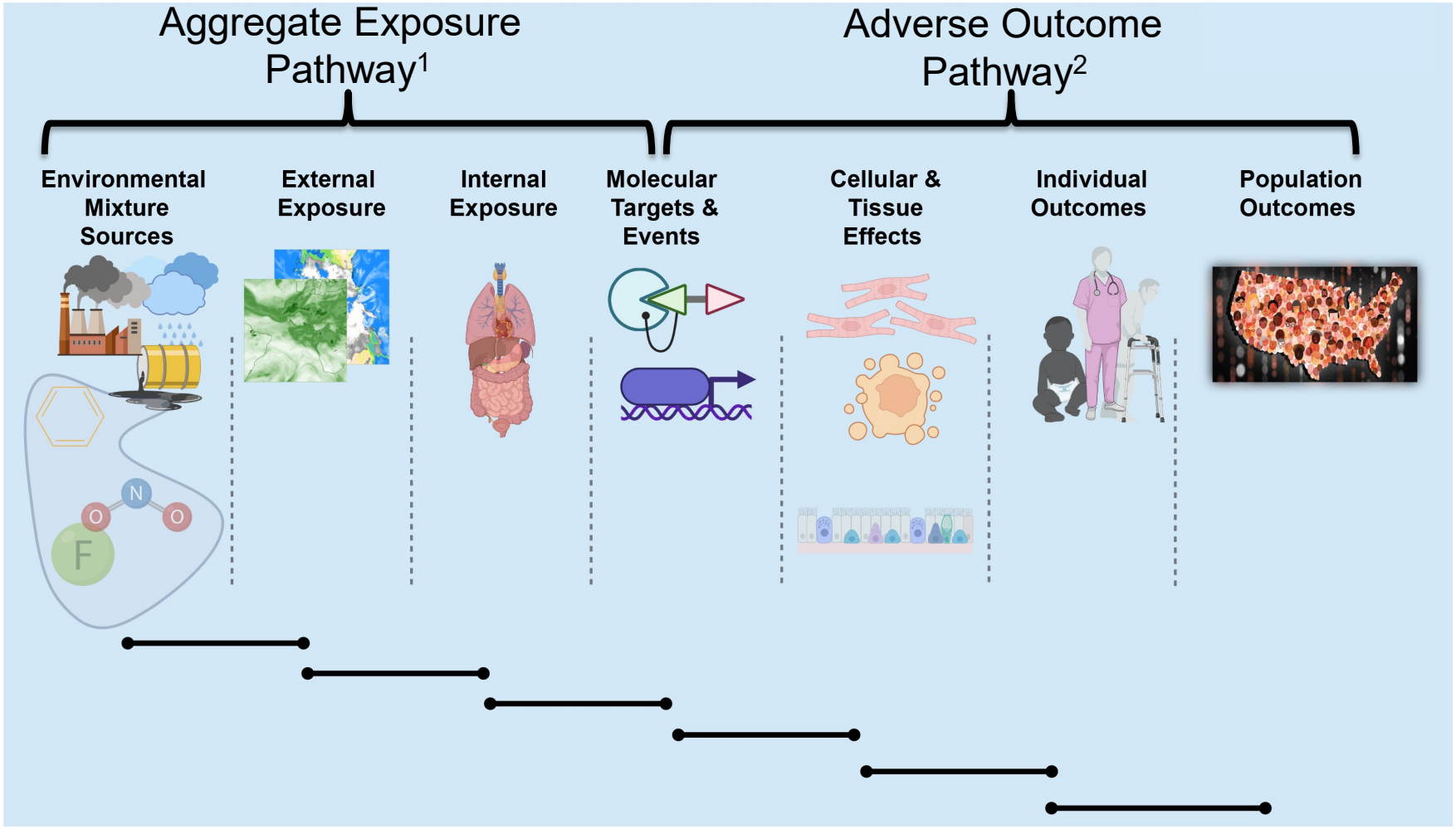
Limitations

- Low Sample Size Throughput
- Expensive
- Batch/Lab Effects
- [Geo] Space/Time Resolution
- [Geo] Space/Time Variability
- Endogenous/Exogenous Specificity

Geospatial Exposomics



Source-to-Outcome Cascade:
Sequential and necessary steps to result in an individual or population health outcome



1. Teeguarden JG, Tan YM, Edwards SW, Leonard JA, Anderson KA, Corley RA, Kile ML, Simonich SM, Stone D, Tanguay RL, Waters KM. Completing the link between exposure science and toxicology for improved environmental health decision making: the aggregate exposure pathway framework.

2. http://aop.wiki.org; Society for the Advancement of Adverse Outcome Pathways

AEP + AOP = **GeoTox**

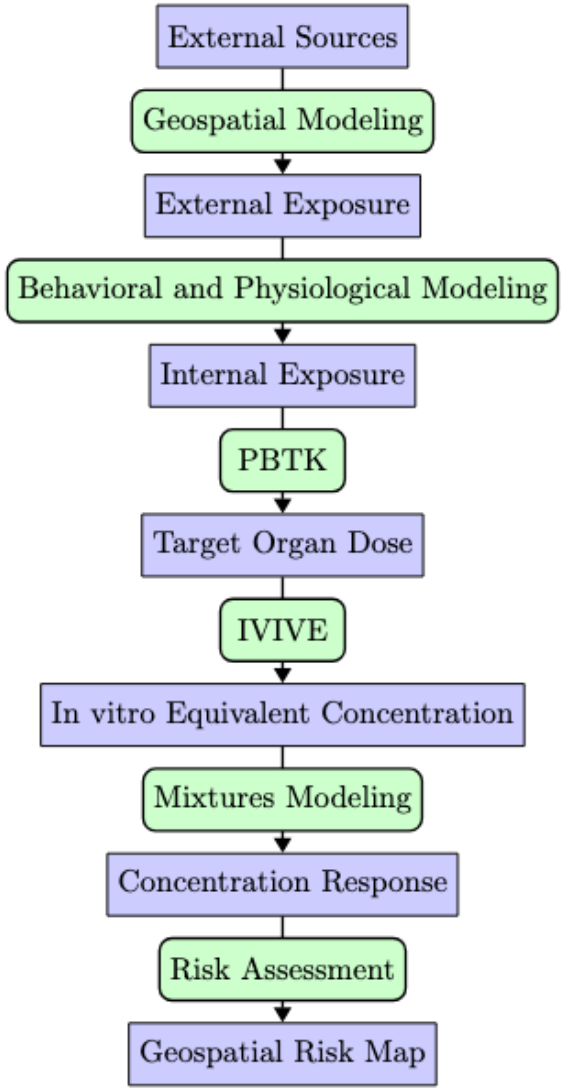
AEP is a comprehensive external analysis of source, media, and transformations

AOPs provide a linkage specific biological target, pathway or process by a stressor and an adverse outcome(s) considered relevant to risk assessment

Source-to-Outcome Workflow

Data Object

Method



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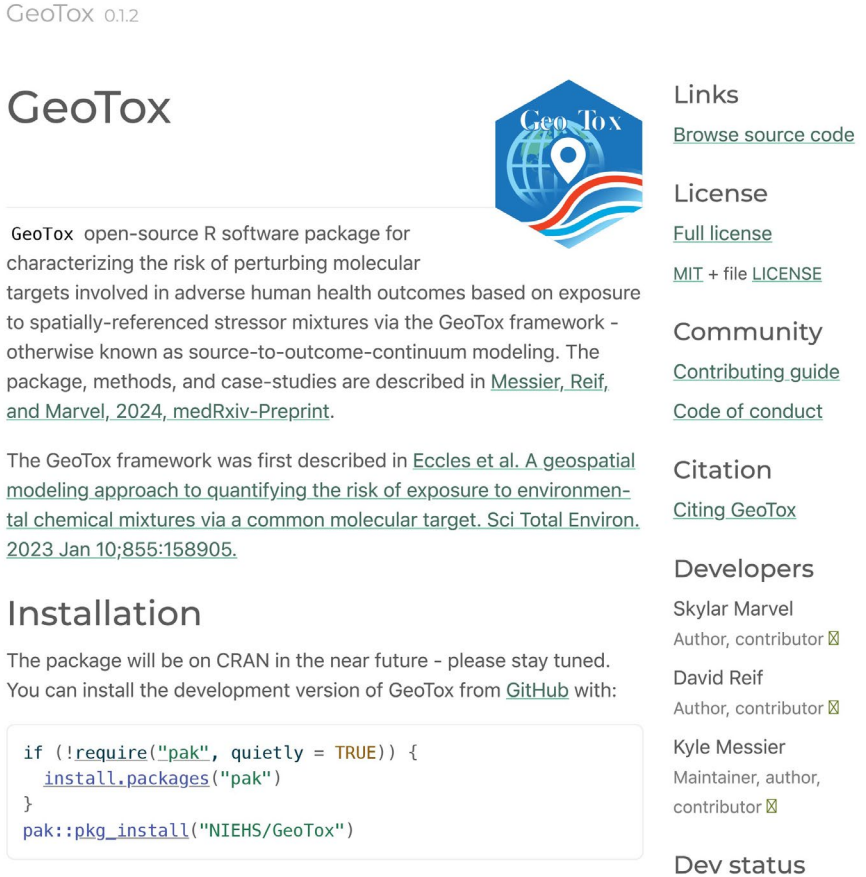
Introducing the *GeoTox* R package

Computational Best Practices

- Open Source
- Test Driven
- Documented
- Extensible

Methodology

- Object Oriented
- Tidyverse, pipe-able, |>
- Tracks exposure, population characteristics, dose-response, spatial boundaries, etc.



GeoTox 0.1.2

GeoTox

`GeoTox` open-source R software package for characterizing the risk of perturbing molecular targets involved in adverse human health outcomes based on exposure to spatially-referenced stressor mixtures via the GeoTox framework - otherwise known as source-to-outcome-continuum modeling. The package, methods, and case-studies are described in Messier, Reif, and Marvel, 2024, medRxiv-Preprint.

The GeoTox framework was first described in Eccles et al. A geospatial modeling approach to quantifying the risk of exposure to environmental chemical mixtures via a common molecular target. Sci Total Environ. 2023 Jan 10;855:158905.

Installation

The package will be on CRAN in the near future - please stay tuned. You can install the development version of GeoTox from GitHub with:

```
if (!require("pak", quietly = TRUE)) {
  install.packages("pak")
}
pak::pkg_install("NIEHS/GeoTox")
```

Links

Browse source code

License

Full license

MIT + file LICENSE

Community

Contributing guide

Code of conduct

Citation

Citing GeoTox

Developers

Skylar Marvel
Author, contributor

David Reif
Author, contributor

Kyle Messier
Maintainer, author, contributor

Dev status

GeoTox Object

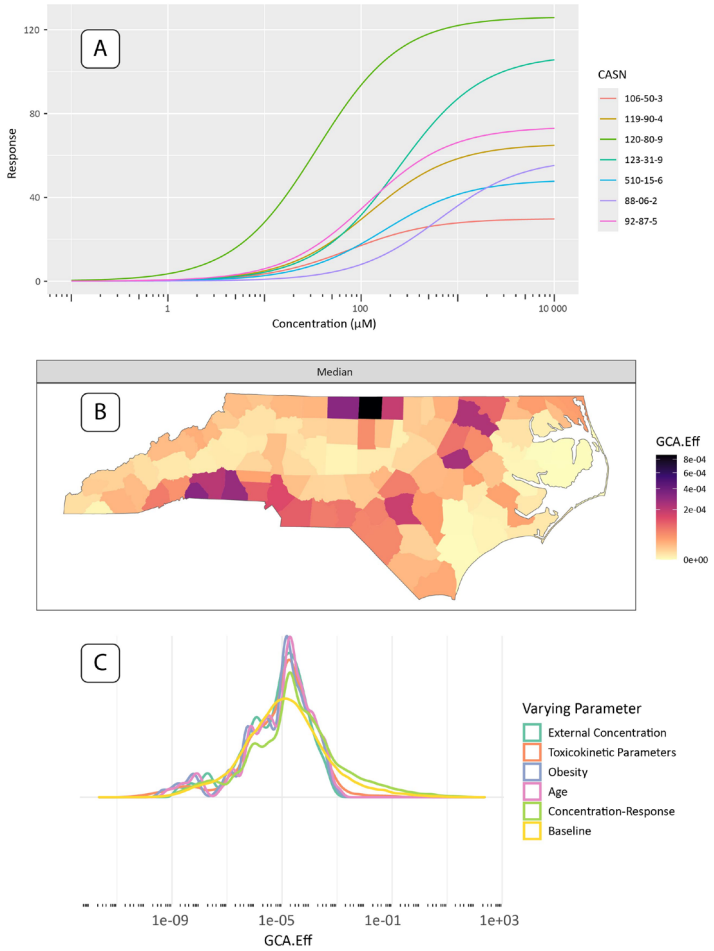
```
set.seed(2357)
geoTox <- GeoTox() |>
  # Set region and group boundaries (for plotting)
  set_boundaries(region = geo_tox_data$boundaries$county,
                 group  = geo_tox_data$boundaries$state) |>
  # Simulate populations for each region
  simulate_population(age           = split(geo_tox_data$age, ~FIPS),
                      obesity       = geo_tox_data$obesity,
                      exposure      = split(geo_tox_data$exposure, ~FIPS),
                      simulated_css = geo_tox_data$simulated_css,
                      n             = n) |>
  # Estimated Hill parameters
  set_hill_params(geo_tox_data$dose_response |>
                    fit_hill(assay = "endp", chem = "casn") |>
                    filter(!tp.sd.imputed, !logAC50.sd.imputed)) |>
  # Calculate response
  calculate_response() |>
  # Perform sensitivity analysis
  sensitivity_analysis()

geoTox
#> GeoTox object
#> Assays: 13
#> Chemicals: 20
#> Regions: 100
#> Population: 250
#> Data Fields:
#>      Name            Class               Dim
#>       age    list(integer)       100 x (250)
#>        IR    list(numeric)       100 x (250)
#>   obesity  list(character)       100 x (250)
#>     C_ext     list(matrix)  100 x (250 x 21)
#>      C_ss     list(matrix)  100 x (250 x 21)
#> Computed Fields:
#>          Name             Class               Dim
#>         D_int      list(matrix)  100 x (250 x 21)
#>     C_invitro      list(matrix)  100 x (250 x 21)
#>          resp  list(data.frame)  100 x (3250 x 6)
#>   sensitivity        list(list)         5 x (100)
#> Other Fields: par, boundaries, exposure, css_sensitivity, hill_params
```

Geospatial Risk Mapping of Chemical Mixtures

Example 1

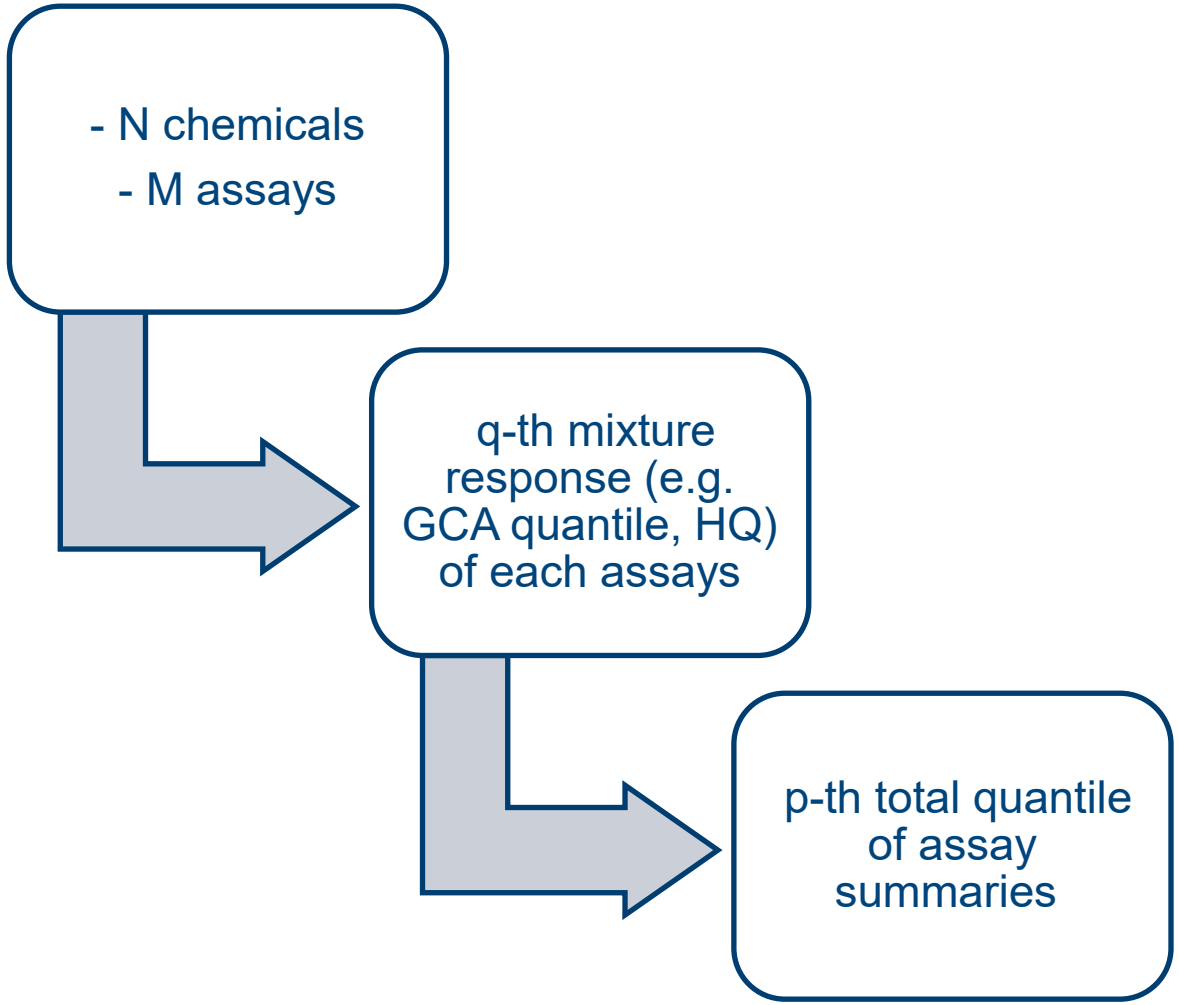
- 7 chemicals in air
- 1 Assay: H2AX Histone Modification
- Generalized Concentration Addition
- Mapped risk as quantified by assay response



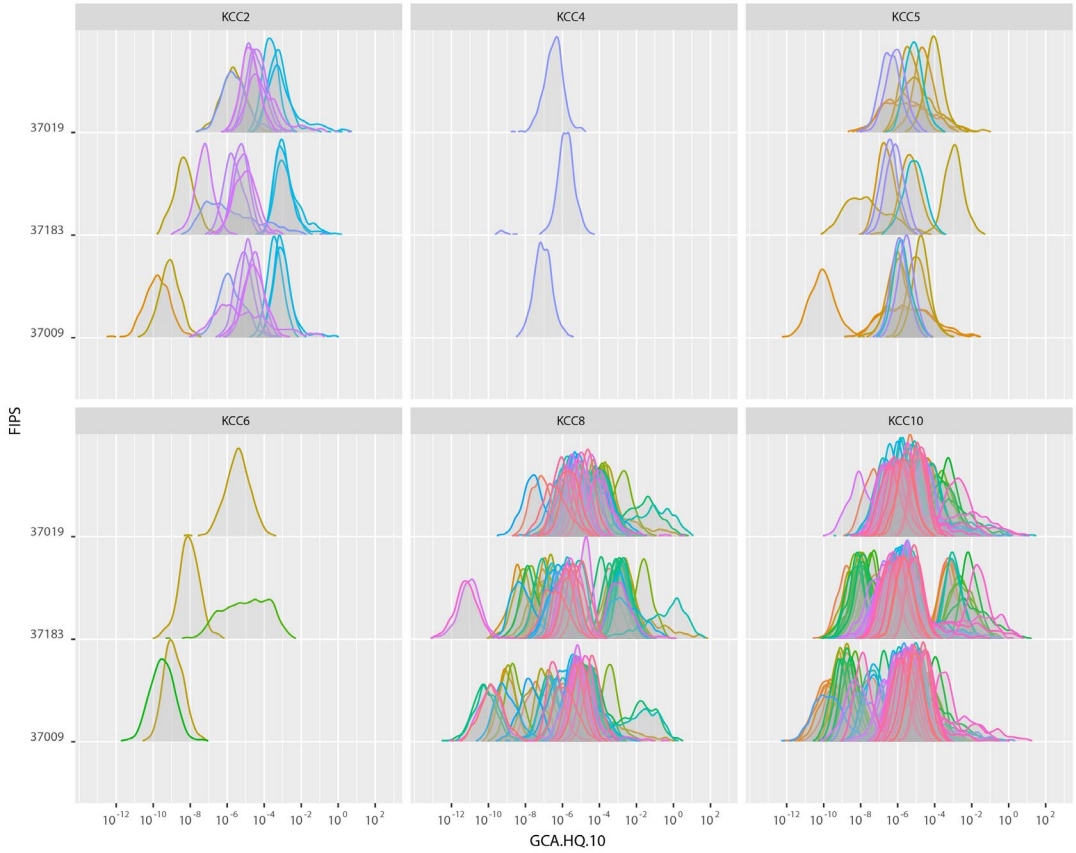
Multi-Assay Risk Mapping of Chemical Mixtures

Example 2

- 40+ chemicals in air
- 200+ Assays Based on Key Characteristics of Carcinogens (KCC)
- GCA, IA, Hazard Quotient
- "*p*-th total quantile of the *q* assay-level quantiles"

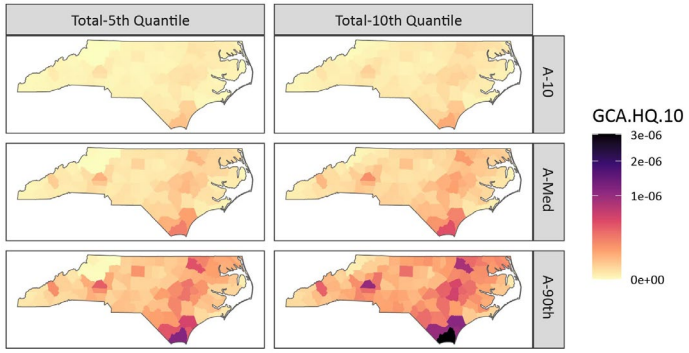


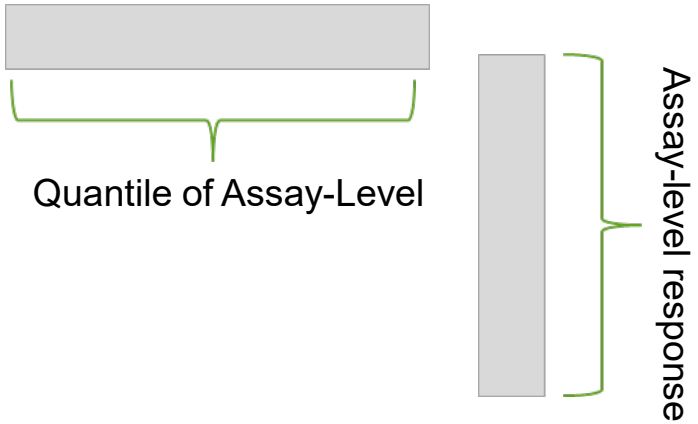
Multi-Assay Risk Mapping of Chemical Mixtures



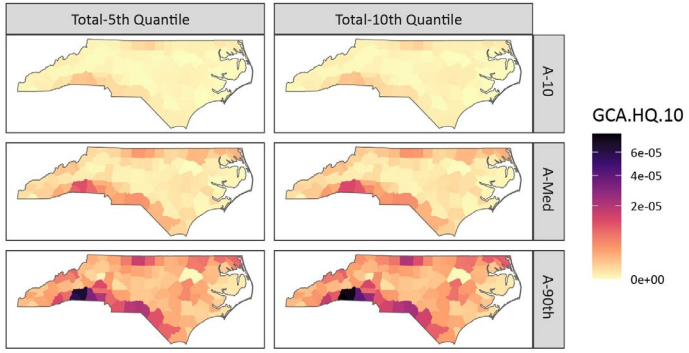
Multi-Assay Risk Mapping of Chemical Mixtures

Multi-Assay Summaries: All KCC Assays

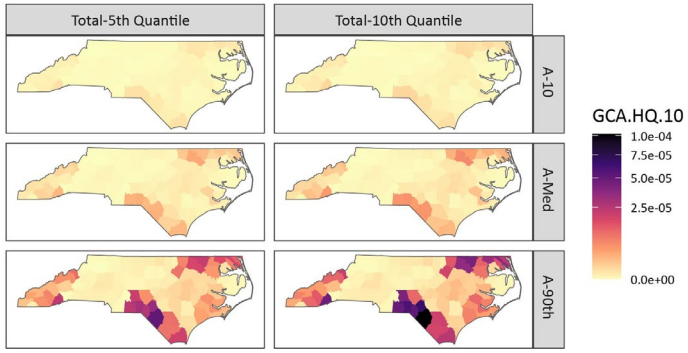




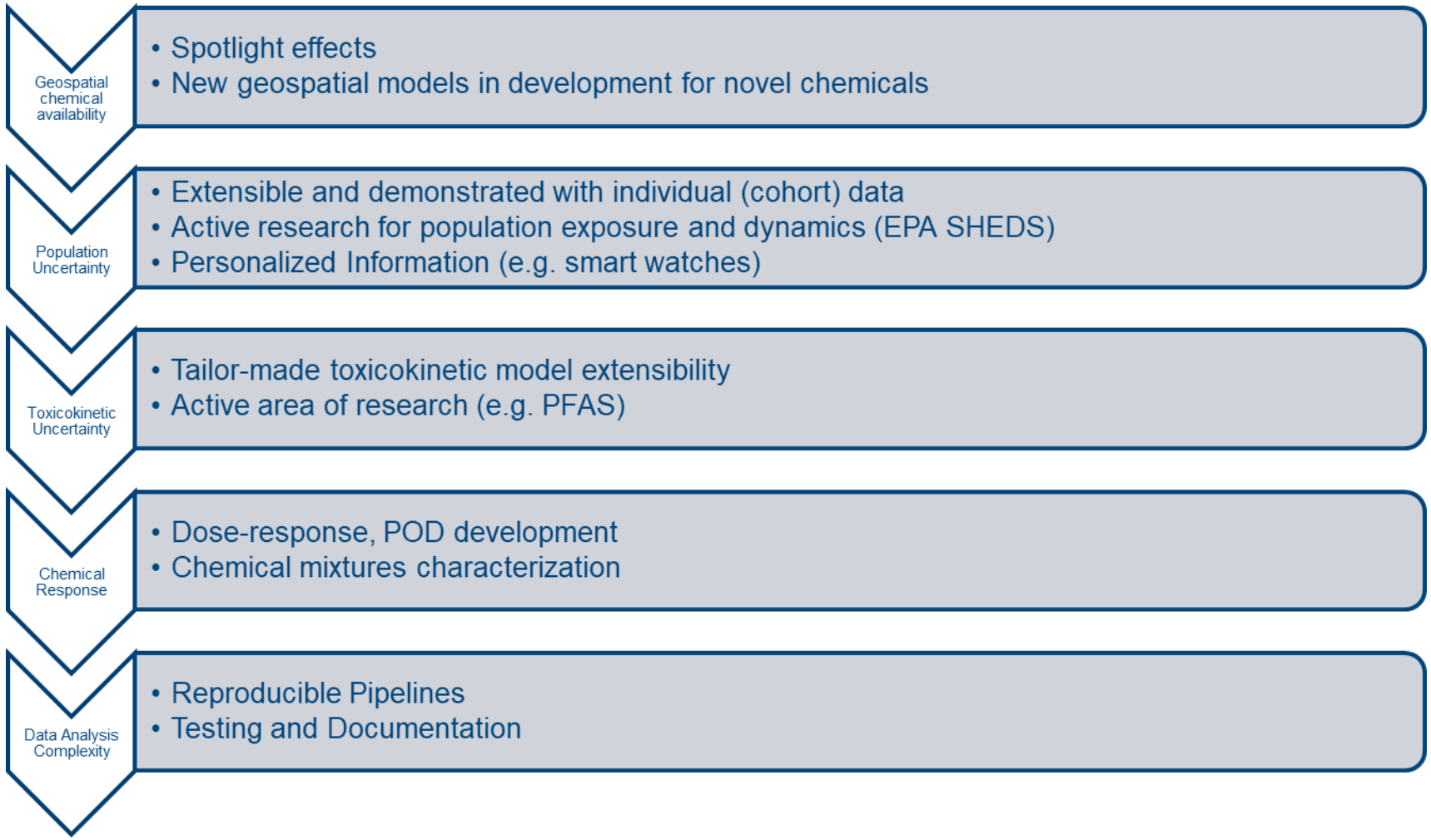
KCC5: Oxidative Stress



KCC2: Genotoxic Effects



Limitations + Future Directions



Software and Computational Best-Practices

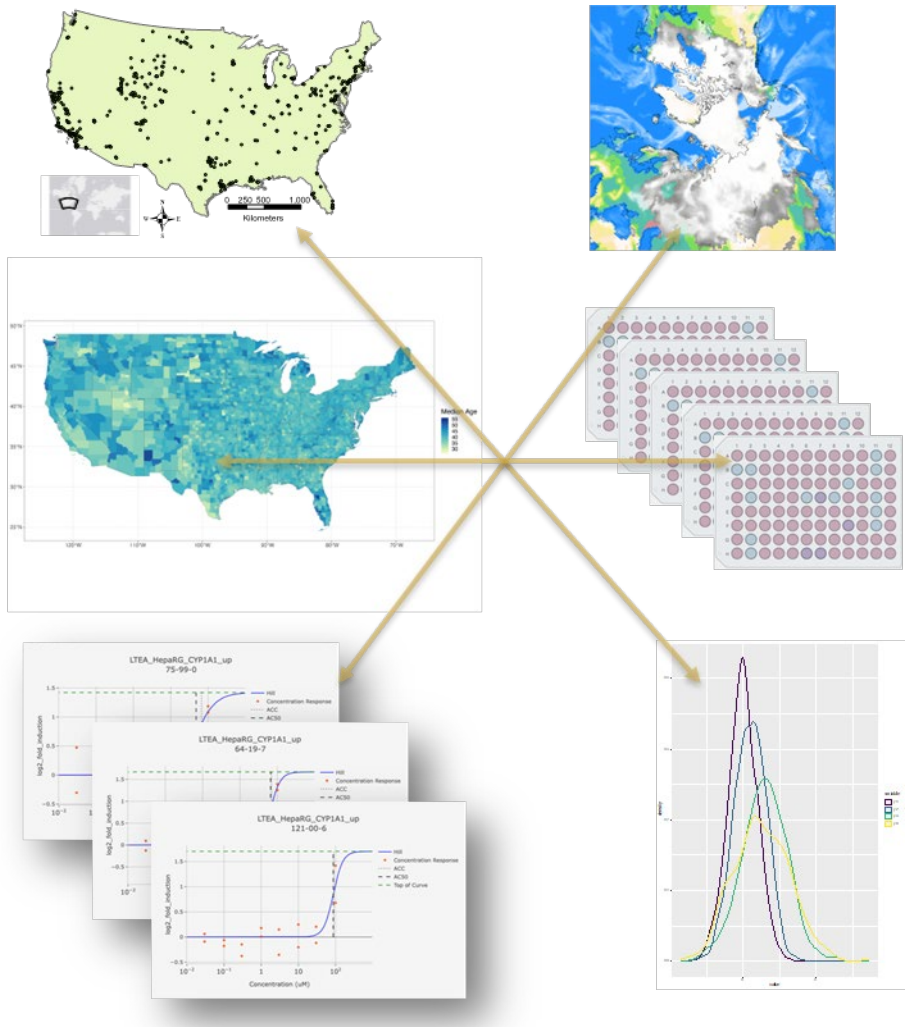
- Test Driven Development
- Continuous Integration
- Build Checks
- Style / Linting
- Workflows / Pipelines

Best Practices are Needed for Complex Environmental Health Pipelines

Geospatial modeling and GeoTox offer a tractable approach for quantifying the exposome health impacts

Need best practices to build towards a very complex analysis and understanding

(1) In-Situ Monitoring Data (2) Atmospheric / Geophysical Data (3) Census Data (4) In-Vitro Screening Data (5) Concentration-Response Modeling (6) Probabilistic Models (7) …



Acknowledgements

- Co-authors on GeoTox package and manuscript: David Reif & Skylar Marvel
- Lead author on GeoTox concept paper and former postdoc: Kristin Eccles
- SET group member: Eva Marques, Mariana Alifa, Mitchell Manware
- NIEHS Computational Support: Office of Scientific Computing

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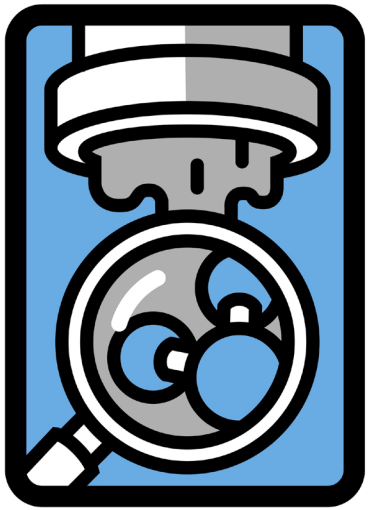
Human and Environmental Exposure Framework for Biosolids

Carsten Prasse, Ph.D.

Department of Environmental Health & Engineering,
Johns Hopkins University

- **Disclaimer**: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Any use of trade, firm, or product names is for descriptive purposed only and does not imply endorsement by the U.S. Government.

Funding



Fate and Transport of Unregulated Organic Contaminants in Biosolids: Development of a Human and Environmental Exposure Risk Framework

EPA Grant: R840247

Project Team









Principal Investigator (PI): Carsten Prasse

Co-PI: Keeve Nachman

Thomas Burke

Research Associates: Sara Lupolt

Matthew Newmeyer

Students: Riley Demo

Noor Hamdan

Kate Burgener

Dominic Sanchez

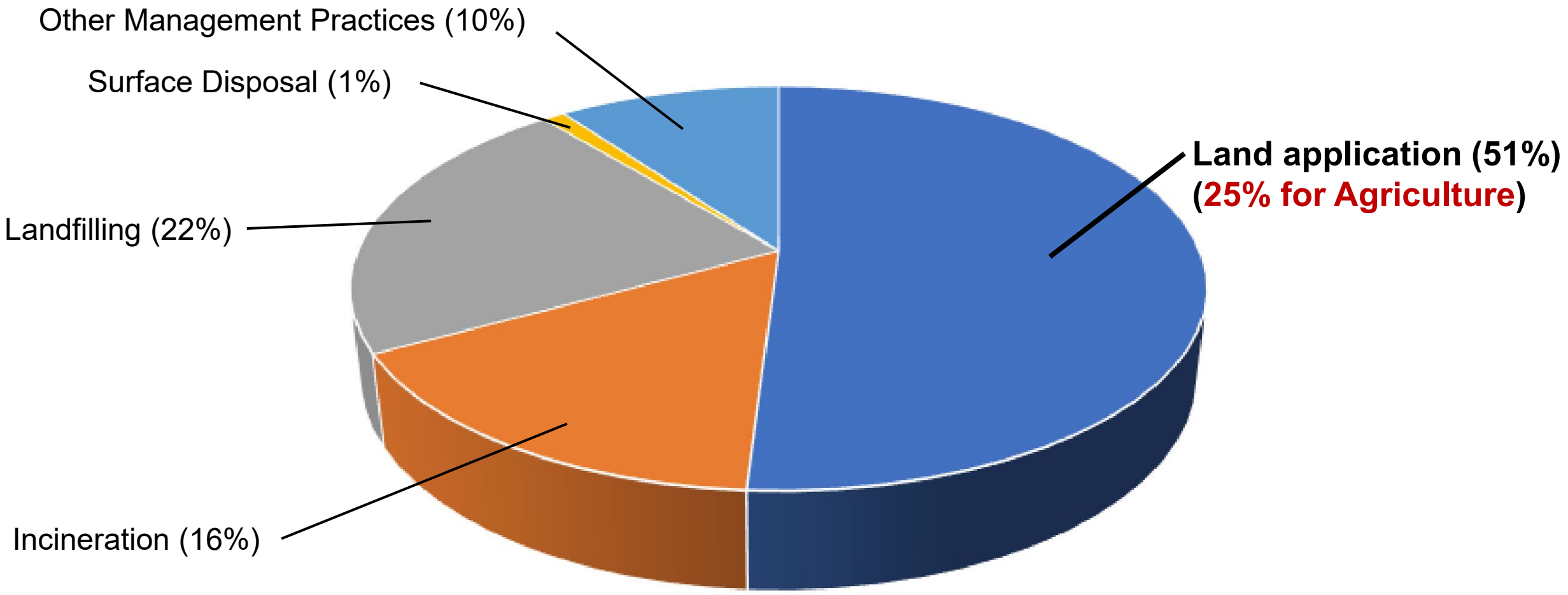
EPA Collaborators: Antony Williams

Jon Sobus

What are biosolids?

→ solid byproduct of sewage treatment processes

4.75 million dry metric tons of biosolids are generated each year



EPA, 2021

Land application of biosolids

Biosolid land application can be beneficial:
- ✓ Improves soil qualities
- ✓ Supplies nutrients
- ✓ Diverts from landfilling & incineration

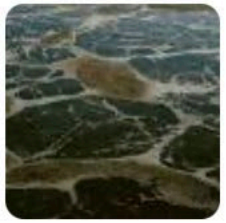
Current federal regulation (40 CFR Part 503) of biosolid quality includes:
- ➢ Limits on 10 heavy metals
- ➢ Requirements for pathogen & vector attraction reduction

→ Currently, no organic contaminants are regulated in biosolids

Example of organic contaminants in biosolids: PFAS

The New York Times

Something's Poisoning America's Land. Farmers Fear 'Forever Chemicals.'



Fertilizer made from city sewage has been spread on millions of acres of farmland for decades. Scientists say it can contain high levels of...

Aug 31, 2024

Nebraska Public Media

This farmer's livelihood was ruined by PFAS-contaminated fertilizer that few Midwest states test for

Biosolids — a type of treated sewage byproduct from wastewater treatment plants — are used as a nutrient-rich fertilizer on farms across the...

Mar 11, 2024

The Guardian

Texas farmers claim company sold them PFAS-contaminated sludge that killed livestock

Two ranches also allege biosolids with 'forever chemicals' ruined crops, polluted drinking water and left their properties worthless.

Mar 1, 2024

Chemical & Engineering News

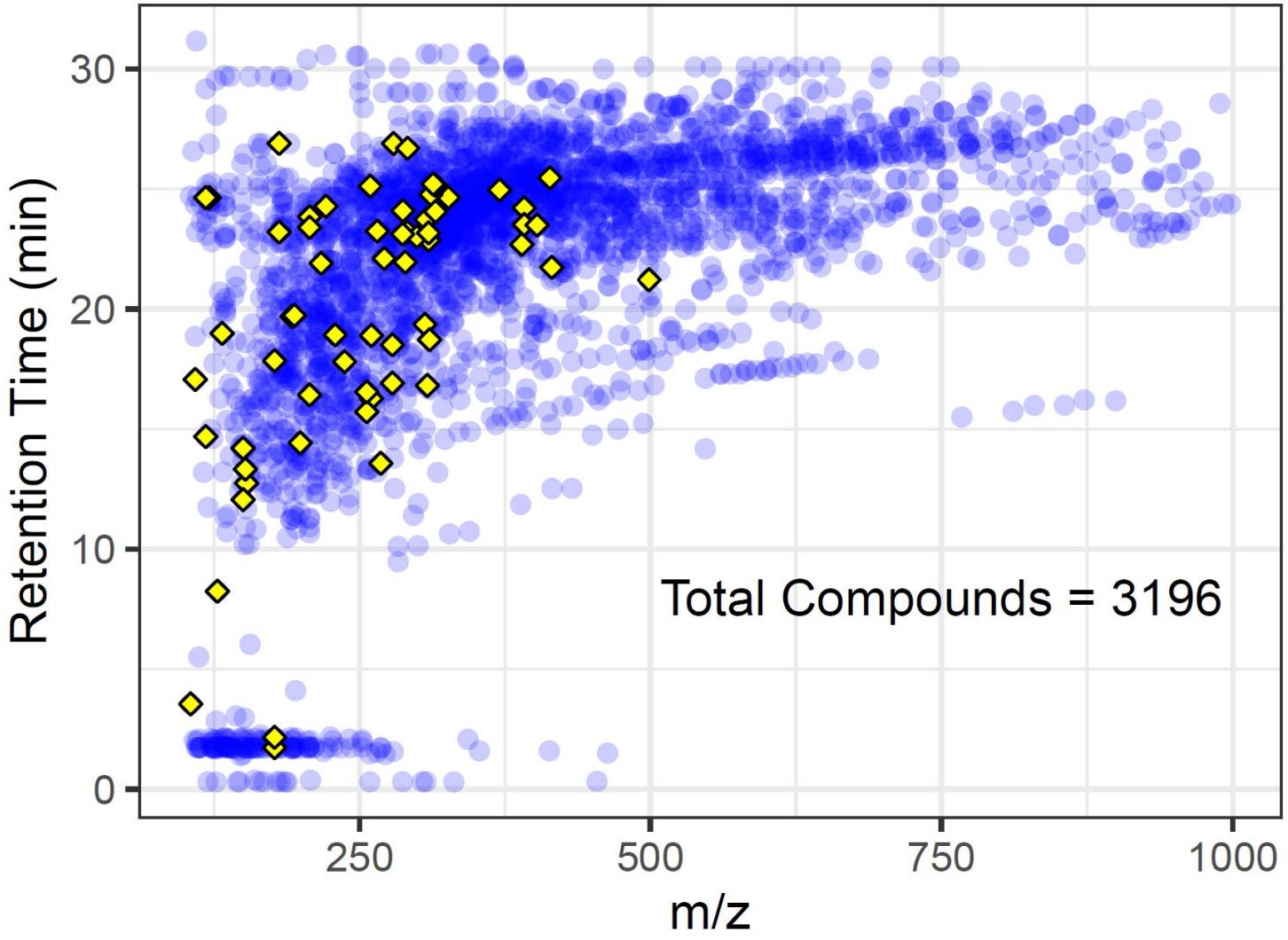
PFAS in biosolids prompt lawsuits

The lawsuit alleges that Synagro failed to warn product users about the adverse health effects associated with exposure to PFAS. Some of the...

Feb 28, 2024

Biosolids contain a large number of unknown organic compounds

(e.g. pharmaceuticals, industrial chemicals, pesticides, naturally occurring compounds, …)



< 5% of compounds are known (highlighted in yellow; based on EPA biosolids list)

High-resolution mass spectrometry system available in Prasse lab.

Biosolids contain a large number of unknown organic compounds

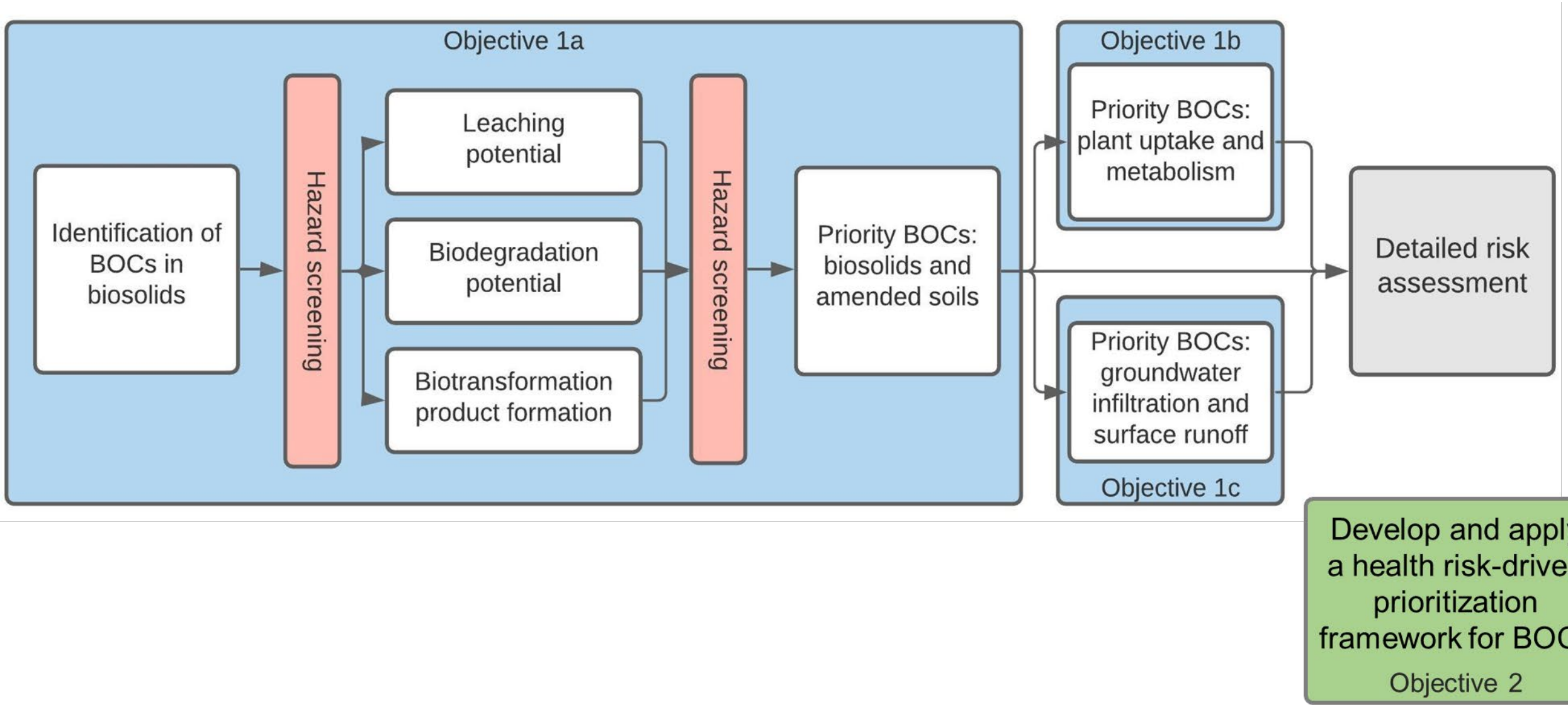
(e.g. pharmaceuticals, industrial chemicals, pesticides, naturally occurring compounds, …)

< 5% of compounds are known (highlighted in yellow; based on EPA biosolids list)

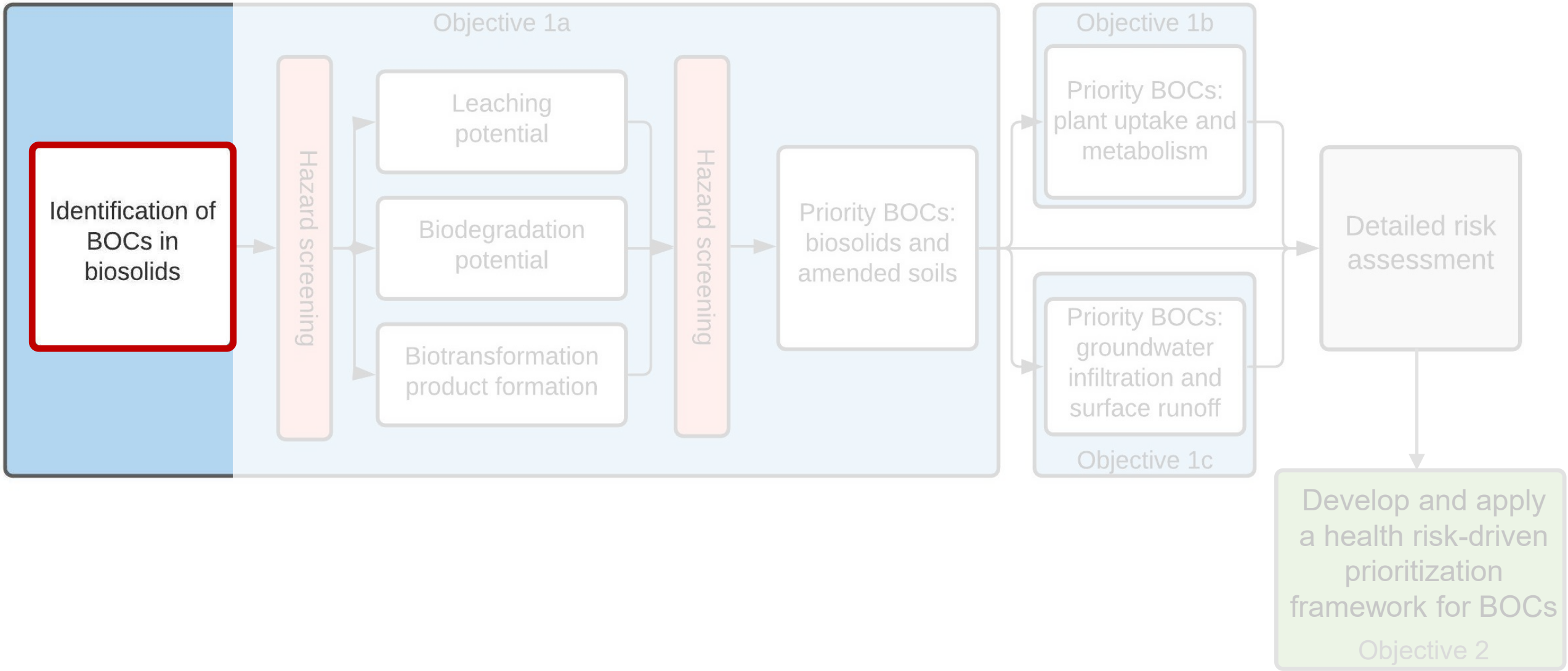
We need to develop approaches that aid in the identification of toxic compounds in complex mixtures

High-resolution mass spectrometry system available in Prasse lab.

Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)

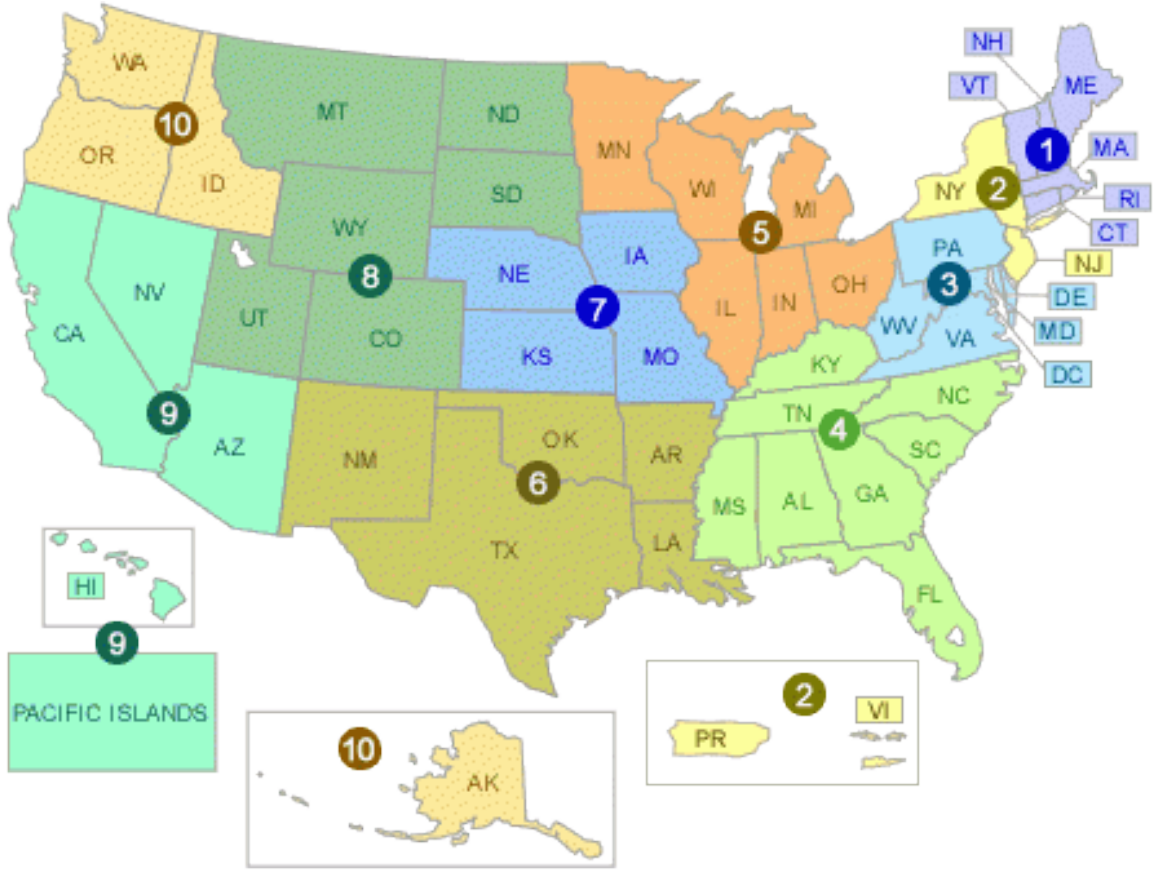


Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)



Biosolid sampling

- 16 samples across U.S. and Canada:
  - U.S.: 13 samples
    - Region 2: 2 samples
    - Region 3: 3 samples
    - Region 5: 2 samples
    - Region 7: 2 samples
    - Region 9: 4 samples
  - Canada: 3 samples



Regional and Geographic Offices. https://www.epa.gov/aboutepa/regional-and-geographic-offices. Accessed 2023 Aug 08

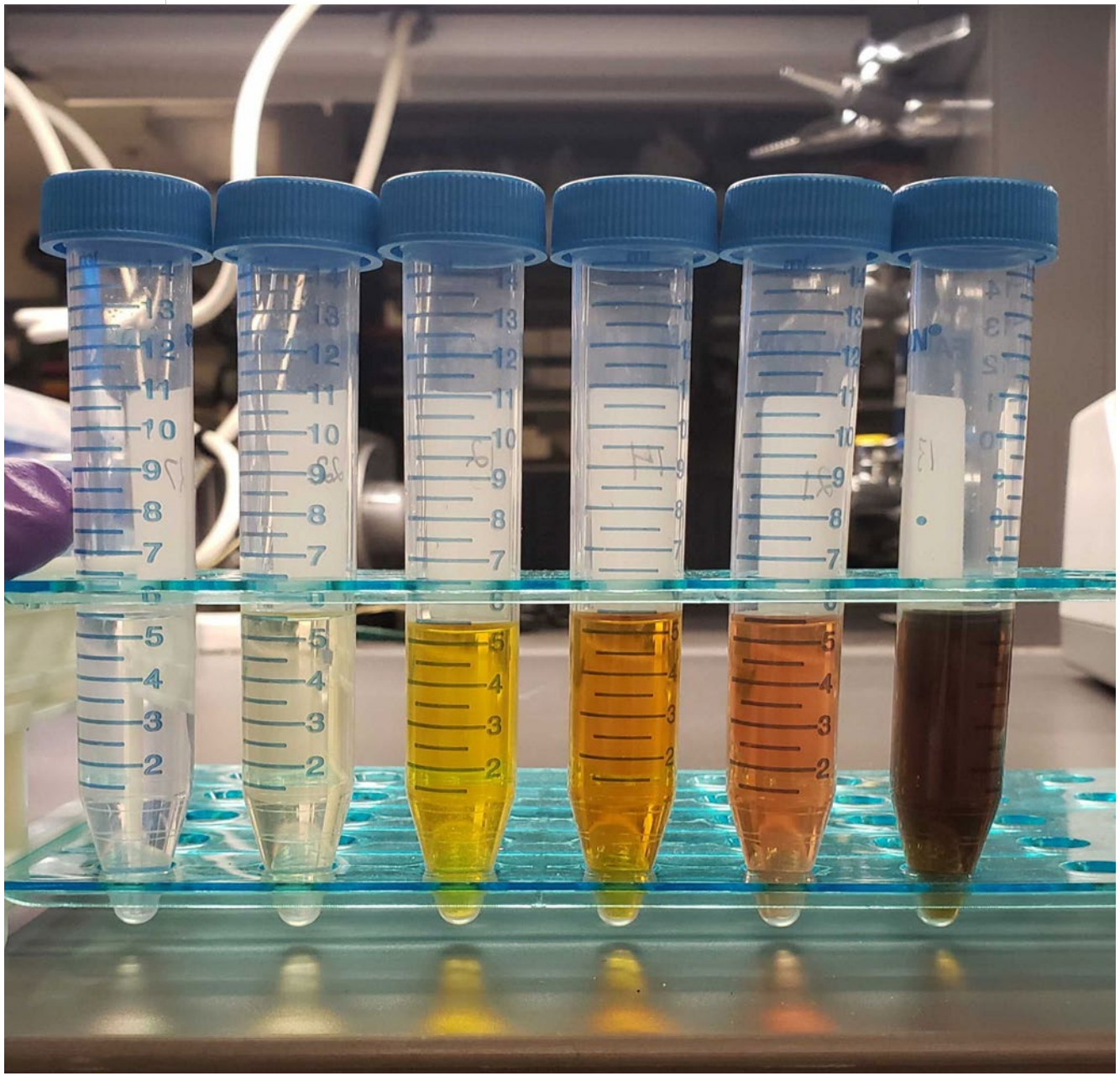
Interested in compounds present in > 80% of samples

Biosolid preparation and analysis

- Specimens were extracted via QuEChERS with dSPE

- Extracts analyzed via LC-HRMS
  - Positive: A) 1 mM ammonium fluoride, B) 0.1% formic acid in methanol
  - Negative: A) 1 mM ammonium fluoride, B) acetonitrile
  - Full Scan/data-dependent MS^2 (top 10)

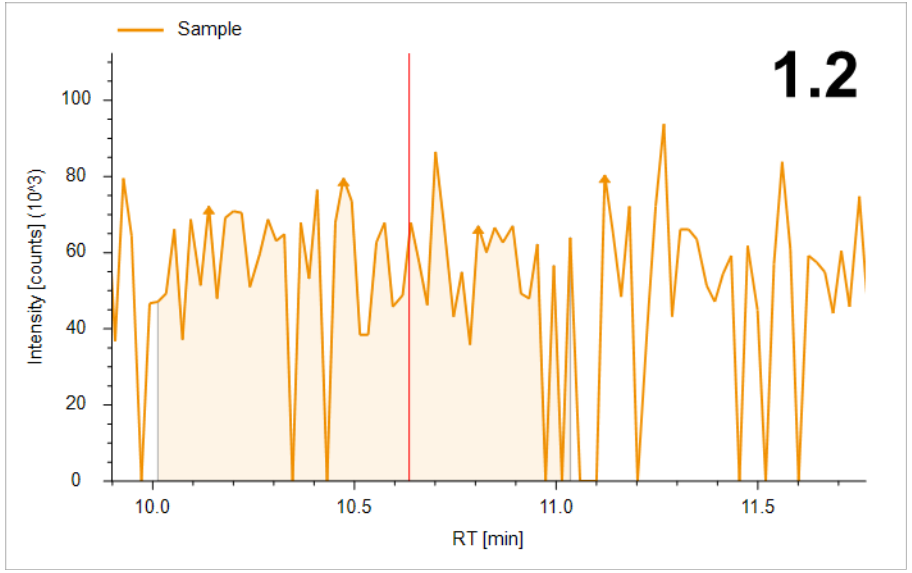
After dSPE and centrifuging

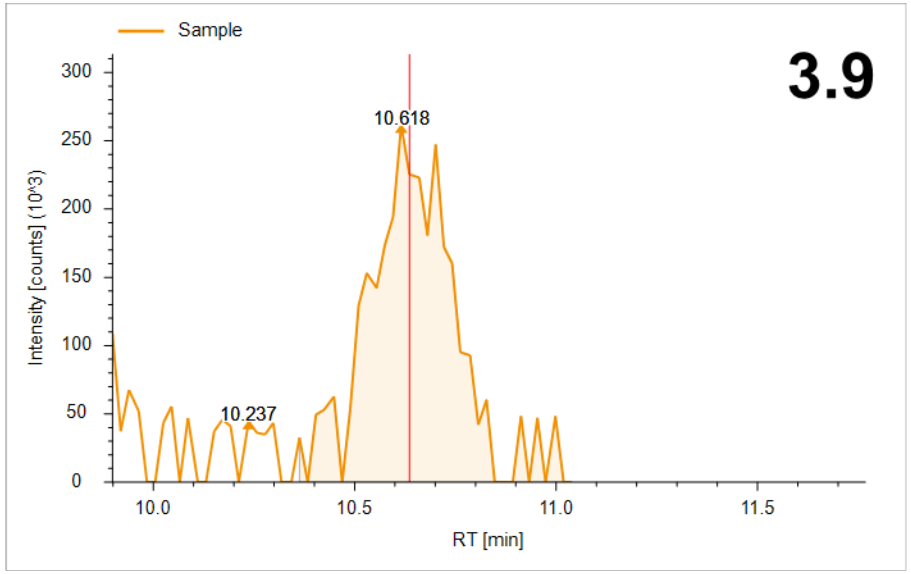


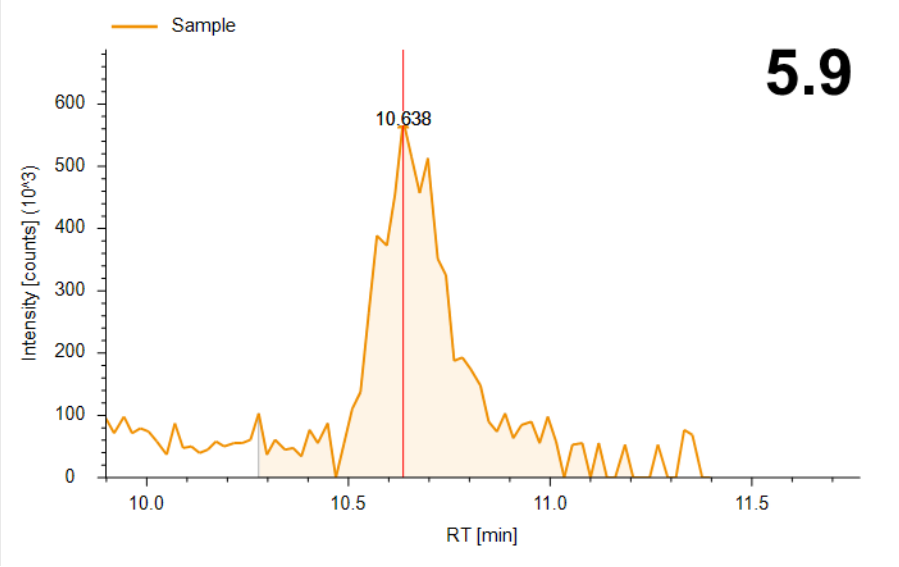
LC-HRMS data analysis

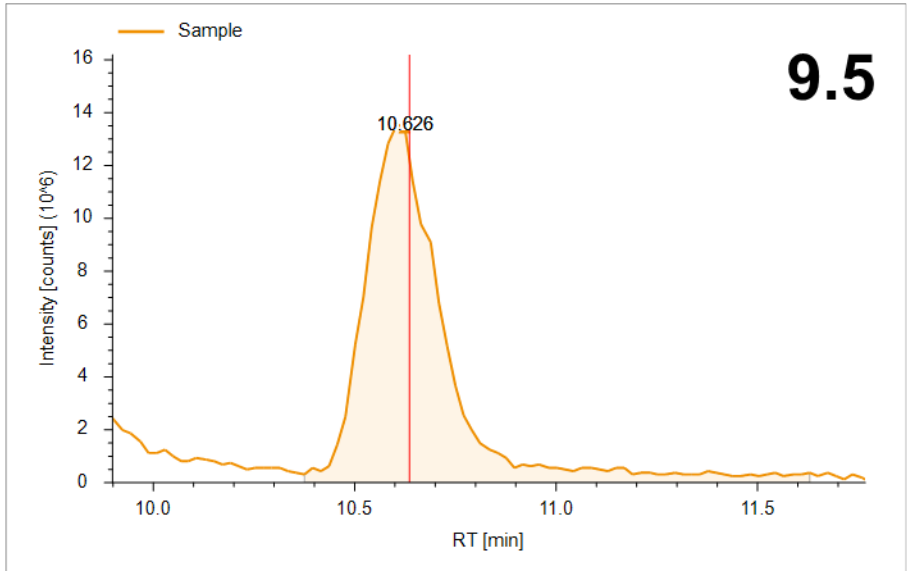
- Perform Quality Control (QC)-based peak area normalization
  - Detection in ≥50% pooled QCs (pooled biosolid samples)
  - %RSD before normalization ≤30%
  - %RSD after normalization ≤25%
- Detected in all pooled QC replicates
- Peak rating ≥6

Peak rating examples for the same feature in different samples (scored on a scale from 0-10)









LC-HRMS data analysis

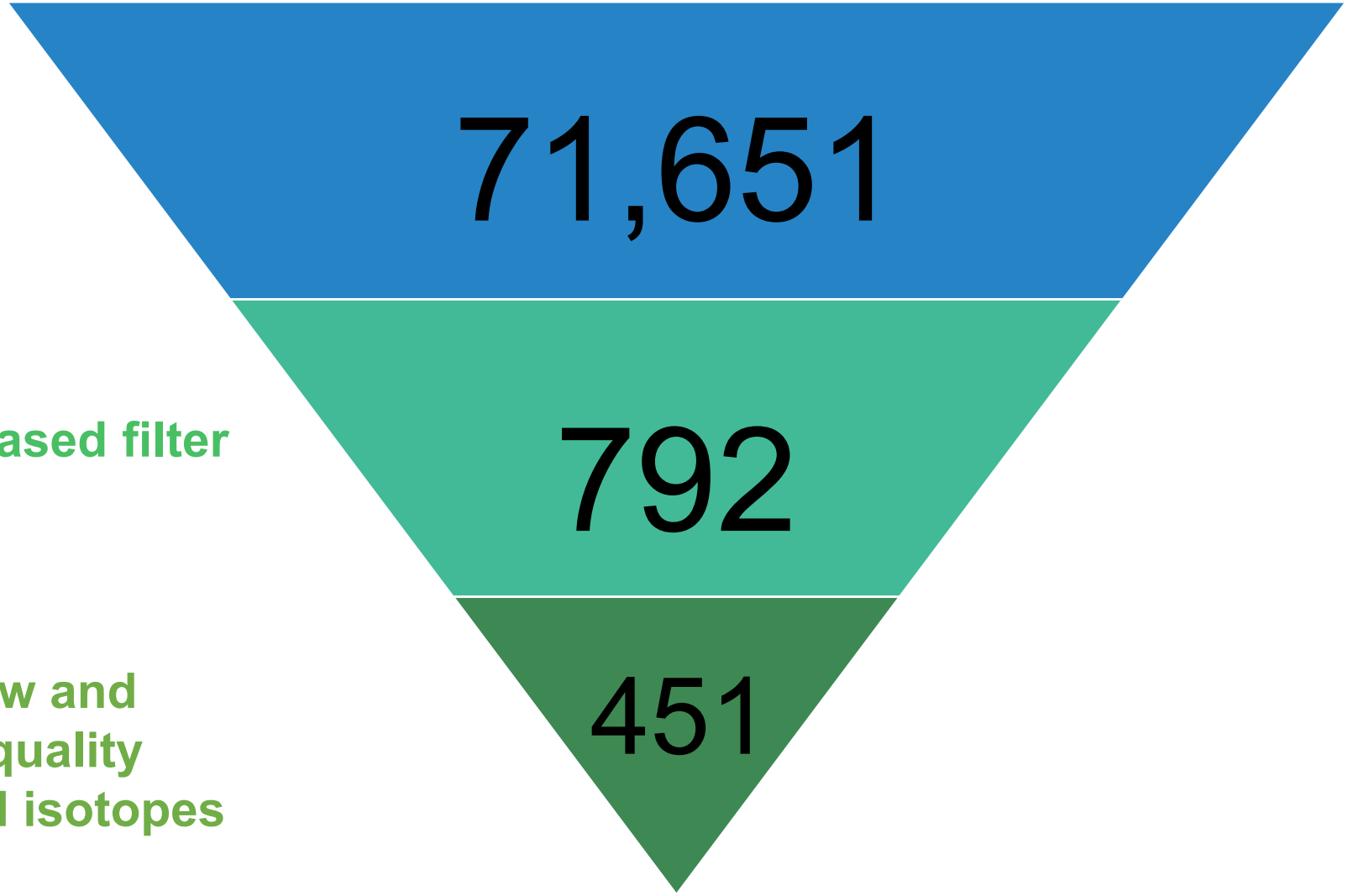
- Perform Quality Control (QC)-based peak area normalization
  - Detection in ≥50% pooled QCs (pooled biosolid samples)
  - %RSD before normalization ≤30%
  - %RSD after normalization ≤25%
- Detected in all pooled QC replicates
- Peak rating ≥6
- **Detected in at least 80% of biosolid samples**

Feature counts after different processing steps

 # Features originally detected

 # Features that fulfilled all QC-based filter criteria

 # Features remaining after review and removing background and low-quality peaks, in-source fragments, and isotopes



Confidence Level assignment (Schymanski criteria)

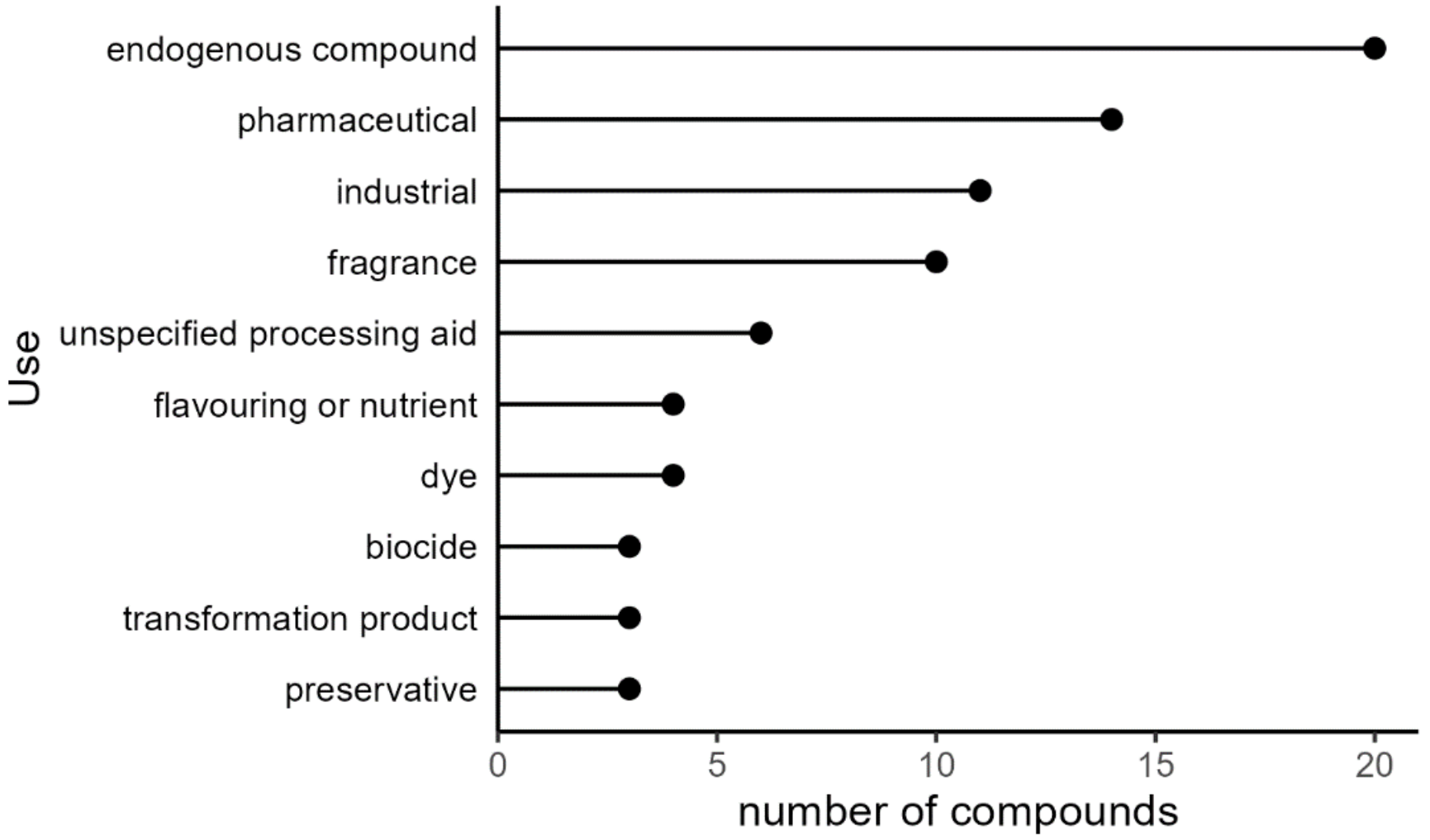
Confidence Level	Notes
Level 1	RT and MS^2 match between sample and reference standard
Level 2 – mzCloud	Spectral library match with score ≥70
Level 2 – MoNA	Spectral library match with score ≥70 and no mzCloud match
Level 3	Multiple plausible spectral library matches with score ≥70
Level 4	-No spectral library matches -Single predicted formula from CD also found in ChemSpider
Level 5	-No spectral library matches -Either: -None of the CD predicted formulas in ChemSpider -Multiple predicted formulas from CD also found in ChemSpider

Confidence Level assignment (Schymanski criteria)

Confidence Level	Notes
Level 1	RT and MS^2 match between sample and reference standard
Level 2 – mzCloud	Spectral library match with score ≥70
Level 2 – MoNA	Spectral library match with score ≥70 and no mzCloud match

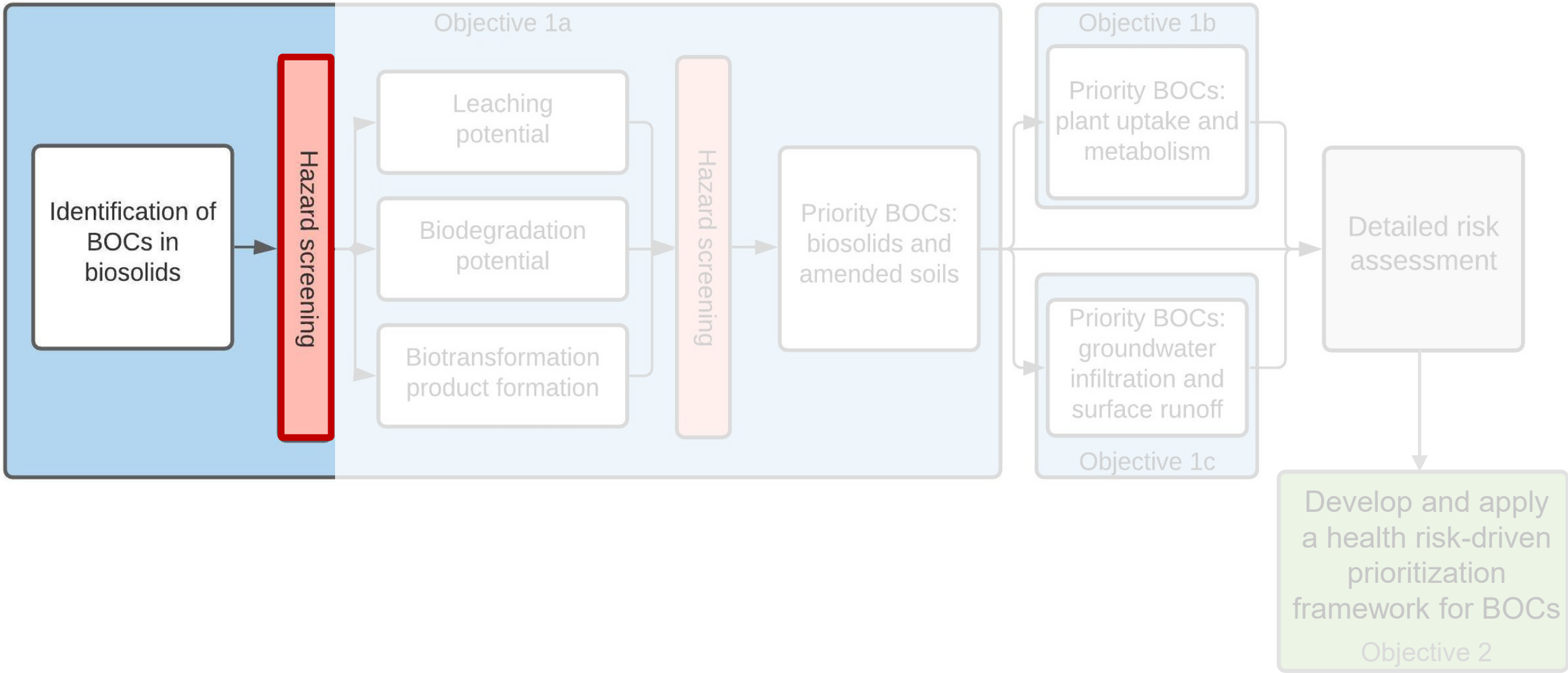
- **92 compounds** assigned confidence Level 1 & 2
  - **58/92 compounds** reported for the first time in biosolids
- **77.4% of compounds** have an unknown structure (Level 3-5)

Top 10 use categories of detected compounds

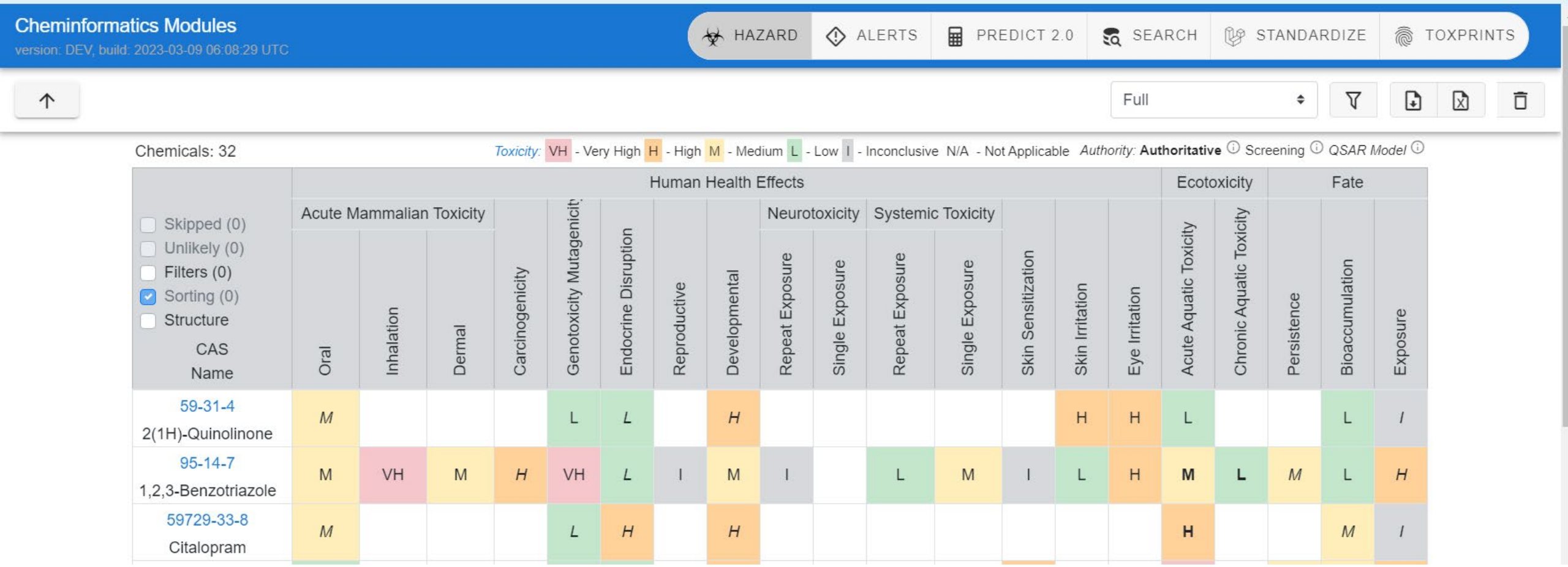


Source: Harmonized Functional Use data from the Chemicals and Products Database (CPDat) via EPA's ChemExpo site (https://comptox.epa.gov/chemexpo/get_data/)

Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)

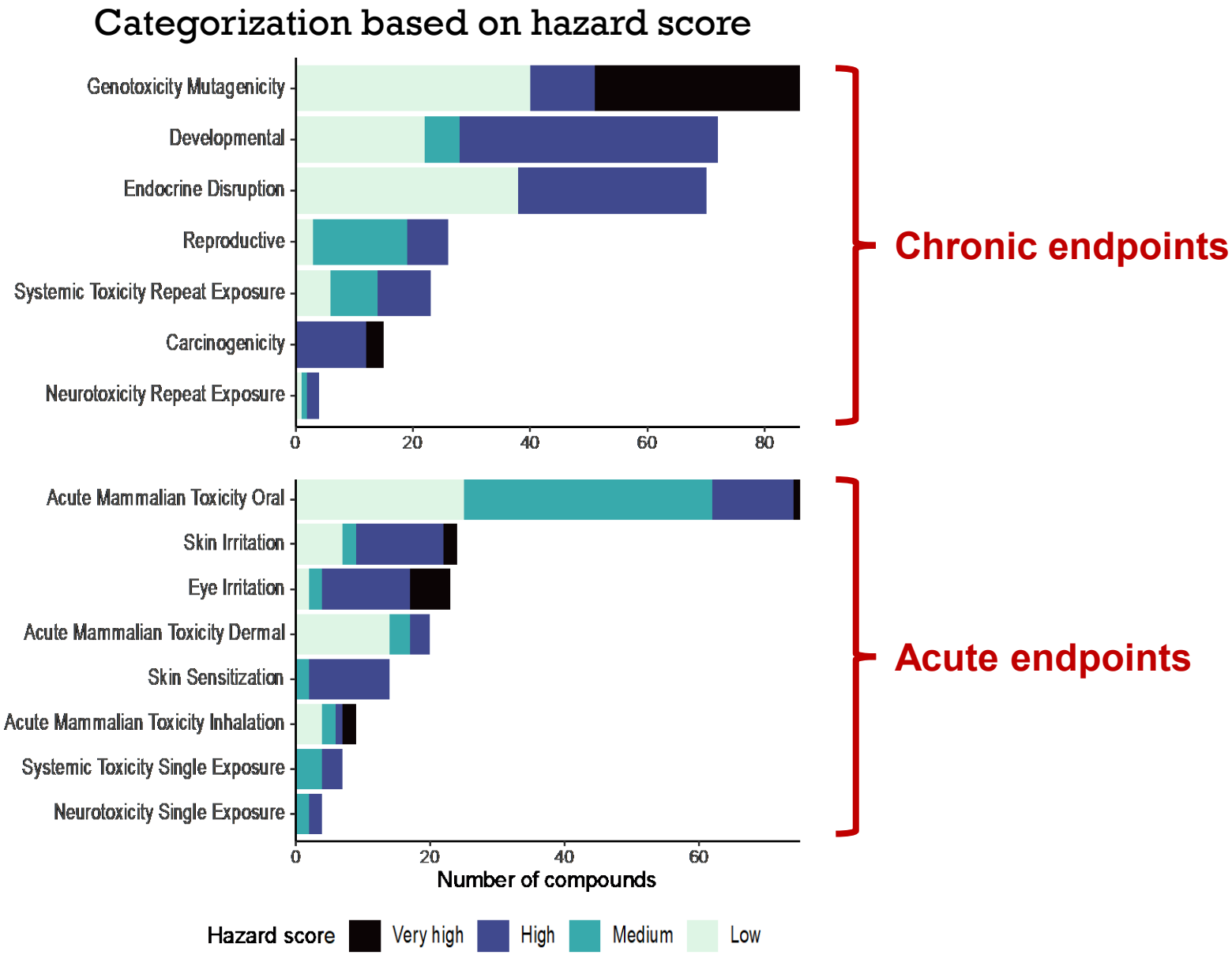


EPA Cheminformatics Hazard Comparison Module (HCM)



https://www.epa.gov/chemical-research/cheminformatics

Hazard assessment – Biosolid samples

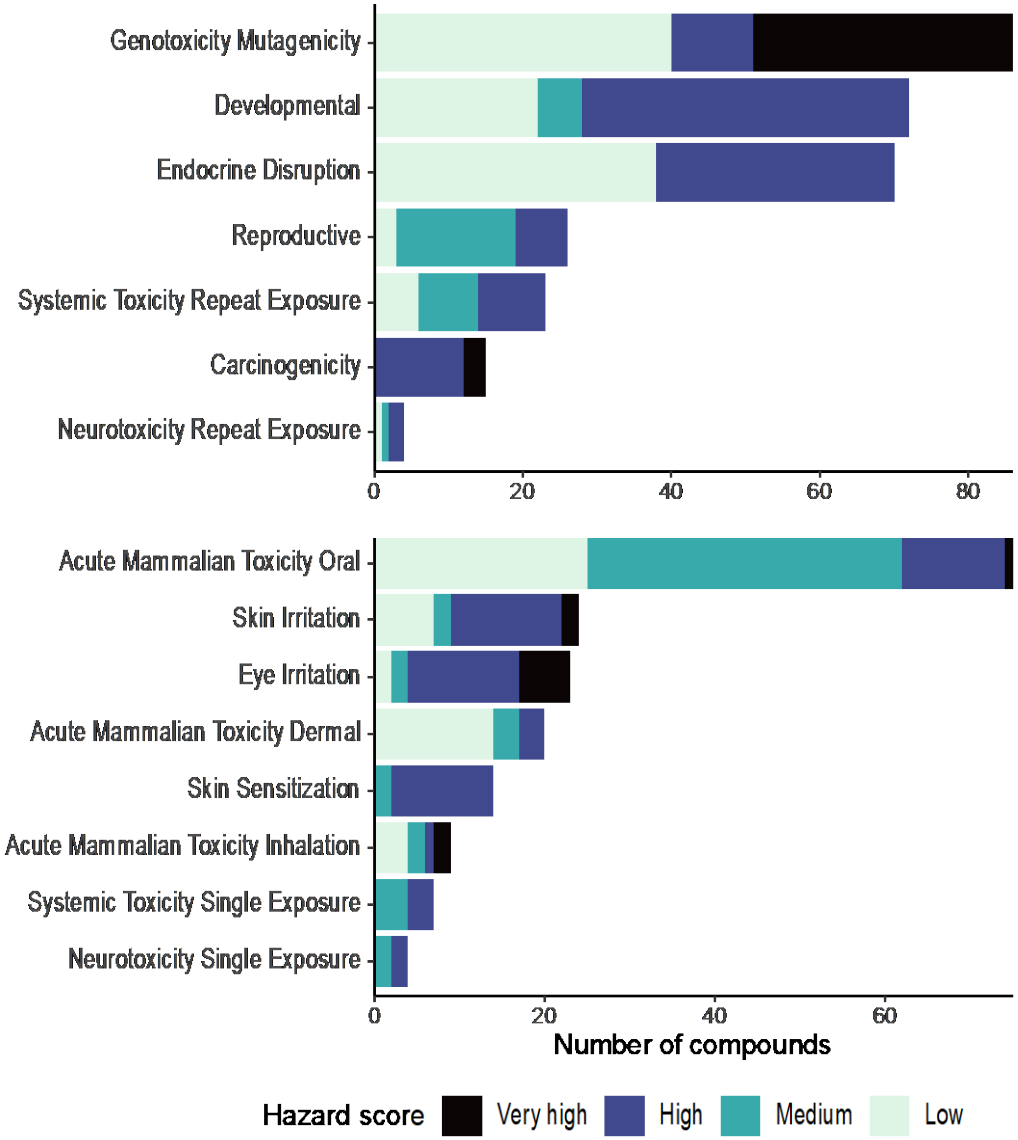


Chronic endpoints

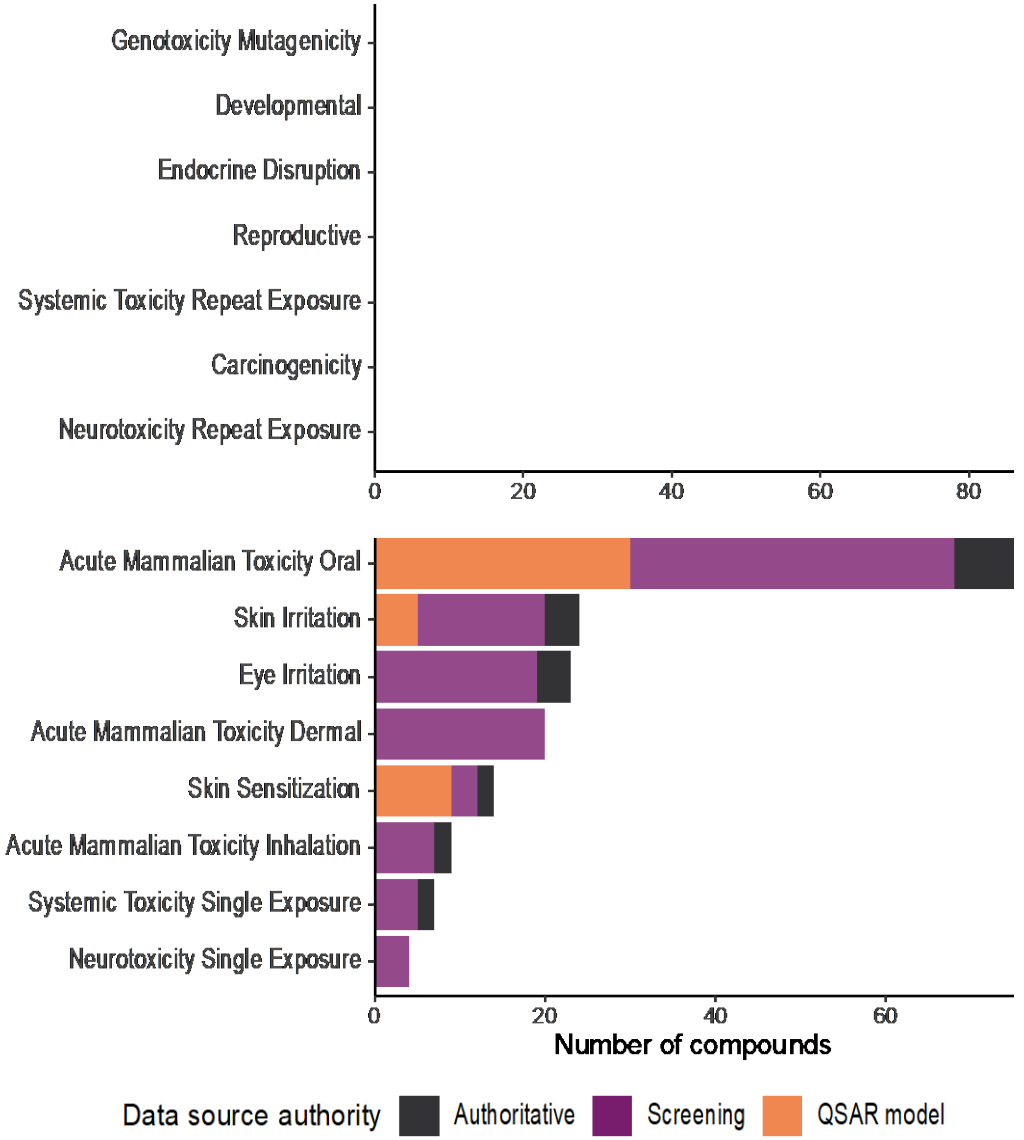
Acute endpoints

Hazard assessment – Biosolid samples

Categorization based on hazard score



Categorization based on data source authority



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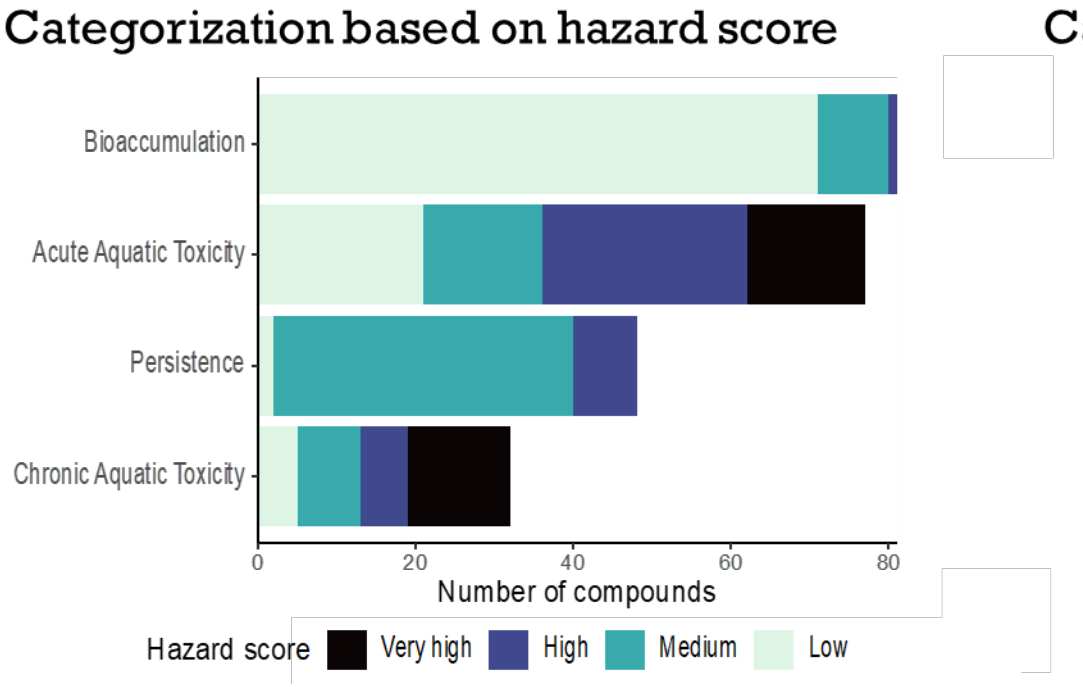

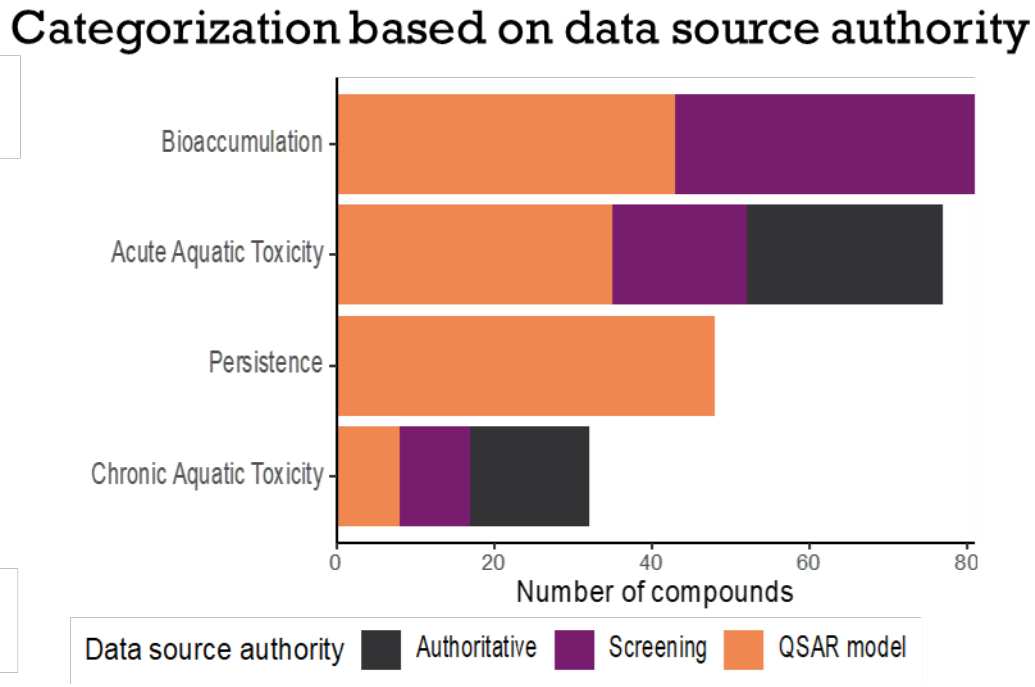

Compound prioritization approach

$$\boldsymbol{Avg.Hazard\ score} = \frac{\sum individual\ hazard\ scores}{number\ of\ endpoints\ with\ data\ available}$$

Hazard score categories: low (1), medium (2), high (3), very high (4)

Compound prioritization approach

$$Avg.Hazard\ score = \frac{\sum individual\ hazard\ scores}{number\ of\ endpoints\ with\ data\ available}$$

$$\boldsymbol{Avg.Quality\ score} = \frac{\sum individual\ quality\ scores}{number\ of\ endpoints\ with\ data\ available}$$

Data quality score categories: QSAR (1), screening (2), authoritative (3)

Compound prioritization approach

$$\boldsymbol{Avg.Hazard\ score} = \frac{\sum individual\ hazard\ scores}{number\ of\ endpoints\ with\ data\ available}$$

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$$\boldsymbol{Completeness\ score} = \frac{number\ of\ endpoints\ with\ data\ available}{number\ of\ endpoints\ searched}$$

Compound prioritization approach - Example

Compound	Endpoint A	Endpoint B	Endpoint C	Hazard Score	Quality Score	Completeness Score	Quality-adjusted Hazard Score
1	**VH Authoritative**	H Screening	*L QSAR*	2.67	2.00	1.00	5.34
2	*H QSAR*	**L Authoritative**	M Screening	2.00	2.00	1.00	4.00
3	*L QSAR*		L Screening	1.00	1.00	0.67	1.00

Hazard prioritization results

Top 5 Prioritized Compounds for Each End Point Group Based on Quality-Adjusted Hazard Scores

compound (confidence level)	quality-adjusted hazard score	completeness group[a]	use[b]
Human Health Effects—chronic Exposure (7 End Points)			
ketoconazole (2b)	8.01	medium low	pharmaceutical
p-cresol (2a)	7.19	high	fragrance
4-androstene-3,17-dione (2a)	6.99	medium low	endogenous
clorophene (2a)	6.72	medium high	biocide
phenolphthalein (2a)	6.68	high	not specified
Human Health Effects—short-term Exposure (8 End Points)			
clorophene (2a)	7.56	high	biocide
p-cresol (2a)	7.14	high	fragrance
indole (2a)	6.99	medium low	fragrance
thymol (1)	6.88	medium high	fragrance
ketoconazole (2b)	6.25	medium low	pharmaceutical
Ecological End Points (4 End Points)			
fludioxonil (2a)	12.00	medium high	biocide
ketoconazole (2b)	8.01	high	pharmaceutical
4-(1,1,3,3-tetramethylbutyl)phenol (2a)	6.75	high	adhesion promoter
triclocarban (2a)	6.75	high	preservative
carbamazepine (1)	6.22	high	pharmaceutical

Hazard prioritization results

Table 2. Top 5 Prioritized Compounds for Each End Point Group Based on Quality-Adjusted Hazard Scores

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Target list for further in-depth investigations

Flame Retardants

- **Triethyl phosphate**
- Tris(2-chloroethyl) phosphate
- **Tris(2-chloroisopropyl)phosphate**
- Triphenyl phosphate

Industrial Chemicals

- **2-Naphthol**
- 4-Nonylphenol
- **N,N'-Diphenylguanidine**
- **Isoquinoline**

Natural Products

- **Androstenedione**
- **Caffeine**
- **Indole**
- **Norharman**
- **Piperine**

UV Filters

- Avobenzone
- **Benzotriazole**
- Octinoxate
- Octocrylene
- Oxybenzone

Antimicrobials

- **4-Methylphenol**
- C12 BAC
- C12 DADMAC
- **Clorophene**
- **Methylparaben**
- **Triclocarban**
- Triclosan
- **Thymol**

Other Biocides

- Atrazine
- **Fludioxonil**
- **Ketoconazole**
- **Thiabendazole**

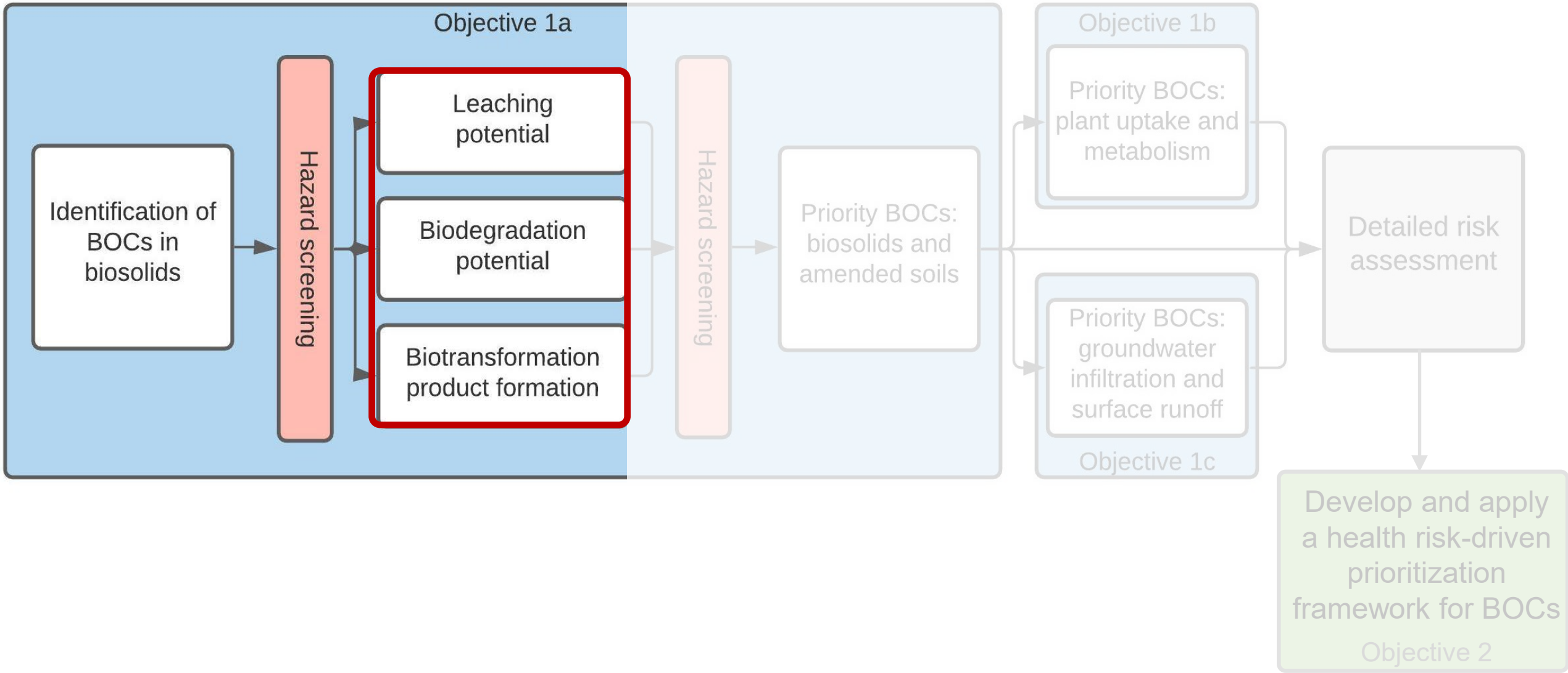
Plasticizers

- **Bisphenol A**
- Bisphenol F
- Bisphenol S

Pharmaceuticals

- Amitriptyline
- **Carbamazepine**
- **Citalopram**
- **Diphenhydramine**
- Fluoxetine
- **Lamotrigine**
- **Metoprolol**
- Spectinomycin
- **Trazodone**

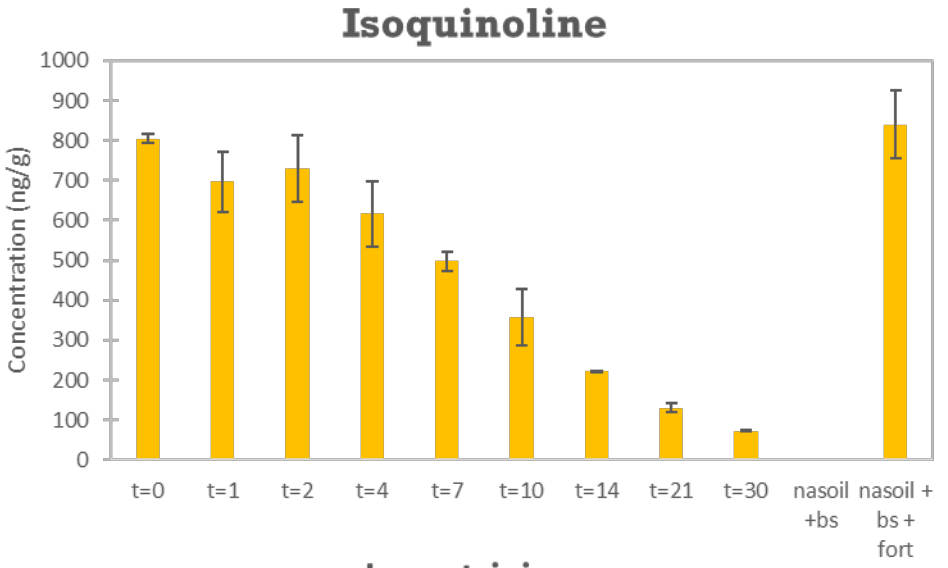
Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)

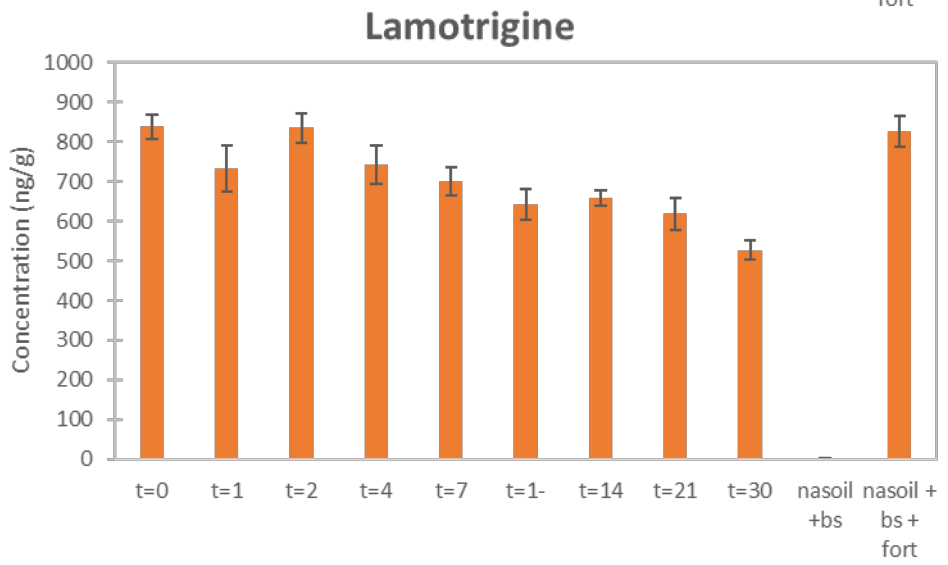


Laboratory experiments - Biodegradation

30 day soil + biosolids batch experiments:

Compound	% Degradation	Compound	% Degradation
4-Methylphenol	100%	Triclosan	41.7%
2-Napthol	98.5%	Bisphenol S	39.9%
Androstenedione	92.6%	Lamotrigine	37.2%
Isoquinoline	90.9%	Piperine	33.5%
Bisphenol F	79.9%	Caffeine	27.5%
Oxybenzone	78.4%	Tris(2-chloroethyl)phosphate	17.1%
Bisphenol A	74.5%	1,3-diphenylguanidine	15.9%
Ketoconazole	69.9%	Amitripytline	15.8%
Clorophene	56.8%		
Trazodone	51.7%		





→ among 31 compounds tested,
18 degraded by >15 %

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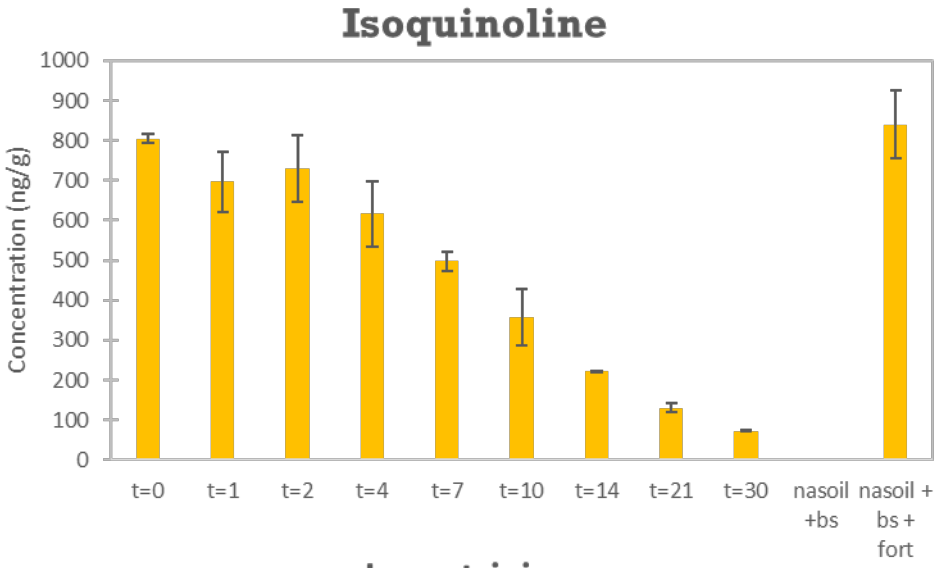

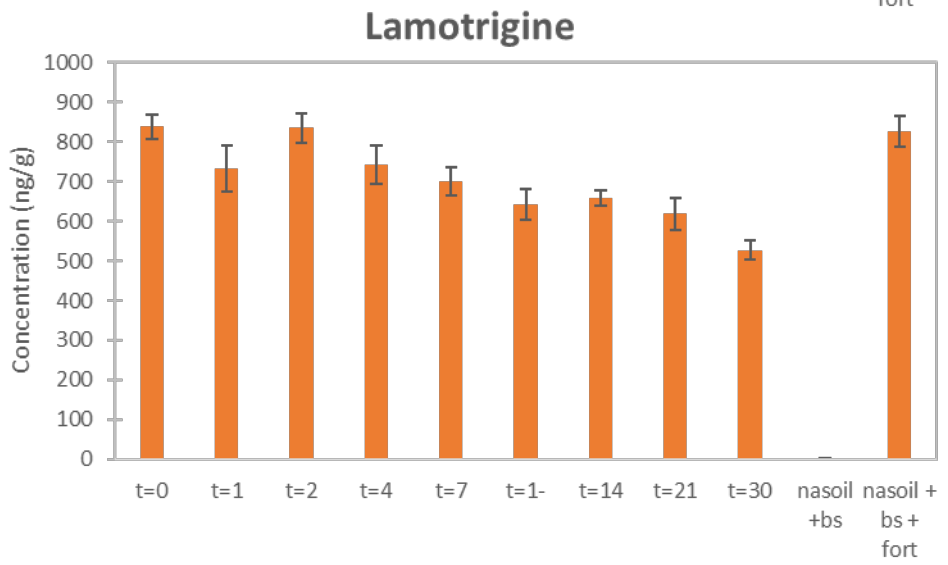

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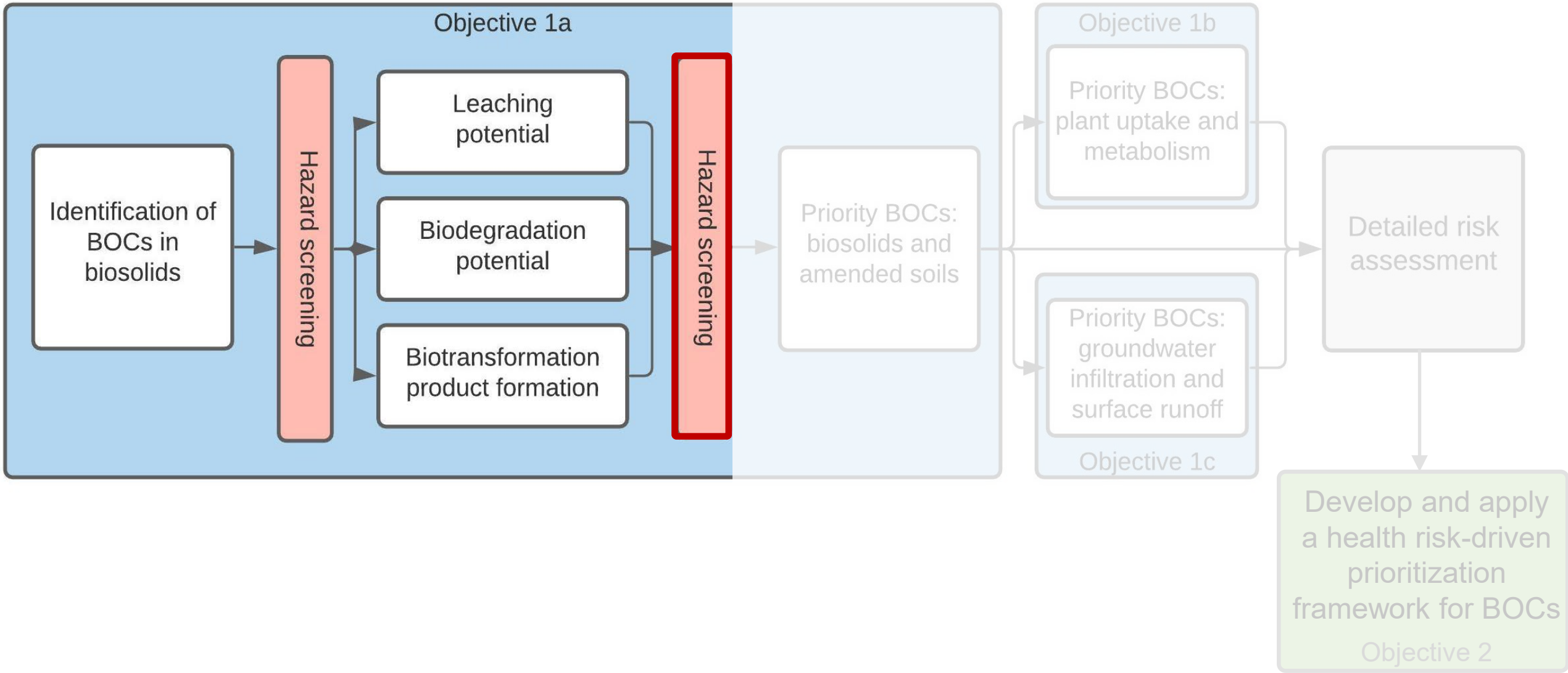
Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)



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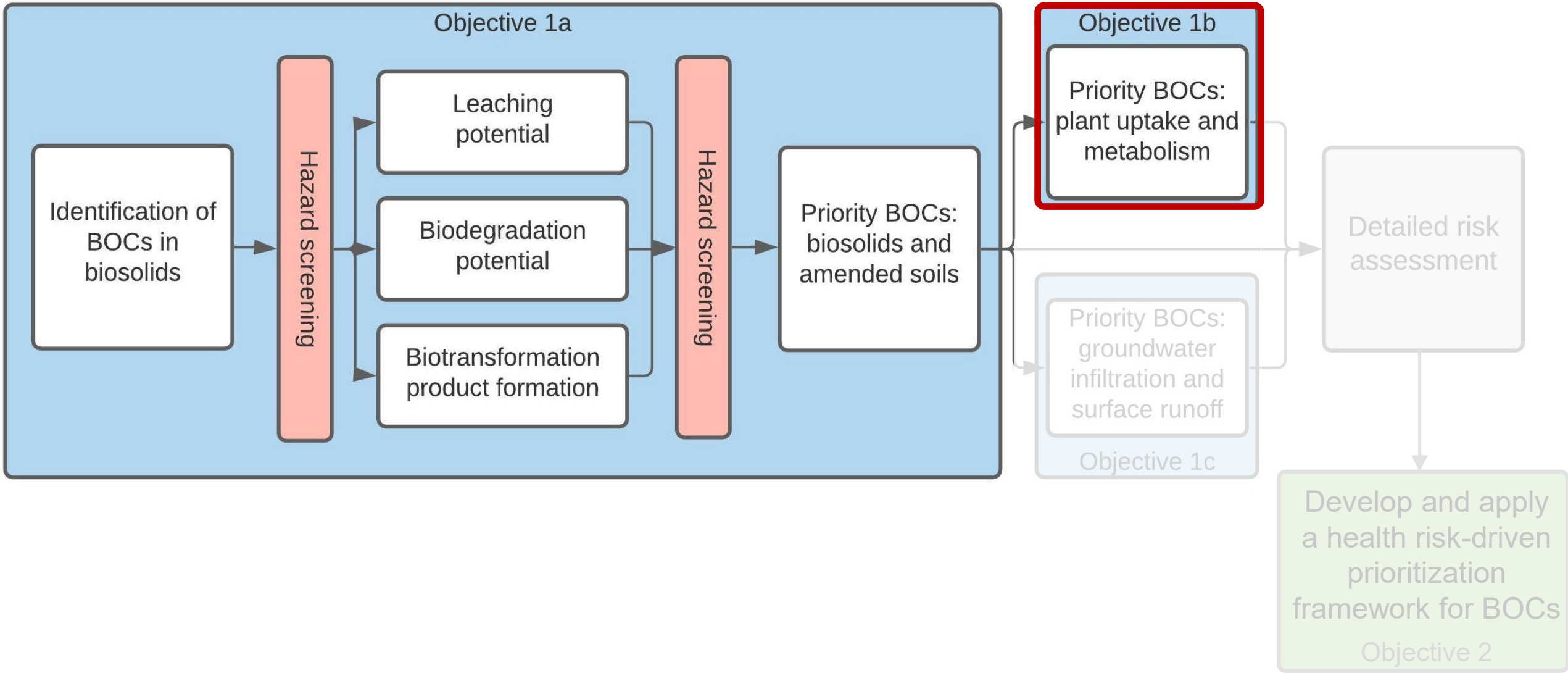
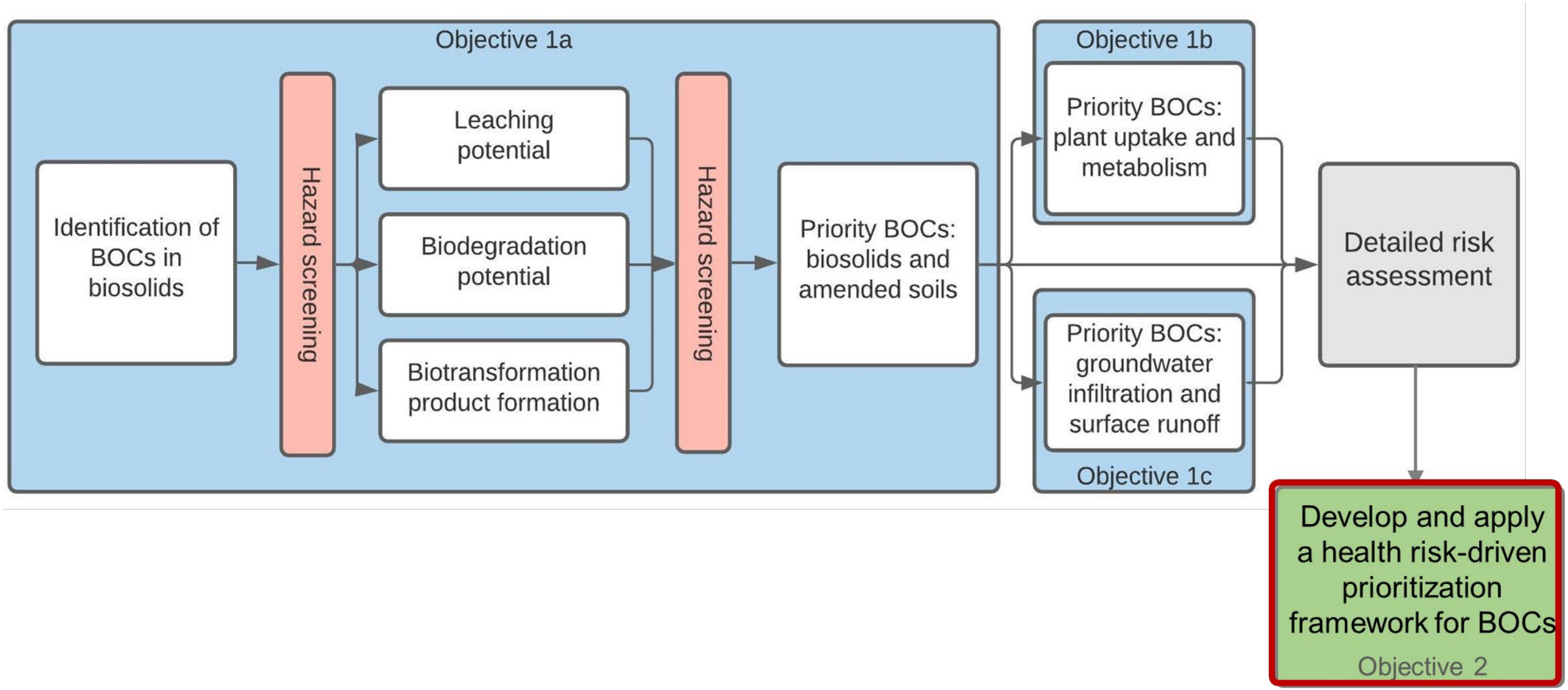


Lab & field experiments – Plant uptake

Characterize the occurrence, fate, transport and risks of novel biosolid-associated contaminants (BOCs)



Pilot Study: Characterization of exposure scenarios and quantification of exposure factors unique to biosolids workers

- Semi-structured in-depth interviews (IDI's) with workers from seven states across the US.
- Land application involves six processes:
  - hauling, loading, spreading, post-application field work, cleaning, and maintenance
  - differing levels of exposure to biosolids based on the used processes
- Findings were used to develop a biosolids exposure questionnaire (BEQ) to quantify exposure factors unique to biosolids workers.
- We are currently administering the questionnaire to biosolids workers.

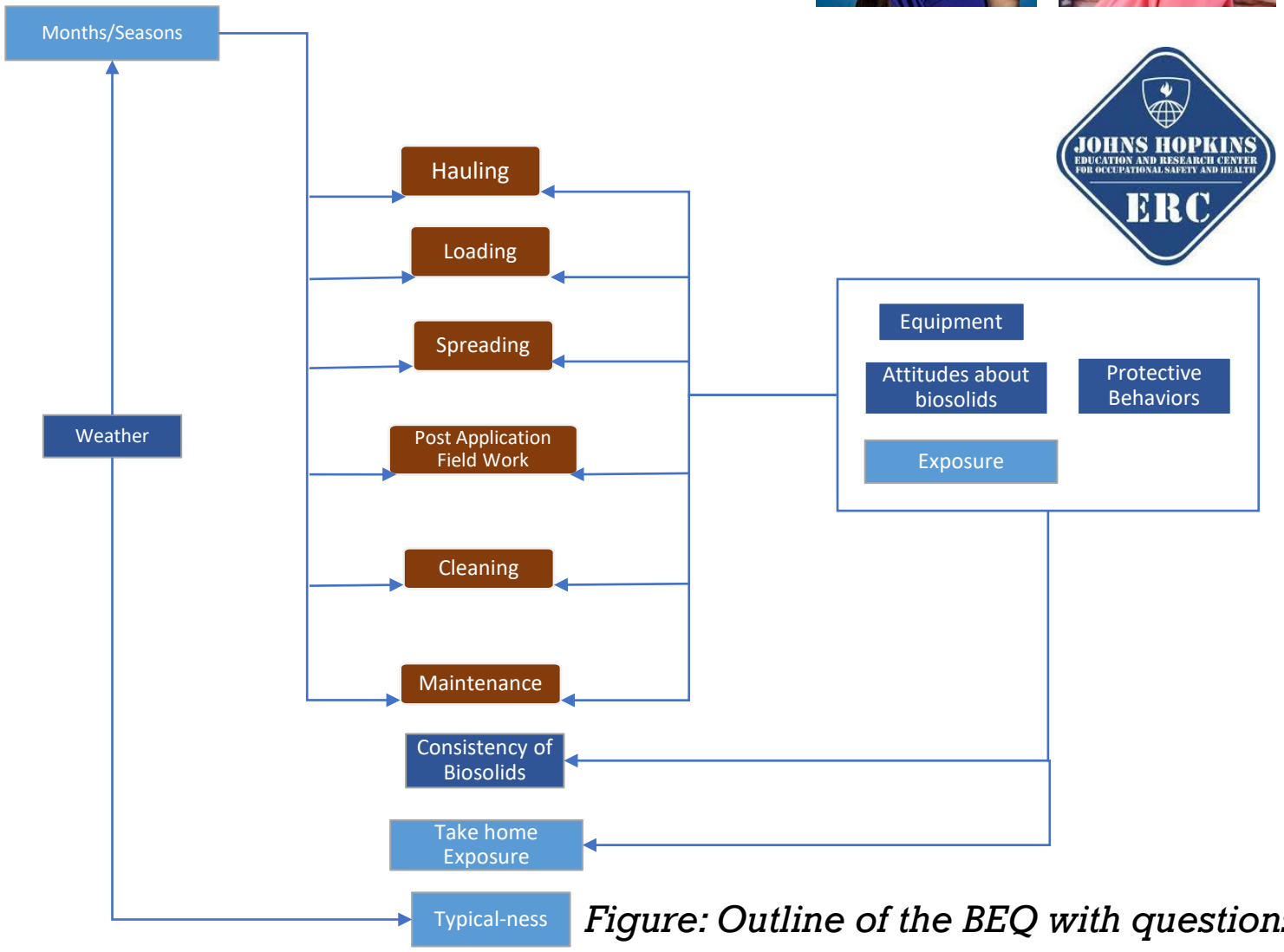


Figure: Outline of the BEQ with questions that leverage findings from the IDIs

Conclusions

- Framework based on high-resolution mass spectrometry and hazard assessment tools is a promising approach for the prioritization of compounds in complex mixtures

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- Prioritization can support:
  - Selection of analytes for identification using reference standards
  - Selection of compounds for in-depth toxicity assessment
  - Selection of compounds for risk assessment

Conclusions

- Framework based on high-resolution mass spectrometry and hazard assessment tools is a promising approach for the prioritization of compounds in complex mixtures
- Prioritization can support:
  - Selection of analytes for identification using reference standards
  - Selection of compounds for in-depth toxicity assessment
  - Selection of compounds for risk assessment
- Major challenge is the limited number of compounds in MS databases

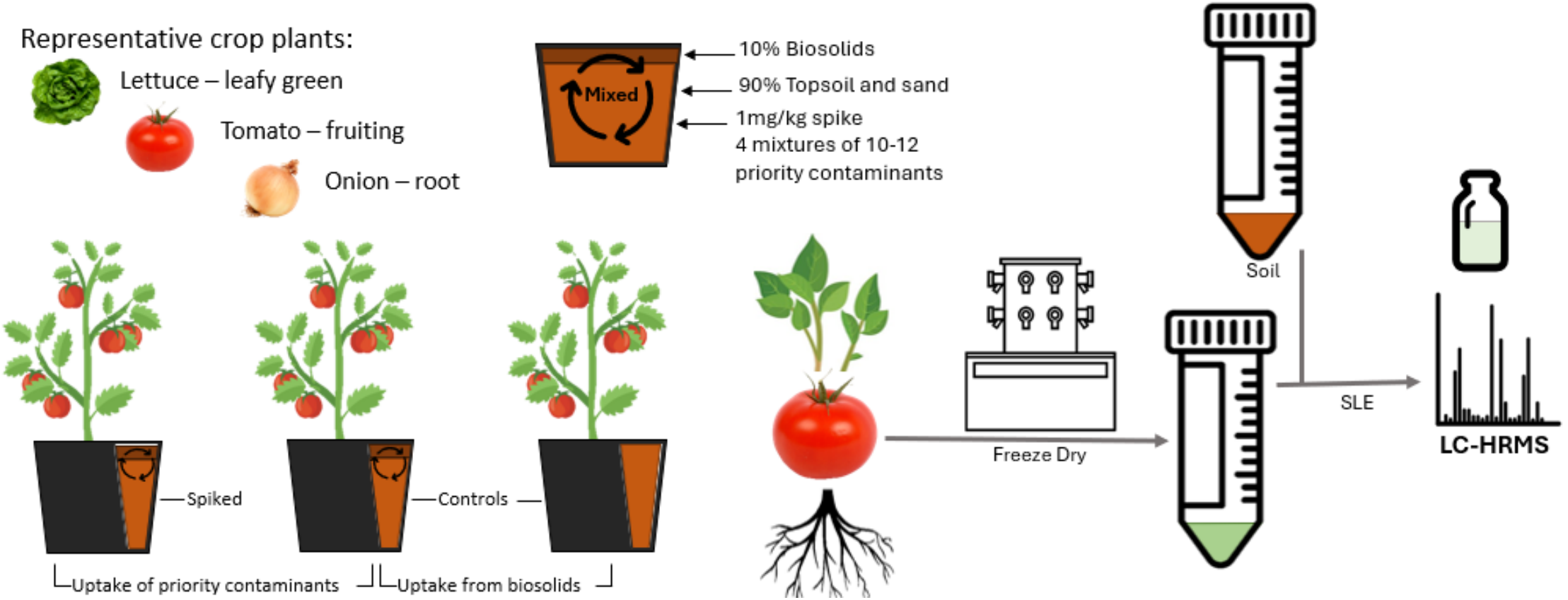
Thank you!

Email: cprasse1@jhu.edu
Lab homepage: www.prasselab.com

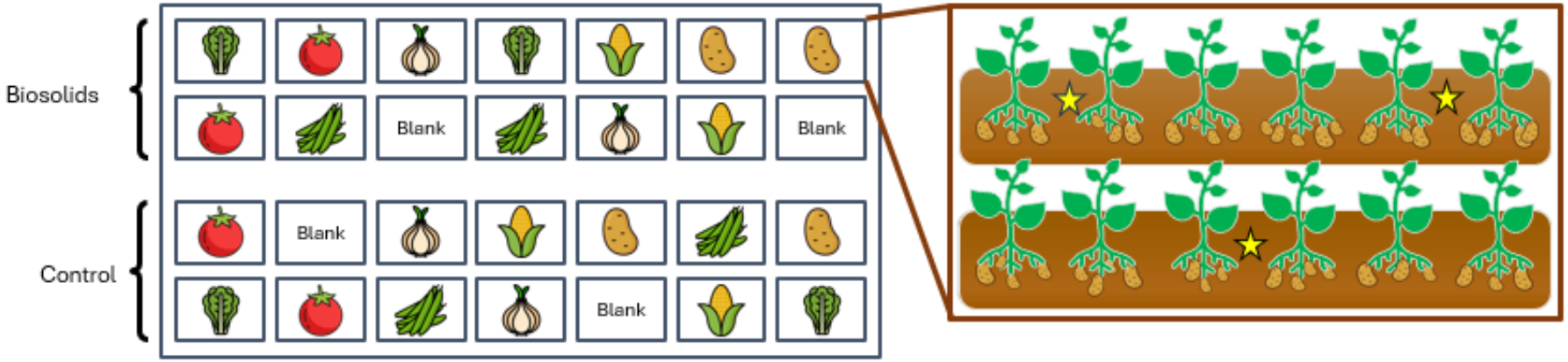
Laboratory experiments – Plant uptake/metabolism

Pot-spike Experiments

- Target and non-target approach with LC-HRMS
- Determine uptake and accumulation of priority contaminants from biosolids-amended soil
- Identify *in-planta* transformation products of priority contaminants

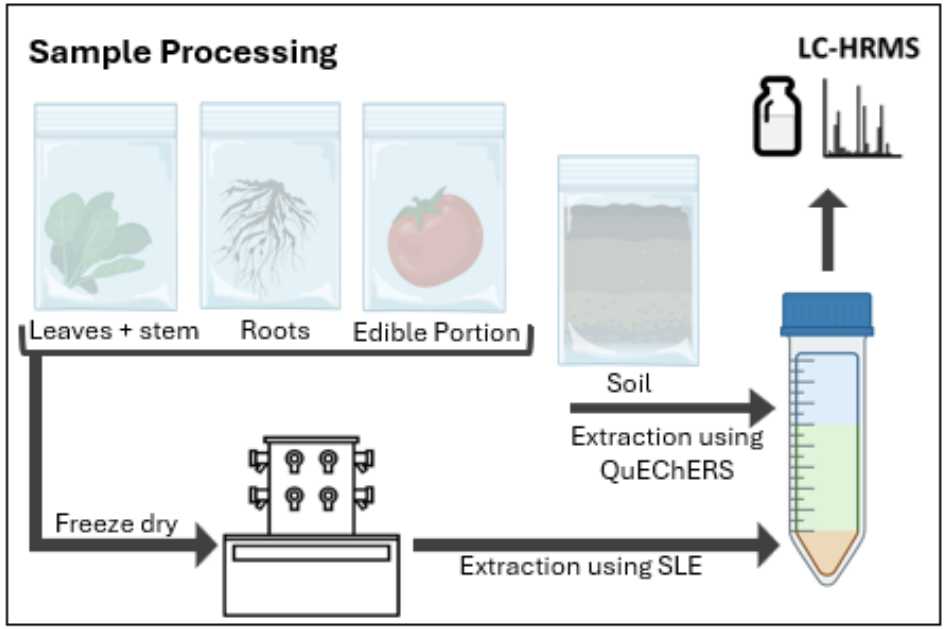


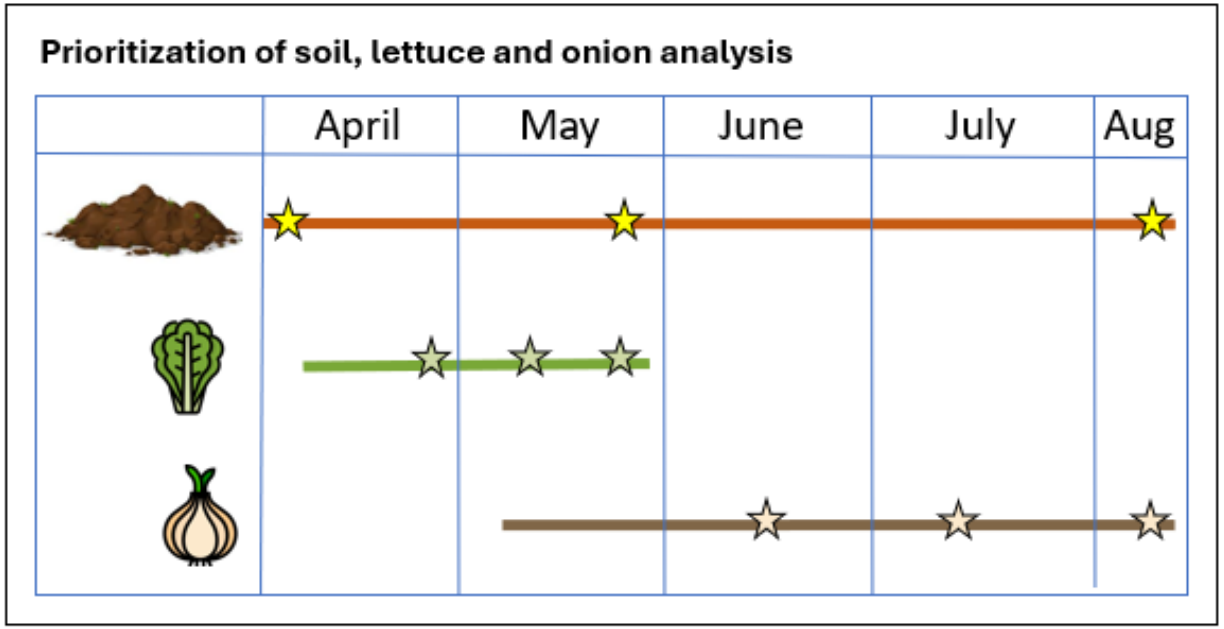
Field experiments – Plant uptake



Soil Sampling ☆
- 3 locations per plot
- 2 time points (biosolid application and harvest)

Plant Sampling
- 3 sampling events
- 3 plants / sampling event
- Up to 3 different tissue types





Collaborative Vision for Omics-Based Chemical Testing

Connie Mitchell
Senior Scientific Program Manager
Health and Environmental Sciences Institute (HESI)

The HESI Model: Bridging Research to Application

One of first and longest standing nonprofits to use multi-sector, int'l model - since 1989!

IMPROVED
SAFETY AND INNOVATION FOR HUMAN AND ENVIRONMENTAL HEALTH

Academic, Clinical, & Research Scientists & Organizations

Industry Research & Development

NGOs, Patient Advocacy Groups, & Foundations

Government Research & Regulation

Emerging Systems Toxicology for the Assessment of Risk (eSTAR)

Committee Mission

- Develop and deliver innovative systems toxicology approaches for risk assessment
- Catalyze adoption of new translational and predictive tools that guide decision-making based on mechanistic understanding of toxicological response

eSTAR Participants

Academia

- Broad Institute
- Cambridge University
- Clemson University
- Cornell University
- Georgetown University
- Kansas University Medical Center
- Indiana University
- McGill University
- Michigan State University
- MIT
- North Carolina State University
- Newcastle University
- Orebro University
- University of Ottawa
- University of Michigan
- University of North Carolina
- University of Pittsburgh

Government

- BC Cancer Agency
- BfArM
- Dutch Medicines Evaluation Board
- Health Canada
- PMRA
- US Army
- US EPA
- US FDA
- US NIH NCI
- US NIH NIEHS

Non-Profit

- PSCI
- Lhasa

Industry

- AbbVie
- Amgen
- AstraZeneca
- Bayer AG
- Boehringer-Ingelheim
- Corteva
- FMC
- GSK
- Janssen
- Merck & Co., Inc
- Merck KGaA
- Newcells Biotech
- Ono
- Pfizer
- Recursion
- Roche/Genentech
- Sanofi-Aventis
- Servier
- Syngenta
- Taconic Biosciences
- Takeda
- TwinStrand Biosciences
- Vertex

eSTAR

Omics: what do I mean

Transcriptomics

Genomics

Proteomics

Metabolomics

Epigenomics

Phenomics (e.g., Cell Painting)

Omics: what are they good for?

- Promise of omics at the turn of the century
  - Improved understanding of toxicology mechanisms, species, and susceptibility
- Difficulty to use omics in regulatory decision making
- “Hairball diagrams” – pathway diagrams, heat maps, long list of differentially expressed genes.
  - What do we do with these data?
- Move towards using omics for decision making
  - Biomarkers for pathways
  - Deriving points of departures

Current eSTAR Working Groups

Molecular POD

Using transcriptomic point of departure for chemical risk assessment.

TGx-DDI

An *in vitro* transcriptomic biomarker to predict probability that an agent is DNA damage-inducing (DDI) or non-DDI.

Carcinogenomics

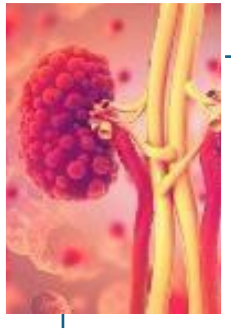
Identifying and evaluating transcriptomic biomarkers for rat-liver tumors to reduce the need for two-year carcinogenicity studies.

Error Correct Sequencing

Exploring the use of error corrected sequencing to detect non-genotoxic carcinogens.

OASIS

Exploring the use of Cell Painting, transcriptomics, and proteomics for safety assessment.



miRNA Biomarkers

Investigating and evaluating the use of miRNAs as biomarkers for renal injury.

HESI Emerging Systems Toxicology for Assessment of Risk (eSTAR) Committee 2024 Annual Meeting

Wednesday 13 November 9 am – 3 pm ET

Free, open meeting to learn about all the projects.

Register Here

Current eSTAR Working Groups

Molecular POD

Using transcriptomic point of departure for chemical risk assessment.

Carcinogenomics

Identifying and evaluating transcriptomic biomarkers for rat-liver tumors to reduce the need for two-year carcinogenicity studies.

OASIS

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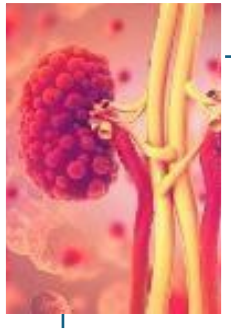
TGx-DDI

An *in vitro* transcriptomic biomarker to predict probability that an agent is DNA damage-inducing (DDI) or non-DDI.

Error Correct Sequencing

Exploring the use of error corrected sequencing to detect non-genotoxic carcinogens.

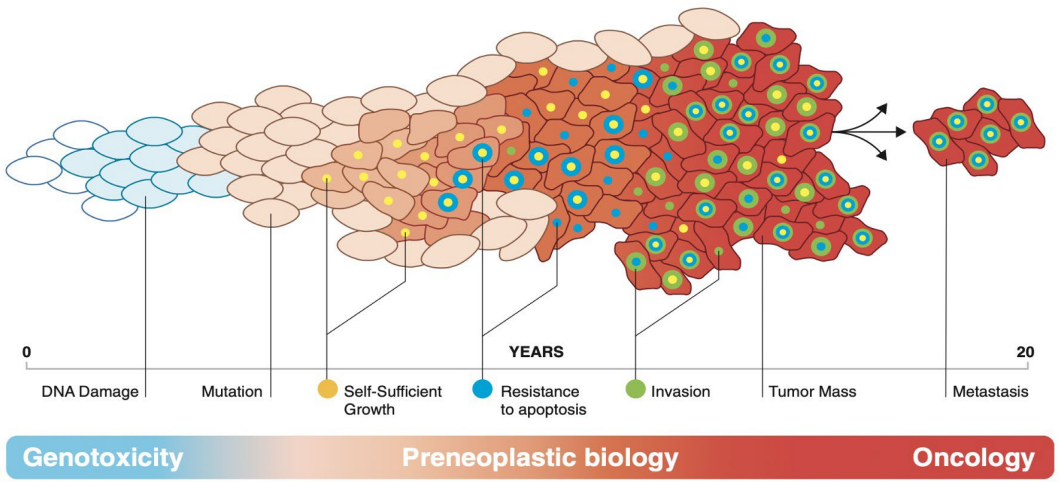


miRNA Biomarkers

Investigating and evaluating the use of miRNAs as biomarkers for renal injury.

Why a TGx-DDI Biomarker?

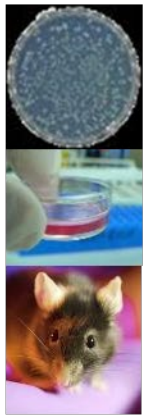
Genotoxicity is a key driver of carcinogenesis



Salk and Kennedy, 2020

Need for high-throughput genotoxicity tests that provide mechanistic information in human-relevant cell culture models.

Standard Tests for Chromosome Damage and Mutation:



Bacterial Reverse Mutation Test (Ames assay)

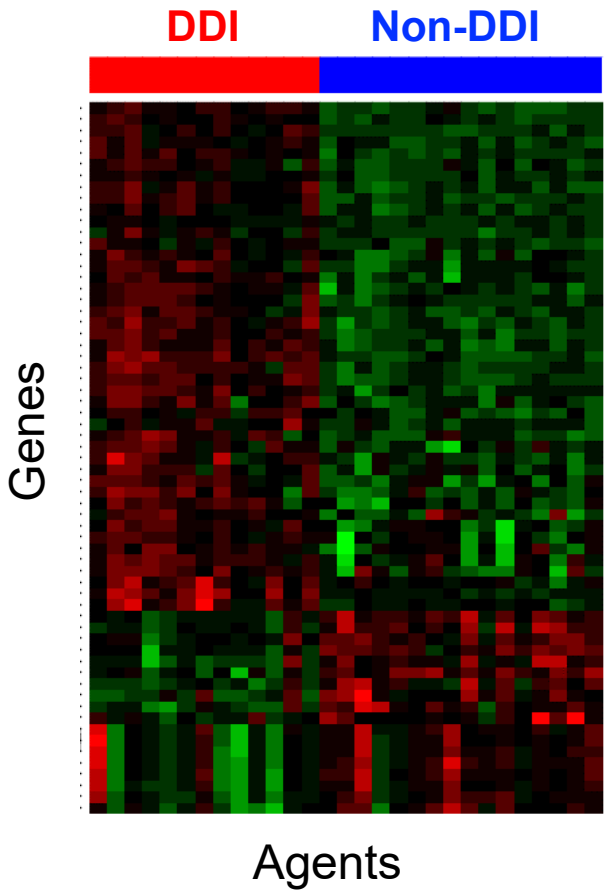
In vitro Mammalian Genetox Assay: chromosome aberrations, micronuclei, gene mutation

In vivo Genetox Assay: chromosome aberrations, micronuclei, transgene mutation

Limitations of current tests:

- Test methods are sensitive but lack specificity leading to high rates of misleading positive results.
- Lack efficiency and do not provide information on the mechanisms causing genotoxicity.

Fit for Purpose Solution: TGx-DDI



An *in vitro* transcriptomic biomarker that predicts the probability that an agent is DDI (DNA damage-inducing) or non-DDI using toxicogenomics (TGx).

- Developed in human TK6 cell cultures
- From exposure to 28 prototype DDI and non-DDI chemicals
- 64 genes identified as being predictive of DDI potential

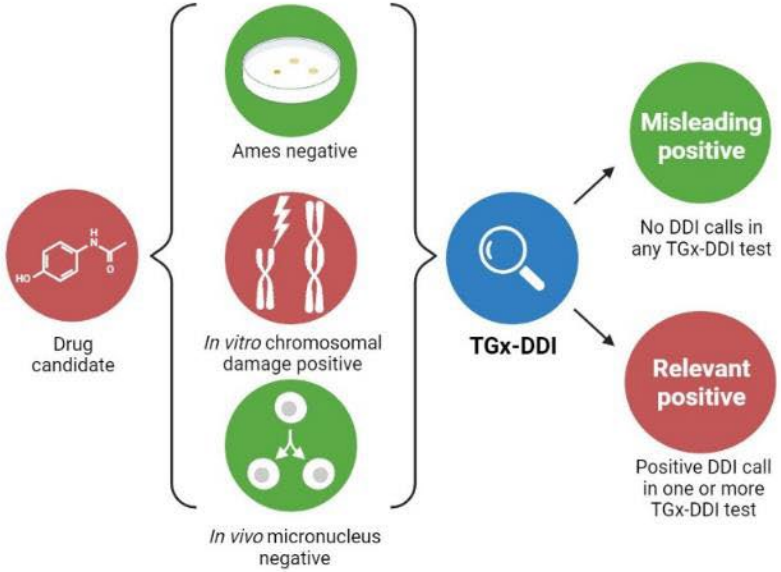
Why do we like it?

- High specificity (correctly identifies 'irrelevant' chromosome damage results)
- Endogenous cellular responses to DNA damage in human cells
    - Biological relevance
- Transferable to other cell types
- Multiplex capacities
- Removes subjectivity of transcriptomic data analysis

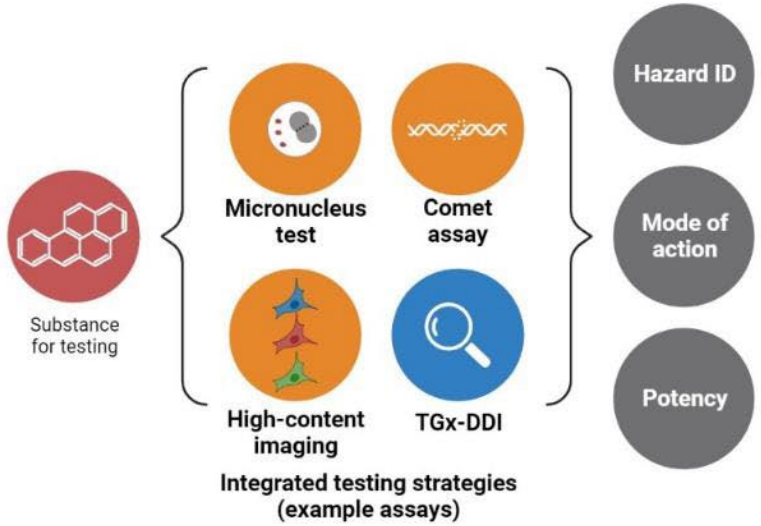
Li, HH et al. *Environ Mol Mutagen* (2015); *PNAS* (2017)
Yauk, et al. *Environ Mol Mutagen* (2016)

The context of use

1. **Drug screening**



2. **Chemical safety assessment**



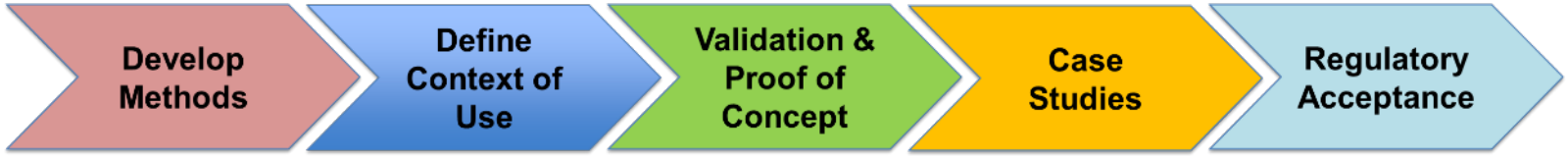
Regulatory tests that TGx-DDI addresses

1. TGx-DDI can be used in weight of evidence analyses to inform irrelevant *in vitro* chromosome damage results (FDA).
2. TGx-DDI can be used in integrated testing to inform potential hazards and mode of action, and for potency assessment.

Li et al., Development and validation of a high-throughput transcriptomic biomarker to address 21st century genetic toxicology needs. *PNAS*, 2017.

A long ride to date…

18 Papers published
NIEHS tool
qPCR array
CRO offering assay!

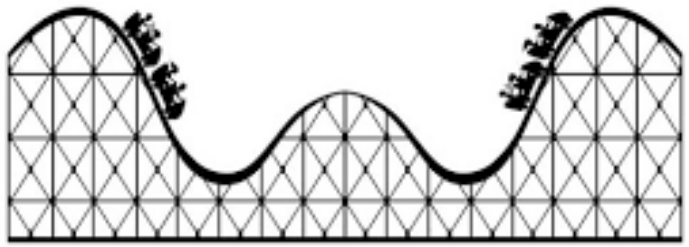


- TGx-DDI analytical pipelines
- TGx-DDI biomarker software tool
- BMD modeling
- HepaRG

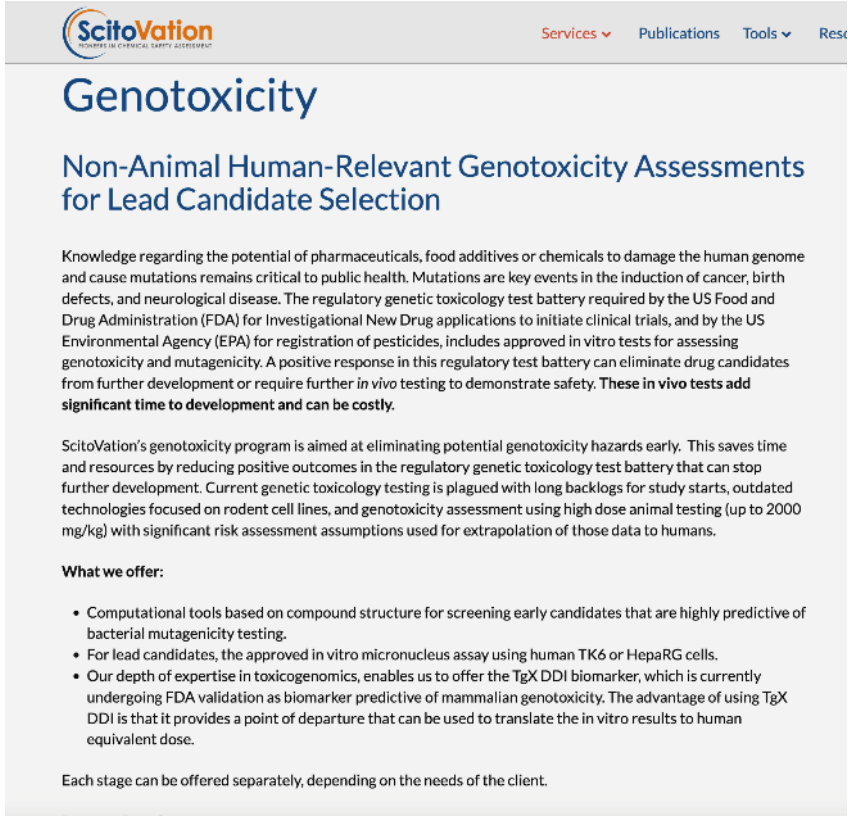
- Approximately 100 chemicals tested
- Metabolic activation
- Cross-platform – Affymetrix, qPCR, NanoString, RNA-seq, TempO-seq

- Three case studies completed

- **FDA Biomarker Qualification Plan**
- Part of Health Canada's GeneTox21 platform
- Integrated Approaches to Testing and Assessment (IATA)



2010 **2024**



ScitoVation

Services Publications Tools Res

Genotoxicity

Non-Animal Human-Relevant Genotoxicity Assessments for Lead Candidate Selection

Knowledge regarding the potential of pharmaceuticals, food additives or chemicals to damage the human genome and cause mutations remains critical to public health. Mutations are key events in the induction of cancer, birth defects, and neurological disease. The regulatory genetic toxicology test battery required by the US Food and Drug Administration (FDA) for Investigational New Drug applications to initiate clinical trials, and by the US Environmental Agency (EPA) for registration of pesticides, includes approved in vitro tests for assessing genotoxicity and mutagenicity. A positive response in this regulatory test battery can eliminate drug candidates from further development or require further *in vivo* testing to demonstrate safety. **These in vivo tests add significant time to development and can be costly.**

ScitoVation's genotoxicity program is aimed at eliminating potential genotoxicity hazards early. This saves time and resources by reducing positive outcomes in the regulatory genetic toxicology test battery that can stop further development. Current genetic toxicology testing is plagued with long backlogs for study starts, outdated technologies focused on rodent cell lines, and genotoxicity assessment using high dose animal testing (up to 2000 mg/kg) with significant risk assessment assumptions used for extrapolation of those data to humans.

What we offer:

- Computational tools based on compound structure for screening early candidates that are highly predictive of bacterial mutagenicity testing.
- For lead candidates, the approved in vitro micronucleus assay using human TK6 or HepaRG cells.
- Our depth of expertise in toxicogenomics, enables us to offer the TgX DDI biomarker, which is currently undergoing FDA validation as biomarker predictive of mammalian genotoxicity. The advantage of using TgX DDI is that it provides a point of departure that can be used to translate the in vitro results to human equivalent dose.

Each stage can be offered separately, depending on the needs of the client.

Recently Completed: Validation Study

Objectives: To assess the cross-laboratory reproducibility of TGx-DDI classification calls involving one platform (NanoString)

Resourcing: *Funding from FDA (1U01FD007473-01) and in-kind effort and materials from HESI partner organizations.*

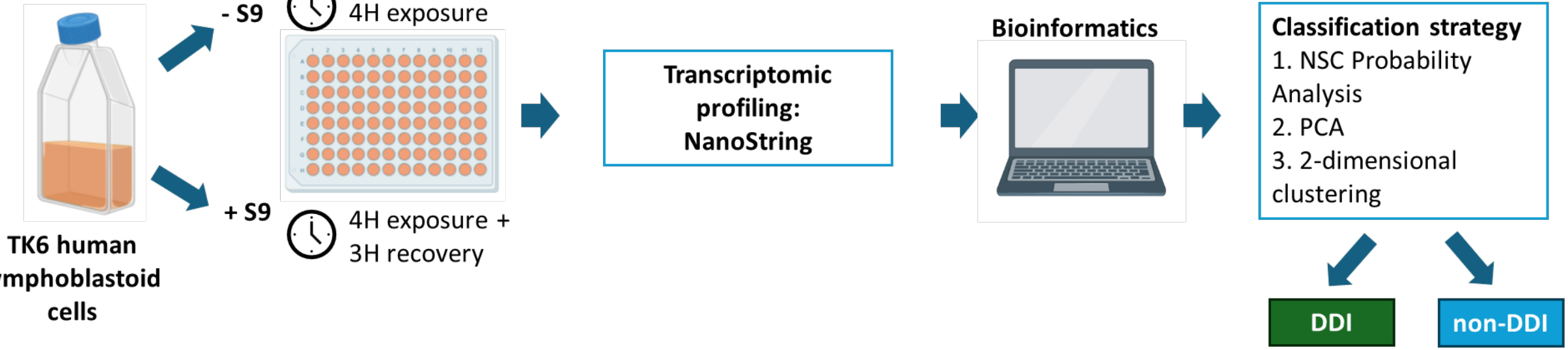
Experimental Plan:

- 4 study sites
- Sites receive test compound in solution (three concentrations and solvent)
- 14 chemicals tested
- TK6 cells, samples in triplicate
- Culture and exposure per the SOP
- Analysis and classification using public software tool

Study design reviewed by FDA and Agency feedback incorporated on multiple occasions.

INSTITUTION		MULTI-SITE STUDY CONTRIBUTIONS				
		Study coordination (meetings, logistics, supply procurement, shipping)	**TGx-DDI Assay** (cell culture, exposure, RNA isolation)	**NanoString** (RNA QC & Transcriptomics)	**Data Analysis, Interpretation & Reporting**	**Data Compilation Presentation and Cross Site Data Analysis**
HESI	**HESI**	X				X
Georgetown University	**Georgetown University**	X	X	X	X	
SANOFI	**Sanofi Laboratories**		X		X	
P&G Procter & Gamble	**Procter & Gamble Laboratories**		X		X	
BRT	**Burleson Research Technologies**		X	X	X	
Children's National	**Children's National Genomics Core**			X		
THE WISTAR INSTITUTE	**Wistar Institute Genomics Core**			X		

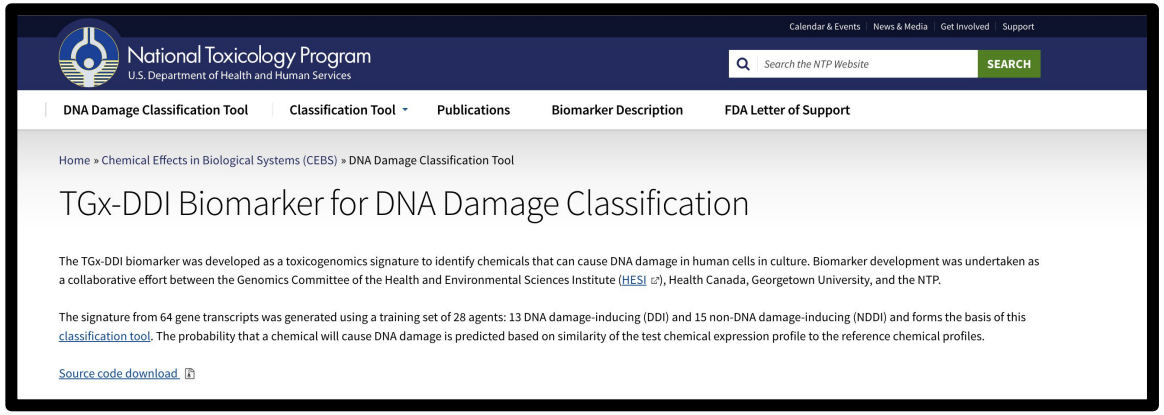
TGx-DDI Ring Trial Experimental Workflow



MTT Assay

Concentration range selected from 24h cytotoxicity

Up to 1 mM or that induces 50% cytotoxicity



Collaboration is key

Long project with team members in each sector

- Development to regulatory acceptance!

Demonstrating uptake of technology

Leveraging industry, government, and in-kind funds

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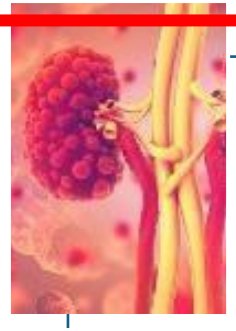

miRNA Biomarkers

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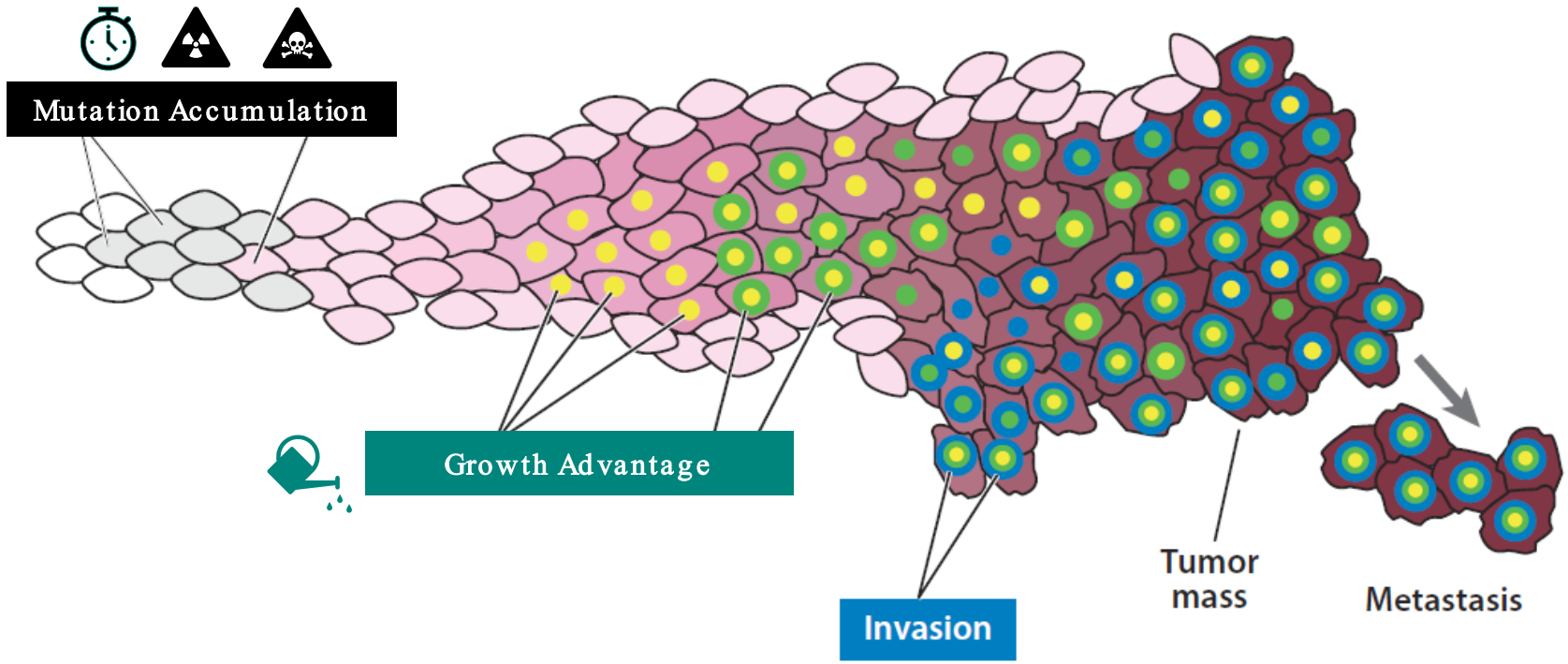
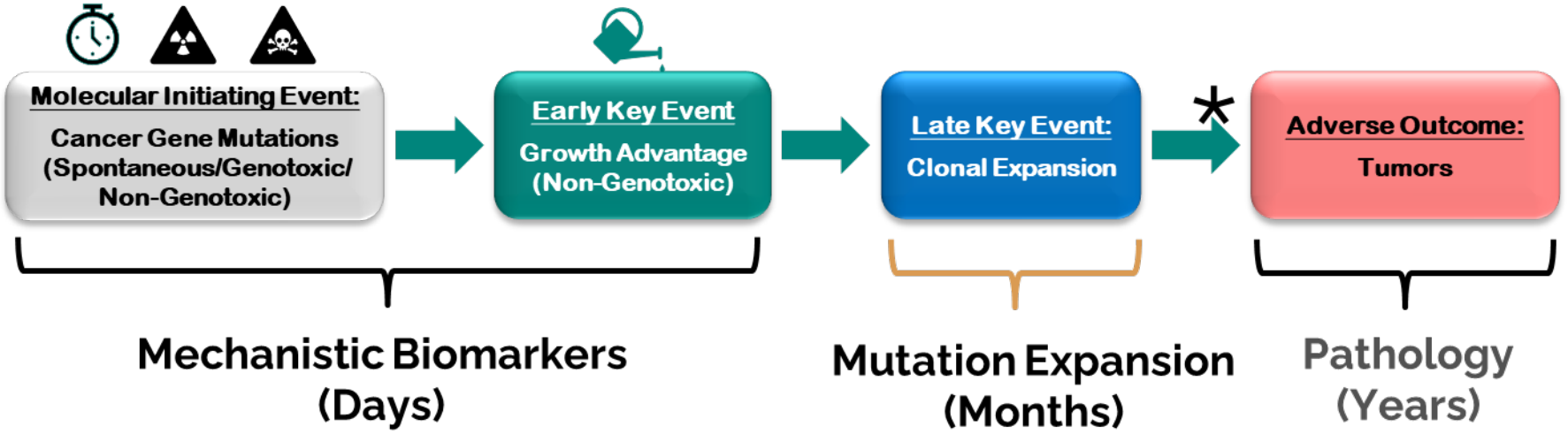
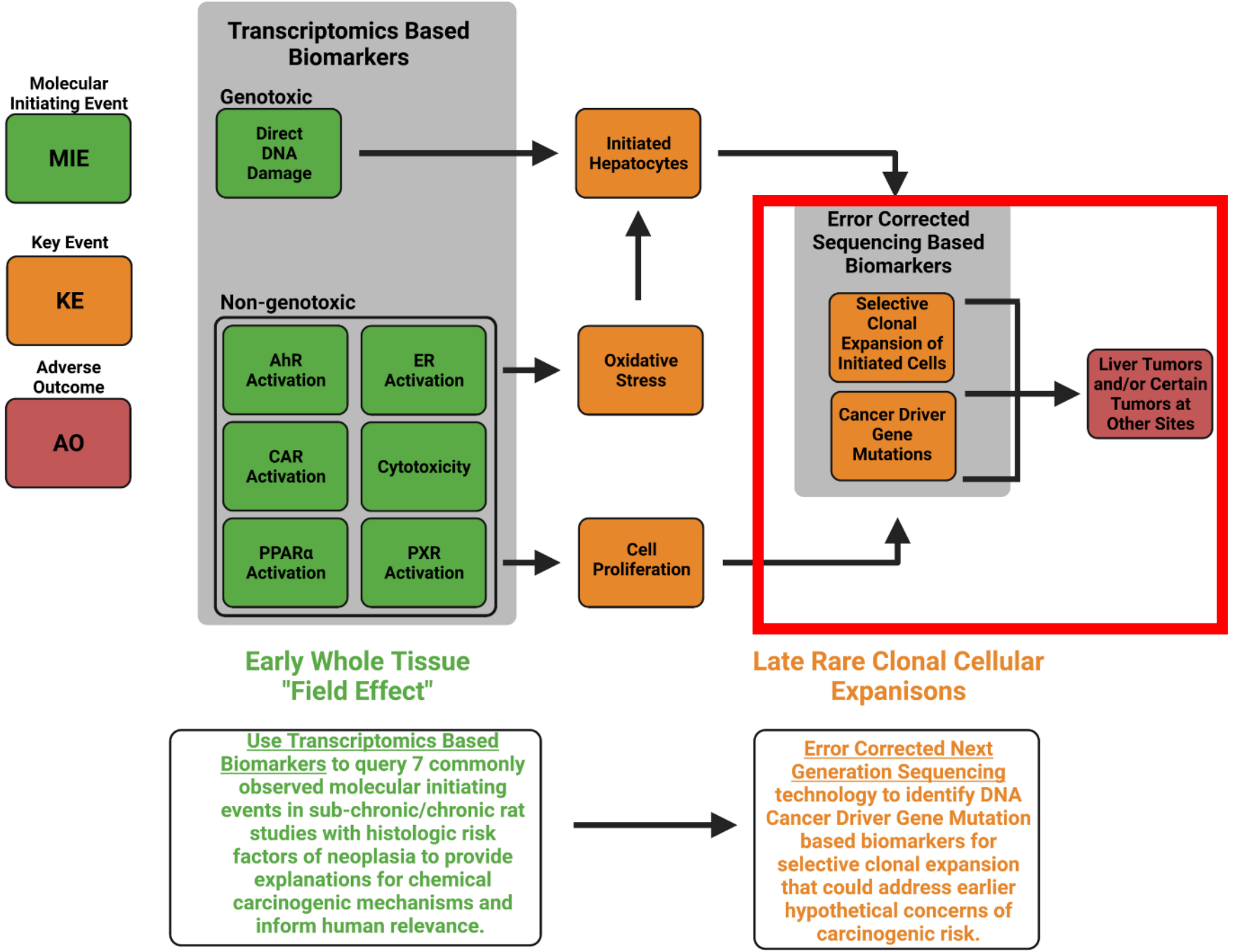
Progression of Cancer Biology: Opportunities for Earlier Detection



- Detection of early tumorigenesis (clonal expansion) could allow assessment of w/in months, prior to gross histopathology
- Combining tools in short-term rodent studies could provide a strong weight-of-evidence alternative to 2yr carcinogenesis studies aligned with ICH S1B (R1) addendum

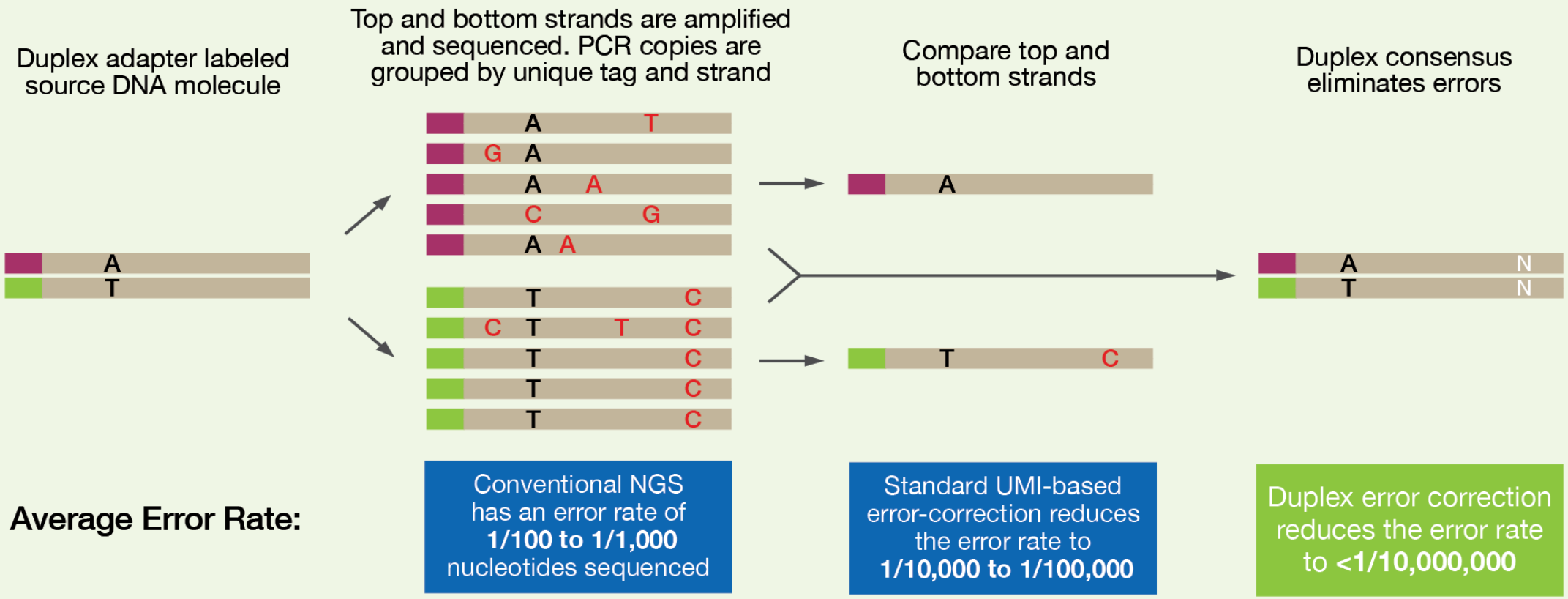


*Challenge - understand the thresholds from initiation, promotion to progression



Corton et al 2022

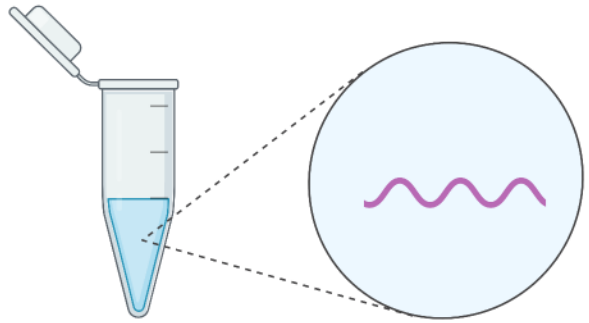
Error Corrected Sequencing (eg. DuplexSeq™) to Measure Clonal Expansion of Cancer Driver Genes



twinstrandbio.com

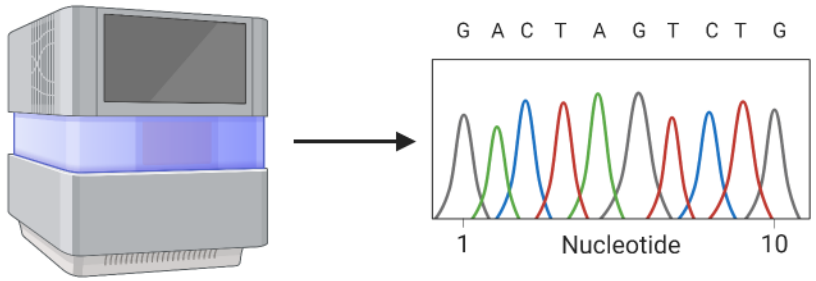
Sequencing Workflow

Step 1:
DNA extraction

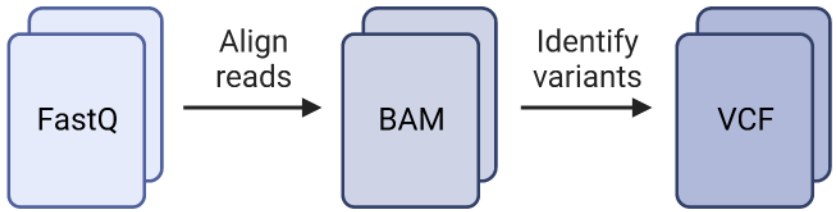


Step 2:
Library preparation

Step 3:
Sequencing



Step 4:
Analysis



How do we build confidence?

1. **Additional Compounds/ Studies:**
   - Studies with additional compounds will help establish sensitivity/ specificity of the approach as well as the ability to distinguish tumorigenic from non-tumorigenic dose levels

2. **Additional Genes:**
   - A sensitive assay will require broad coverage of potential driver genes across different NGT mechanisms
   - Studies with additional compounds will help identify gaps in existing panels and WES on these samples can be used to identify novel driver genes.

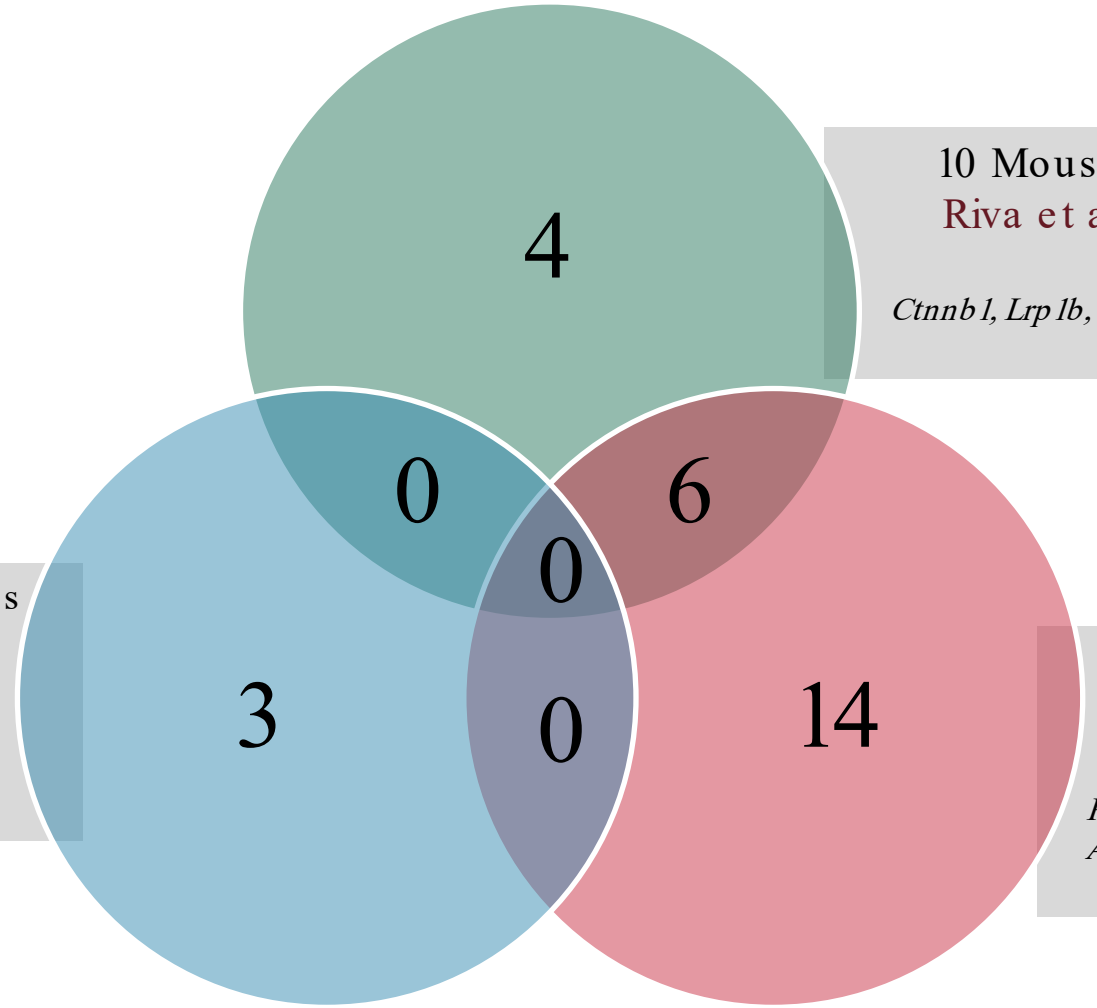
3. **Establish Thresholds:**
   - Establish normal baseline of expanded clones across time points, and normalized thresholds of concern to enable early risk assessment of novel compounds

4. **Regulatory Acceptance:**
   - Ultimate goal is to use as part of a weight-of-evidence approach for carcinogenic risk assessment and potential waivers of long-term carcinogenesis studies

HESI.

Mouse carcinogenesis panel v1

Panel design covers 27 cancer driver genes that were identified in Tg-rasH2 tumors, Riva et al. (Wt-mouse), and CarcSeq (human)



10 Mouse Cancer Driver Genes
Riva et al., Nat Genetics (2020)

Ctnnb1, Lrp1b, Cnot3, Egfr, Fgfr2, Kras, Hras, Braf, Trp53, Epha3

3 Tg-rasH2 Cancer Driver Genes (putative)
PRJ00183

*Human HRAS, Cdh2, Tsg101**

20 Human Cancer Driver Genes
Harris et al., Tox Sci (2021), CarcSeq

Pik3ca, Trp53, Stk11, Kras, Hras, Braf, Egfr, Lrp1b, Nfe212, Apc, Setbp1, Tert, Rb1, Axin1, Cdkn21, Pten, Acvr2a, Foxl2, Kmt2c, Nras

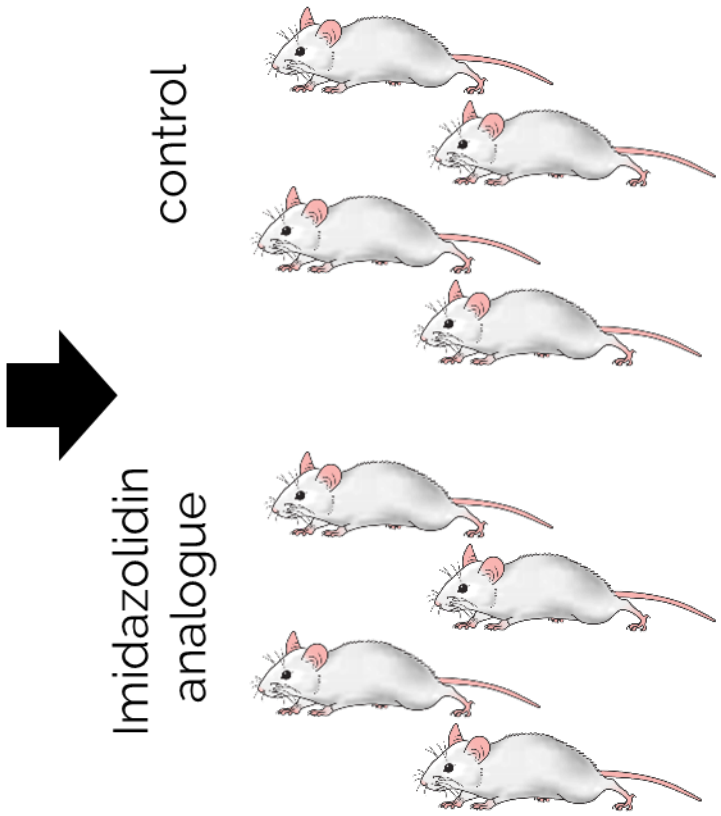
*Not known cancer driver gene

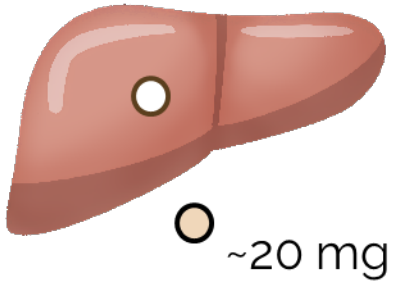
HESI.

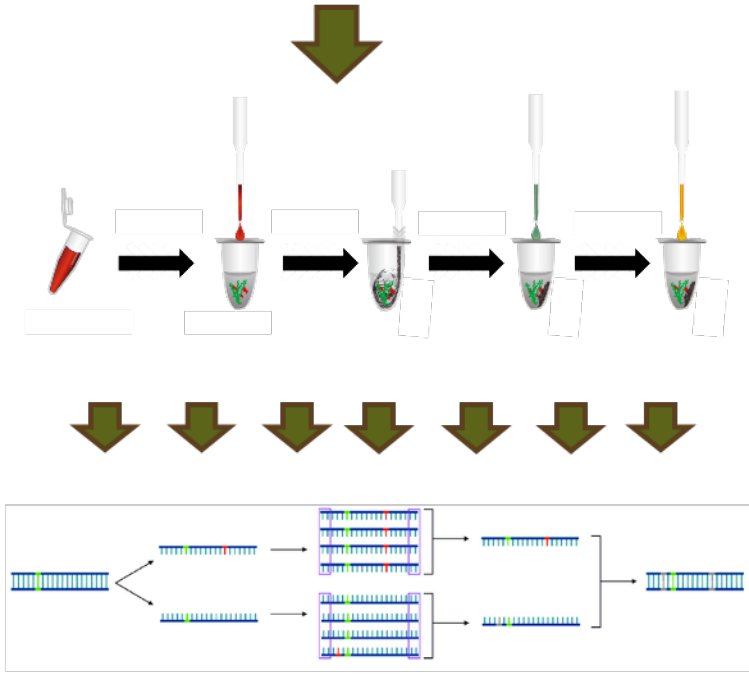
Pilot Study Design

Goals: (1) Evaluate if DuplexSeq can detect CDMs
(2) Establish sample prep and sequencing parameters

- Male CD1 mice
- (OECD 408) 90-day study: Liver weight change and centrilobular hepatocellular hypertrophy
- Vehicle control and 114 mg/kg (dose higher than the carcinogenic dose at 2-year bioassay)

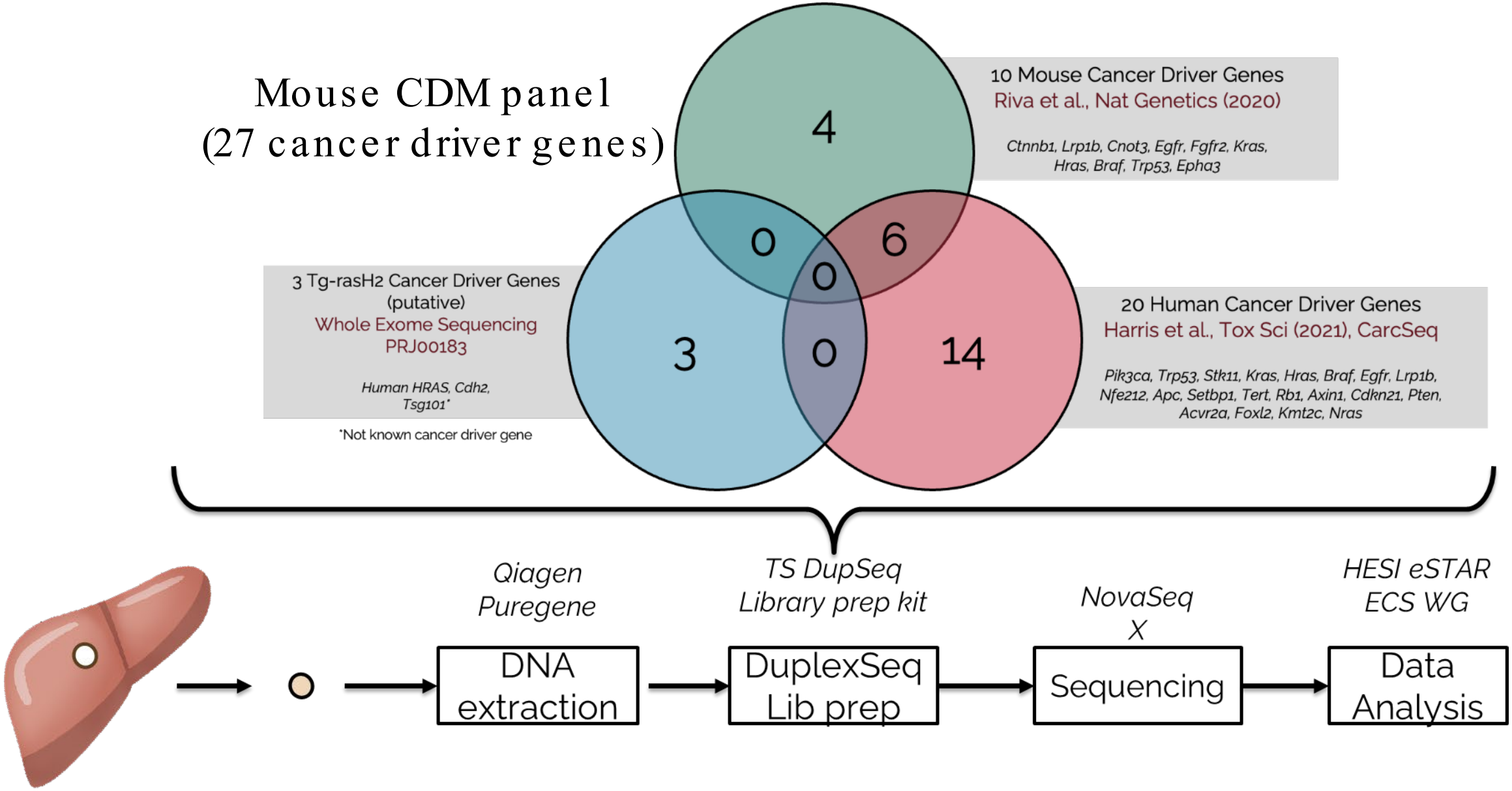






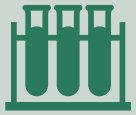
*Syngenta study

Overall Study Workflow



Collaboration is key

Share vision to explore the use of a tool



Multi-site experiment using existing samples

Data Analysis done by multiple groups to agree upon best practices

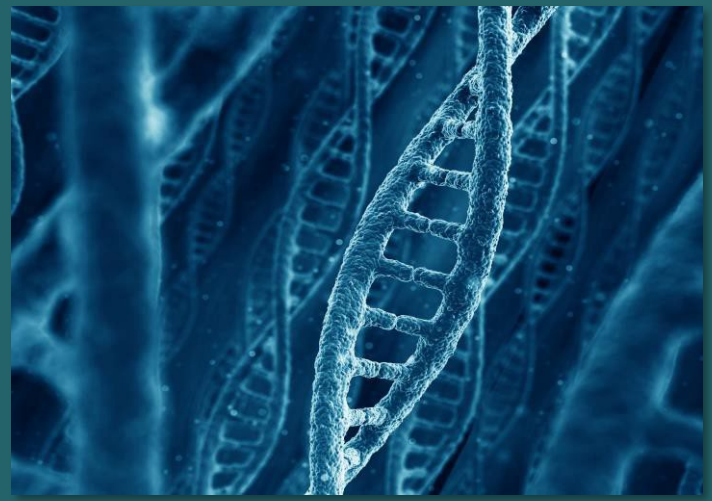
Email me:

Connie Mitchell

(cmitchell@hesiglobal.org)

Development and Application of Transcriptomics at EPA

Logan J. Everett, Ph.D. – Bioinformatics Scientist, US EPA / ORD

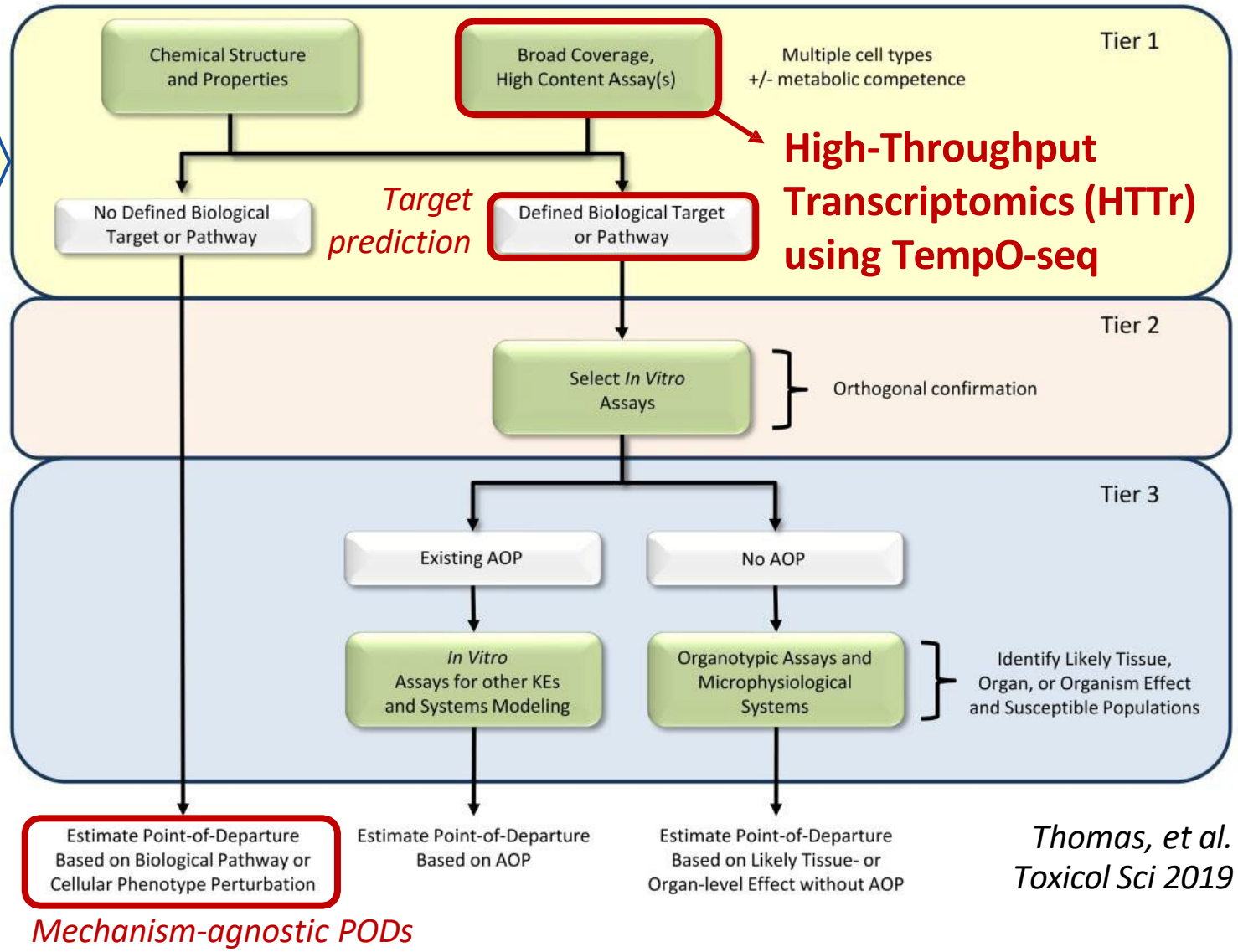


The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA.
Company or product names do not constitute endorsement by U.S. EPA.

Tiered Chemical Safety Testing Strategy

Tier 1 Primary Goals:

- Prioritize chemicals by bioactivity & potency
- Predict biological targets for chemicals



Mechanism-agnostic PODs

Thomas, et al. Toxicol Sci 2019

EPA Transcriptomic Assessment Product (ETAP)

Short-term *in vivo* exposures with transcriptomic profiling

Automated *in vitro* Chemical Screening Strategy

Cryopreserved Cell Stocks

Cell Expansion

Cell Plating

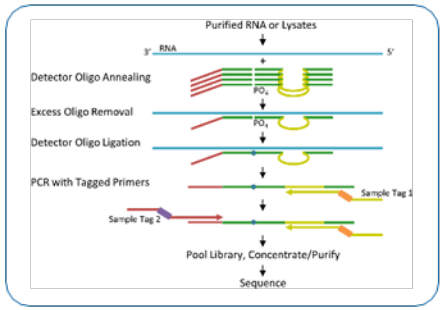
BioTek MultiFlo™ FX

Dispensing Test Chemicals

LabCyte Echo® 550 Liquid Handler

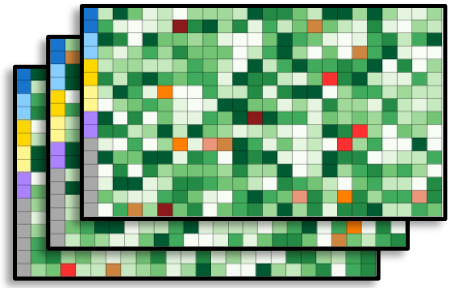
6 - 24 hr exposures

TempO-seq: Targeted sequencing for changes in gene expression



Yeakley, *et al. PLoS ONE* (2017)
DOI: 10.1371/journal.pone.0178302

Cell Line Examples:	
MCF-7	Breast epithelium
U-2 OS	Bone
HepaRG	Liver



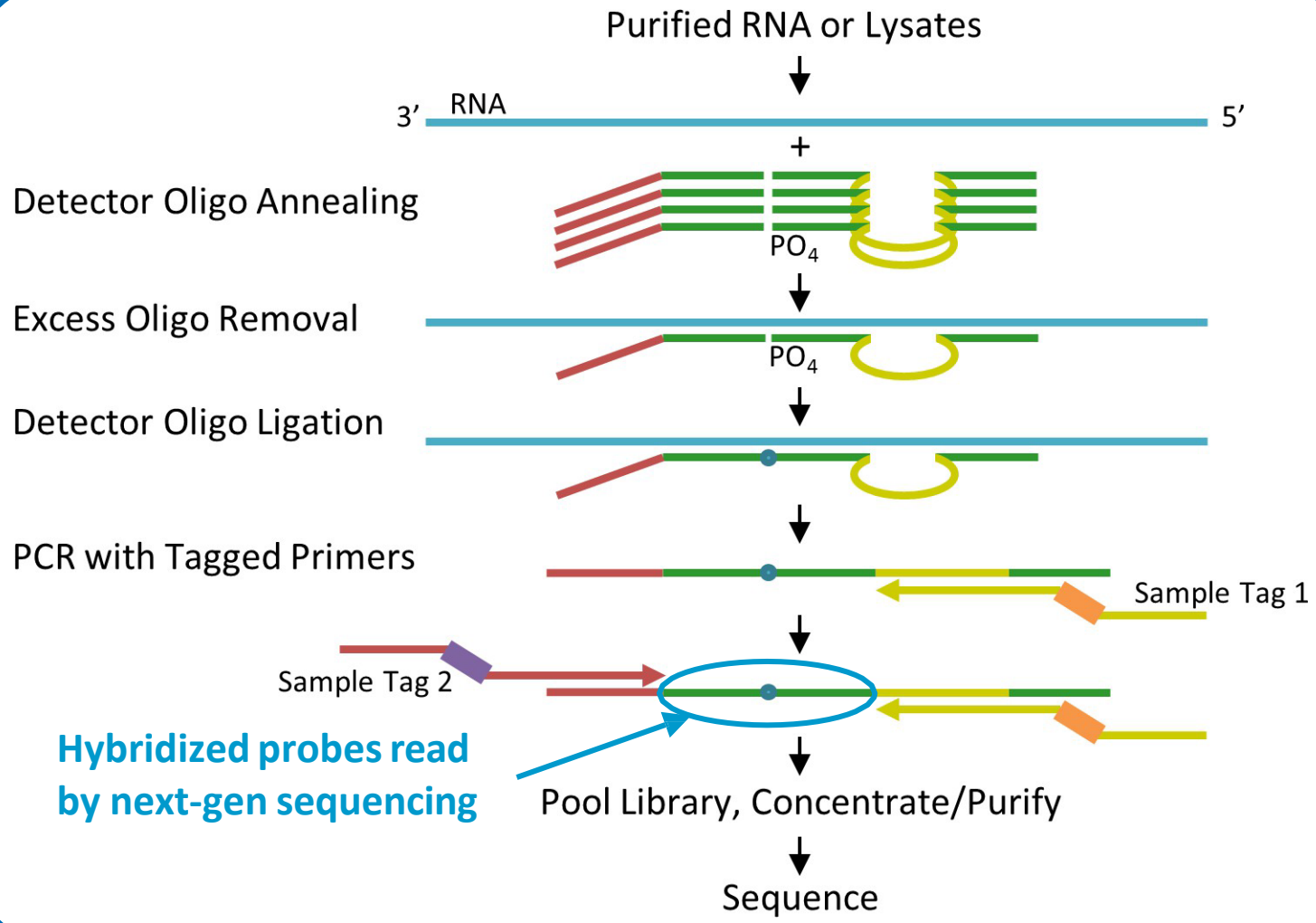
384-well test plates run in triplicate with:

- ~40 test chemicals x 8 concentrations (half-log spacing)
- Multiple vehicle controls, reference chemicals & QC samples on every plate to track assay performance
- Treatment positions randomized on each plate
- Independent culture batch on each plate

See Harrill, et al. Tox Sci 2021
DOI: 10.1093/toxsci/kfab009

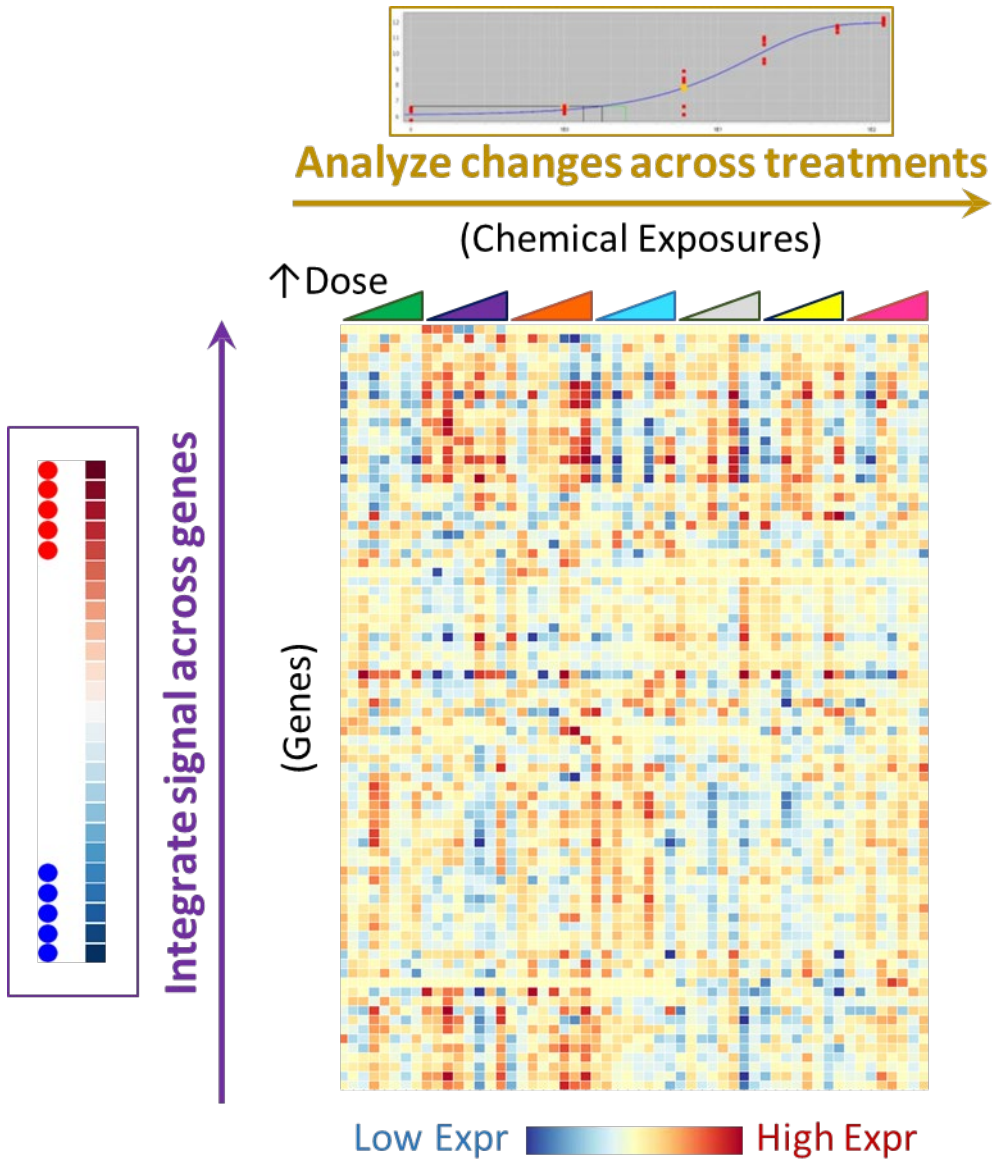
Targeted RNA-seq Assay (TempO-seq)

- Next-Gen sequencing of targeted probes hybridized to expressed transcripts
- Whole transcriptome coverage (>20,000 genes)
- Captures gene expression at lower cost than RNA-seq or microarrays
- Compatible with raw cell lysates – ***ideal for large-scale screening***



Yeakley, *et al. PLoS ONE* (2017) DOI: 10.1371/journal.pone.0178302

Transcriptomic Dose-Response Models



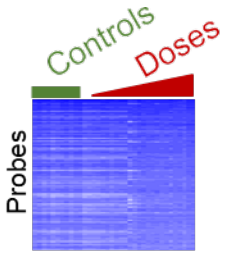
- Different genes may respond at different doses of a given exposure!
- Need to analyze both:
  - Dose-responsive trends
  - Coordinated changes in gene expression
- Gene-level data noisier in transcriptomics than targeted measurements (e.g. RT-qPCR)
- Dose-response modeling thousands of features (e.g. mRNA levels) leads to computational & statistical challenges

Many Analysis Choices!

No single "best" method for transcriptomic dose response modeling

- Are you **interested in mechanism**, or just want a threshold for general bioactivity?
- Is it more important to be **predictive** or **protective** of hazard level *in vivo*?
- What other data is available for the same/analogous chemicals?
- Different technologies require different statistical models, quality control, etc.
- Experimental design *(# of replicates, doses, etc.)* impacts analysis choices!

Dose-Response Modeling of Gene Sets/Signatures



Concentration Series of Whole Transcriptome Profiles + Vehicle Controls

Love et al. (2014)

DESeq2 Moderated Fold-Changes

Curated Signature Collection

Barbie et al. (2009)

Gene Set Enrichment Analysis

Sheffield et al. (2022)

Benchmark Dose Modeling

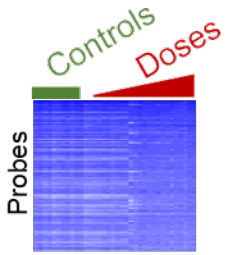
Catalog of gene set signatures with toxicological relevance, annotated for known molecular targets

- **Bioplanet** (*Huang, et al. Front Pharmacol 2019*)
- **CMap** (*Subramanian, et al. Cell 2017*)
- **DisGeNET** (*Piñero, et al. Database 2015*)
- **MSigDB** (*Liberzon, et al. Cell Syst 2015*)

Open Source: github.com/USEPA/CompTox-httrpathway

- EPA/CCTE method for summarizing large-scale transcriptomic screening studies
- Integrates signal across known gene set (a.k.a. signature) ***before*** dose-response modeling

Dose-Response Modeling of Gene Sets/Signatures



Concentration Series of Whole Transcriptome Profiles + Vehicle Controls

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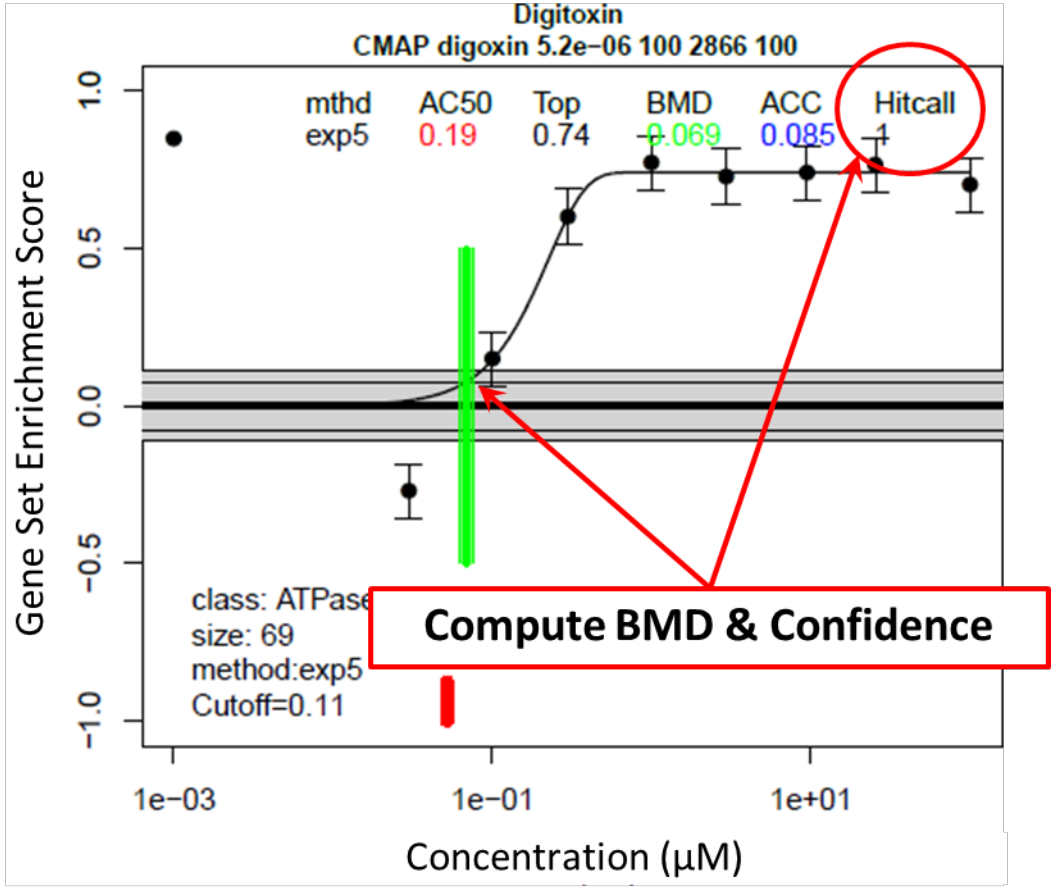
Sheffield et al. (2022)

Benchmark Dose Modeling

Active Signatures have HitCall > 0.9



CRAN.R-project.org/package=tcplfit2

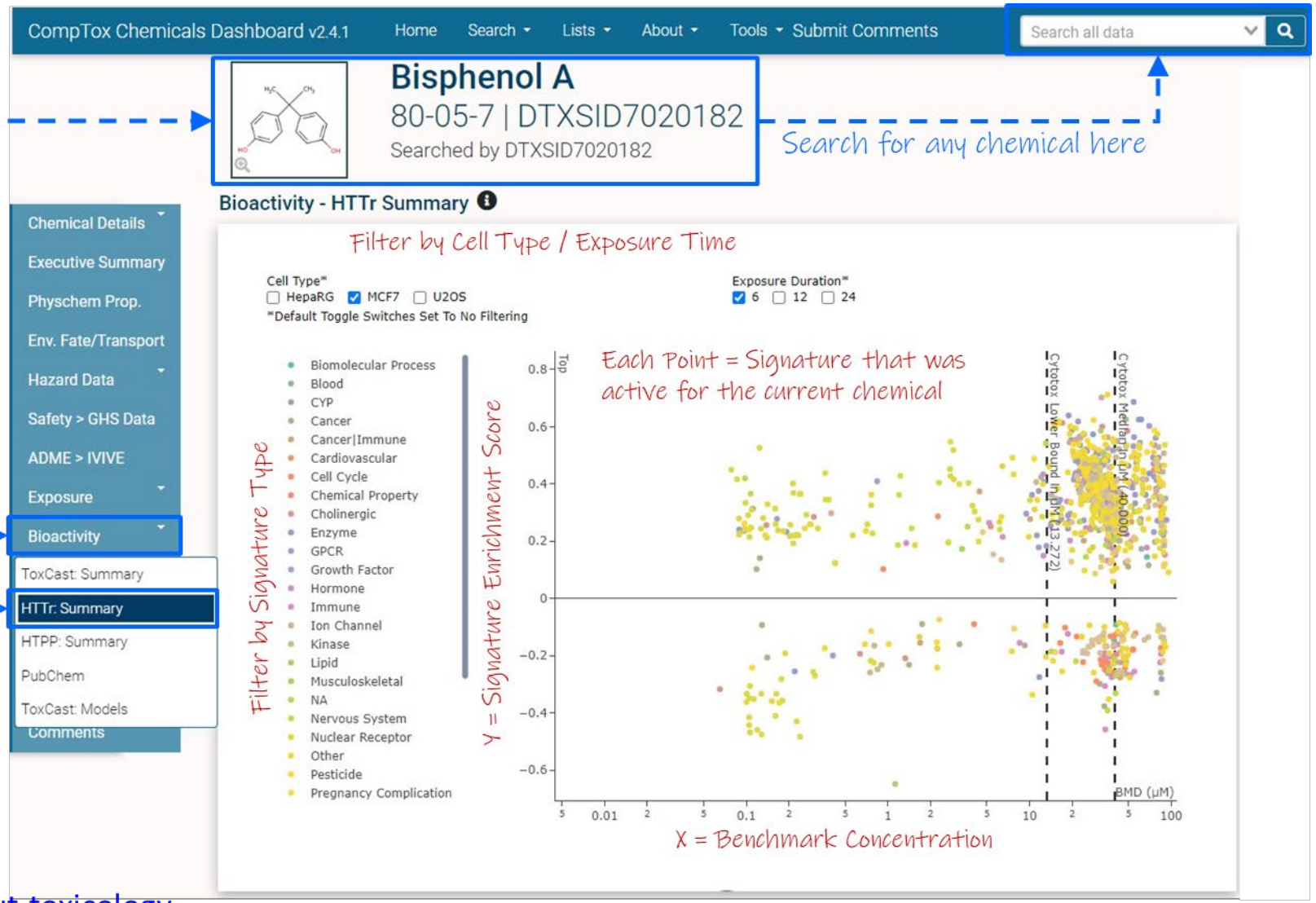
Public Release of HTTr Data

Cell Line/Model	Chemicals Screened	Publications
MCF7: *Breast adenocarcinoma cell line, sensitive to multiple endocrine disruptors*	44 well-characterized chemicals (pilot study)	Harrill, et al. *Tox Sci* 2021 DOI:10.1093/toxsci/kfab009 Harrill, et al. *Toxicology* 2024 DOI:10.1016/j.tox.2023.153694
	1,751 ToxCast chemicals	Harrill, et al. *Tox Sci* 2024 DOI:10.1093/toxsci/kfae108
U-2 OS: *Osteosarcoma cell line with complementary Cell Painting data*	1,201 ToxCast chemicals	Bundy, et al. *TAAP* 2024 DOI:10.1016/j.taap.2024.117073
HepaRG: *Hepatoma cell line, differentiated into metabolically competent hepatocyte-like cells*	1,201 ToxCast chemicals	Rogers, et al. *in review* Shah, et al. *in preparation*

HTTr Results Available on CompTox Chemicals Dashboard

comptox.epa.gov/dashboard

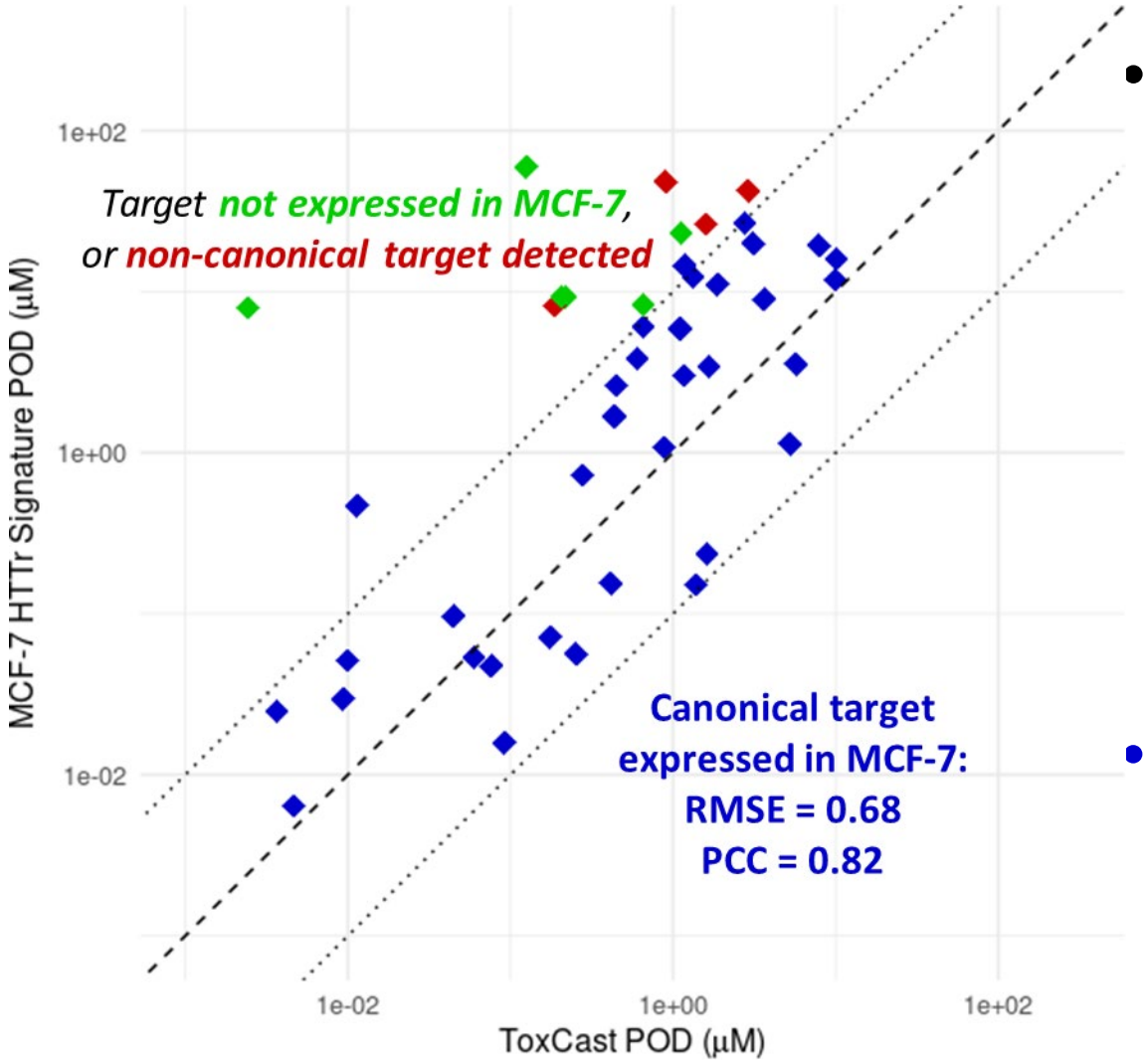
1. Search for a specific chemical
2. All *in vitro* NAMs data available here
3. HTTr data available here



← More Info Available Here

epa.gov/chemical-research/high-throughput-toxicology

HTTr tPODs Are Concordant With ToxCast PODs



- Computed 5th percentile PODs from:
  - Pilot study of 44 well-characterized chemicals in MCF-7 cells, 6h exposure
    Harrill, et al. *Toxicol Sci* (2021)
    DOI: 10.1093/toxsci/kfab009
  - ToxCast targeted assay results *(multiple cell types, assays, and exposure lengths)*
    Paul-Friedman, et al. *Toxicol Sci* (2020)
    DOI: 10.1093/toxsci/kfz201

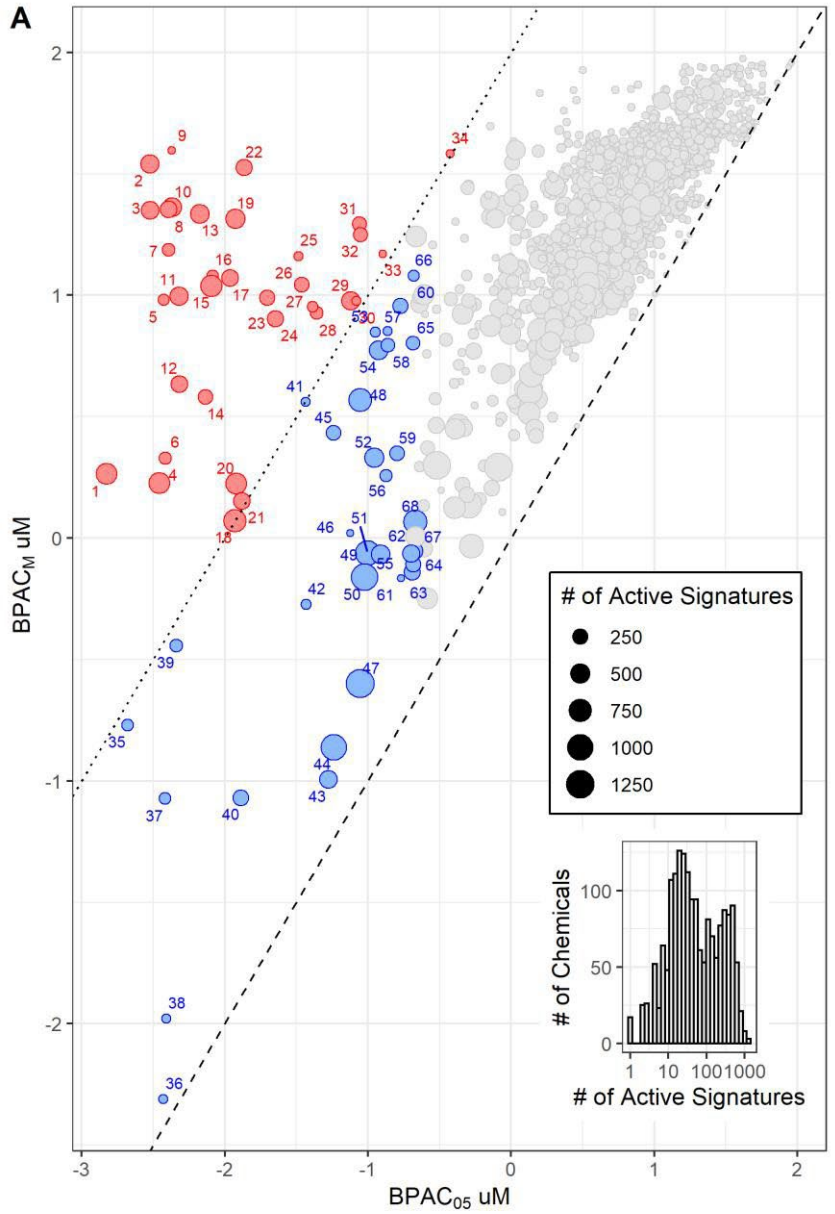
- Signature-based PODs are highly concordant with ToxCast results for the majority of test chemicals in pilot study

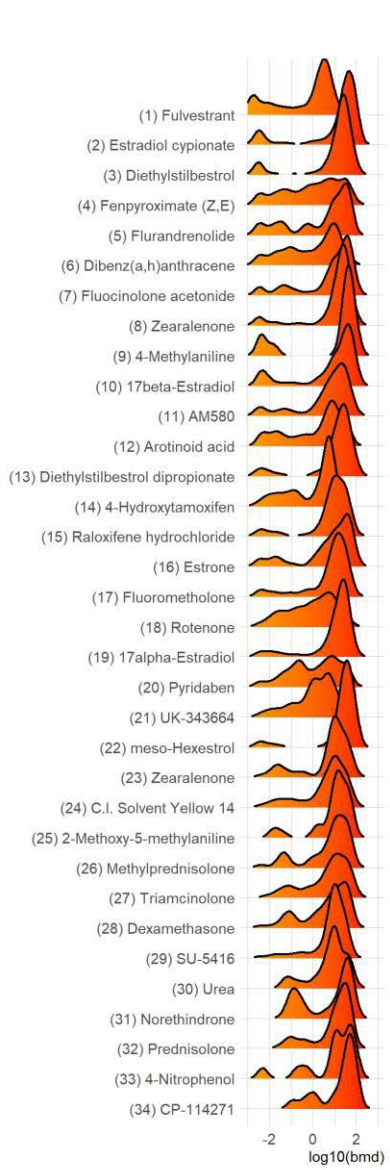
Signature BMC Distributions Vary Across Chemicals

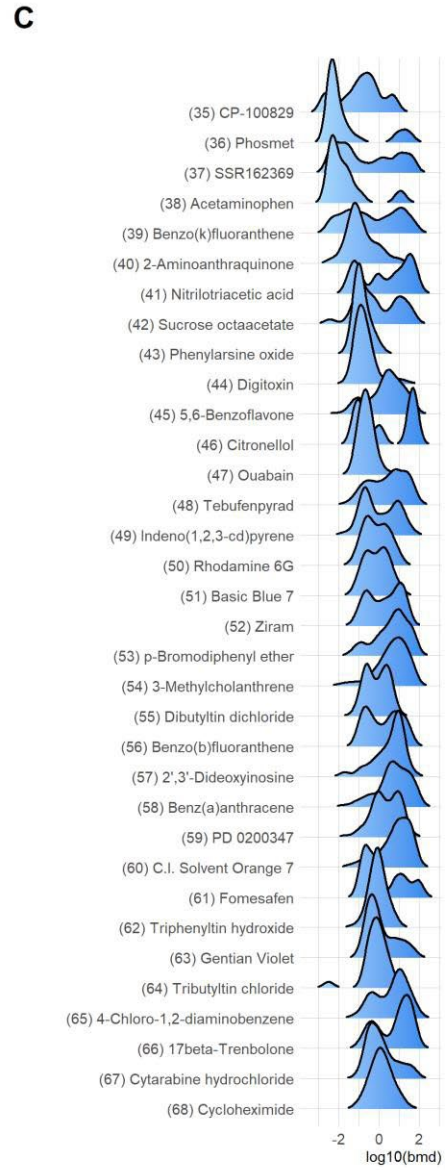
Results from first large screen of 1,751 chemicals in **MCF7 cells**

BPAC = Biological Pathway Altering Concentration.

Chemicals with known molecular target specificity tended to have $BPAC_{05}$ much more potent than the median BPAC (red bubbles)





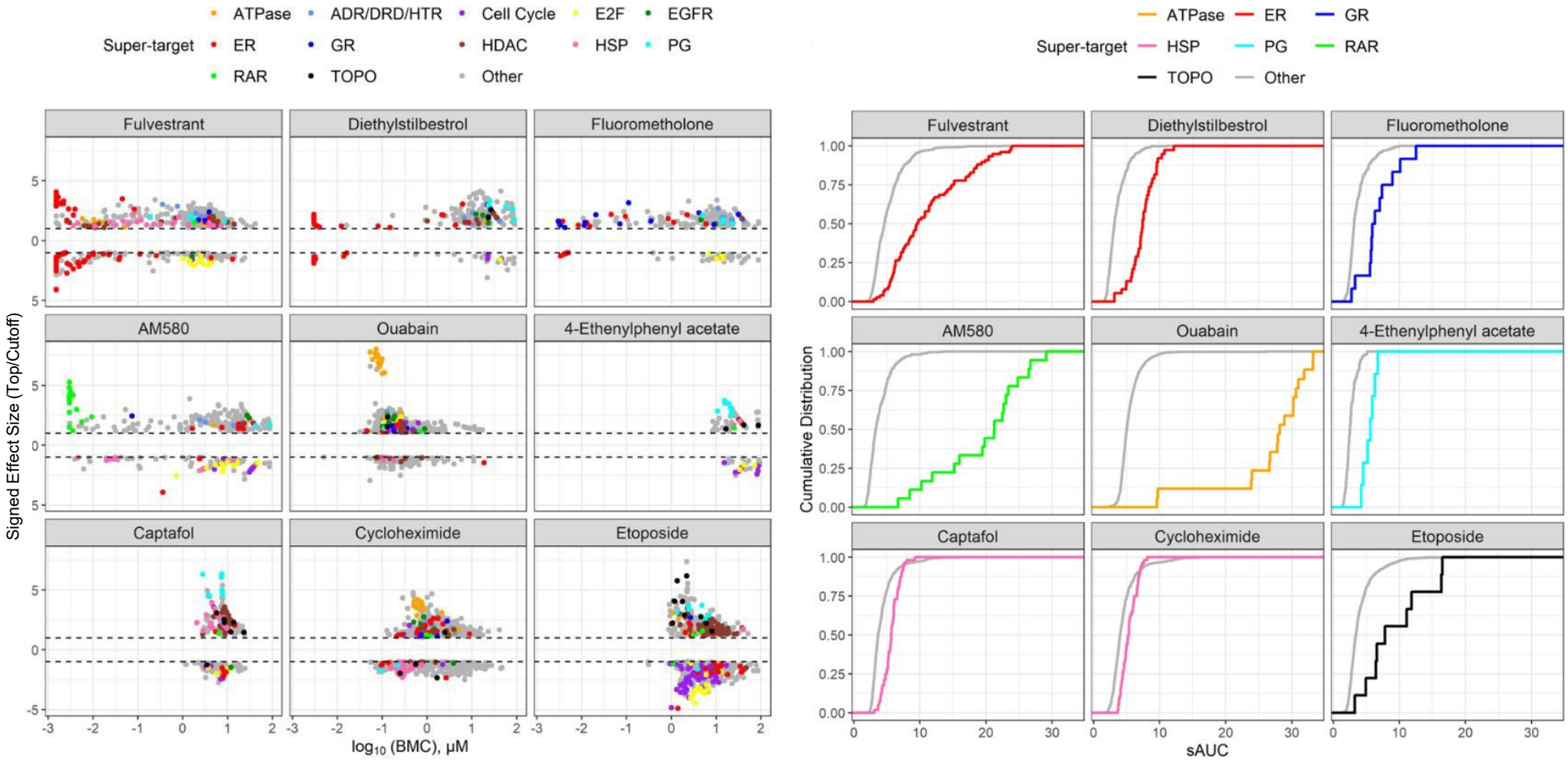


Harrill et al. Tox Sci (2024)
DOI:10.1093/toxsci/kfae108

Distribution of Signature BMCs Indicative of Molecular Target Specificity

Target annotations in existing signature catalog identify target specificity for some chemicals.

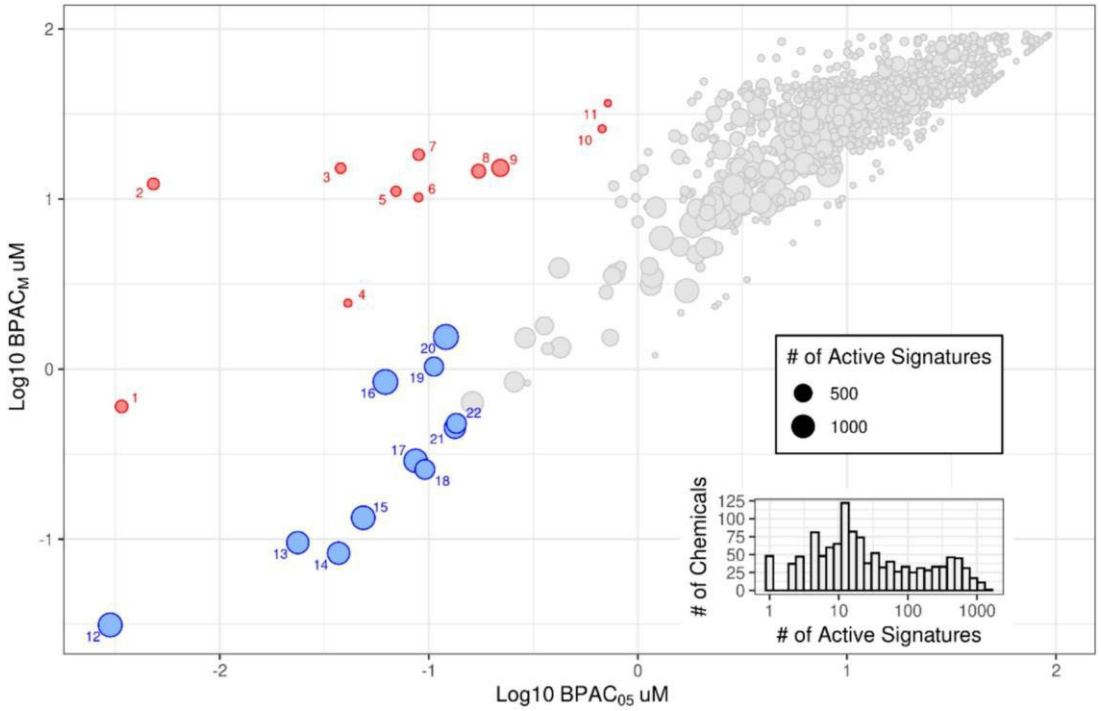
Tested for enrichment of signatures with same target annotation by K-S test on AUC values (lower BMD or greater effect size)

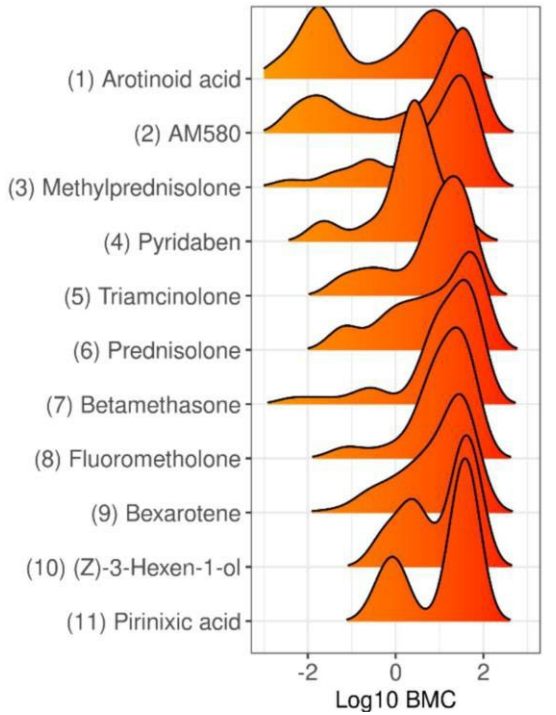


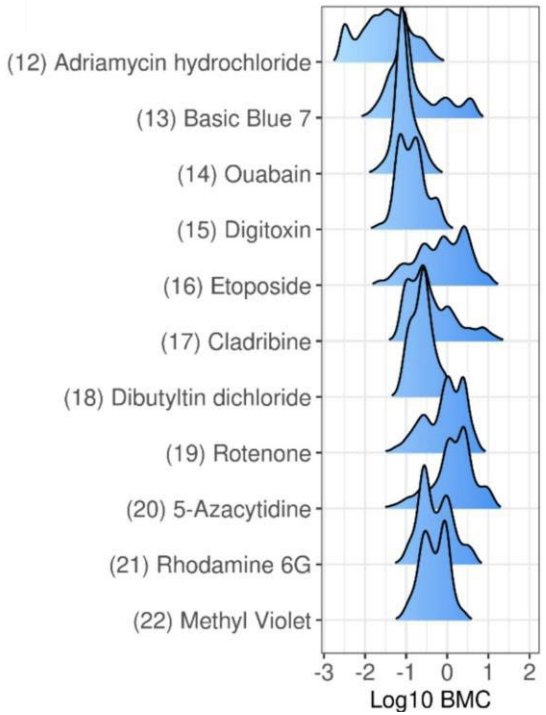
Harrill et al. Tox Sci (2024) DOI:[10.1093/toxsci/kfae108](https://doi.org/10.1093/toxsci/kfae108)

Credit: Joshua Harrill (EPA ORD)

Application of Signature Analysis Method to Additional Cell Types







Observed similar distributions of BPAC05 and BPACM in **U-2 OS cells** as in MCF7:

- Some chemicals have clear delineation between target-specific and non-specific activity (red)
- Other chemicals have potent but non-specific activity (blue)

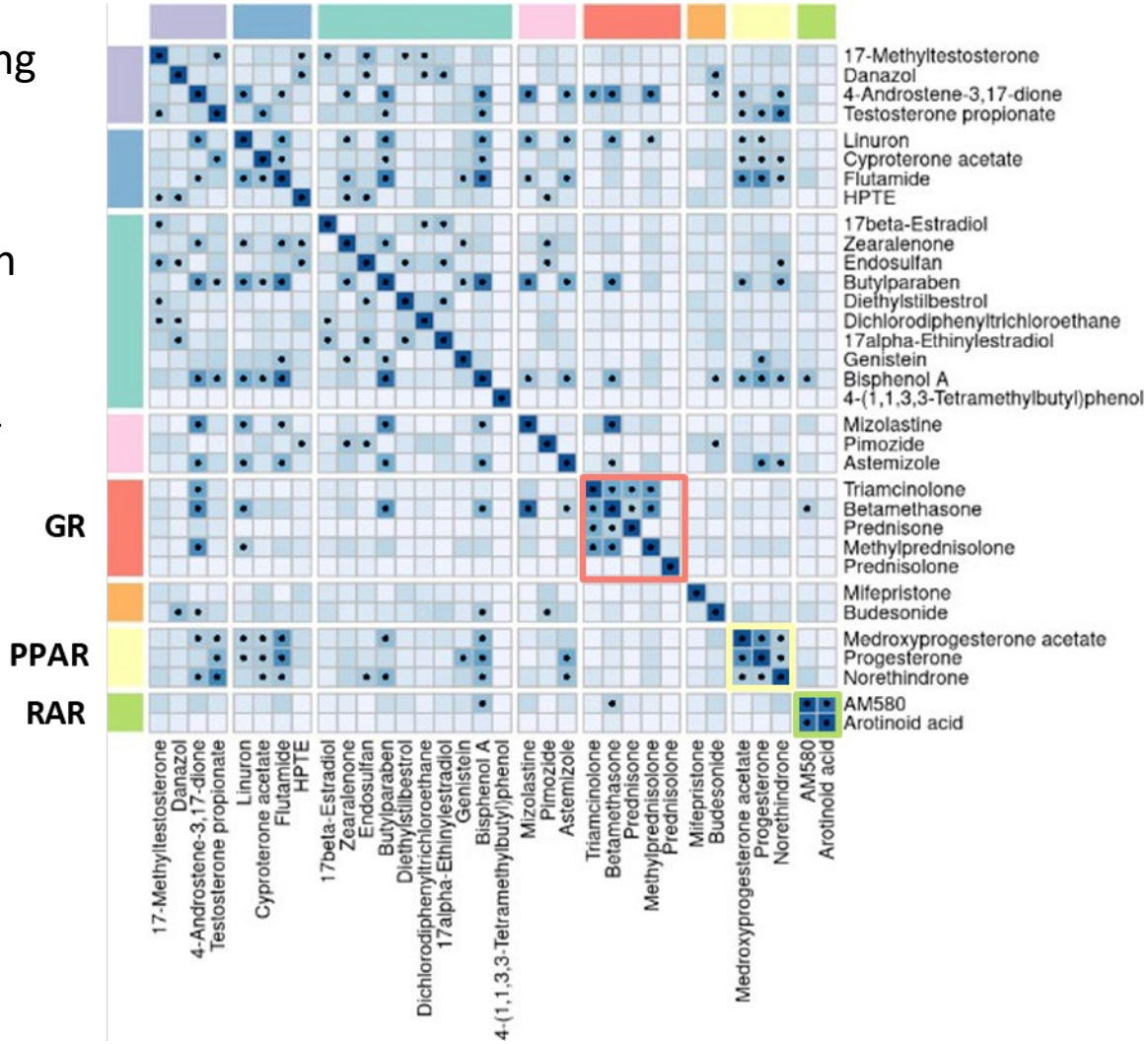
Bundy et al. TAAP (2024) DOI:10.1016/j.taap.2024.117073

Credit: Joshua Harrill (EPA ORD)

Predicting Molecular Targets Using Signature Modeling and Reference Chemicals

- Existing signature annotations are less effective at inferring chemical target in other cell types
  (many signatures derived from studies in MCF-7)
- However, similarity of overall signature activity profile can still be used to group chemicals sharing certain common targets
  Pairwise similarity = Jaccard Index of active signature hits
  Results shown from U-2 OS (osteosarcoma cell line)

Similarity of Signature Hits by Chemical in U-2 OS Cells

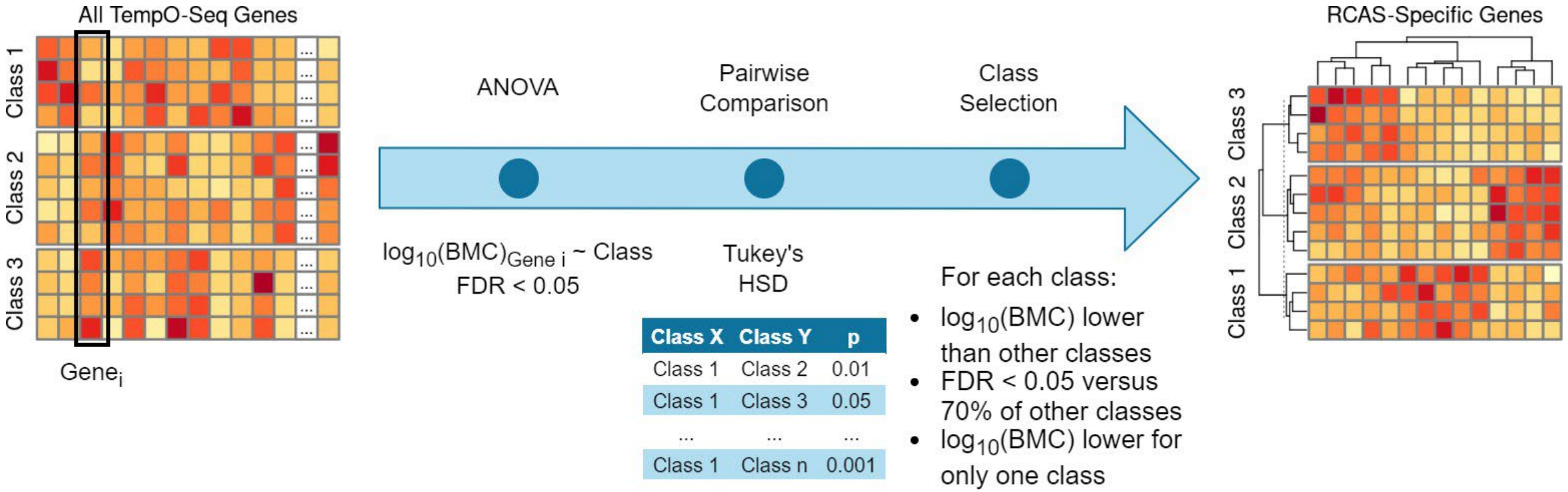


Bundy et al. TAAP (2024) DOI:10.1016/j.taap.2024.117073

Credit: Jesse Rogers (EPA ORD)

Reference Class-Associated Signatures (RCAS) for Profiling Chemical Mechanisms-of-Action

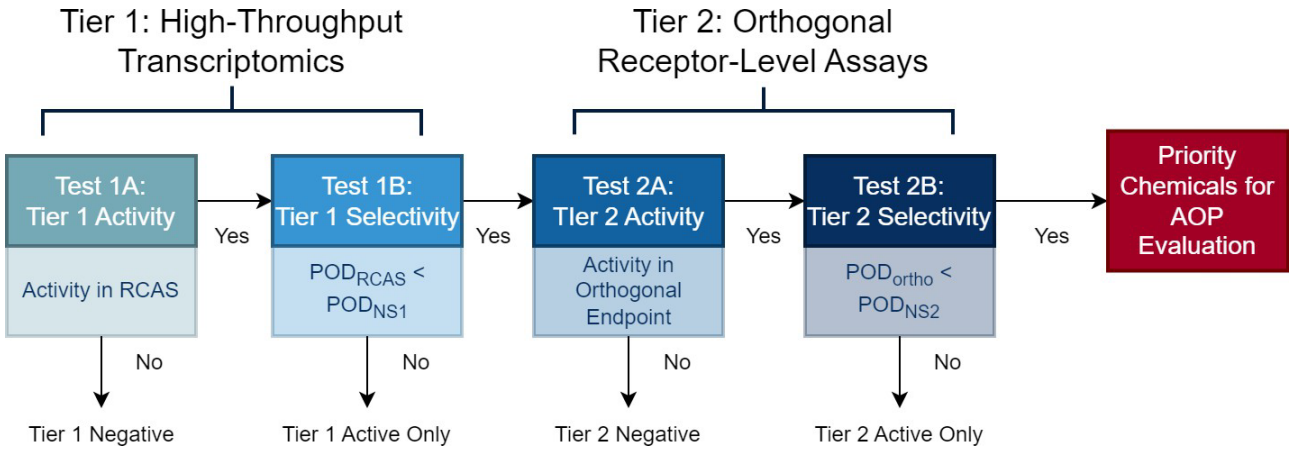
- **Goal: Develop new target-specific signatures tailored to each cell line** *(applied to U-2 OS and HepaRG so far)*
- Genes uniquely sensitive for individual molecular targets selected via univariate analysis
- Tailored signatures identify selective chemical effects during Tier 1 screening



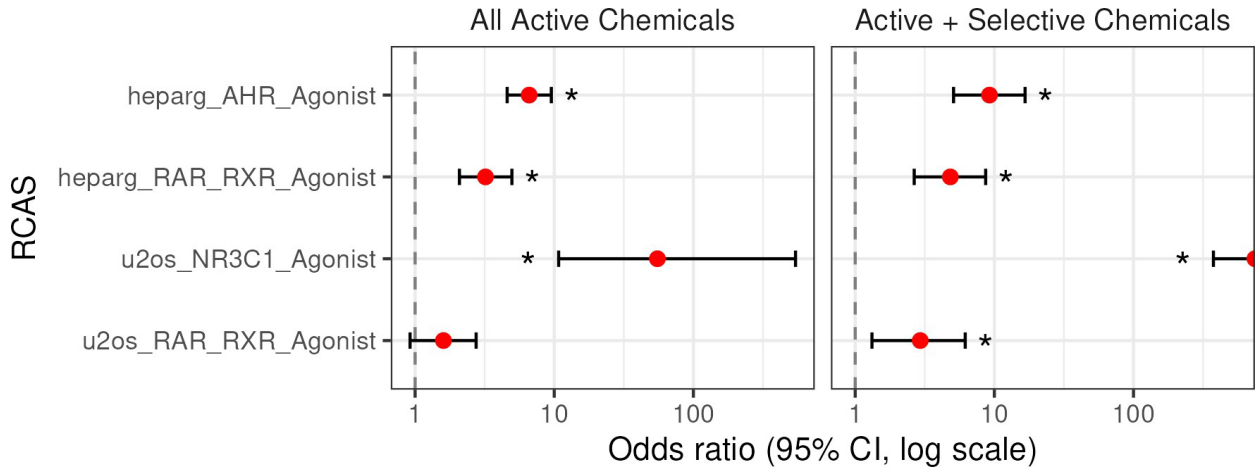
Rogers, et al. Manuscript submitted

Credit: Jesse Rogers (EPA ORD)

Integration of TempO-Seq Readouts for Chemical Prioritization Framework



RCAS-derived potencies compared to non-selective point of departure (POD_{NS}) estimated from public signature collection



Chemicals with selective effects on molecular targets identified via RCAS and validated using high-throughput screening assay data from US EPA's ToxCast program

Rogers, et al. Manuscript submitted

Credit: Jesse Rogers (EPA ORD)

Summary

- High-Throughput Transcriptomics (HTTr) applied to human cells *in vitro* captures a wide variety of biological perturbations and can be used to:
  - Derive an overall “mechanism-agnostic” point of departure (POD/BPAC)
  - Detect perturbation of specific targets/mechanisms
- Ongoing work at US EPA:
  - Screening in additional cell types (e.g. hTERT lines)
  - Benchmarking/harmonization of analysis methods in specific contexts
  - Development of additional signatures/models for mechanistic inference

Acknowledgements

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HTTr Team

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Laura Taylor
James Johnson
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Clinton Willis
Thomas Sheffield
Khan Inan
Monique Hazemi
Joshua Witten

Questions?

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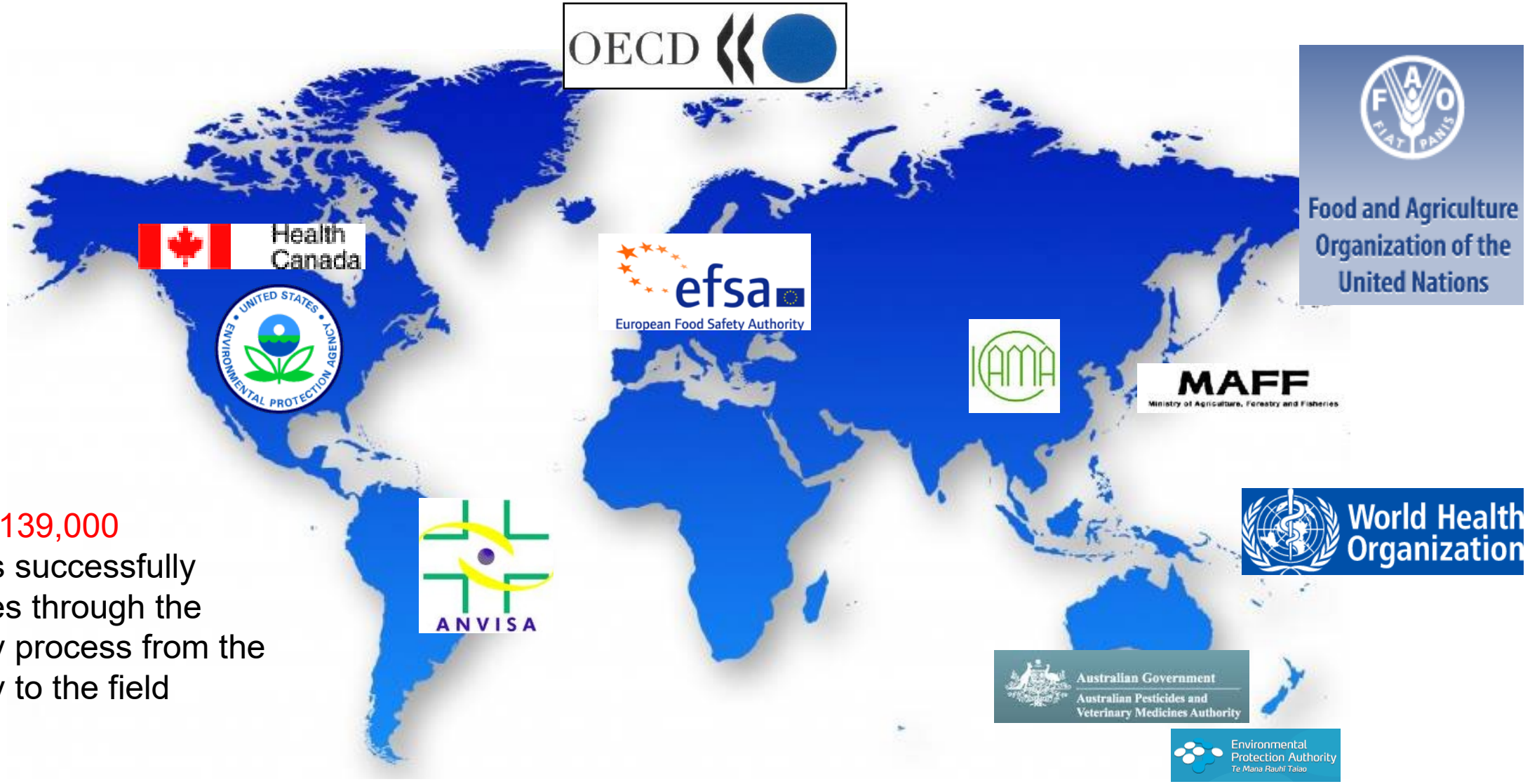
Use of ‘Omics to Inform Development and Safety of New Pesticide Active Ingredients

JESSICA LAROCCA
CORTEVA AGRISCIENCE

Global Regulatory Agencies



Only 1 in 139,000 chemicals successfully progresses through the regulatory process from the laboratory to the field

Agrochemical *In Vivo* Mammalian Toxicology Requirements

Task Description	OECD Guidelines	Species	Males		Females	
			Min	Max	Min	Max
Global Toxicokinetic Registration PK/Met (Default study)	OECD 417 (2010)	Rat	8	32	8	32
Acute Oral Toxicity (Default Rat)	OECD 423 (2001)	Rat	0		3	24
Acute Dermal Toxicity	OECD 402 (2017)	Rat	0		1	7
Acute Inhalation Toxicity	OECD 403 (2009)	Rat	3	15	3	15
Acute Dermal Irritation	OECD 404 (2015)	Rabbit	0		1	3
Acute Eye Irritation	OECD 405 (2017)	Rabbit	0		1	3
Dermal Sensitization (LLNA)	OECD 429 (2010)	Mouse	0		25	33
Acute Neurotoxicity (Rat)	OECD 424 (1997)	Rat	40		40	
28-Day Mouse Oral + Palatability	OECD 407 (2008)	Mouse	20	30	20	30
28-Day Rat Oral	OECD 407 (2008)	Rat	20	40	20	40
13-Week Dog Oral	OECD 409 (2018)	Dog - Beagle	16	24	16	24
13-Week Mouse Oral	OECD 408 (2018)	Mouse	40	50	40	50
13-Week Rat Oral	OECD 408 (2018)	Rat	40	50	40	50
SubChronic Neurotoxicity	OPPTS 870.6200	Rat	120	150	120	150
28-Day Rat Dermal	OECD 410 (1981)	Rat	20	25	20	25
1-Year Chronic Dog	OECD 452 (2018)	Dog - Beagle	16	21	16	21
In vivo Mouse Micronucleus	OECD 474 (2016)	Mouse	25	35	25	35
2-Year Chronic Rat/Carcinogenicity	OECD 453 (2018)	Rat	220	310	220	310
18-Month Carcinogenicity Mouse	OECD 451 (2018)	Mouse	200	265	200	265
Dev Screen OECD 421	OECD 421 (2016)	Rat	68		68	
Reproduction Study (2 gen)	OECD 416 (2001)	Rat	120		120	
Rat Dev Tox Full (Teratogenicity)	OECD 414 (2018)	Rat	0		88	
Rabbit Dev Tox Full (Teratogenicity)	OECD 414 (2018)	Rabbit	0		25	
28-Day Stand-Alone Immunotoxicity (Sheep Red Blood Cell)	OPPTS 870.7800	Mouse or Rat	0	50	50	

NAMs for Predictive Toxicology Studies to Reduce Animal Use

Discovery

Pre-Development

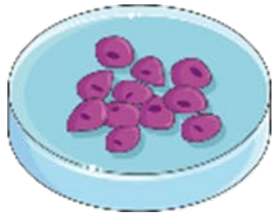
Development

New Approach Methodologies (NAMs)

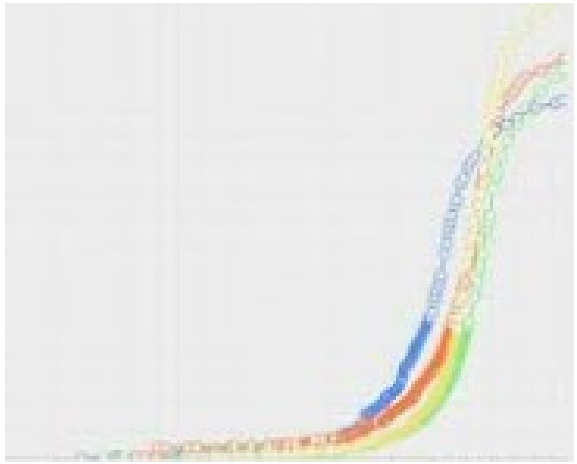
In Silico

In Vitro and Aquatic

In Vivo



Transcriptomics to Predict Points of Departure

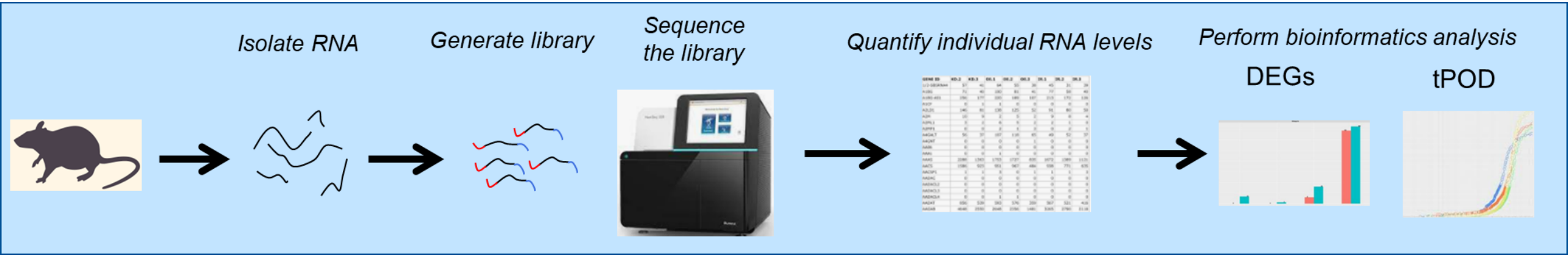


Transcriptomics Is a Tool to Comprehensively Examine Change in Gene Expression (RNA levels)

Central Dogma of Molecular Biology

DNA → RNA → Protein

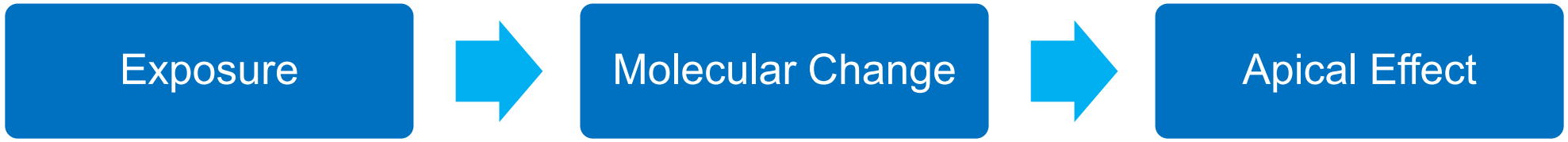
Typically, 13,000 – 16,000 unique RNA molecules are observed per organ



DEG: Differentially Expressed Gene

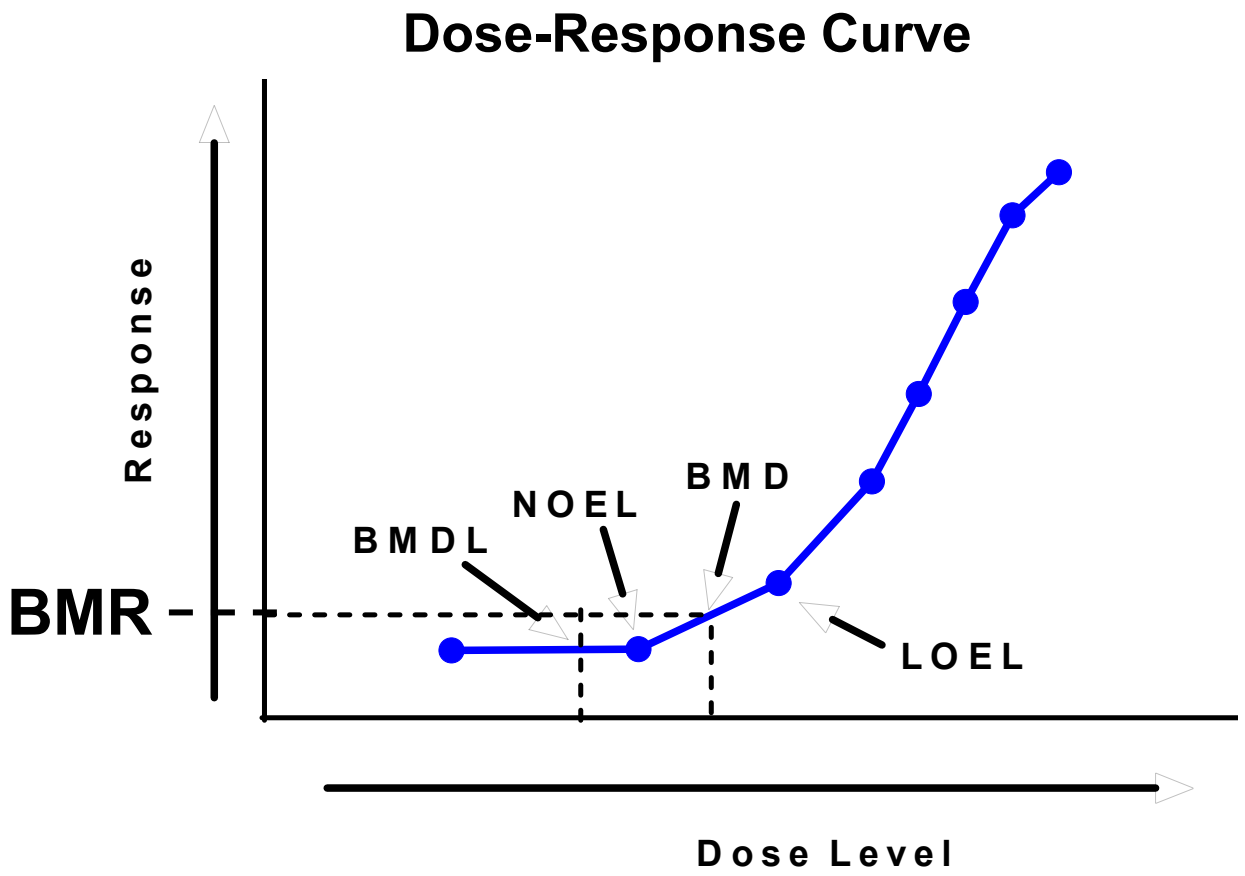
All Apical Effects Result From A Prior Change At The Molecular Level

Generic Adverse Outcome Pathway



Therefore, changes observed in *comprehensive* molecular data (like toxicogenomics) will detect any apical effect (however, the identity of the apical effect is unknown).

A Point of Departure Can Be Determined Using NOEL or BMD Approaches



NOEL: No Observed Effect Level

LOEL: Lowest Observed Effect Level

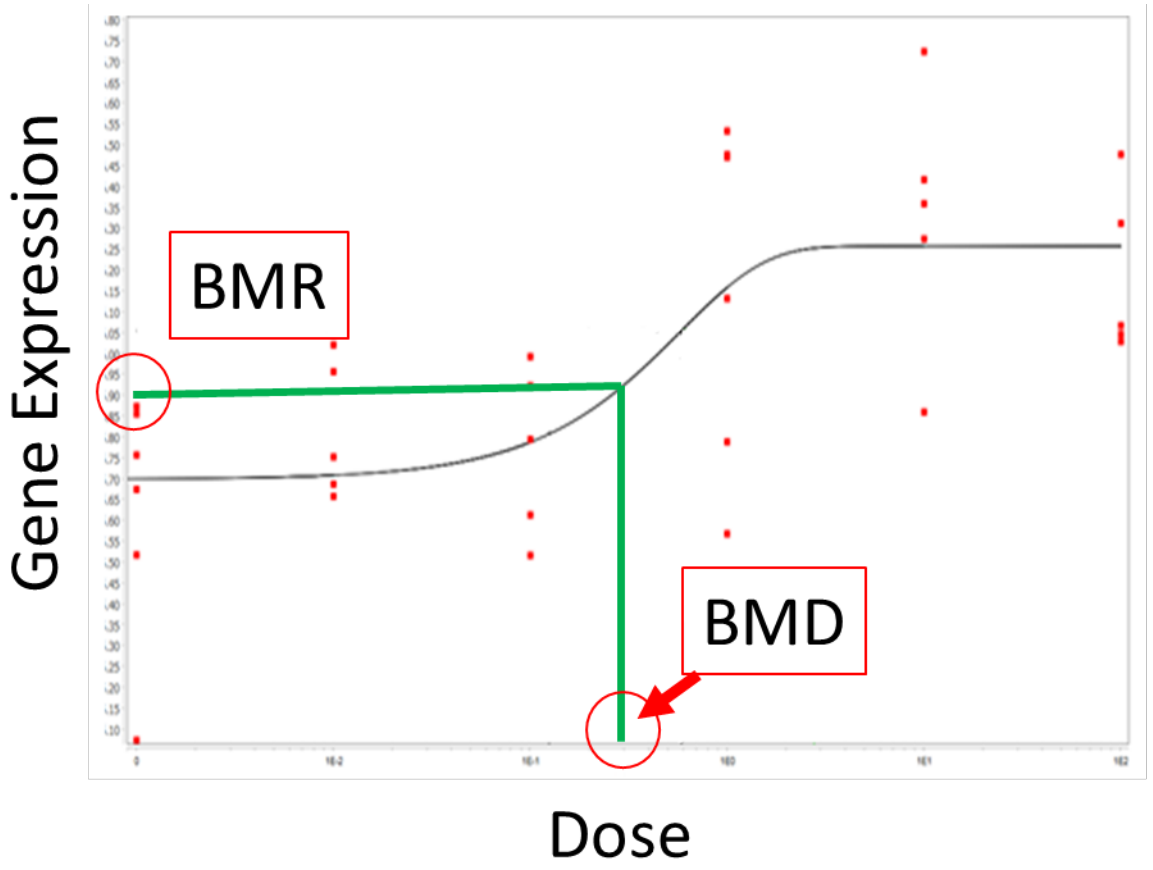
BMD: Benchmark Dose

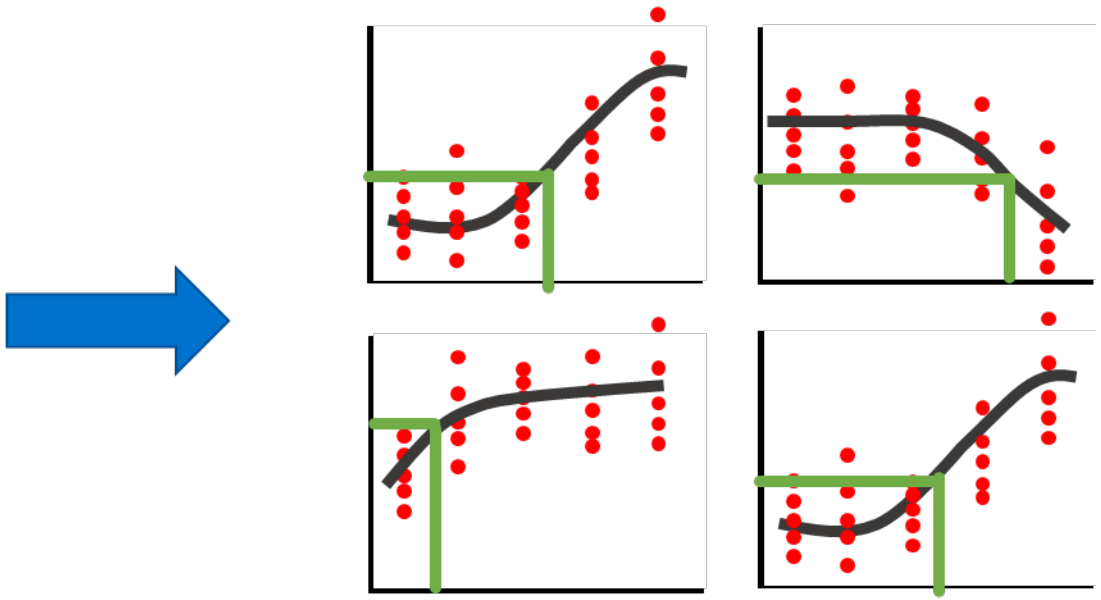
BMDL: Benchmark Dose Lower Confidence Limit

BMR: Benchmark Response

What is a Transcriptome Point of Departure (tPOD)?

- Estimates the lowest dose for a “concerted” change in gene expression

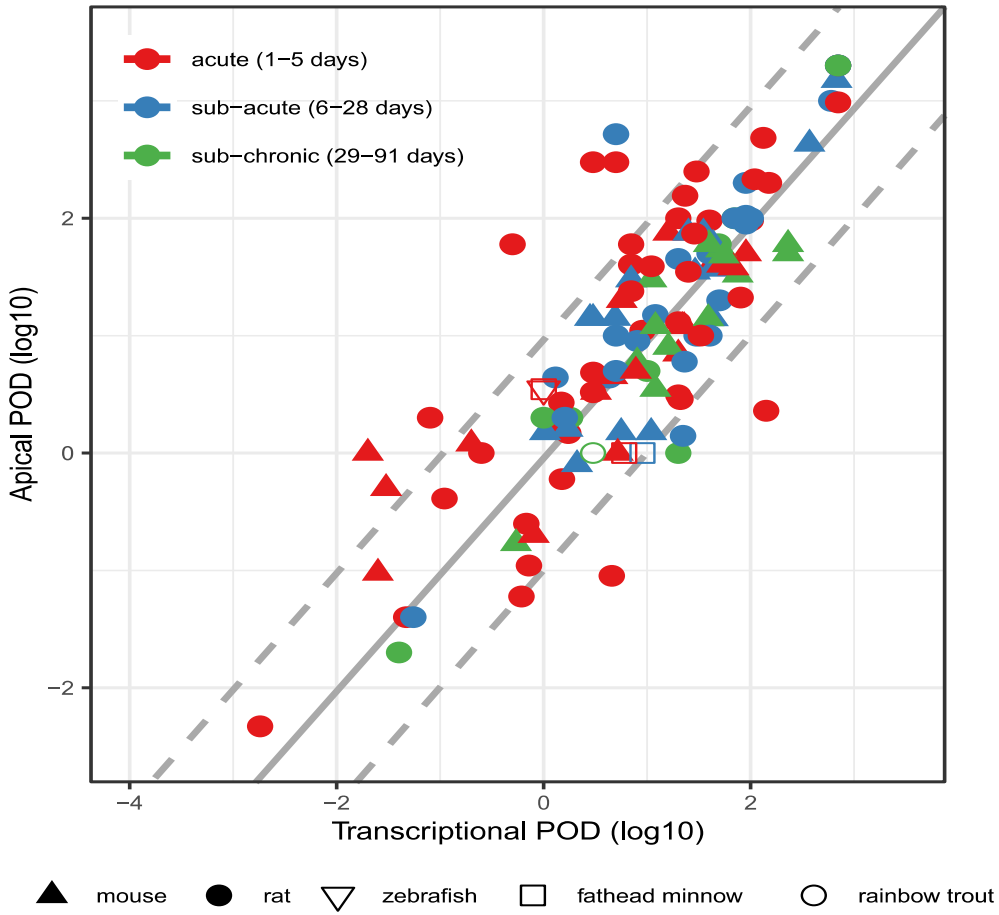




A tPOD Accurately Estimates a Traditional Apical Endpoint POD

Systematic literature review of >100 articles (from Jason O'Brien)

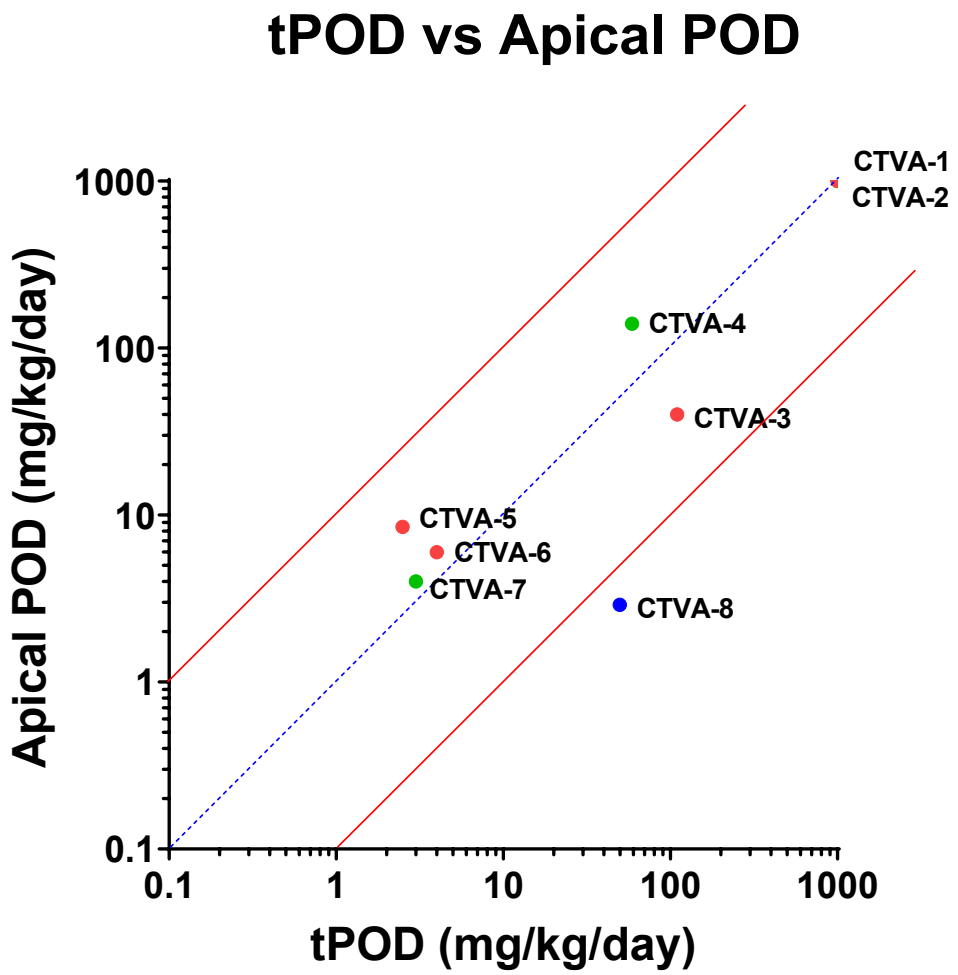
- $R^2 > 0.80$
- 95% within 10-fold of traditional POD
- >88% within 5-fold of traditional POD
- High correlation after **1-5 days!!**



- Concordance of tPOD and a traditional POD is similar to the concordance of traditional PODs from repeating a guideline study

tPods Accurately Predict Apical PODs for Corteva Agrochemicals

Short-term transcriptomic studies with tPODs can approximate the apical POD from long-term studies (90-day rat, cancer bioassay, two-gen, etc).



5-day Toxicogenomic Study Design to Inform Target Organs and PoDs

- **Standardized study design that can be used for novel compounds with unknown hazard and PODs**
- Short-term study can be used to inform PODs resulting from chronic exposure (and inform risk assessments)
- **Rat 5-Day TGx Study Design** Dietary exposure
  - Males only (animal reduction purposes) (5/group)
  - Organ weights collected from all organs in TGx study and perform histopathology on liver, kidney, and expected target organs, if known
  - Seven default organs analyzed via RNAseq – can be modified based upon prior information
  - 6 – 7 dose levels tested

Organs collected for RNAseq			
Liver (49%)	Kidney (19%)	Thyroid (14%)	Testis (3%)
Adrenal (3%)	Spleen (2%)	Epididymis	Pituitary

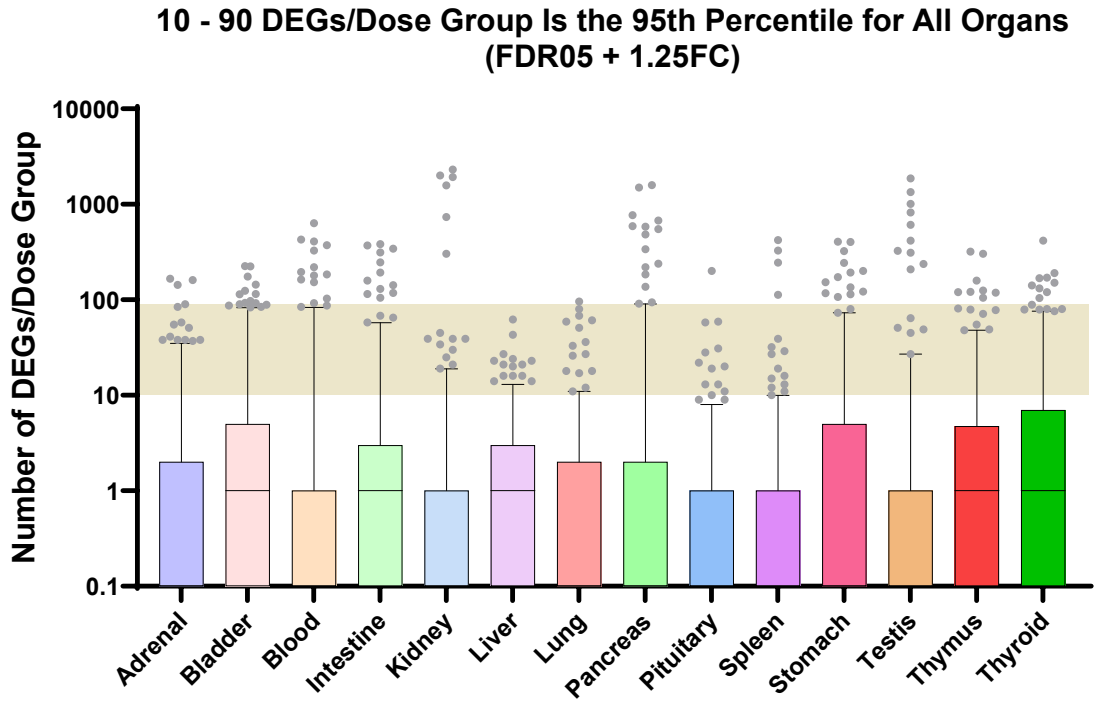
Number in parentheses is the % of 435 pesticides that target that organ.

Rat 5-Day Toxicogenomics Study Control Data Analysis

If a tPOD is identified in an organ, it will be concluded that an apical effect (adaptive or adverse) is present after 5-days of exposure or will occur with a longer duration of exposure.

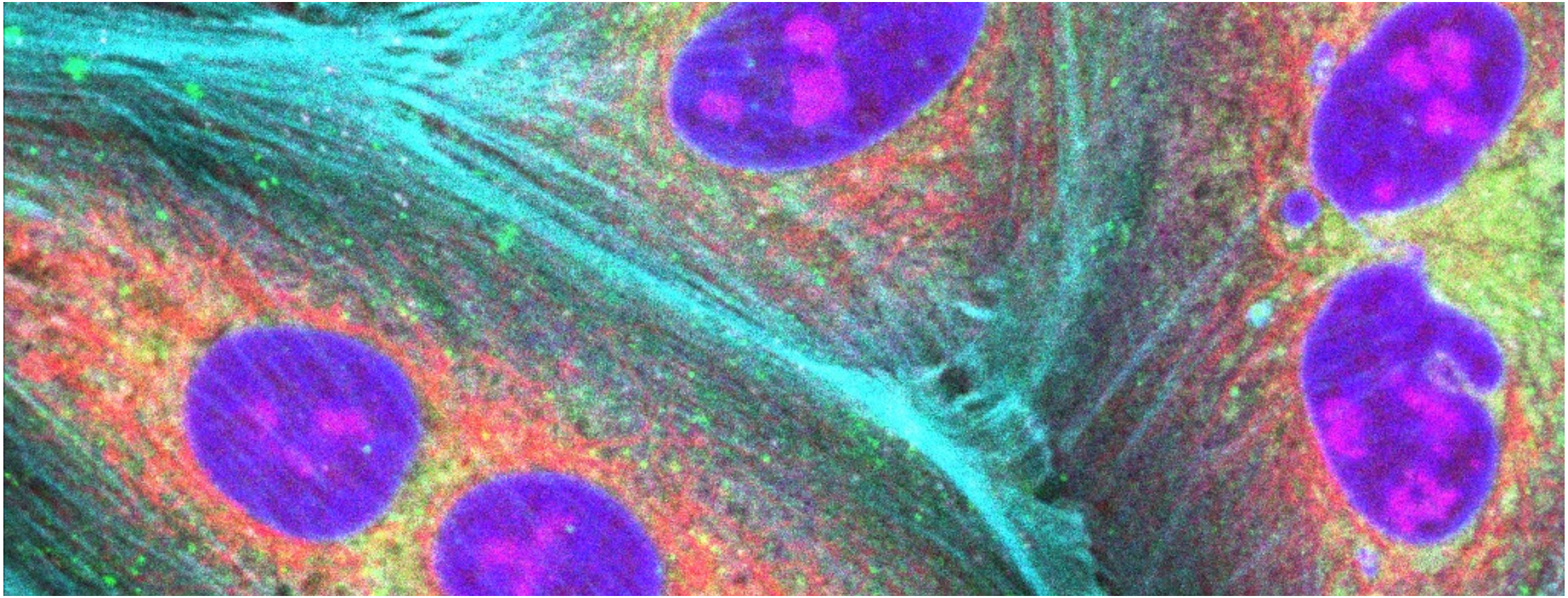
A tPOD will be derived if more than 90 DEGs are identified in an organ at the highest dose level tested.

- Data in graph below are from a control (unexposed) rat multi-organ RNAseq study (i.e. no exposure) performed at Corteva.
- In all organs, identification of 90 DEGs was the 95th percentile upper bound in control rat organ RNAseq



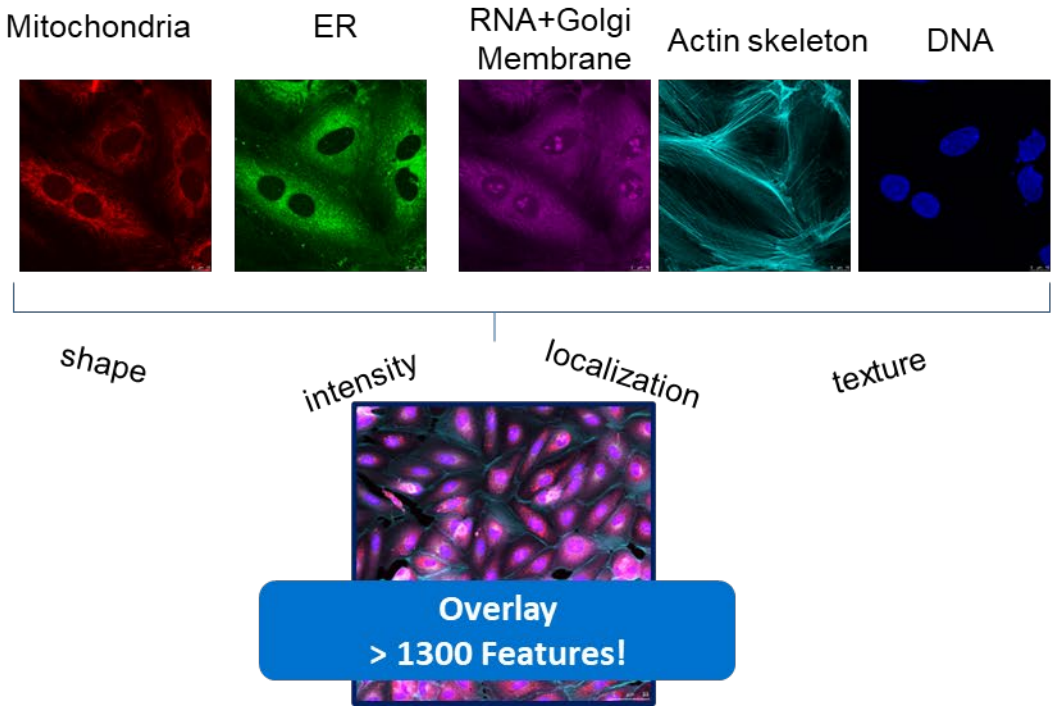
DEG: Differentially Expressed Gene

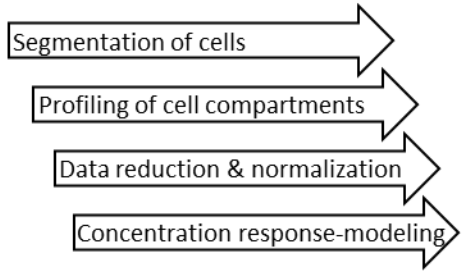
Cell Painting Toxicophenomics to Predict PODs

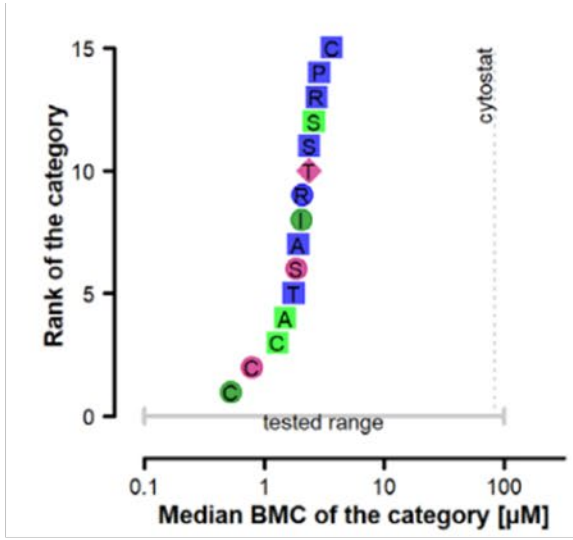


Cell Painting: A high-throughput method to inform toxicological-induced cellular perturbations

- Cell painting is a HCI technique that involves staining of various cell organelles with fluorescent dyes.
  - Developed by Broad Institute *Nat Protoc. 2016*





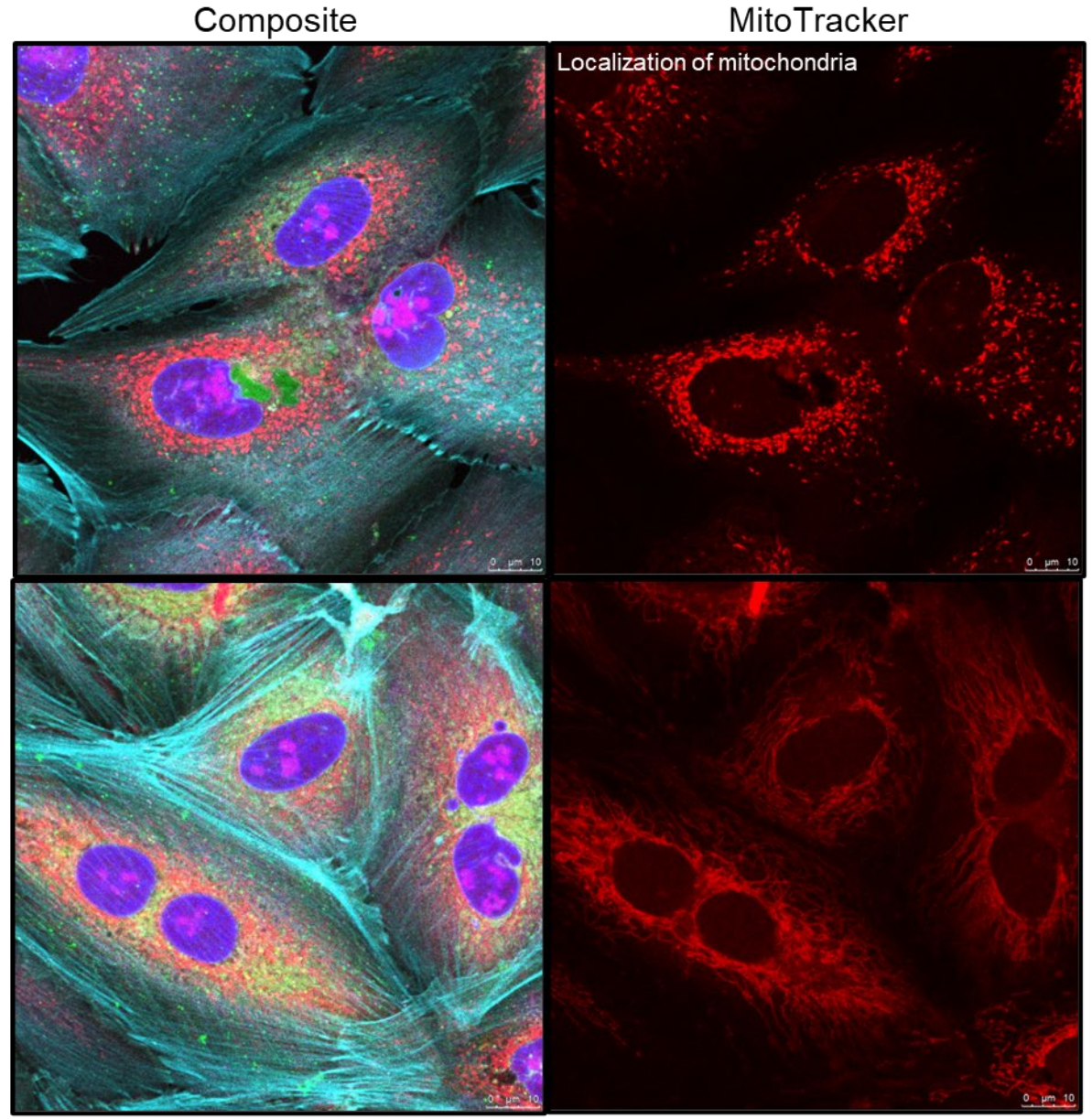


Adapted from Willis et al. SLAS DISCOVERY. 2020;25(7):755-69.

Liver Cell Painting

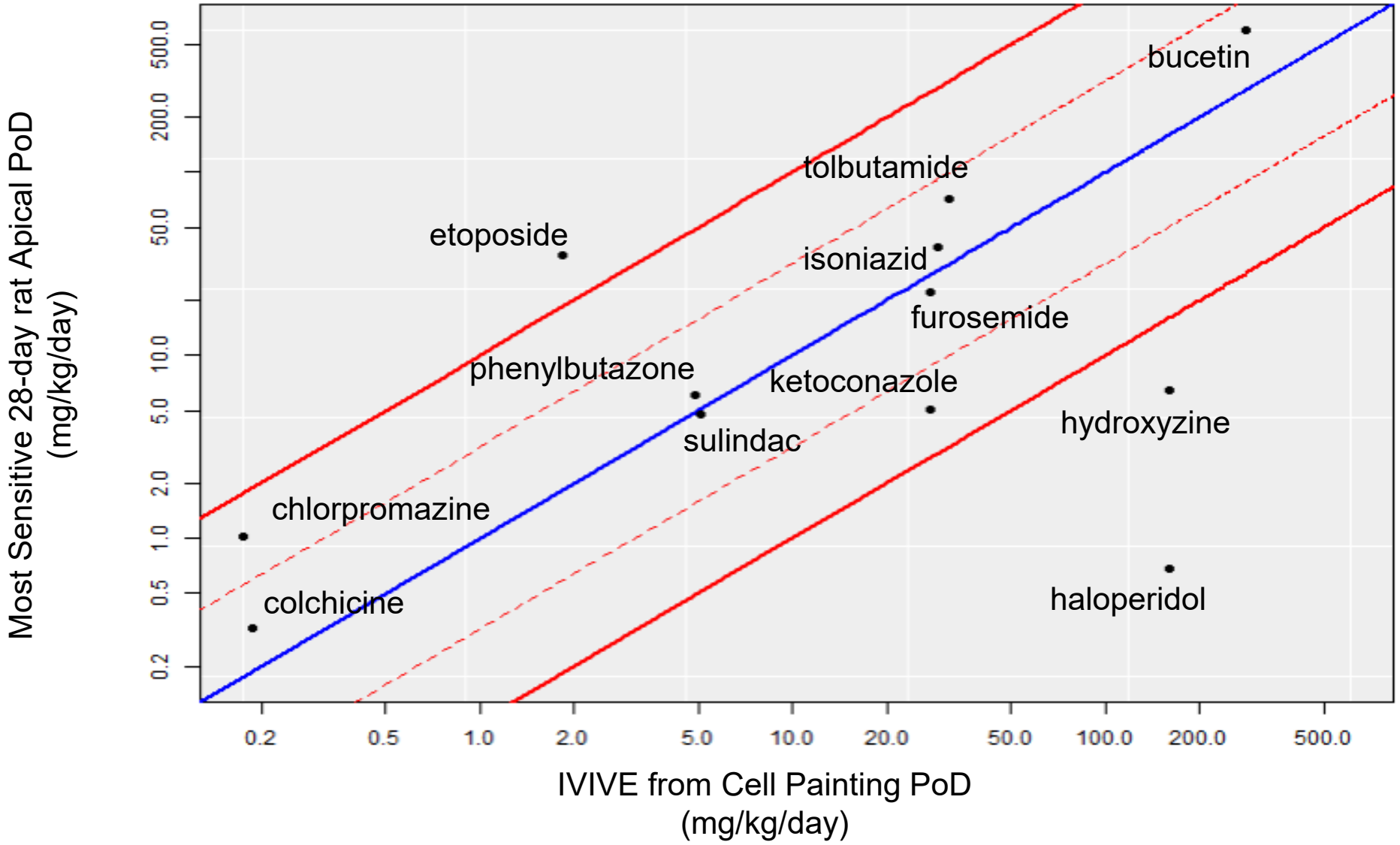
- Liver is the #1 target organ for agrochemicals.
- Many agrochemicals are extensively metabolites, therefore metabolically competent cells are more relevant.
- Therefore, HepaRG liver cells were selected for predictive tox

Channel	Stain	Organelles
405/435-480	Hoechst 33342	Nucleus
488/500-550	Concanavalin A; SYTO14	ER, Nucleoli/RNA
561/570-630	Wheat Germ Agglutinin, Phalloidin	Actin skeleton, Golgi, Plasma membrane
640/650-760	MitoTracker™ Deep Red FM	Mitochondria



HepaRG Cell Painting IVIVE Approximates In Vivo Pharmaceutical PODs

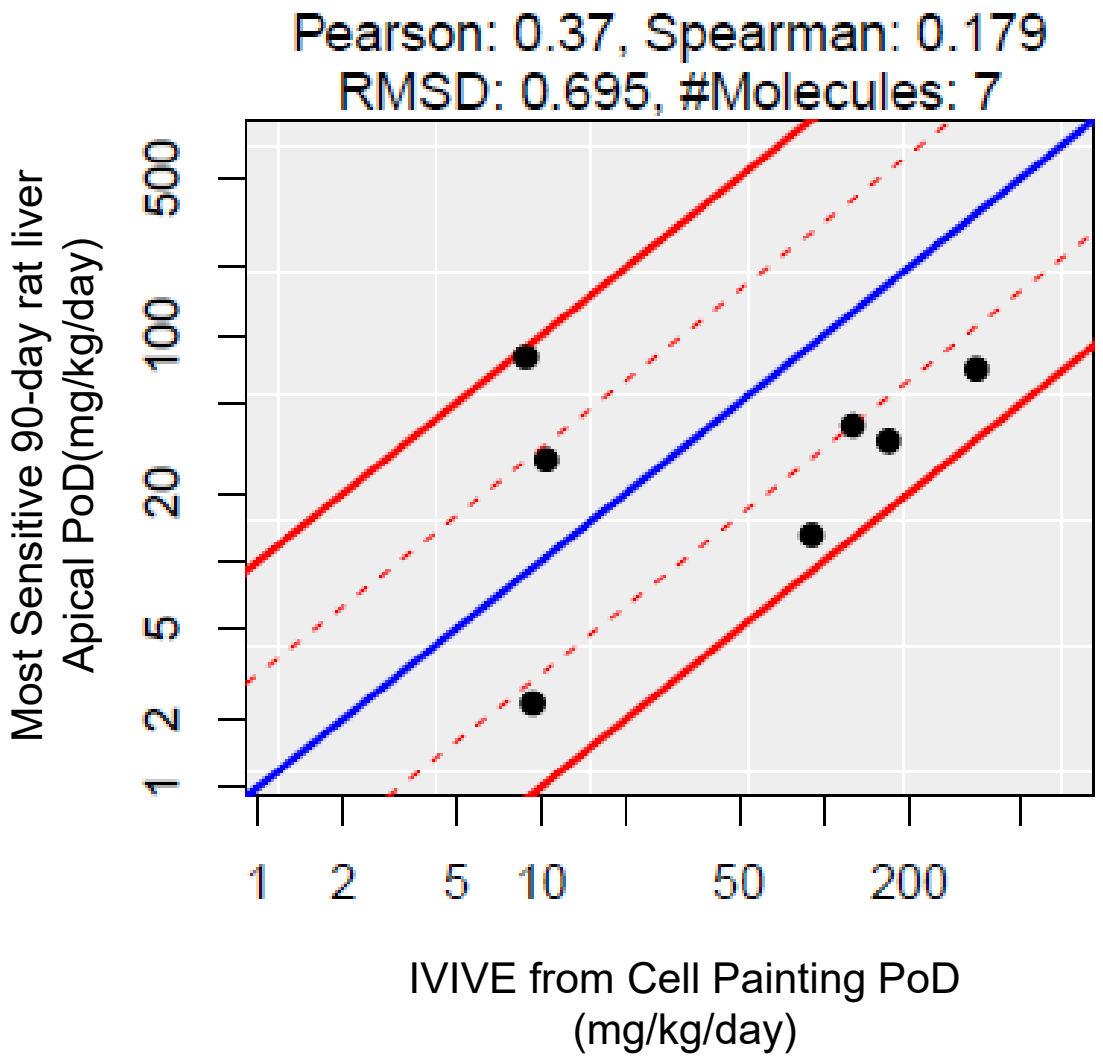
Comparison of cell paint POD with IVIVE with most sensitive 29-day apical endpoint POD (mg/kg/day)



HepaRG Cell Painting IVIVE Approximates In Vivo Agchem Subchronic PODs

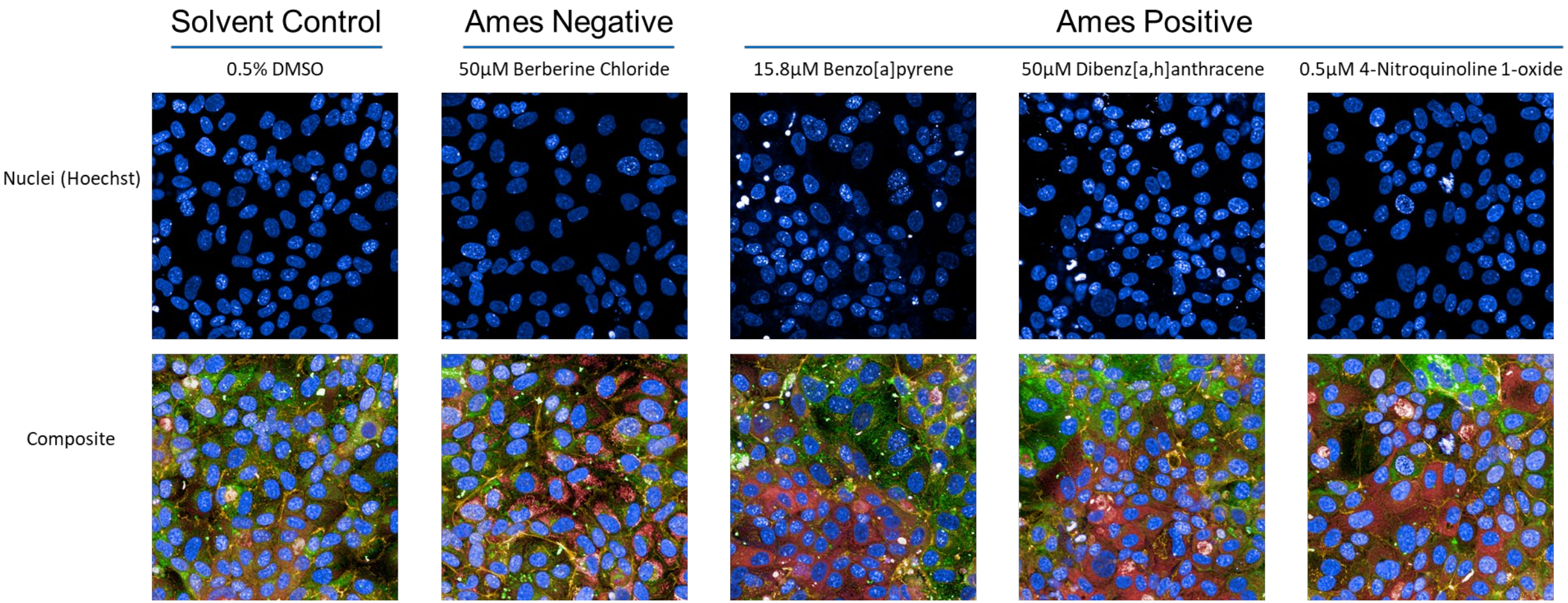
Comparison of cell paint POD with IVIVE with most sensitive liver 90-day apical endpoint POD (mg/kg/day).

Molecules with cell paint POD > highest concentration tested were excluded



Machine Learning with Cell Painting to Predict Hazard

Can We Use Machine Learning to Predict Specific Toxicity Mechanisms/Adverse Outcomes?



Cell Painting Machine Learning Can Predict Ames Classification

Training data (well level): 918 Positive V.S. 899 Negative
(compound level: 104 Pos V.S. 107 Neg)

Compound_ label		Reference	
		Positive	Negative
Predicted	Positive	**84**	27
	Negative	20	**80**

Sensitivity/Recall	Specificity	Precision	F1	Balanced Accuracy
0.808	0.748	0.757	0.781	0.777

HESI OASIS

Mission: Gain confidence in the combination of Cell Painting, transcriptomics and proteomics for safety assessment using hepatotoxicity as use-case.

OASIS
Omics for Assessing Signatures for Integrated Safety Consortium

HESI®

Objective: Benchmark in vivo hepatotoxicity (from rodent or clinical trial data) induced by a series of compounds against informatically aligned molecular & phenotypic cell-based assays.

Public and private compounds with associated in vivo and/or human data

- 200 donated from industry partners
- 1300 from public domain

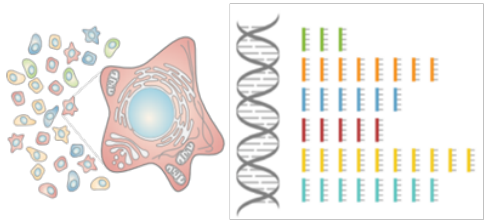
Cell model systems of increasing complexity, physiological relevance, and cost

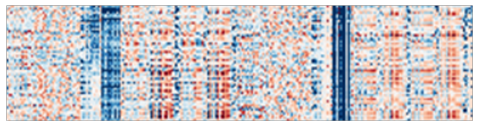
- U2OS human osteosarcoma immortal cell line
- HepaRG human primary hepatocyte-like cell line
- Primary rat hepatocytes

Image Profiling (Cell Painting)

mRNA Profiling (transcriptomics)

Protein Profiling (nELISA)





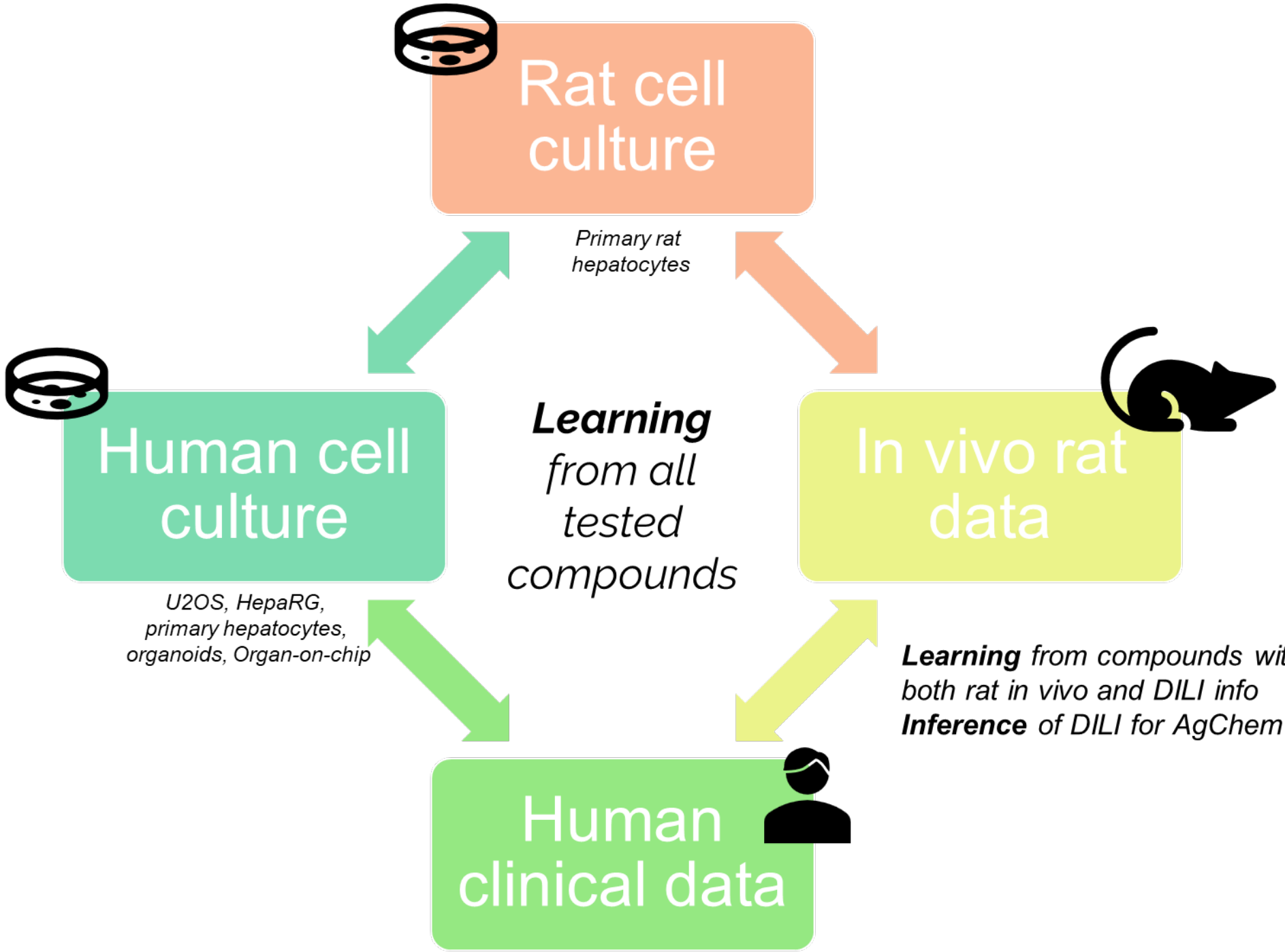
Comprehensive Data Analysis

- Translating *in vitro* to *in vivo* concentrations (qIVIVE)
- Distinguishing & interpreting mechanisms
- Integrating evidence across multiple data types/cell models

Publicly accessible data

Published methodologies

- Community of Practice


Rat cell culture
Primary rat hepatocytes
Human cell culture
U2OS, HepaRG, primary hepatocytes, organoids, Organ-on-chip
Learning from all tested compounds
In vivo rat data
Learning from compounds with both rat in vivo and DILI info
Inference of DILI for AgChem
Human clinical data

Discovery

Pre-Development

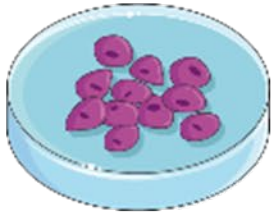
Development

New Approach Methodologies (NAMs)

In Silico

In Vitro and Aquatic

In Vivo



Acknowledgements

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Paul Deford
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Zachary Sutake
Eric Sherer
Jiaqi Xu
Yang Li
Sagar Ksheera
Dillon Aberasturi

US EPA
Joshua Harrill
Jo Nyffeler

Broad Institute
Anne Carpenter
Shantanu Singh

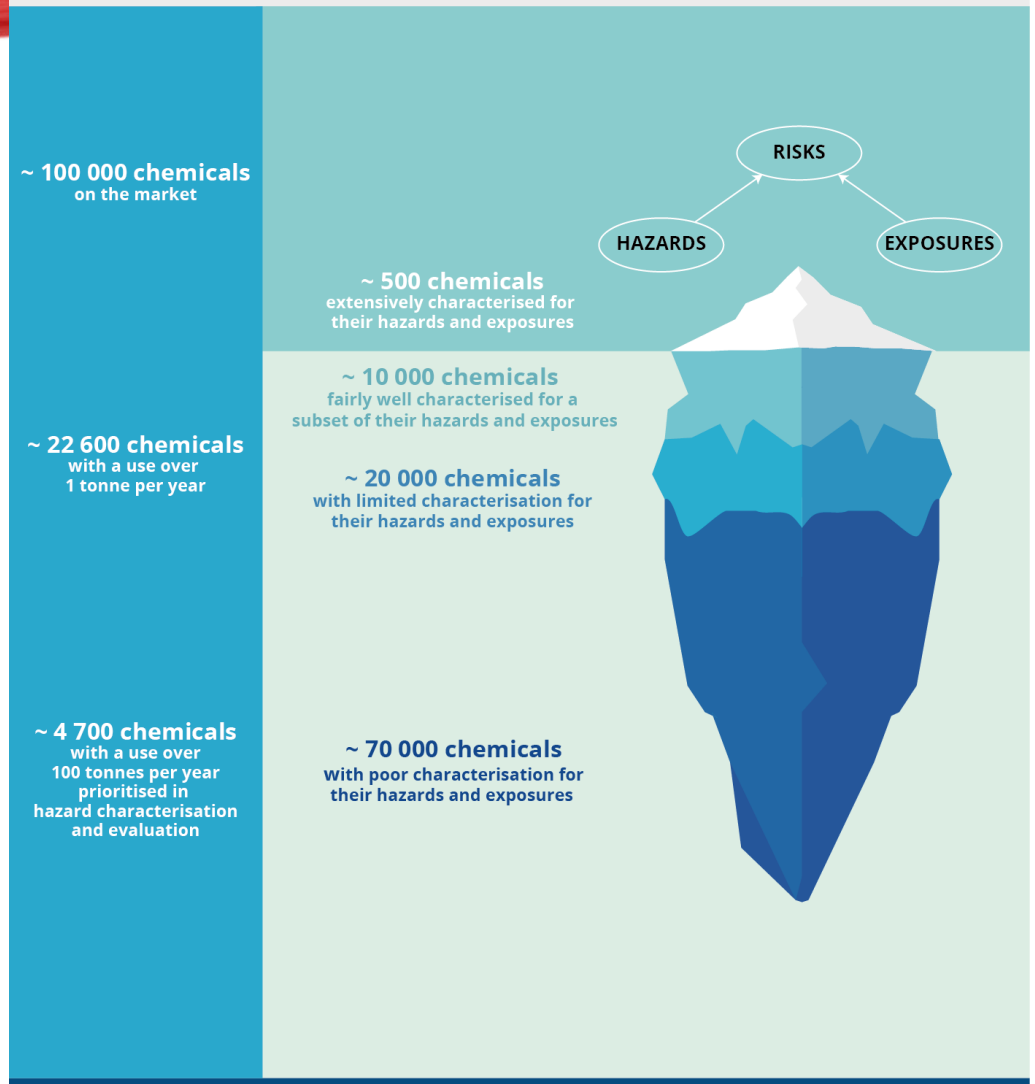
HESI OASIS Steering Team

Knowledge gaps and opportunities for omics data in environmental assessments

Chris Martyniuk, Ph.D.
Department of Physiological Sciences
College of Veterinary Medicine
EPA NAMs workshop, November 5-6, 2024

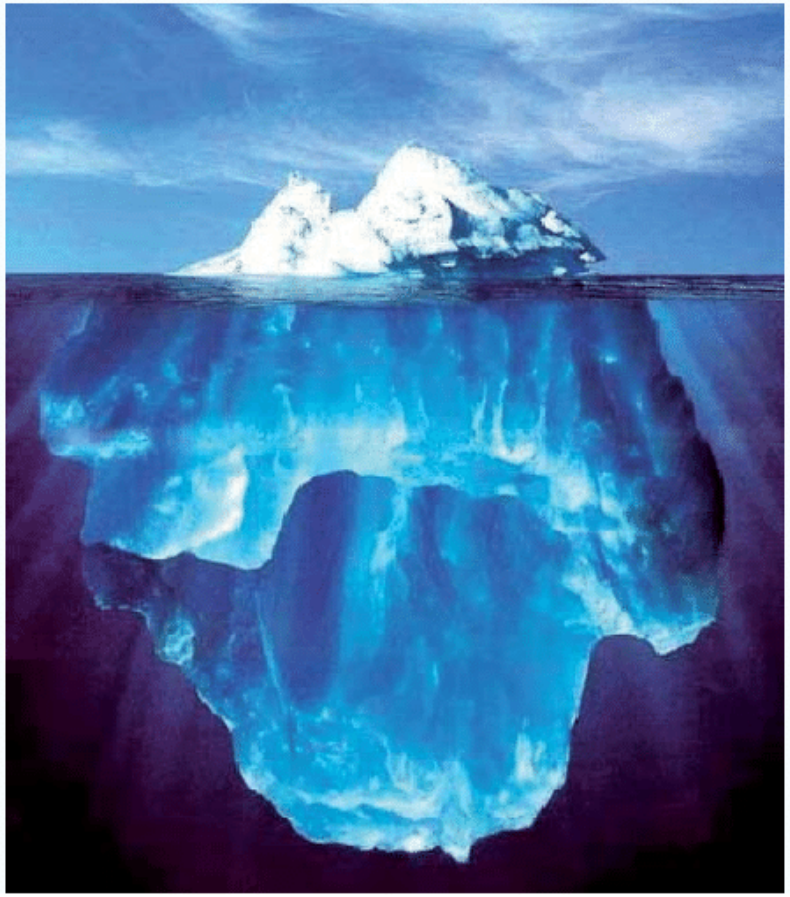
The unknown territory of chemical risks

There are many chemicals on the market and only a small fraction of these have been extensively studied for their risks. Designing safe products with a smaller number of different chemicals is one way of reducing potential risks.



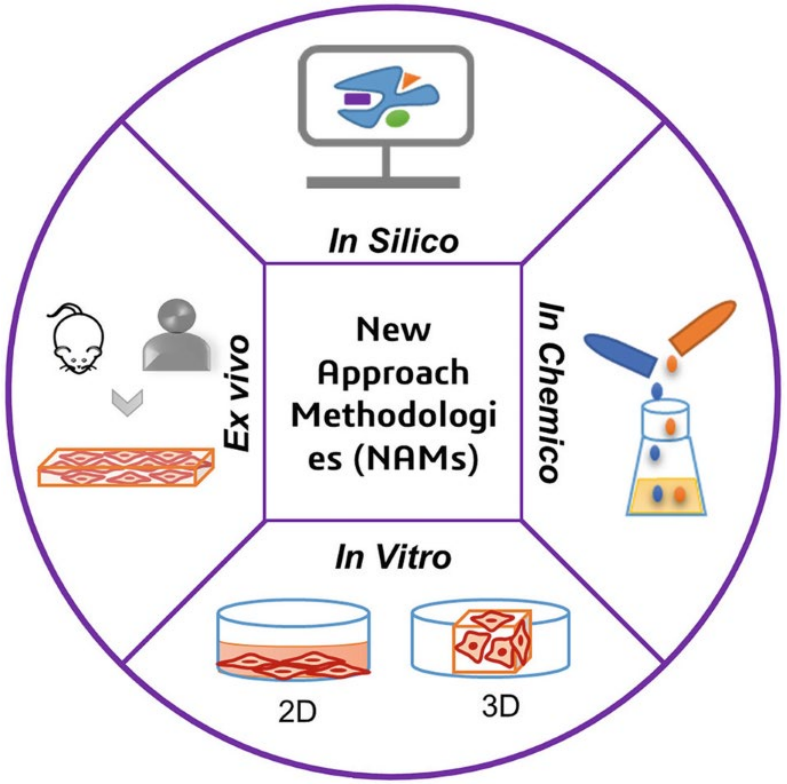
Source: EEA report - The European environment — state and outlook 2020[1].

THE CHALLENGE FOR REGULATORY AGENCIES



Why important?

Risk Evaluations for Existing Chemicals



Prioritization

Which one do we test?

Evaluation

How do we measure the toxicity?

Management

Which ones do we regulate?

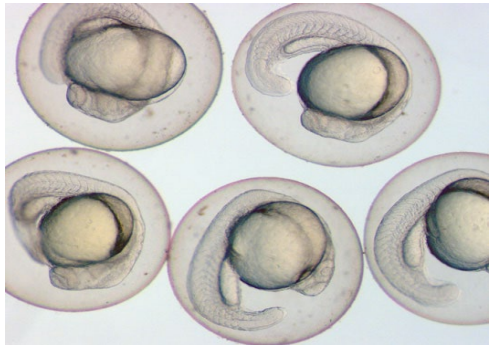
When:
22 & 23 November

Current Status of New Approach Methodologies (NAMs) for Regulatory Purposes

Online
Lhasalimited.org/events

THE ZEBRAFISH MODEL FOR HIGH THROUGHPUT TOXICITY TESTING

- Relatively cheap to house and readily accessible
- Rapid development with transparent developmental stages
- Replication: High numbers of individuals can be examined at once
- Amendable to mechanistic studies with chemicals of concern (AOPs)



OECD/OCDE 236
Adopted:
26 July 2013

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Fish Embryo Acute Toxicity (FET) Test

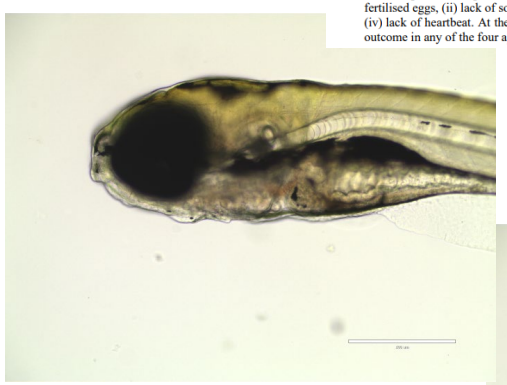
INTRODUCTION

1. This Test Guideline (TG) 236 describes a Fish Embryo Acute Toxicity (FET) test with the zebrafish (*Danio rerio*). This test is designed to determine acute toxicity of chemicals on embryonic stages of fish. The FET-test is based on studies and validation activities performed on zebrafish (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14). The FET-test has been successfully applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities (reviewed in 15 and 16).

2. Definitions used in this Test Guideline are given in Annex 1.

PRINCIPLE OF THE TEST

3. Newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs, up to four apical observations are recorded as indicators of lethality (6): (i) coagulation of fertilised eggs, (ii) lack of somite formation, (iii) lack of detachment of the tail-bud from the yolk sac, and (iv) lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC_{50} is calculated.



Nan Wu photo credit

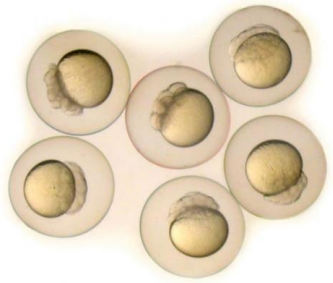
Long term efforts: Defining AOPs for contaminants of concern

Evidence from omics

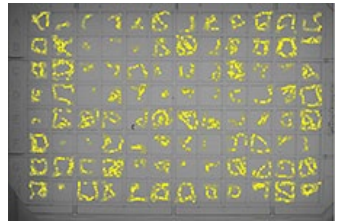
Molecular Initiating Event

Mitochondrial dysfunction → Oxidative stress → Hatch Rate Deformity → Locomotor Activity

Adverse Outcome



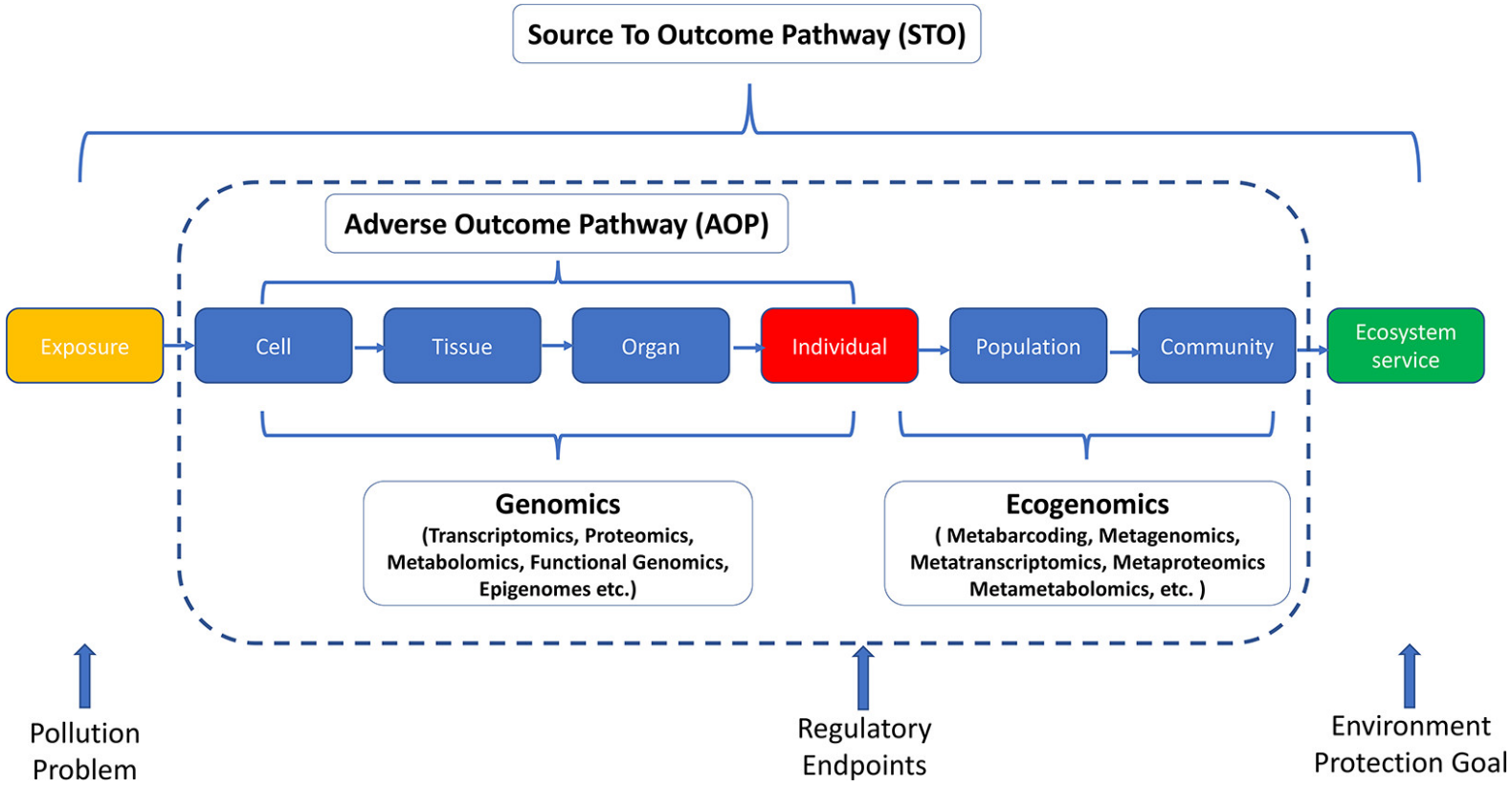
Reactive Oxygen Species



High throughput screening of chemicals

Predictive toxicology, but zebrafish are not ecotox. relevant

Opportunities for omics in Risk Characterization and Assessment?



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

TOXICOLOGICAL SCIENCES, 158(2), 2017, 252–262
doi: 10.1093/toxsci/kfx097
Advance Access Publication Date: May 19, 2017
Forum

FORUM

The Role of Omics in the Application of Adverse Outcome Pathways for Chemical Risk Assessment

Erica K. Brockmeier,* Geoff Hodges,†,1 Thomas H. Hutchinson,‡ Emma Butler,† Markus Hecker,§ Knut Erik Tollefsen,¶ Natalia Garcia-Reyero,‖,|| Peter Kille,|| Dörthe Becker,# Kevin Chipman,# John Colbourne,# Timothy W. Collette,** Andrew Cossins,* Mark Cronin,†† Peter Graystock,a Steve Gutsell,† Dries Knapen,b Ioanna Katsiadaki,c Anke Lange,d Stuart Marshall,† Stewart F. Owen,e Edward J. Perkins,‖ Stewart Plaistow,* Anthony Schroeder,f Daisy Taylor,g Mark Viant,# Gerald Ankley,h and Francesco Falciani*

Zhang et al., 2018
Environ. Sci. Technol. 2018, 52, 7, 3842−3851

Prioritization

Which one do we test?

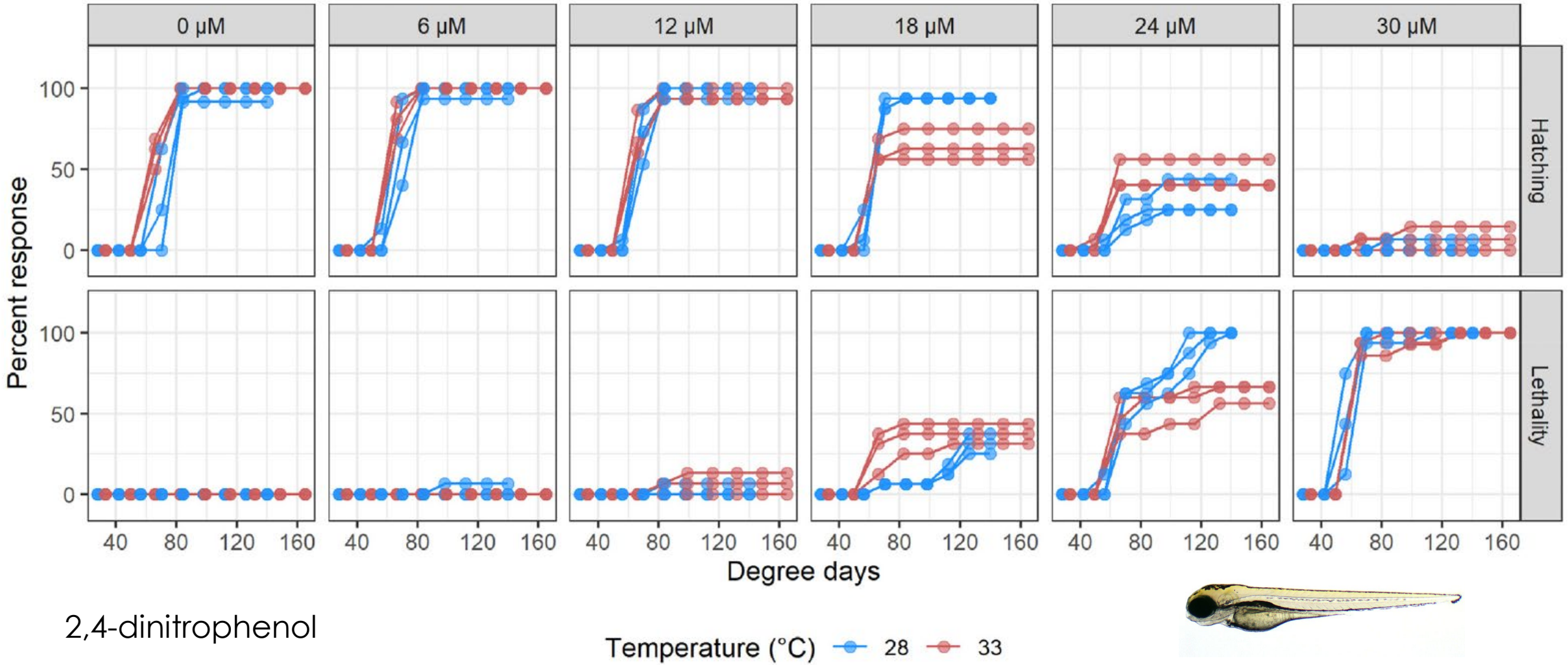
Evaluation

How do we measure the toxicity?

Management

Which ones do we regulate?

Temperature, omics, and a mitochondrial toxicant



2,4-dinitrophenol

Fig. 1. Percent hatching and lethality as a function of exposure duration (degree days) for each clutch, temperature, and 2,4-Dinitrophenol concentration.

Dreier DA, Nouri MZ, Denslow ND, Martyniuk CJ. Lipidomics reveals multiple stressor effects (temperature × mitochondrial toxicant) in the zebrafish embryo toxicity test. Chemosphere. 2021 Feb;264(Pt 1):128472. doi: 10.1016/j.chemosphere.2020.128472. Epub 2020 Sep 30. PMID: 33039916.

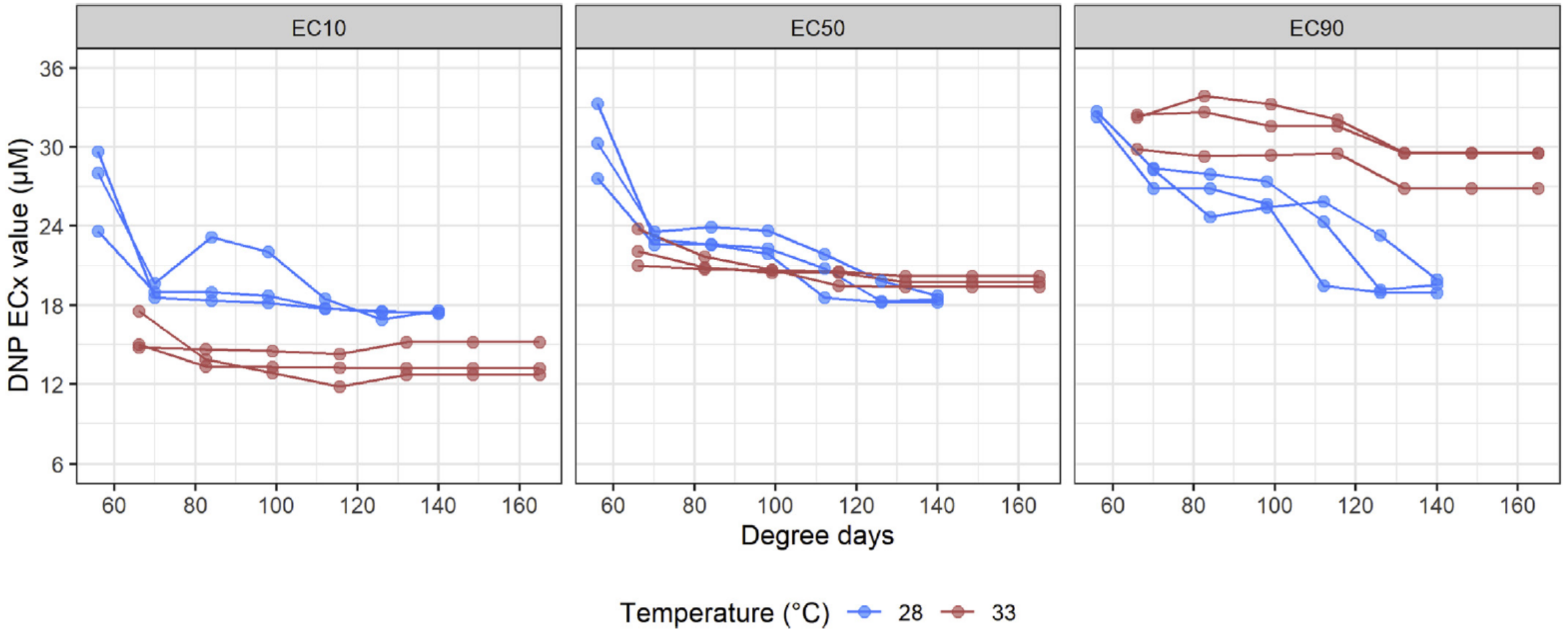
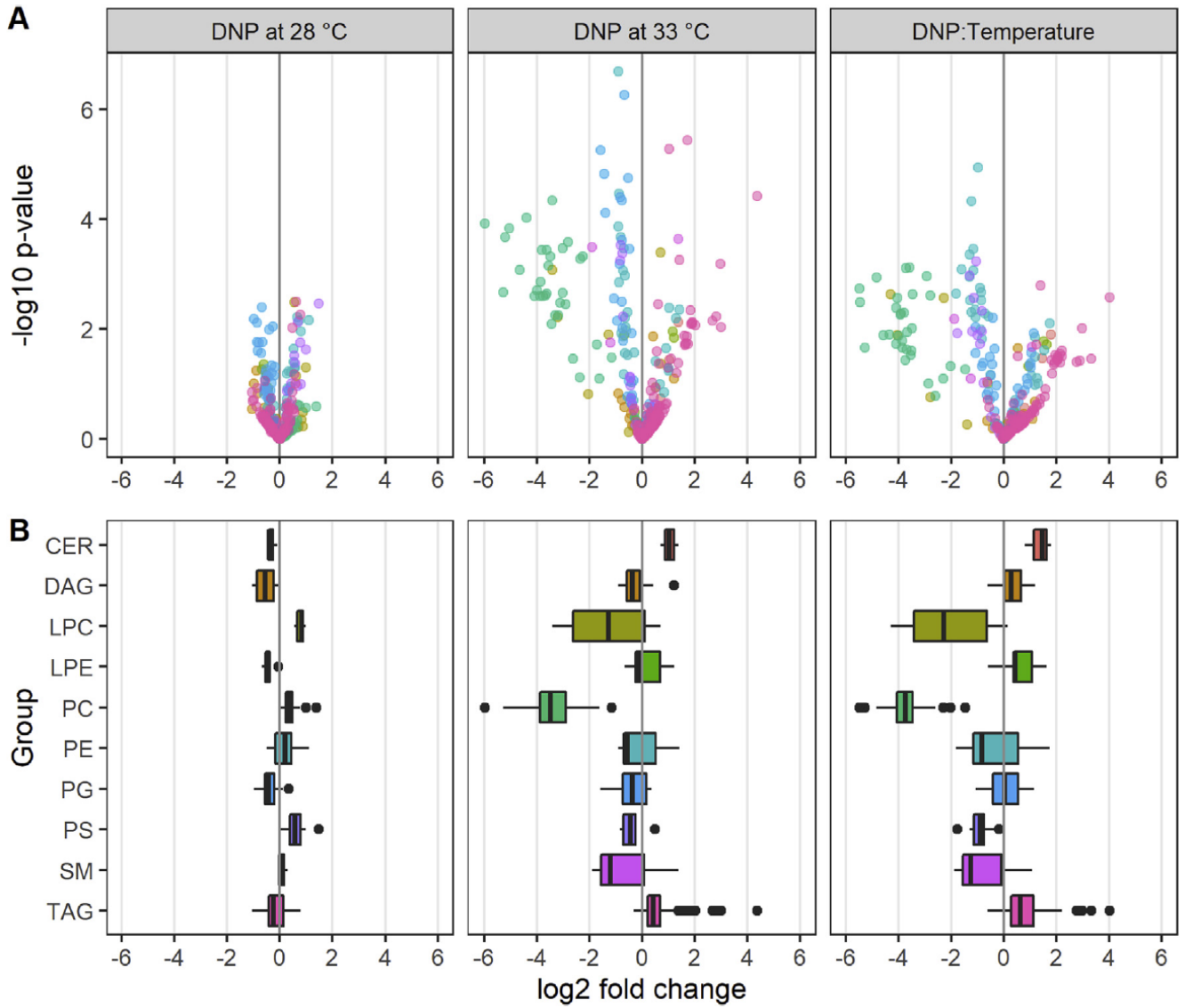


Fig. 2. 2,4-Dinitrophenol (DNP) ECx values as a function of exposure duration (degree days) for each clutch (connected points) and temperature.

Dreier DA, Nouri MZ, Denslow ND, Martyniuk CJ. Lipidomics reveals multiple stressor effects (temperature × mitochondrial toxicant) in the zebrafish embryo toxicity test. Chemosphere. 2021 Feb;264(Pt 1):128472. doi: 10.1016/j.chemosphere.2020.128472. Epub 2020 Sep 30. PMID: 33039916.



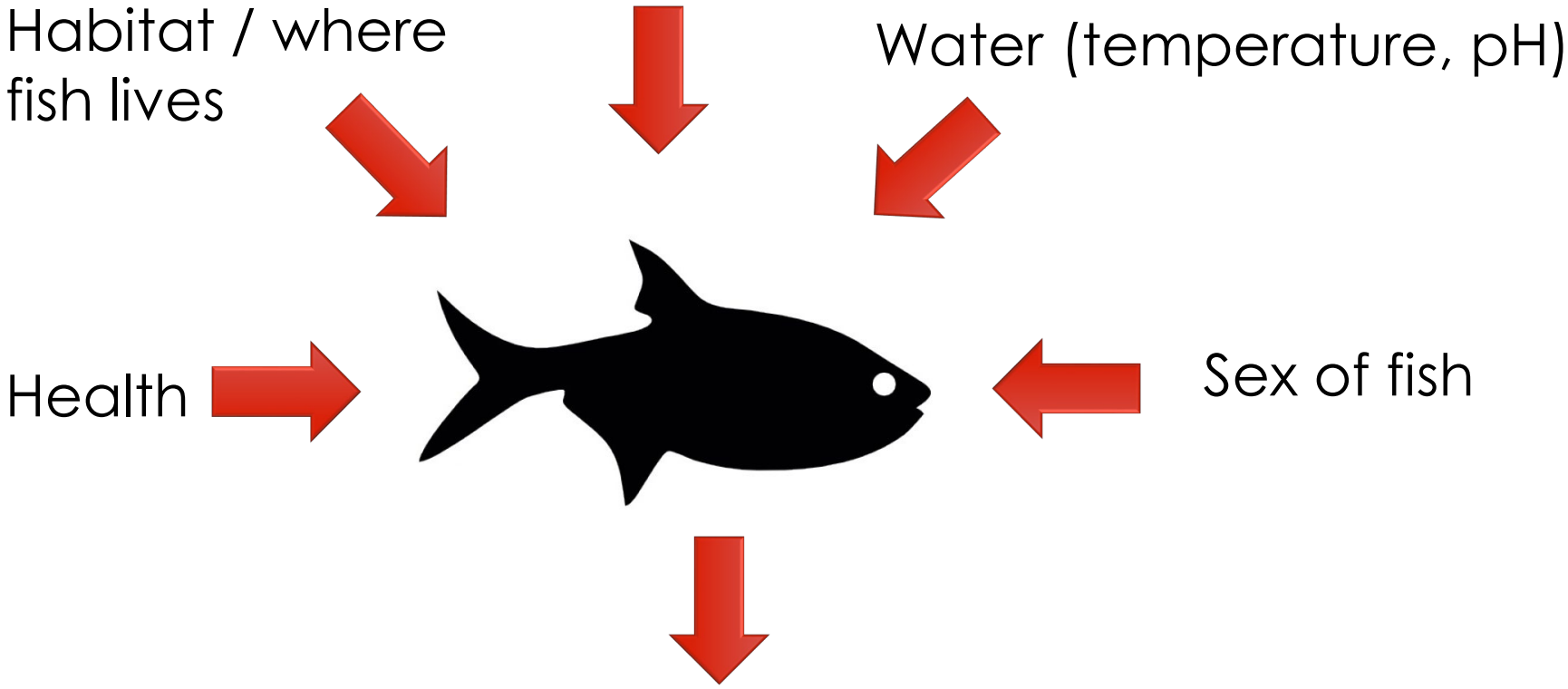
Lipidomics data set

Fig. 4. Differential lipid composition for linear contrasts of interest (DNP at 28 °C, DNP at 33 °C, DNP:Tempearture) visualized as a (A) volcano plot and (B) boxplot.

Dreier DA, Nouri MZ, Denslow ND, Martyniuk CJ. Lipidomics reveals multiple stressor effects (temperature × mitochondrial toxicant) in the zebrafish embryo toxicity test. Chemosphere. 2021 Feb;264(Pt 1):128472. doi: 10.1016/j.chemosphere.2020.128472. Epub 2020 Sep 30. PMID: 33039916.

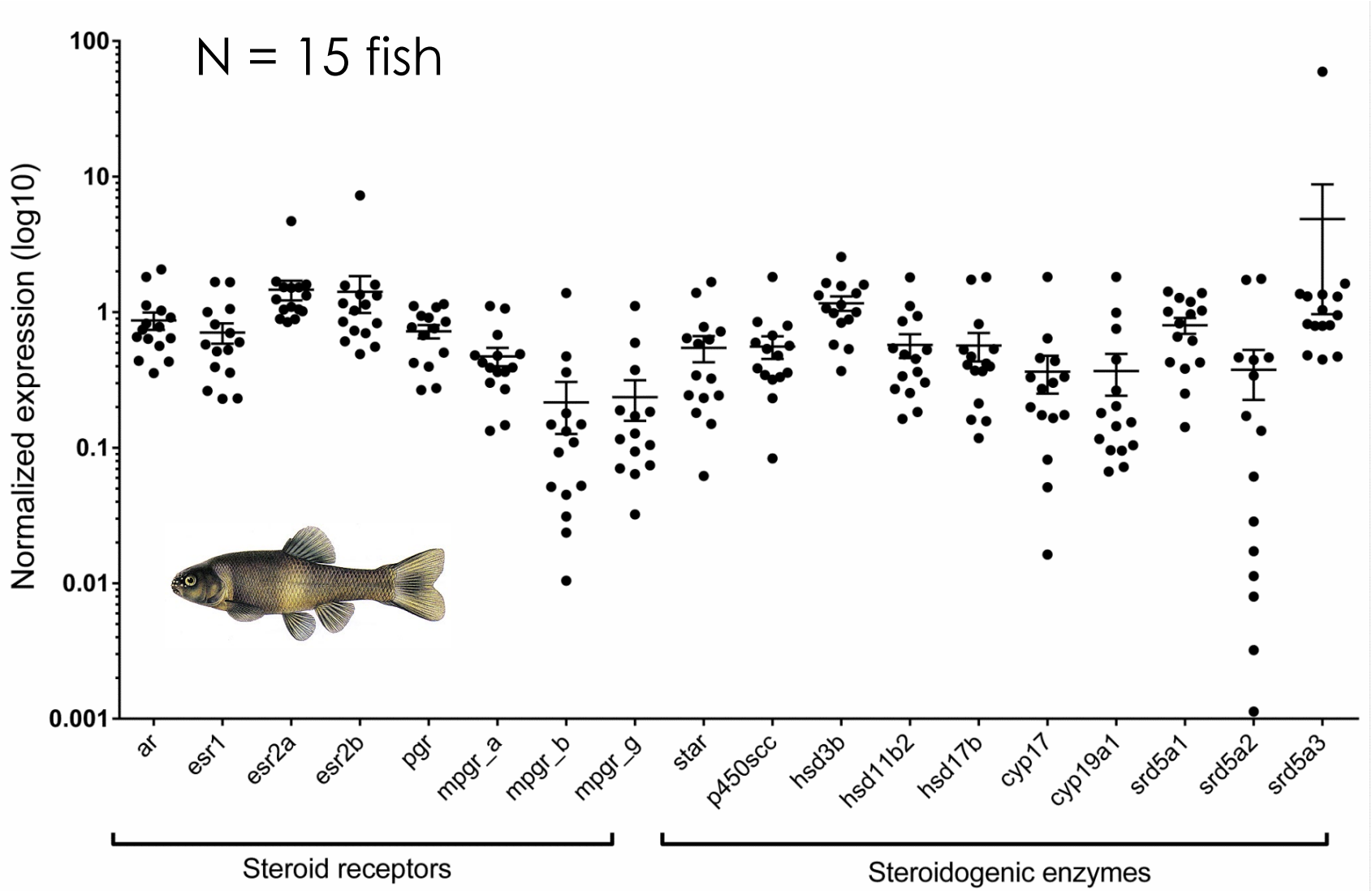
THE CHALLENGE MOVING INTO THE ENVIRONMENT…

Ecological Interactions



Individual Response

INDIVIDUAL VARIABILITY IN GENE EXPRESSION



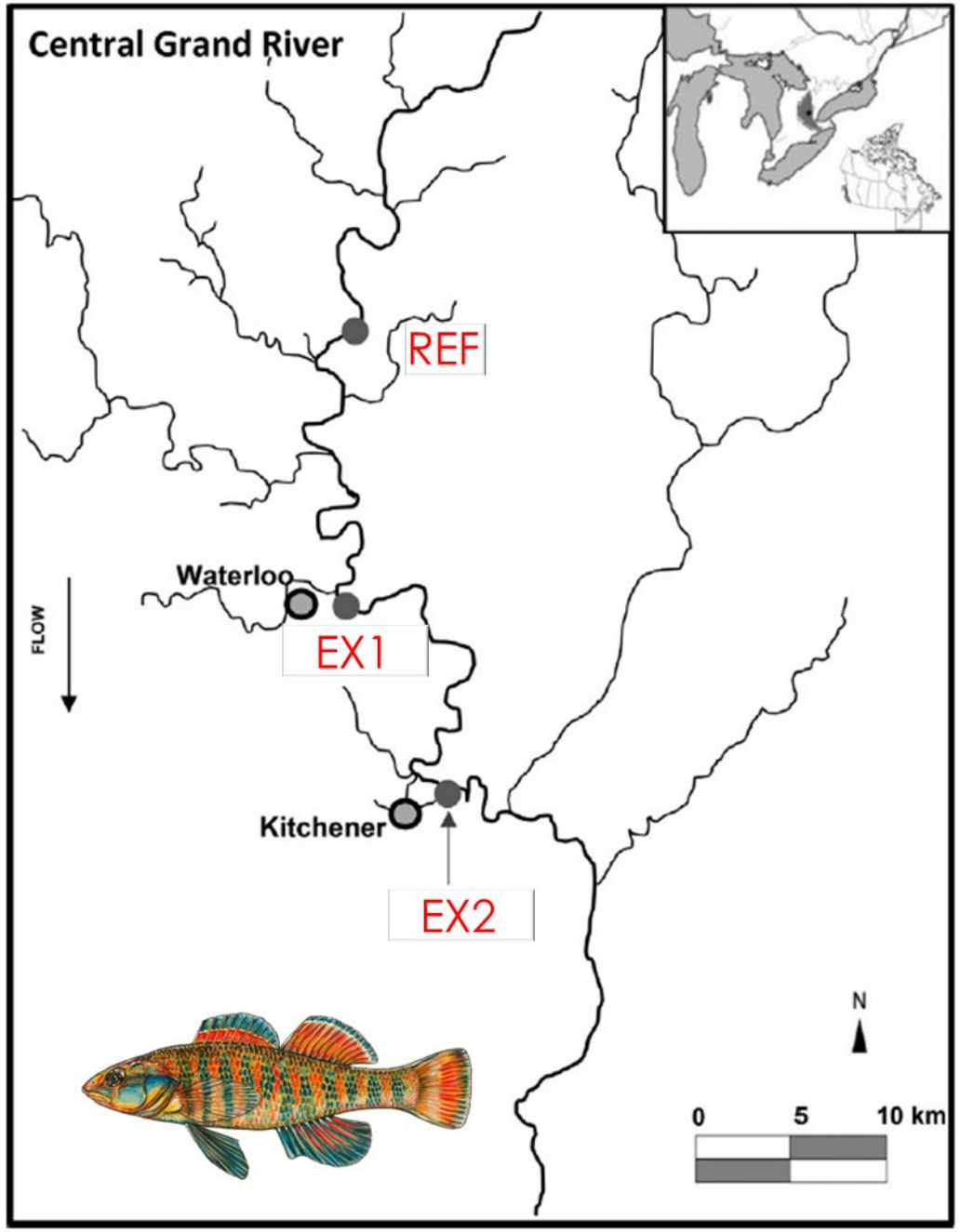
Cowie et al. Comp Biochem Physiol B Biochem Mol Biol. 2015

Largest watershed in southern Ontario, Canada

This system receives discharges from 30 Sewage Plants

Upgrades at the Kitchener Plant

Ref = upstream
EX1 = downstream Waterloo
EX2 = downstream Kitchener



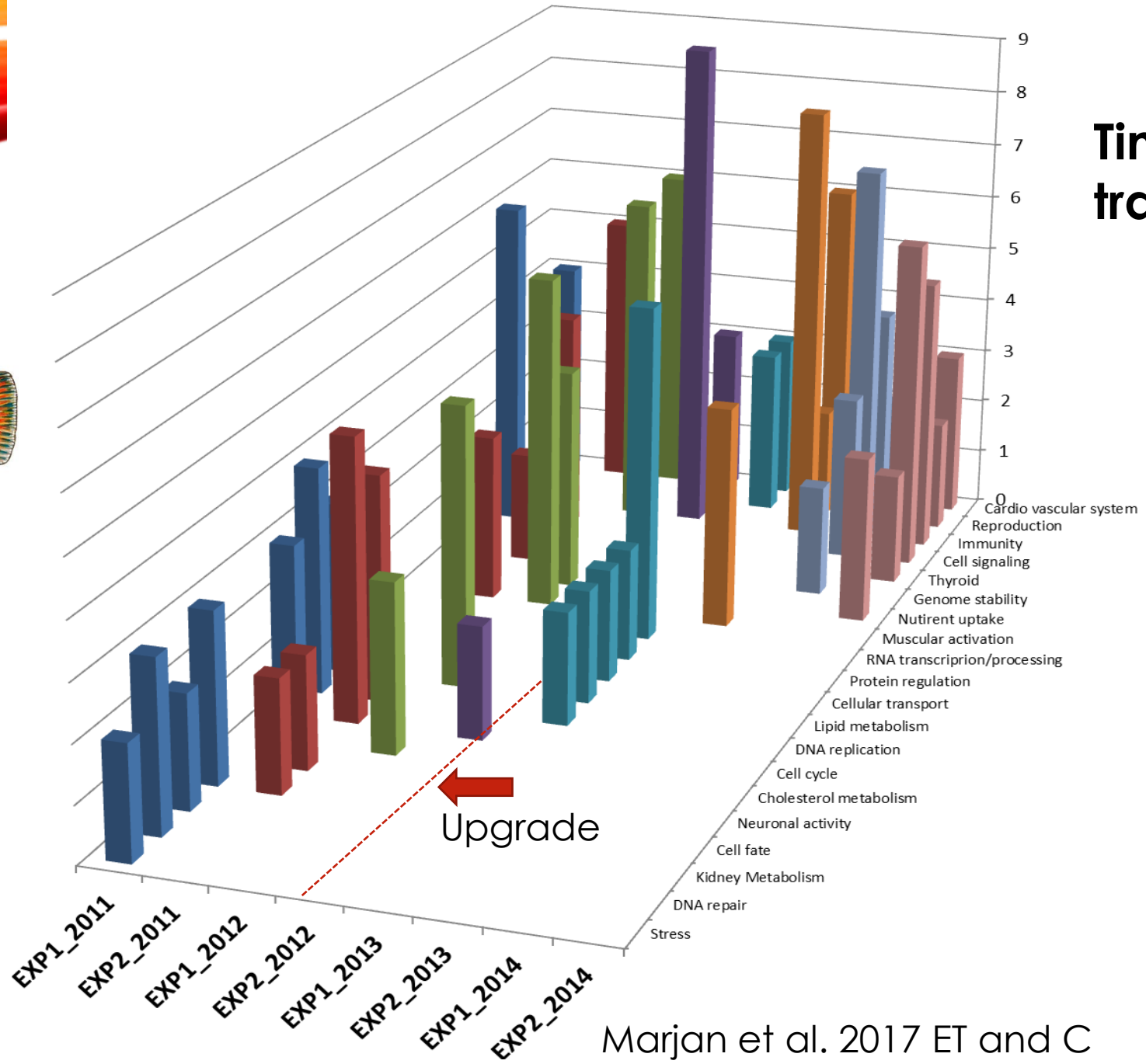
Marjan P, Bragg LM, MacLatchy DL, Servos MR, Martyniuk CJ. How Does Reference Site Selection Influence Interpretation of Omics Data?: Evaluating Liver Transcriptome Responses in Male Rainbow Darter (Etheostoma caeruleum) across an Urban Environment. Environ Sci Technol. 2017 Jun 6;51(11):6470-6479. doi: 10.1021/acs.est.7b00894. Epub 2017 May 17. PMID: 28489360.

Time and transcriptomes

Cell Fate

DNA Repair

Stress



Marjan et al. 2017 ET and C

Time and transcriptomes

Cell Fate

DNA Repair

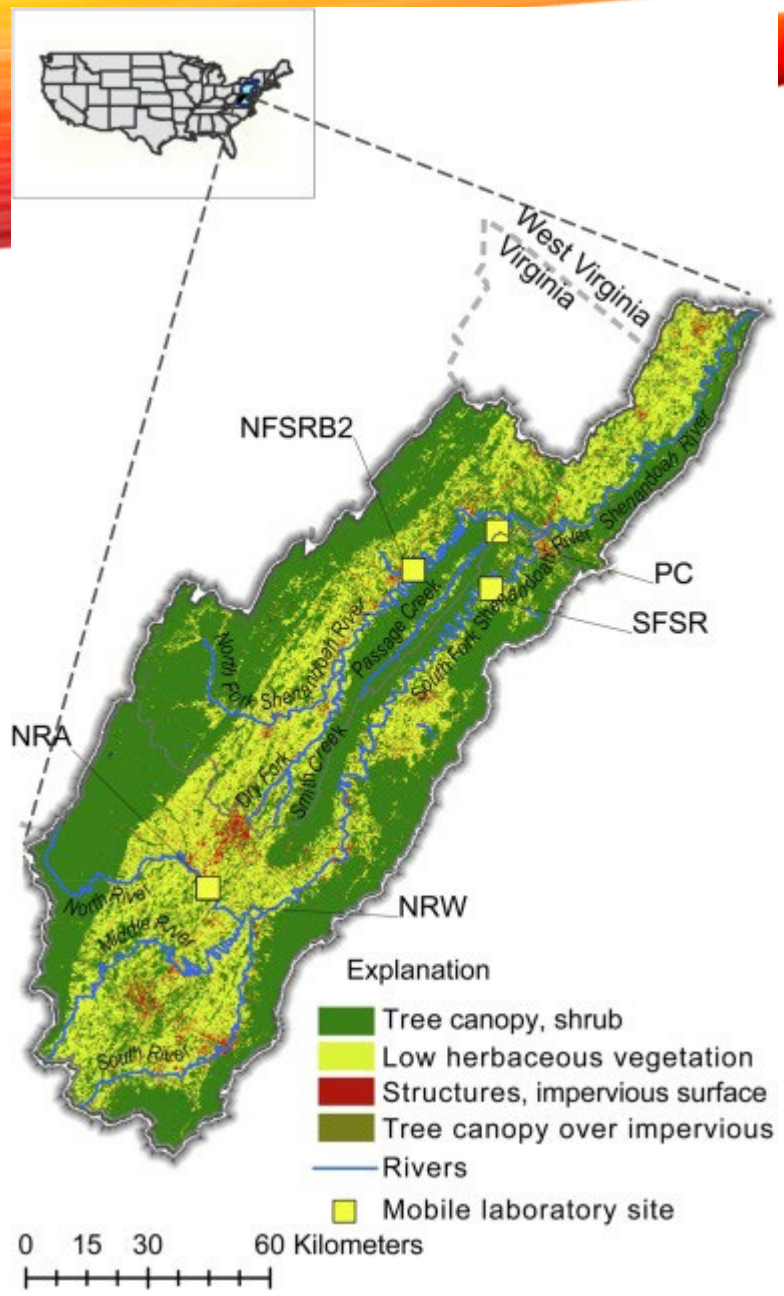
Stress

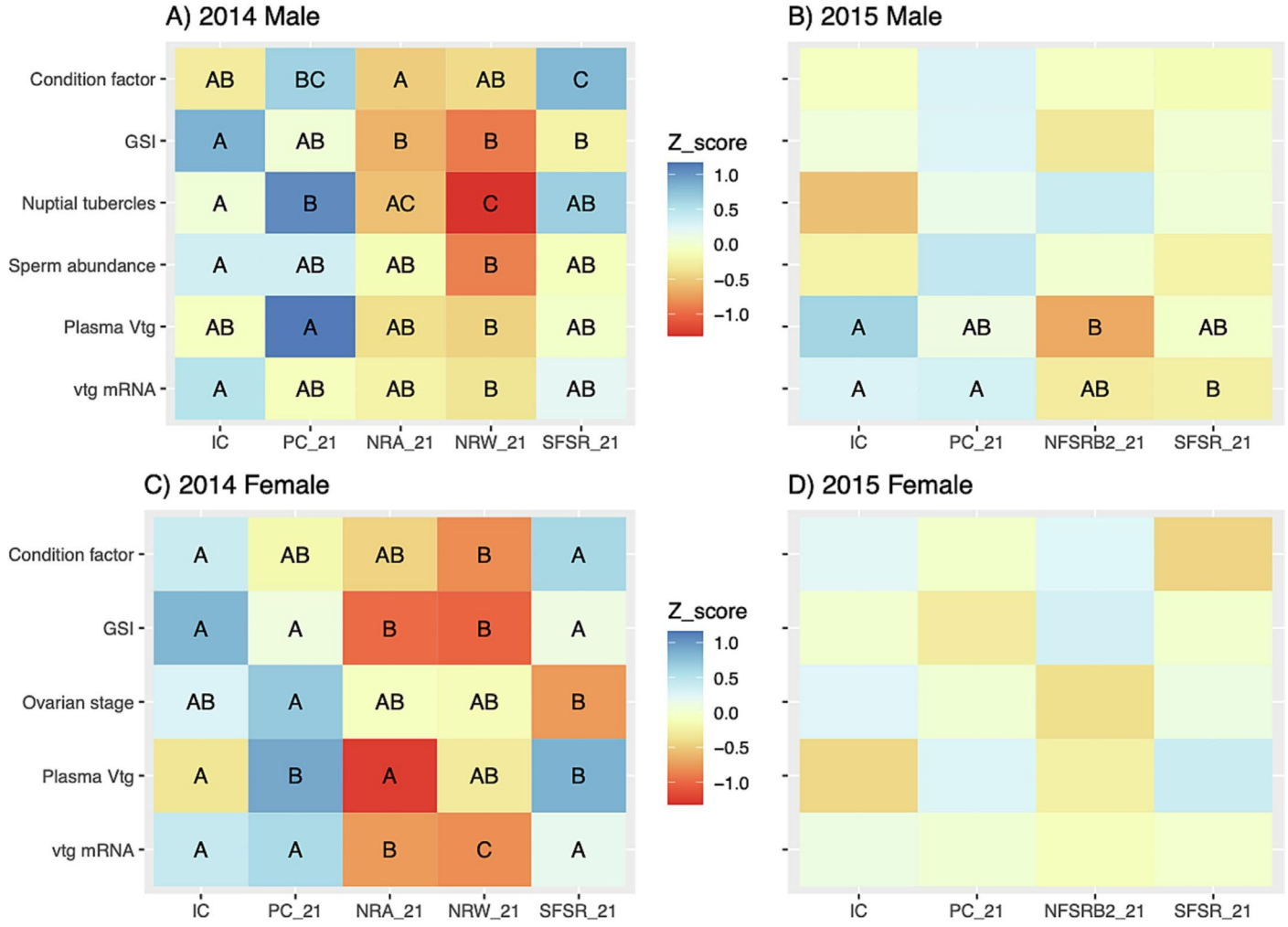
Genome Stability

Protein Regulation

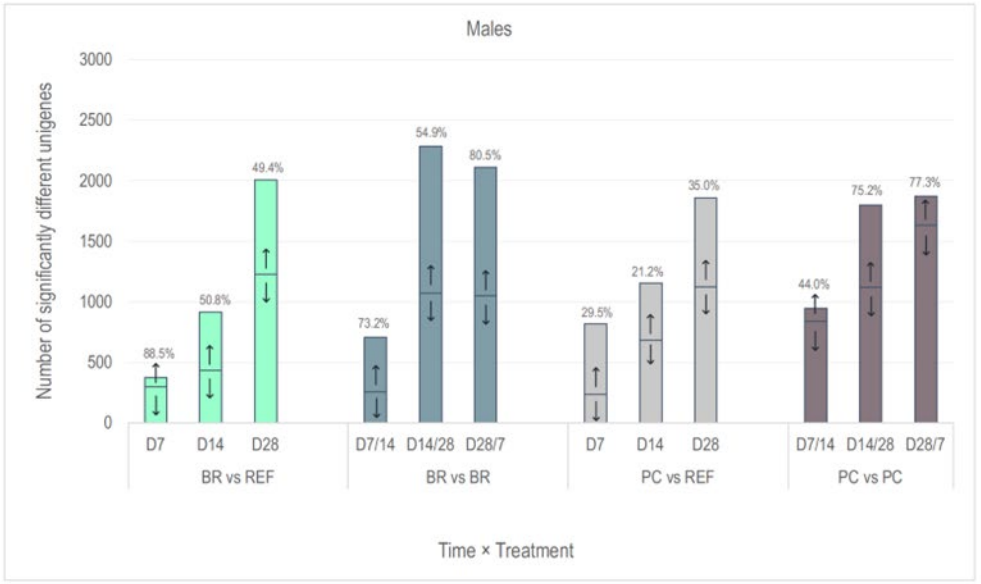
Reproduction

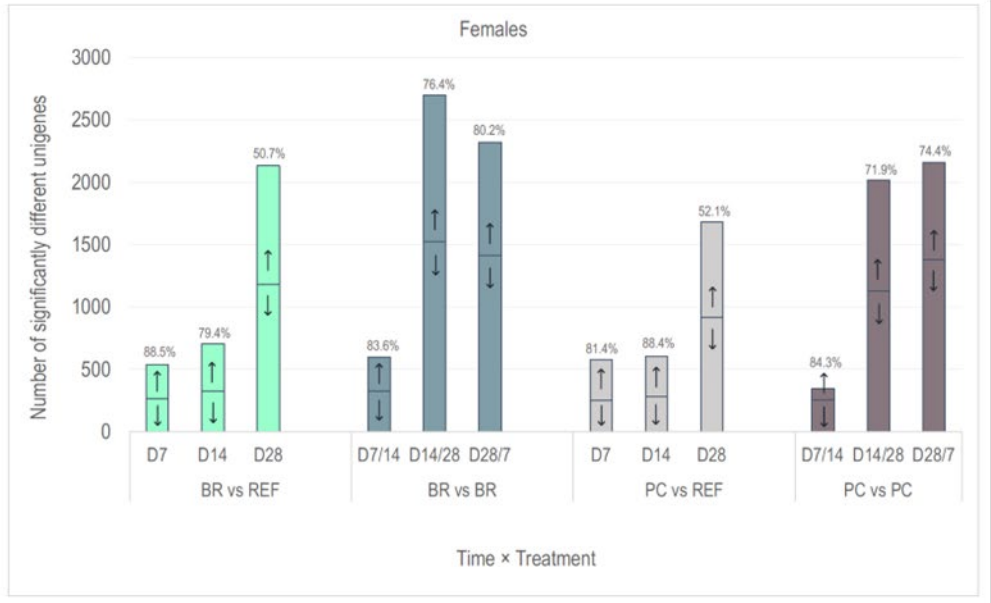
Marjan et al. 2017 ET and C





Bertolatus DW, Barber LB, Martyniuk CJ, Zhen H, Collette TW, Ekman DR, Jastrow A, Rapp JL, Vajda AM. Multi-omic responses of fish exposed to complex chemical mixtures in the Shenandoah River watershed. Sci Total Environ. 2023 Dec 1;902:165975. doi: 10.1016/j.scitotenv.2023.165975. Epub 2023 Aug 1. PMID: 37536598; PMCID: PMC10592118.





"While there are several putative biomarkers identified in hubs related to gene sets, temporal responses in the hepatic transcriptome made it challenging to elucidate definitive response patterns that could be used in field-based ecotoxicogenomic studies on the impacts of well-treated MWWE."

Marjan P, Martyniuk CJ, Arlos MJ, Servos MR, Ruecker NJ, Munkittrick KR. Identifying transcriptomic indicators of tertiary treated municipal effluent in longnose dace (Rhinichthys cataractae) caged under semi-controlled conditions in experimental raceways. Sci Total Environ. 2024 May 1;923:171257. doi: 10.1016/j.scitotenv.2024.171257. Epub 2024 Feb 27. PMID: 38417510.

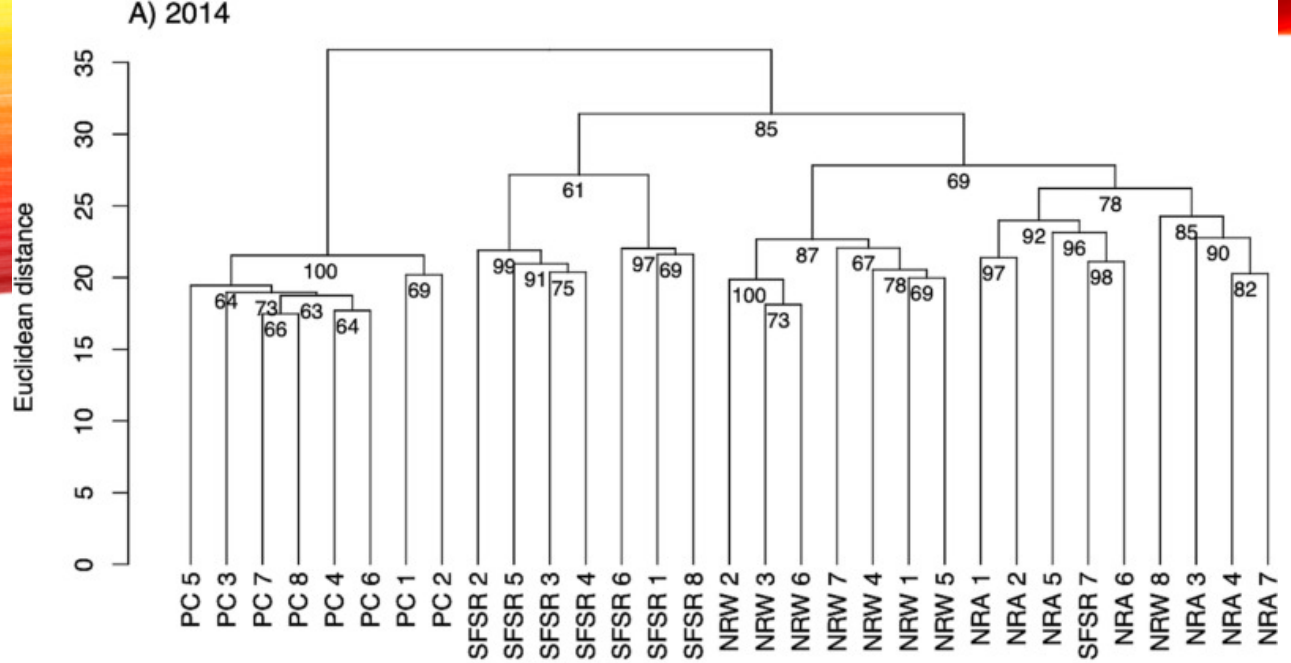
Critical Review

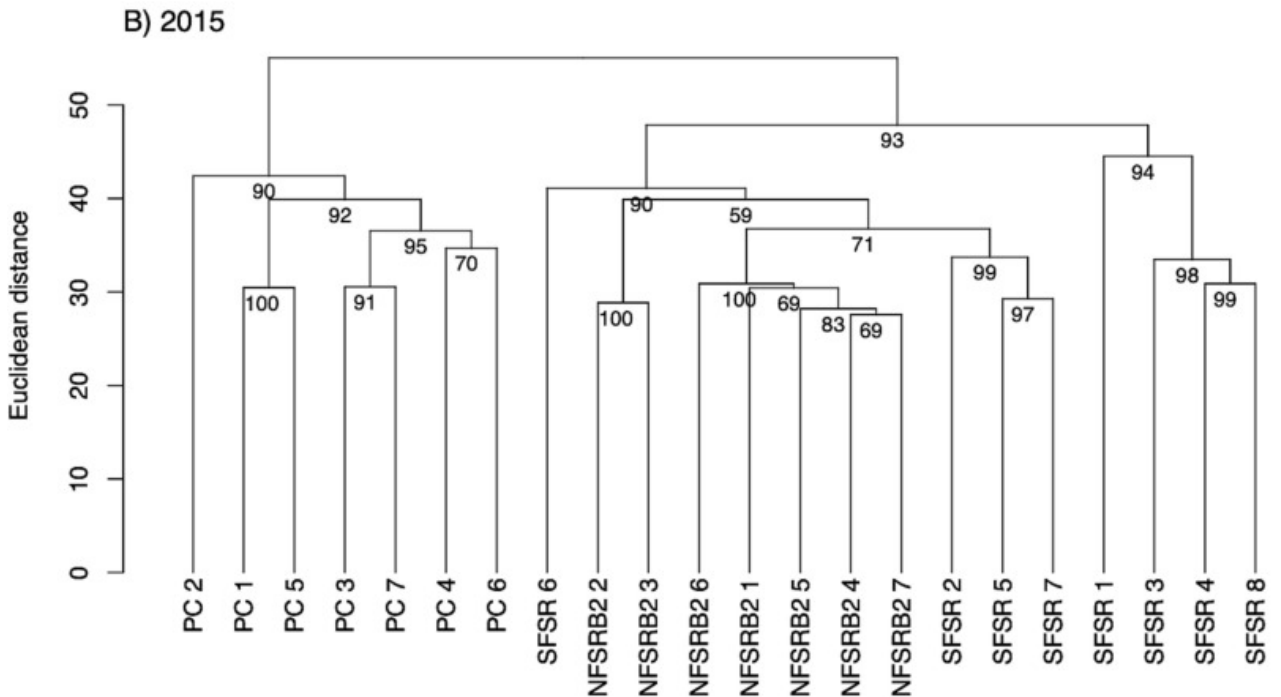
DEFINING THE ROLE OF OMICS IN ASSESSING ECOSYSTEM HEALTH: PERSPECTIVES FROM THE CANADIAN ENVIRONMENTAL MONITORING PROGRAM

PAULINA A. BAHAMONDE, APRIL FESWICK, MEGHAN A. ISAACS, KELLY R. MUNKITTRICK, and CHRISTOPHER J. MARTYNIUK*
Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, New Brunswick, Canada

(Submitted 28 March 2015; Returned for Revision 16 May 2015; Accepted 20 August 2015)

- DEFINING NORMAL: AN IMPORTANT EEM CONSIDERATION
- Sampling period and temporal variability
- Spatial scale variation in field studies
- Understanding magnitude of abiotic, non-chemical factors
- Physiological responses

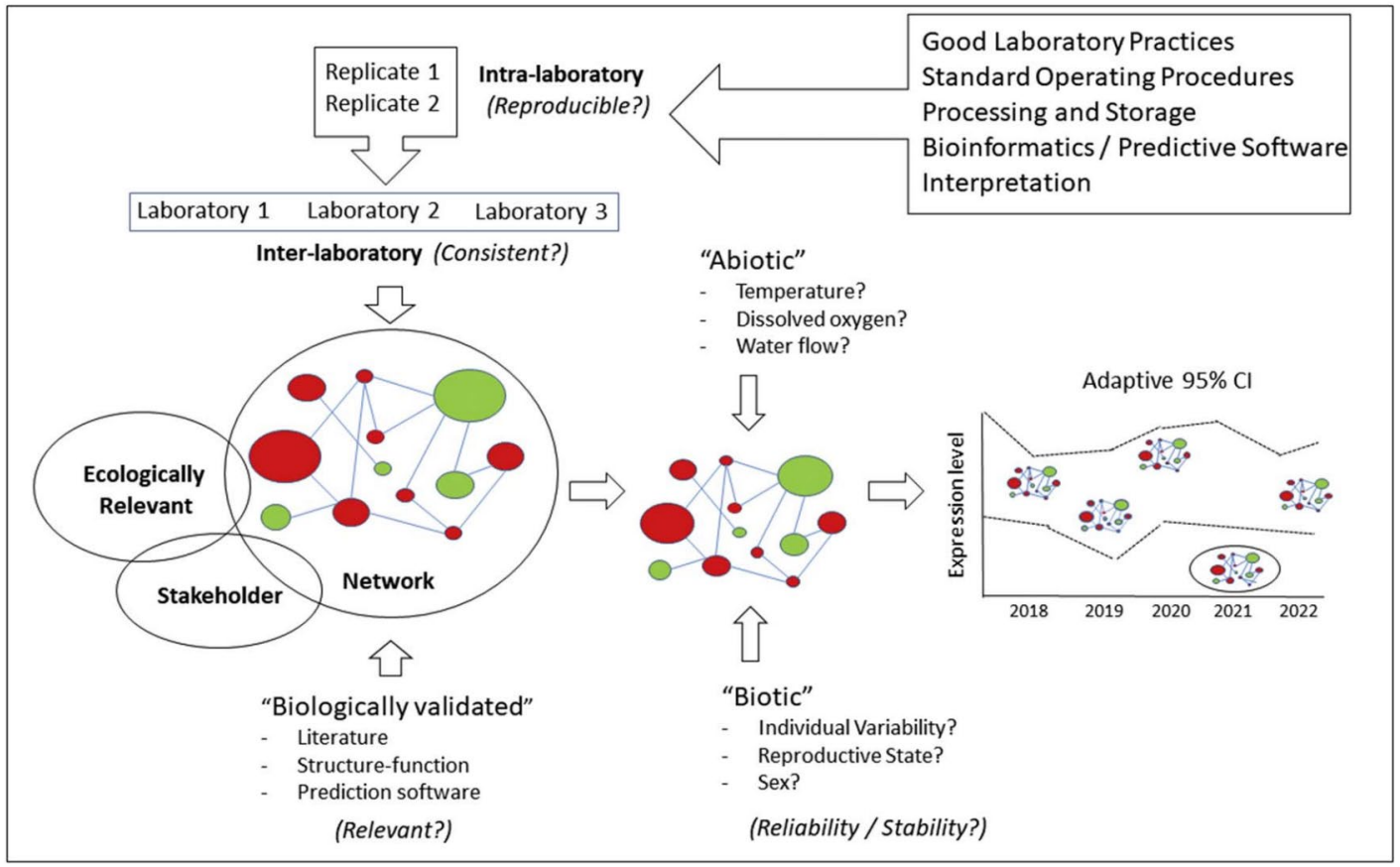




Omics: Good at predicting individual habit

But….

Relative response due to chemical vs. other variables?



Key points to be addressed prior to implication of omics technologies into a monitoring program.

Key Points

- Baseline data for omics responses in ecologically relevant species to be assessed.
- Data to identify the range of what is biologically normal within a given system to be monitored.
- Develop a level of standardization, consistency, and rigor that will allow interpretation of the relevance of response across broader scales.
- Leverage the AOP framework to bridge the gap between molecular responses and apical endpoints relevant for environmental monitoring.
- Discussion on what constitutes a meaningful change in a molecular network.

Martyniuk CJ. Are we closer to the vision? A proposed framework for incorporating omics into environmental assessments. Environ Toxicol Pharmacol. 2018 Apr;59:87-93. doi: 10.1016/j.etap.2018.03.005. Epub 2018 Mar 8. PMID: 29549817.

Prioritization

Which one do we test?

Evaluation

How do we measure the toxicity?

Management

Which ones do we regulate?

ACKNOWLEDGEMENTS

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Nancy D. Denslow, U Florida
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Kelly R. Munkittrick, U Calgary

David Bertolatus, Adams State
Alan Vajda, U. Colorado Denver

MARTYNIUK LABORATORY

Canadian Water Network

NSERC CRSNG

FINNOVATION

UF Physiological Sciences
College of Veterinary Medicine

Canada Research Chairs / Chaires de recherche du Canada

Center for Undergraduate Research — UF Undergraduate Research for All
GO GATORS | GO RESEARCH

UF University of Florida
College of Veterinary Medicine

NSF


Thank
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Health Canada Santé Canada

Canada

Fostering transparency and reproducibility using the OECD Omics Reporting Framework

Presented at the 4th U.S. Environmental Protection Agency NAMs Conference:
State of Science on Development and Use of NAMs for Chemical Safety Testing (November 5-6, 2024)

Matthew J. Meier, Research Scientist, Health Canada

Contributors to the OORF

- **Members of EAGMST, ESCA, and Omics Expert Group for valuable feedback**
- **Case study partners**
- **Tim Gant (Univ. of Leicester)**
- **Carole Yauk (Univ. of Ottawa)**
- **Joshua Harrill (US EPA)**
- **Mark Viant (Univ. of Birmingham)**
- **Ksenia Groh (Eawag)**
- **Matthew Meier (Health Canada)**
- **Magda Sachana (OECD secretariat)**
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- Lyle Burgoon
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- Pim E.G. Leonards
- Mirjam Luijten
- Alberto Martini
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- Alexandra Schaffert
- Oliver Schmitz
- Tomasz Sobanski
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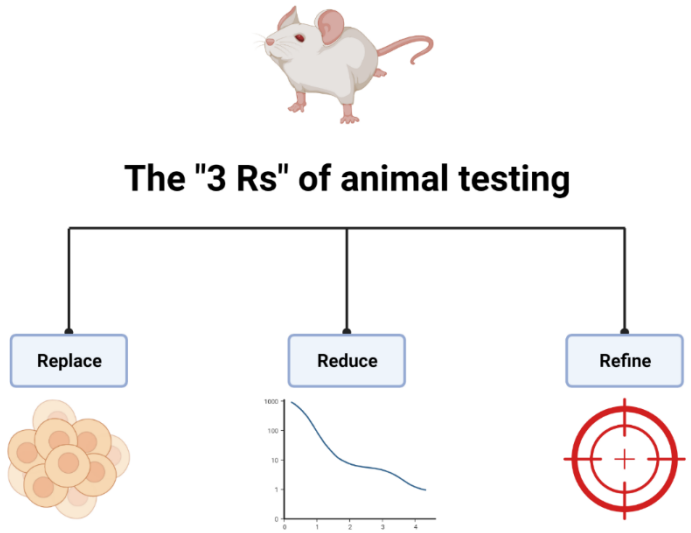
- Vikrant Vijay
- Ralf J.M. Weber
- Antony J. Williams
- Andrew Williams
- Maurice Whelan
- David Crizer
- Tom Lawson
- N. Cabaton
- M. Trembley-Franco
- C. Canlet
- T. Schock
- K. Hagiwara
- Leah Wehmas
- Sarah Davidson
- Alison Harrill
- Beena Vallanat
- Nil Basu/Doug Crump

Evaluating thousands of chemicals for potential toxicity: easier said than done

- Disadvantages of historically used single-endpoint tests:
  - Slow 
  - Expensive 
  - Use animals 

- New Approach Methods (NAMs)
  - Regulatory agencies worldwide are reducing reliance on tests in animals (EU and cosmetics, 2013; US EPA; Canada has legislated changes in CEPA)

- Omics plays a critical role in NAM-based tests
  - Compatibility with non-animal tests: e.g., human cell lines, micro-physiological systems – increasingly complex *in vitro* assays

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MENU

Canada.ca › Health › Health system and services › Health-related consultations

› Consultation: Draft strategy to replace, reduce or refine vertebrate animal testing

Draft Strategy to Replace, Reduce or Refine Vertebrate Animal Testing under the Canadian Environmental Protection Act, 1999

Environment and Climate Change Canada
Health Canada

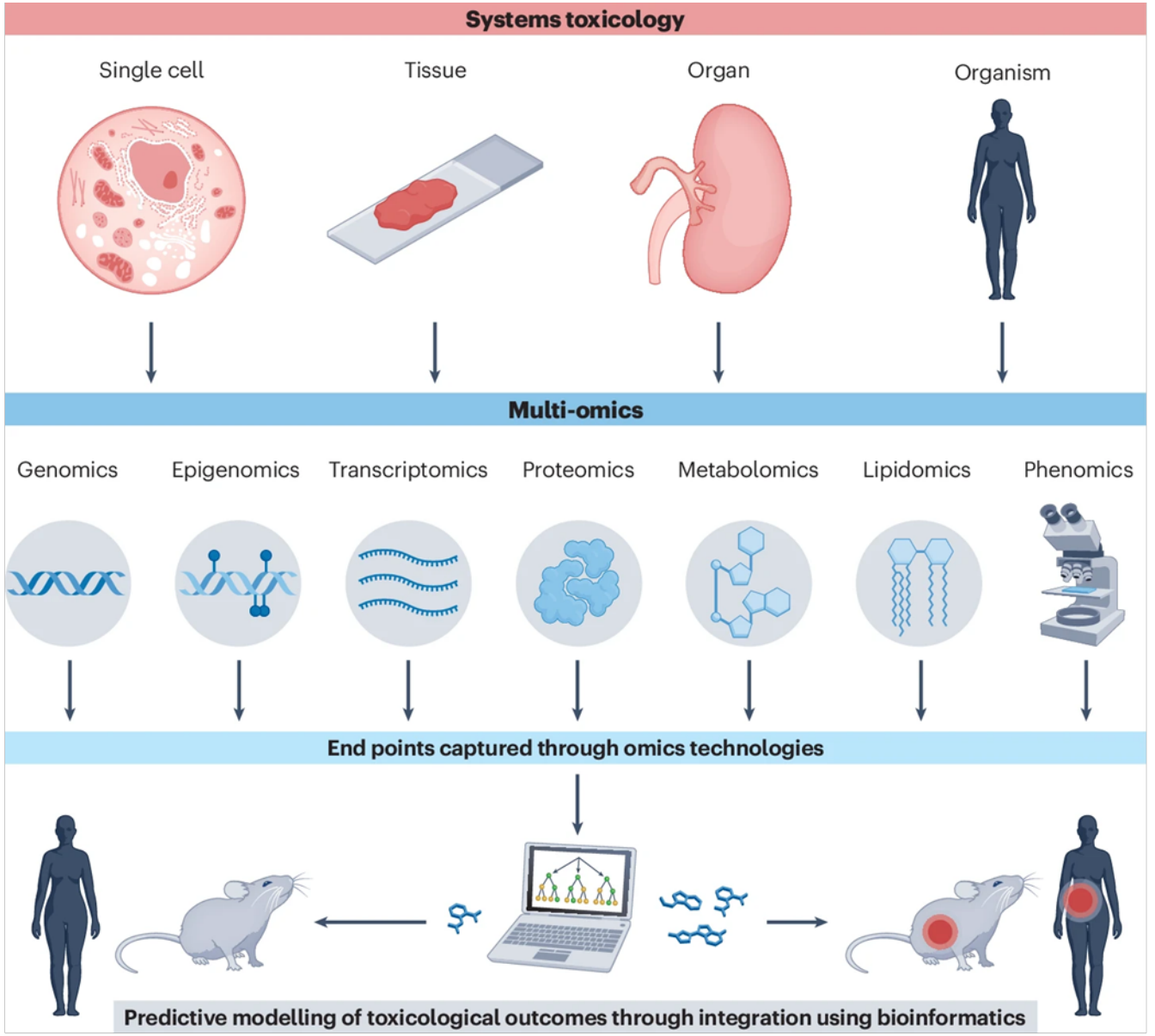
September 2024

Using omics to explore biological responses

Exposure

Response

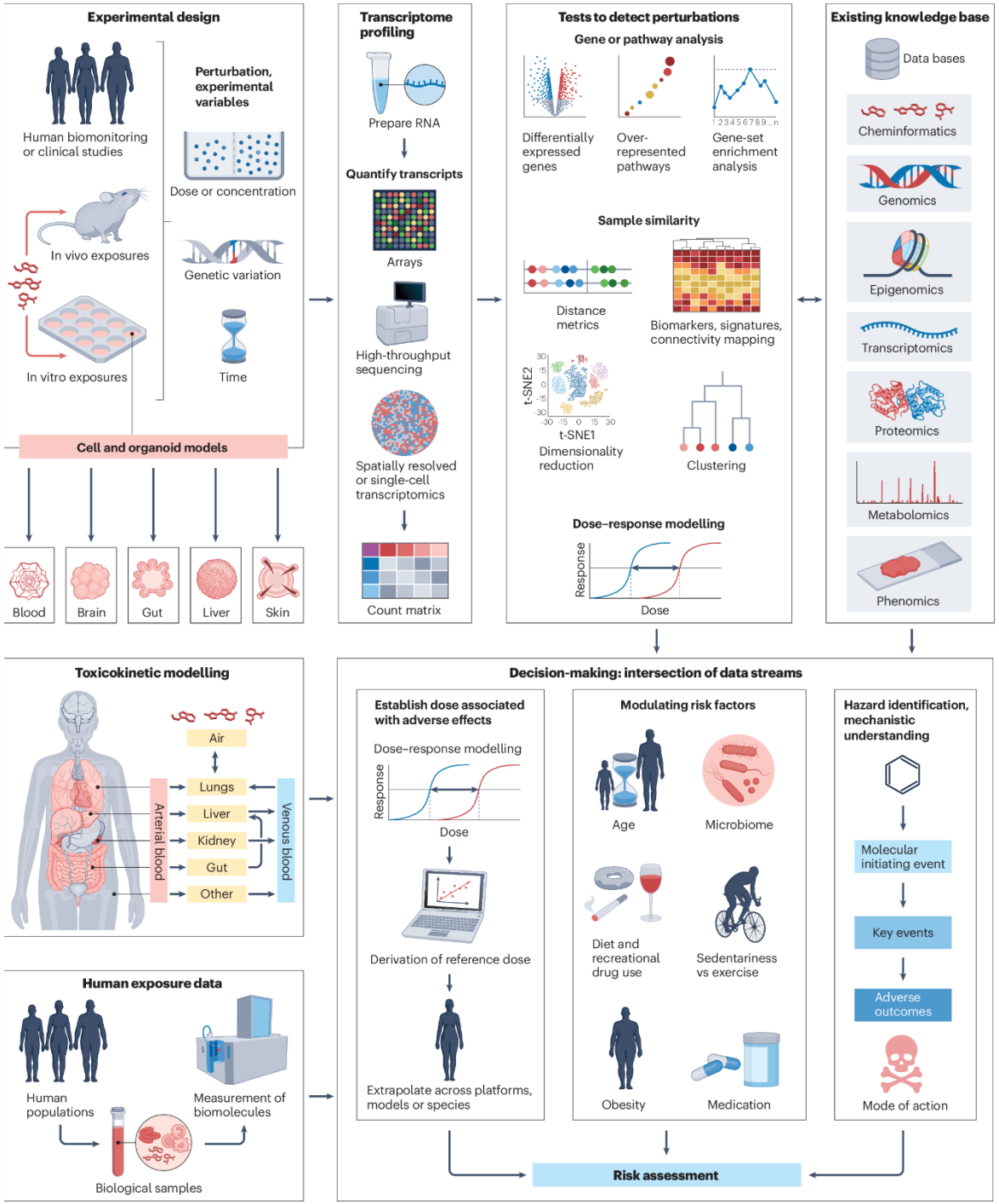
Disease



Meier, M.J., Harrill, J., Johnson, K., Thomas, R.S., Tong, W., Rager, J.E., Yauk, C.L. *Nat Rev Genet* (2024).

The promise of toxicogenomics for human health protection

- Molecular alterations occur before changes in apical endpoints
- Omics provides significant advantages over traditional toxicology tests
  - Rapid & cost-effective data generation
  - Reduction in animal use



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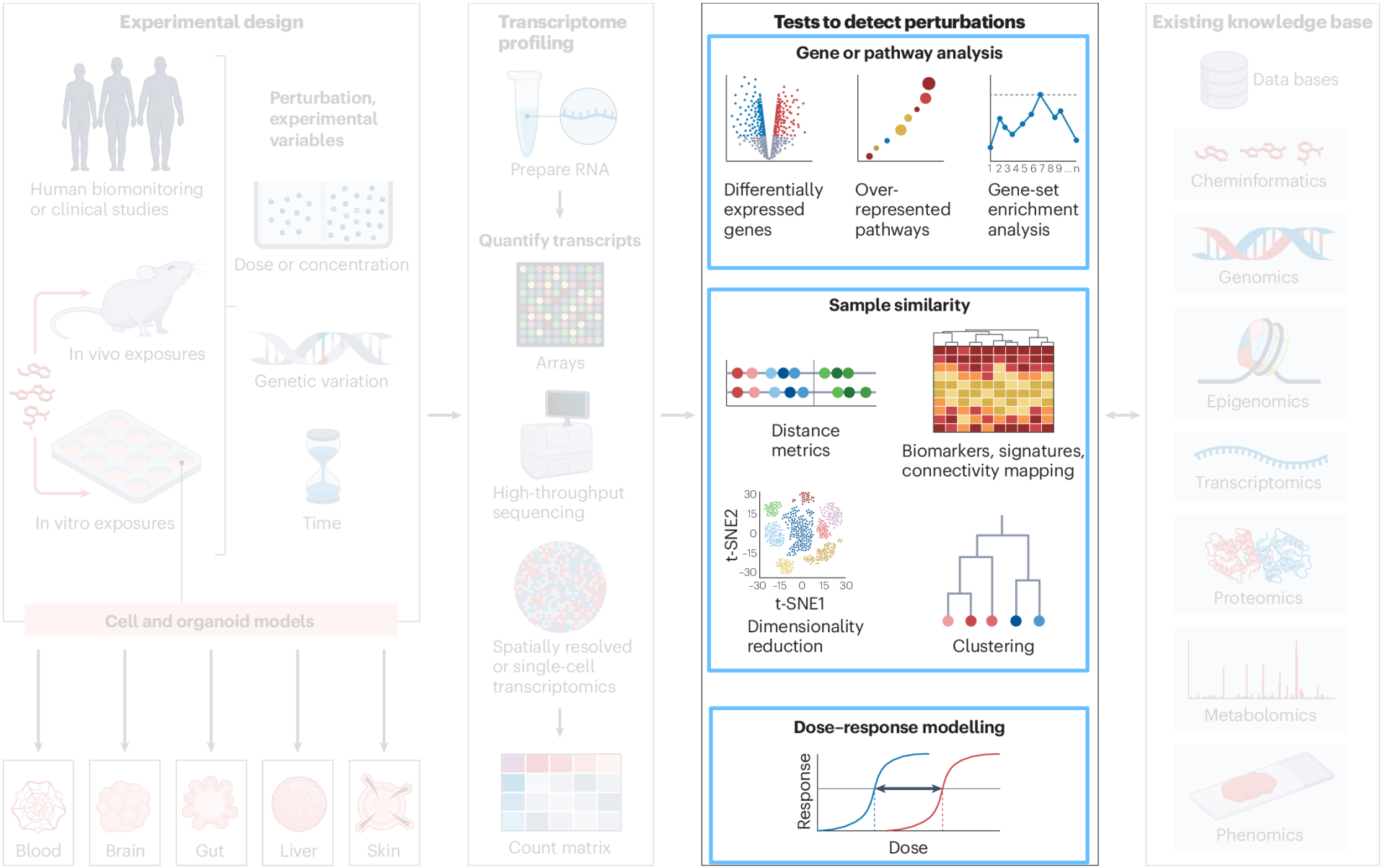
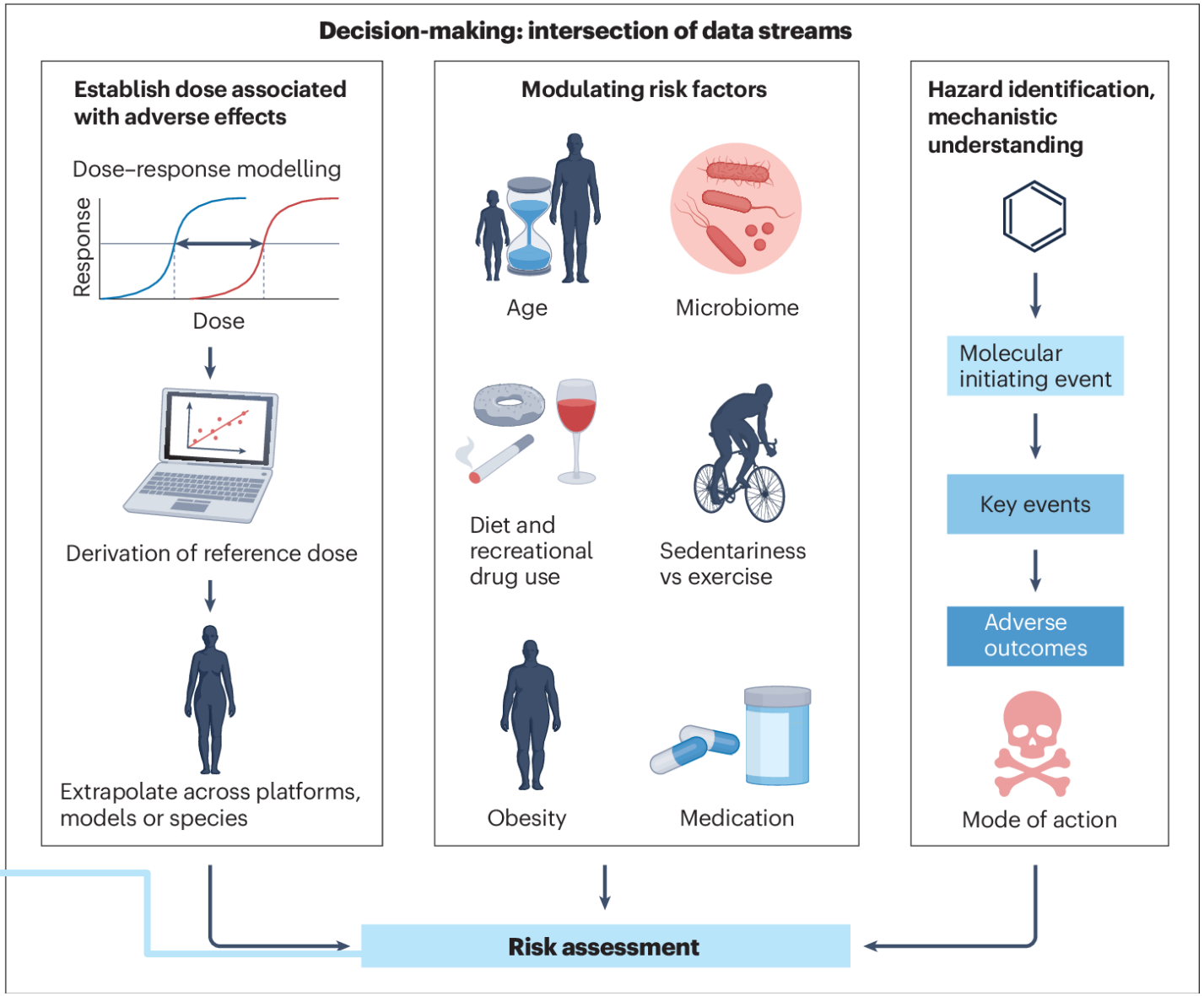


Implementing toxicogenomics in decision-making

- Data streams converge on risk assessment



The fine print: government, industry, and academia must agree on acceptable criteria for data use

“Torture the data, and it will confess to anything.”

-Ronald Coase, British economist

Challenges applying omics in risk assessment

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Commentary

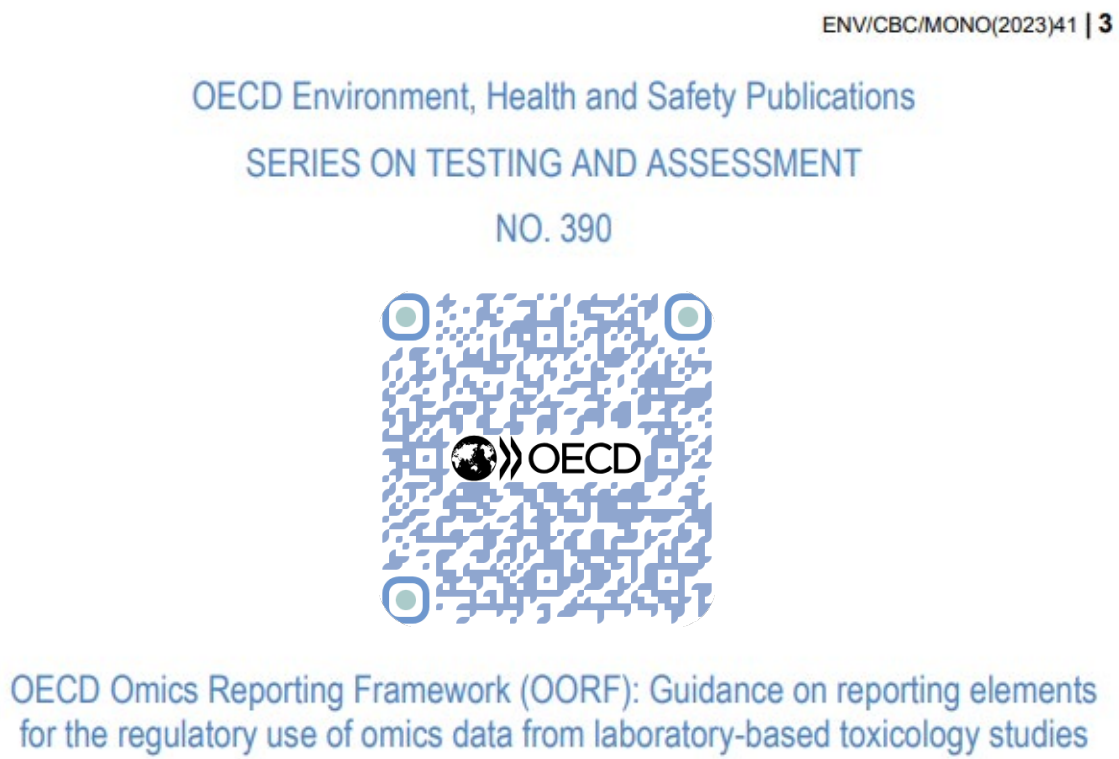
Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology

Joshua A. Harrill [a,1,*], Mark R. Viant [b,c,**,1], Carole L. Yauk [d,1,***], Magdalini Sachana [e], Timothy W. Gant [f], Scott S. Auerbach [g], Richard D. Beger [h], Mounir Bouhifd [i], Jason O'Brien [j], Lyle Burgoon [k], Florian Caiment [l], Donatella Carpi [m], Tao Chen [h], Brian N. Chorley [a], John Colbourne [b,c], Raffaella Corvi [m], Laurent Debrauwer [n,o], Claire O'Donovan [p], Timothy M. D. Ebbels [q], Drew R. Ekman [r], Frank Faulhammer [s], Laura Gribaldo [m], Gina M. Hilton [t], Stephanie P. Jones [j], Aniko Kende [u], Thomas N. Lawson [c], Sofia B. Leite [m], Pim E.G. Leonards [v], Mirjam Luijten [w], Alberto Martin [i,2], Laura Moussa [x], Serge Rudaz [y,z,aa], Oliver Schmitz [ab], Tomasz Sobanski [i], Volker Strauss [s], Monica Vaccari [ac], Vikrant Vijay [h], Ralf J.M. Weber [b,c], Antony J. Williams [a], Andrew Williams [ad], Russell S. Thomas [a], Maurice Whelan [m]

- Lack of transparency in data generation and processing
- Lack of standardisation in study parameters and reporting of results
- Lack of case studies and guidance describing acceptable (and ultimately best) practices

→ Impacts on experimental or analytical reproducibility → Reduced regulatory utility

OECD Omics Reporting Framework (OORF)



- Recognition in international community that omics provides significant advantages over traditional tests
  - However - regulators don't want to drink from a fire hose
- Growing interest in regulatory applications of omics must be supported by guidelines and frameworks
- The OECD Omics Reporting Framework (OORF) is now publicly available for use (Nov. 2023)
  - Began under the Extended Advisory Group for Molecular Screening and Toxicogenomics (EAGMST), now the OORF and related projects are within the Omics Expert Group, reporting to the Advisory Group on Emerging Science in Chemicals Assessment (ESCA), under the Working Party on Hazard Assessment (WPHA)

https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/omics-technologies-chemical-testing.html

OECD Omics Reporting Framework (OORF)

Objective

Develop a framework to standardize **reporting** of omics data generation and analyses, so regulators can **understand and interpret** omics studies.

Why do we need the OORF?

To ensure that sufficient information is available to **enable an evaluation of the quality of the experimental data and interpretation, and support reproducibility.**

(The 5 Rs: Regulatory Ratification Requires Reproducible Research)

× **NOT** to stipulate the methods of data analysis or interpretation

✓ **Rather**, provide guidance on reporting of information that fosters transparency and reproducibility

Modular structure of the OORF

Study Summary Reporting Module (SSRM)

Toxicology Experiment Reporting Module (TERM)

Expanding the scope of the OORF

- Early OORF development focused on transcriptomics and metabolomics
- There is a need to expand and re-evaluate language to encompass all omics
- Ksenia Groh (Eawag) and Alexandra Schaffert (Medical University Innsbruck) are co-leading the integration of proteomics modules, and reviewing what components should be updated, creating new DAPRMs

Development of Proteomics Modules for the OECD Omics Reporting Framework

eawag aquatic research

Ksenia Groh[1], Premkumari Kumarathasan[2], Steve U. Ayobahan[3], Davide Degli-Esposti[4], Verónica I. Dumit[5], Sebastian Eilebrecht[3], Vera Engelbrecht[6], Salvador Fernandez Arroyo[7], Nico Jehmlich[8], Predrag Kukic[9], Isabel Karkossa[8], Thibaut Leger[10], Arnaud Salvador[11], Johannes Schmidt[12], Kristin Schubert[8], Joshua Harrill[13], Magdalini Sachana[14] and Alexandra Schaffert[15]

[1]Eawag, Swiss Federal Institute of Aquatic Sciene and Technology, 8600 Duebendorf, Switzerland; [2]Analytical Biochemistry and Proteomics Laboratory, Mechanistic Studies Division, Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Canada; [3]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Department Ecotoxicogenomics, 57392 Schmallenberg, Germany; [4]INRAE (National Research Institute for Agriculture, Food and Environment), Centre de Lyon-Grenoble Auvergne-Rhone-Alpes, Unité de recherches RiverLy, Ecotox team, 69625 Villeurbanne Cedex, France; [5]Federal Institute for Risk Assessment (BfR), Berlin, Germany; [6]PETA Science Consortium International e.V.; [7]EURECAT, Center for Omics Sciences, Reus, Tarragona, Spain; [8]Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany; [9]Unilever, Safety and Envrionmental Assurance Centre, Computational and in vitro Toxicology, Colworth Science Park, Bedfordshire, UK; [10]ANSES – French Agency for Food, Environmental and Occupational Health & Safety, Fougères Laboratory, France; [11]University of Lyon, CNRS, Universite Claude Bernard Lyon 1, Institute des Sciences Analytiques, UmR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France; [12]Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany; [13]US EPA, Center for Computational Toxicology and Exposure (CCTE), RTP, NC27709, USA; [14]OECD Environment Health and Safety Division, Environment Directorate, Paris, France; [15]MUI Medical University Innsbruck, Regulatory Toxicology, Institute of Medical Biochemistry, Innsbruck, Austria.

Reporting templates

1. Study Summary Reporting Module

Red = Recommended
Blue = Optional

1.1. Study Identifiers

1.1.1. Abstract — Recommended

INSTRUCTIONS TO DATA PROVIDERS: Use Column E for data entry. Text in italics are instructions (e.g. reporting tips) for the data submitter for individual reporting fields. Text in bold italics are

2. Toxicology Experiment Reporting Module

Red = Recommended
Blue = Optional

REPORTING CATEGORY

2.1. Study Rationale

Toxicology Experiment Mo
1.2. Study Rationale

2.2 Test and Control items

SSRM

INSTRUCTIONS TO DATA PROVIDERS: Use Column D for data entry. Text in italics are instructions (e.g. reporting tips) for the data submitter for individual reporting fields. Text in bold italics are instructions for the data submitter that are applicable to all reporting elements in a section. For additional clarification regarding a reporting field, please refer to the

3.2. Data Acquisition and Processing Reporting Module (DAPRM) for RNA Seq and Targeted RNA-Seq Data

Red = Recommended
Blue = Optional

REPORTING CATEGORY

3.2.1. Technology

3.2.2. Transcriptomics Experime

SSRM

INSTRUCTIONS TO DATA PROVIDERS: Use Column D for data entry. Text in italics are instructions (e.g. reporting tips) for the data submitter for individual reporting fields. Text in bold italics are instructions for the data submitter that are applicable to all reporting elements in a section. For additional clarification regarding a reporting field, please refer to the corresponding guidance document for this reporting template.

4.1. Data Analysis Reporting Module (DARM) for Discovery of Differentially Abundant Molecules - Univariate Methods

Red = Recommended
Blue = Optional

INSTRUCTIONS TO DATA PROVIDERS: Use Column D for data entry. Text in italics are instructio
tips) for the data submitter for individual reporting fields. Text in bold italics are instructions for
that are applicable to all reporting elements in a section. For additional clarification regarding a
please refer to the corresponding guidance document for this reporting template.

REPORTING CATEGORY	REPORTING ELEMENT	RECOMMENDED / OPTIONAL	INPUT
4.1.1. Inputs	4.1.1.1. Data Input(s)	Recommended	
	4.1.1.2. Metadata Inputs(s)	Recommended	see "metadata" tab
4.1.2. Software Documentation	4.1.2.1. Software	Recommended	R version 4.0.3 (2020-10-10)
	4.1.2.2. Operating System	Recommended	Platform: x86_64-pc-linux-gnu (64-bit) running under: Ubuntu 20.04.2 LTS
	4.1.2.3. Additional Libraries used	Recommended	https://www.r-project.org/, https://www.bioconductor.org/
	4.1.2.4. Software Availability	Optional	
4.1.3. Contrasts for Which Differentially Abundant Molecules were Identified	4.1.3.1. Contrasts	Recommended	See "contrasts" tab
4.1.4. Assay Experimental Design			
	4.1.4.1. Group Sizes	Recommended	See "metadata" tab
	4.1.4.2. Covariance	Recommended	Samples were processed in plate-based batches. See "metadata" tab.
	4.1.4.3. Technical Replicates	Recommended	Technical replicates were used as different wells for library building. They derive from different ali exposures.
4.1.5. Statistical Analysis to Identify Differentially Abundant Molecules			
	4.1.5.1. Statistical Approach	Recommended	DESeq2
	4.1.5.2. Data Transformation	Recommended	Size factor normaliziation
	4.1.5.3. Effects Models	Recommended	Negative Binomial Generla Linear Model (GLM) fitting performed using default settings in DESeq2 contrast. Batches were used as covariates in the formula.
	4.1.5.4. Modeling Inputs	Recommended	Wald statistics calculated using DESeq2 with default settings
	4.1.5.5. Bayseian Approaches	Recommended	NA
	4.1.5.6. Decision Criteria	Recommended	R-ODAF criteria were used as the basis.
	4.1.5.7. Other	Recommended	multiple testing correction performed '- Adjusted p-value cutoff = 0.05; MTC = FDR in DESeq2; note: Cook's cutoff was disabled '- 1.5-fold linear fold change cutoff '- Filters applied simultaneously following DESeq results tabular output

1.2.1. Background Information	Recommended	concerns over exposure to bisphenol A (BPA) and human health, particularly during early life stages implementation of regulatory actions towards use chemical for commercial applications. This action in a rise in the use of BPA alternatives in recent ye to the potential for regrettable substitution in cor products considered "BPA-free". This work uses tra data, in supporting the development of an Integra Approaches to Testing and Assessment (IATA), to a
1.2.2. Objectives	Recommended	Concentration response experiments using TempC (BioSpyder Technologies) were conducted in order 16 bisphenols relative to bisphenol A in MCF7-cell objective was to explore potencies across the bisp using benchmark concentration analysis, and simi differences in altered pathways/upstream regulat pathway analysis and transcriptomic biomarkers,
1.2.3. Test item		
a. Test item name	Recommended	Bisphenol A (BPA), Bisphenol C (BPC), 4,4'-Bisphen BPF), 2,4'-Bisphenol F (2,4'-BPF), Bisphenol AP (BPA Bisphenol S (BPS), 2,4'-Bisphenol S (2,4'-BPS), TGSA AF (BPAF), DCDPS or BCPS, BADGE, BPS-MPE, D-8, BPS

SSRM

3.2.1.1. Type and version of the sequencing platform (e.g. Illumina HiSeq2500)	Recommended	Ill
3.2.1.2. Size and type of sequencing (e.g. 100 bp paired-end)	Recommended	50
3.2.1.3. Flow cell used (type and catalogue number)	Recommended	Ne Pr 40 Ca
3.2.1.4. Targeting probe annotation, including list of attenuated genes (if any) (for targeted	Recommended	Te
3.2.1.5. Library type (e.g. mRNA libraries)	Recommended	Hu
3.2.1.6. Purpose (e.g. target gene expression, quality control, etc.)	Recommended	W
3.2.1.7. Other	Recommended	W
3.2.2.1. RNA Processing		*De*
a. RNA Extraction	*see reporting fields below*	
i. Type of extracted RNA (e.g. total RNA, mRNA, miRNA, etc.)	Recommended	Ce
ii. Extraction and purification techniques	Recommended	
iii. Procedures for mRNA enrichment (if applicable), or other enrichment procedures	Recommended	
iv. Storage conditions	Recommended	Ce
b. Quantification and Qualification of RNA	*see reporting fields below*	
i. Tool for RNA assessment	Recommended	
ii. RNA quality	Recommended	
iii. RNA quantity	Recommended	
3.2.2.2. Library Preparation		*Pr*
a. Library preparation applied	*see reporting fields below*	
i. Manual library preparation or automated systems (if yes, which automation system)	Recommended	Re Th bu
ii. Fragmentation strategy (if applicable)	Recommended	
iii. Probe manifest (if applicable)	Recommended	

DAPRM

TERM

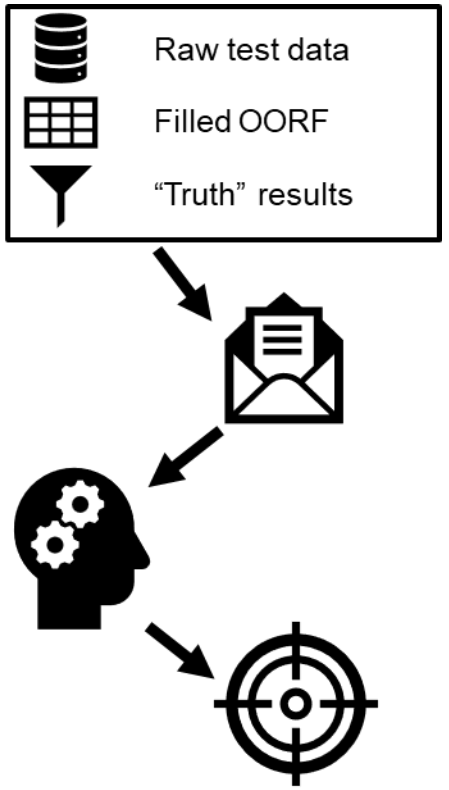
2.1.5. Model selection
a. For *in vivo* studies, describe why the selected animal spec
b. Rationale for the species and strain used
interest
2.1.6. Dose / concentration level and interval selection
2.1.7. Route of administration
2.1.8. Time point selection
2.1.9. Samples and Replicates
a. Biological replicate number
b. Number of technical and analytical replicates
2.1.10. Limitations
2.2.1. Test item name

DARM

REPORTING ELEMENT	RECOMMENDED / OPTIONAL	INPUT
4.1.1.1. Data Input(s)	Recommended	
4.1.1.2. Metadata Inputs(s)	Recommended	see "metadata" tab
4.1.2.1. Software	Recommended	R version 4.0.3 (2020-10-10)
4.1.2.2. Operating System	Recommended	Platform: x86_64-pc-linux-gnu (64-bit) running under: U
4.1.2.3. Additional Libraries used	Recommended	https://www.r-project.org/, https://www.bioconductor
4.1.2.4. Software Availability	Optional	
4.1.3.1. Contrasts	Recommended	See "contrasts" tab
4.1.4.1. Group Sizes	Recommended	See "metadata" tab
4.1.4.2. Covariance	Recommended	Samples were processed in plate-based batches. See "m
4.1.4.3. Technical Replicates	Recommended	Technical replicates were used as different wells for libr exposures.
4.1.5.1. Statistical Approach	Recommended	DESeq2
4.1.5.2. Data Transformation	Recommended	Size factor normaliziation
4.1.5.3. Effects Models	Recommended	Negative Binomial Generla Linear Model (GLM) fitting p contrast. Batches were used as covariates in the formul

OORF trials: how do we develop modules?

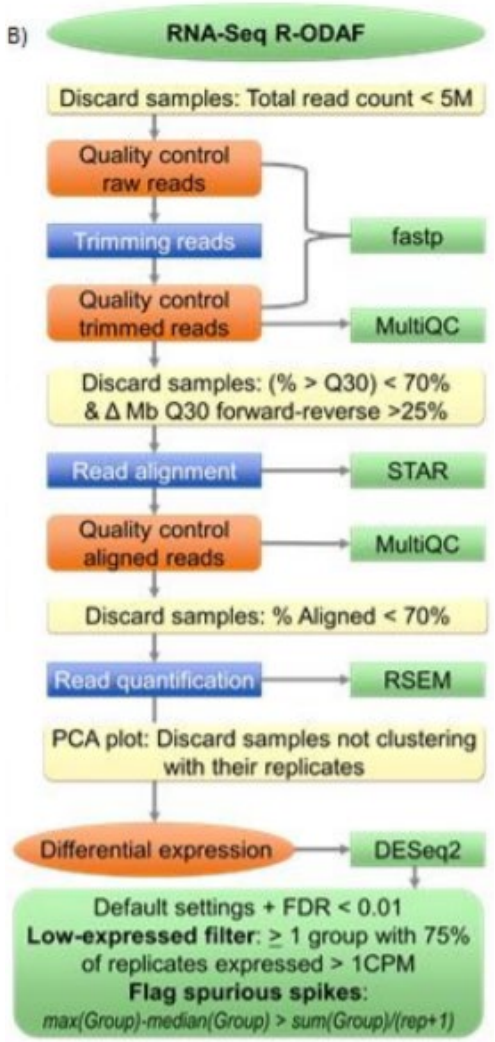
Participant	Step	Actions
Data provider:	1	I. Identify omics dataset II. Process and analyze dataset III. Populate reporting fields in one or more modules of the OORF IV. Write an ease-of-use commentary
Trial coordinator:	2	I. Review report for completeness (return to data provider if incomplete) II. Truncate and blind results and send to 'end user'
End user:	3	I. Use blinded reporting template to reprocess and reproduce the original analysis II. Write a second ease-of-use commentary
Trial coordinator:	4	I. Review report for completeness (return to end user if incomplete) II. Concordance analysis of two completed OORF reporting templates
Omics expert group:	5	I. Review concordance analysis and two ease-of-use commentaries II. Update TRF/MRF reporting templates as required



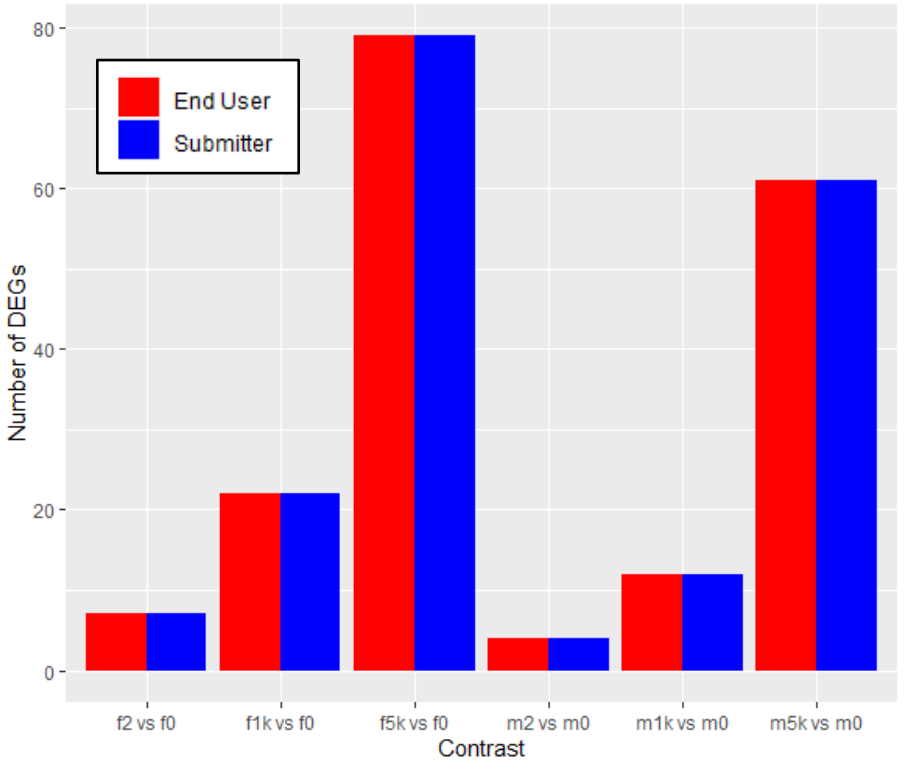
Examples of OORF trials

Platform	Study Description	Module Tested	Computing Environment	Submitter	End User	Status
Agilent Microarray	Four-point concentration-response of furan in male and female Fisher rat liver (GEO GSE62805)	**DAM** (Custom workflow)	R	Andrew Williams (Health Canada)	Leah Wehmas (US EPA)	Complete
RNA-Seq	Three-point concentration-response of hexabromo-cyclododecane in male and female Fisher rat liver (PRJNA395549)	**DAM** (ODAF)	R	Matt Meier (Health Canada)	US EPA	Complete
		BMD (BMDExpress)	R	Andrew Williams (Health Canada)	Sarah Davidson (US EPA)	Complete
		EARM (Reactome)	R	Andrew Williams (Health Canada)	John Stead (Carleton University)	Complete
RNA-Seq	Japanese quail exposed to trenbolone (0, 1 ppm, and 10 ppm; unpublished data)	**DAM** (Eco-Omics Analyst)	Web-based tools	Krittika Mittal (McGill University)	John Martinson (US EPA)	In process
Microarray	Doxorubicin treatment in mice (3 mg/week for 8 wks; n=4)	**EARM** (Ingenuity Pathway Analysis)	Desktop software	Vikrant Vijay (US FDA)	Eunnara Cho (Health Canada)	In process
qPCR	African clawed frog exposed to benzo[a]pyrene (16.6 and 50 µg/L)	**qPCR** Module (EcoToxXplorer)	Web-based tools	Doug Crump (Environment Canada)	Jacob Collins, Dan Villeneuve (US EPA)	In process

Trial results: Differentially Abundant Molecules DARM

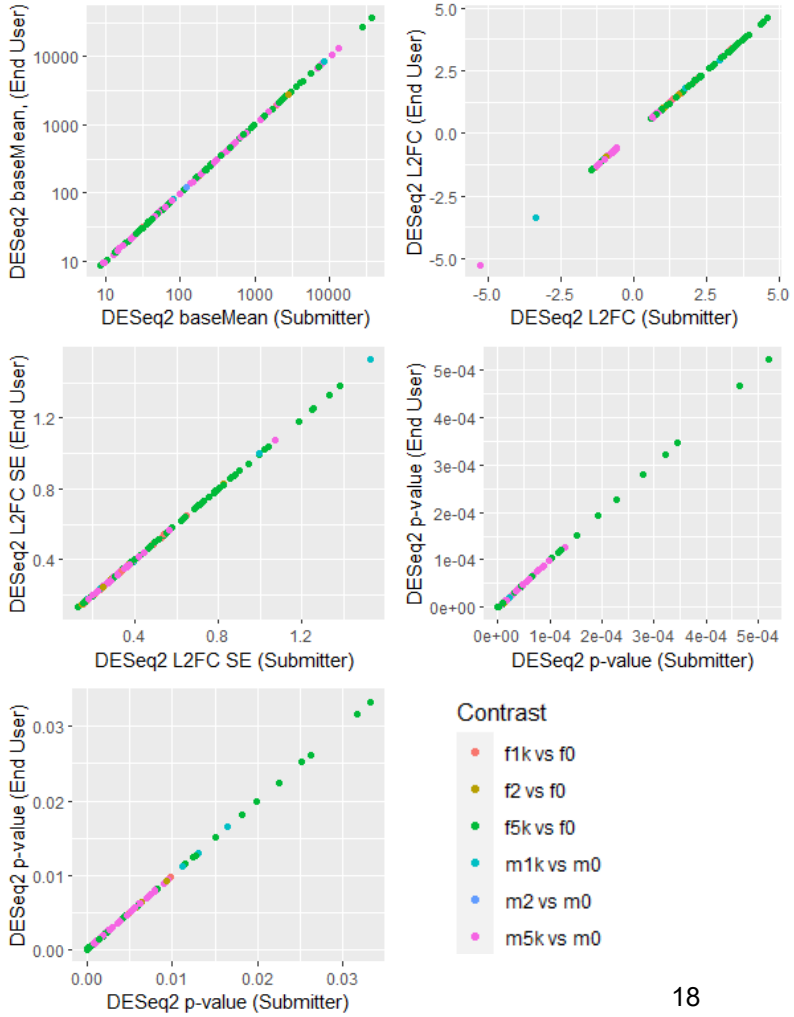


Number of Differentially Expressed Genes



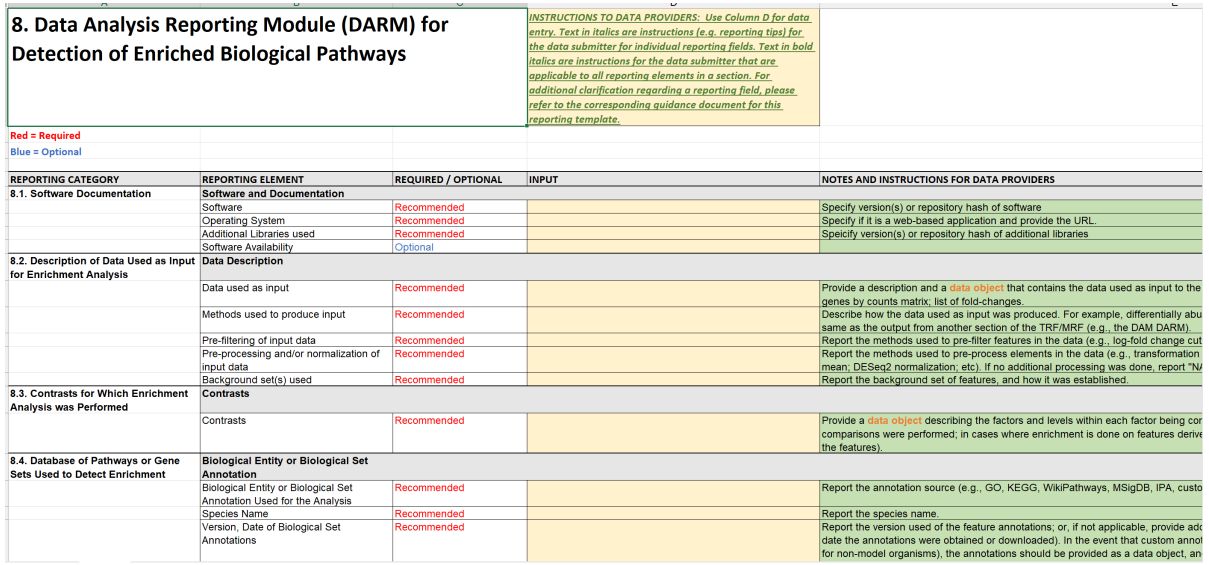
- Highly comparable results from Submitter and End User

Correlation of DESeq2 Outputs



Reporting templates – DARMs – enrichment analysis, in development

- Expert group met to reach consensus on parameter inclusion
  - Participants: Richard Beger, Carole Yauk, Timothy Ebbels, Joshua Harrill, Pim Leonards, Mark Viant, Oliver Schmitz, Magda Sachana, Vikrant Vijay, Andrew Williams
- Module & guidance drafted to capture elements across different enrichment analysis experiments
- Reviewed by experts in metabolomics, proteomics, and transcriptomics
- Conducted trials to test module
- Comments from Omics Expert Group now being addressed



8. Data Analysis Reporting Module (DARM) for Detection of Enriched Biological Pathways

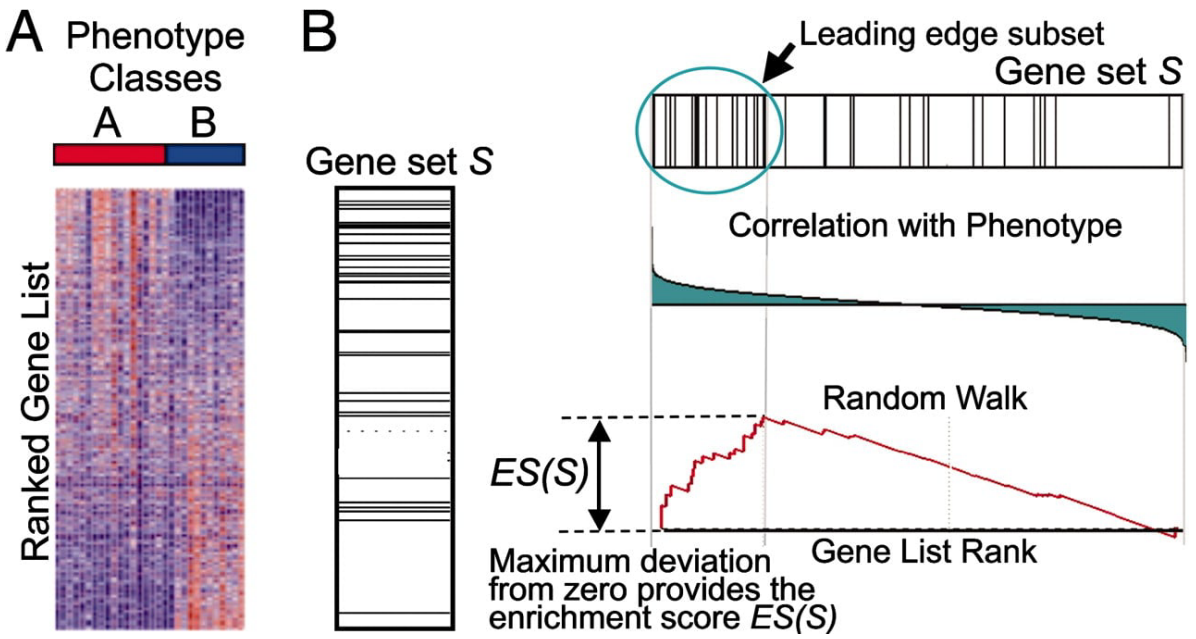
INSTRUCTIONS TO DATA PROVIDERS: Use Column D for data entry. Text in italics are instructions (e.g. reporting tips) for the data submitter for individual reporting fields. Text in bold italics are instructions for the data submitter that are applicable to all reporting elements in a section. For additional clarification regarding a reporting field, please refer to the corresponding guidance document for this reporting template.

Red = Required
Blue = Optional

REPORTING CATEGORY	REPORTING ELEMENT	REQUIRED / OPTIONAL	INPUT	NOTES AND INSTRUCTIONS FOR DATA PROVIDERS
8.1. Software Documentation	**Software and Documentation**			
	Software	Recommended		Specify version(s) or repository hash of software
	Operating System	Recommended		Specify if it is a web-based application and provide the URL.
	Additional Libraries used	Recommended		Specify version(s) or repository hash of additional libraries
	Software Availability	Optional		
8.2. Description of Data Used as Input for Enrichment Analysis	**Data Description**			
	Data used as input	Recommended		Provide a description and a data object that contains the data used as input to the genes by counts matrix; list of fold-changes.
	Methods used to produce input	Recommended		Describe how the data used as input was produced. For example, differentially abu same as the output from another section of the TRF/MRF (e.g., the DAM DARM).
	Pre-filtering of input data	Recommended		Report the methods used to pre-filter features in the data (e.g., log-fold change cut
	Pre-processing and/or normalization of input data	Recommended		Report the methods used to pre-process elements in the data (e.g., transformation mean; DESeq2 normalization; etc). If no additional processing was done, report "N/
	Background set(s) used	Recommended		Report the background set of features, and how it was established.
8.3. Contrasts for Which Enrichment Analysis was Performed	**Contrasts**			
	Contrasts	Recommended		Provide a data object describing the factors and levels within each factor being cor comparisons were performed; in cases where enrichment is done on features derive the features).
8.4. Database of Pathways or Gene Sets Used to Detect Enrichment	**Biological Entity or Biological Set Annotation**			
	Biological Entity or Biological Set Annotation Used for the Analysis	Recommended		Report the annotation source (e.g., GO, KEGG, WikiPathways, MSigDB, IPA, custo
	Species Name	Recommended		Report the species name.
	Version, Date of Biological Set Annotations	Recommended		Report the version used of the feature annotations; or, if not applicable, provide ad date the annotations were obtained or downloaded). In the event that custom annot for non-model organisms), the annotations should be provided as a data object, an

Development of Enrichment Analysis DARM (transcriptomics point of view)

- **Features** to be tested for enrichment could be derived from many sources:
  - DEGs
  - Fold changes in expression
  - Gene clusters (e.g., WGCNA modules)
  - Dose-responsive genes
- Common **Statistical Methods**
  - GSEA (Gene Set Enrichment Analysis)
  - ORA (Over-Representation Analysis)
- Common **Databases**
  - Reactome
  - WikiPathways
  - MSigDB
  - CLUE (CMap and LINCS Unified Environment)
  - GO
  - KEGG
  - IPA (Ingenuity pathway analysis: proprietary database and software, Qiagen)



OORF trial case study

Trial module: enrichment analysis DARM

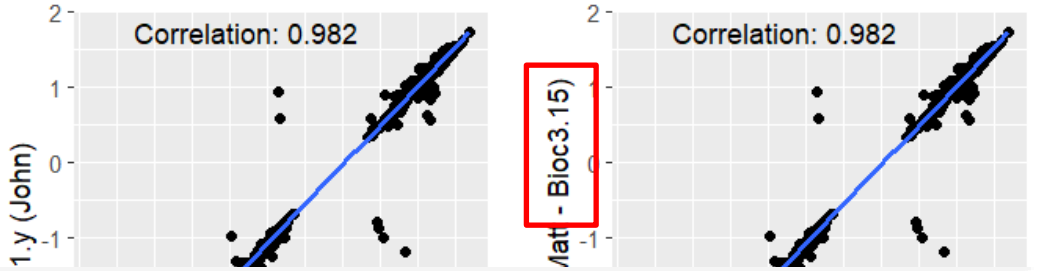
Topic: hexabromocyclododecane (HBCD) exposure dataset with RNA-Seq results (male and female rat liver, PRJNA395549)

Data submitter: Andrew Williams produced a GSEA analysis using the *fgsea* R package

End user: John Stead (Carleton University) reproduced the analysis

Challenges in trials

- GSEA: Andrew provided code alongside completed EA DARM and raw data
  - John successfully ran the code
  - Result: tables of normalized enrichment scores (NES) for all Reactome pathways



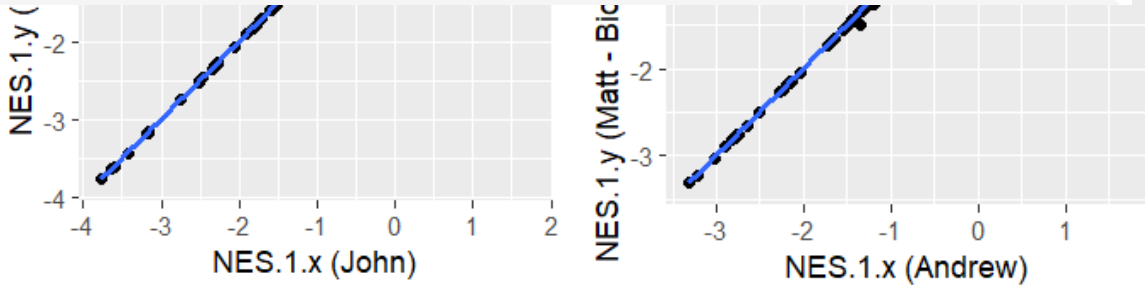
I work in an analysis core facility, and we often need to re-run old analyses. So we have R installs going back to R-3.5.0 or so, along with the associated library dirs. And I am old and stuff, so I use emacs/ESS with various scripts so M-x R will start the correct R version. So it's easy for me to do small additional things to existing analyses without having to update entirely. Which is good, primarily for annotations, because people don't like it when their results change. Genes come into and out of existence with some regularity, and losing the top gene just because NCBI no longer thinks it's a thing is hard to explain to some people.

0

ADD REPLY • link

2.4 years ago James W. MacDonald 64k

- The main determinant in this example turned out to be the reactome.db version: **however – big caveat – the version used by Andrew (1.79, correctly reported in the reporting module) was no longer available! Anywhere!!**



Lessons learned from OORF trials: analysis reproducibility

Analyses in **open-source computing environments** (R, Python, etc.)

- Reproducibility depends less on reporting fields and more on code/scripts (versions still important)
- Issue: users may not have sufficient expertise with open-source computing environments (easier for end users with coding skills to reproduce)
- No financial or licensing barriers for accessing tools

Analyses using **freeware analysis software or web applications** (BMDExpress)

- Reproducibility depends on clear and precise reporting in the OORF documentation and/or a configuration file the end user could follow
- More user friendly and require less technical/statistical expertise compared to open-source computing
- No “pay wall”

Analyses using **proprietary software** (Partek, Ingenuity, etc.):

- Reproducibility depends on clear and precise reporting in the OORF documentation and/or a configuration file the end user could follow
- End user needs access to the same software (and version)
- “Pay wall” issues

Lessons learned from OORF trials: big picture

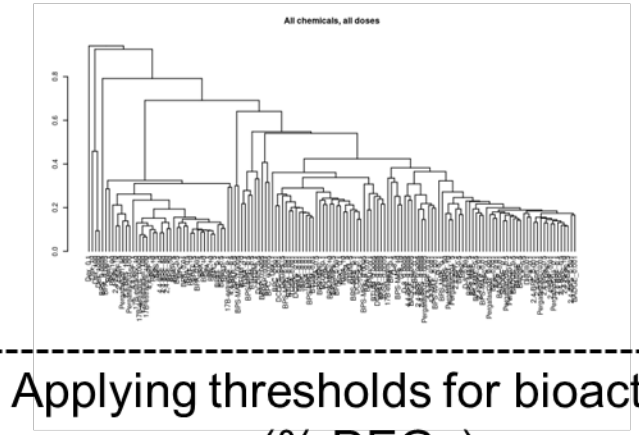
- Trials have demonstrated where clarifications/revisions in the OORF were needed
- High degree of concordance observed in the trial results (i.e., the framework works)
  - **Minor differences** in pipelines that implement permutation analyses (expected but not major differences), and different database versions (also expected)
  - **Differences would not change study conclusions**

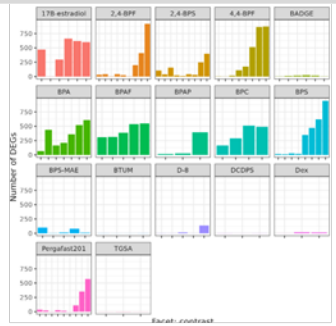
- Challenges include finding submitters and end-users that have access to, or are willing to apply, the same software or pipeline
  - Paywall issues with some software
  - Complexity of using other people's pipelines
    - GitHub repositories and Omics Data Analysis Framework for Regulatory Application (R-ODAF) or EPA's httrpl are solutions

- Experience with regulatory partners demonstrates utility for increasing transparency and reproducibility of the omics analyses

Application Reporting Module case study: chemical grouping using gene expression data for a common group of chemicals (BPA and alternatives)

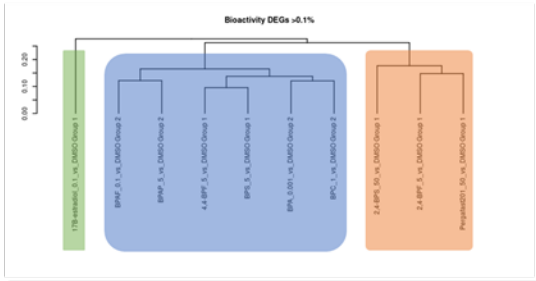
- Qualitative Analysis:
  - *Bioactivity profile-based grouping* using the complete set of features (top figures)
  - Established thresholds to focus on chemicals with demonstrated bioactivity after exposure
- Grouping Analysis:
  - *Bioactivity profile-based grouping* using a subset of pre-filtered differentially expressed genes
  - *Omics signature-based grouping* using subset of genes from a published estrogen receptor biomarker
- The methods, approaches, and results are being used to develop and trial the reporting module

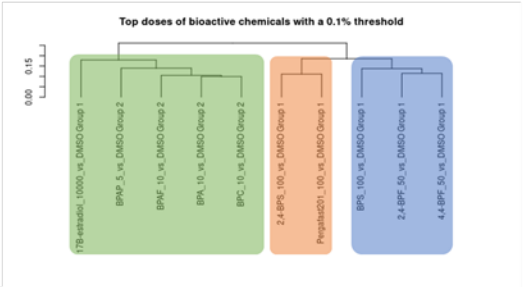




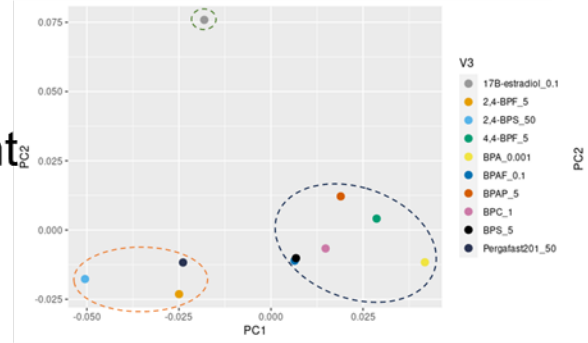
Applying thresholds for bioactivity (% DEGs)

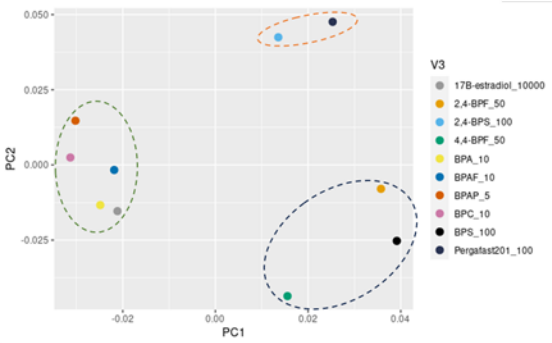
Hierarchal Cluster Analysis (HCA)





Principal Component Analysis (PCA)



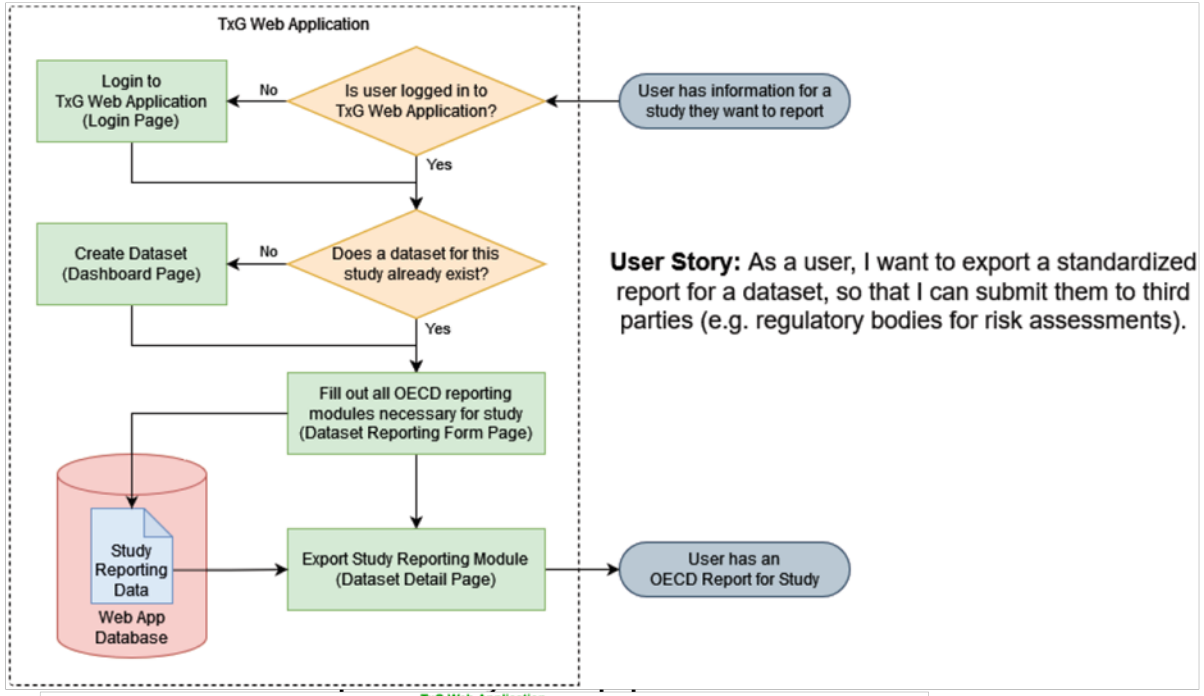


Lowest Conc.

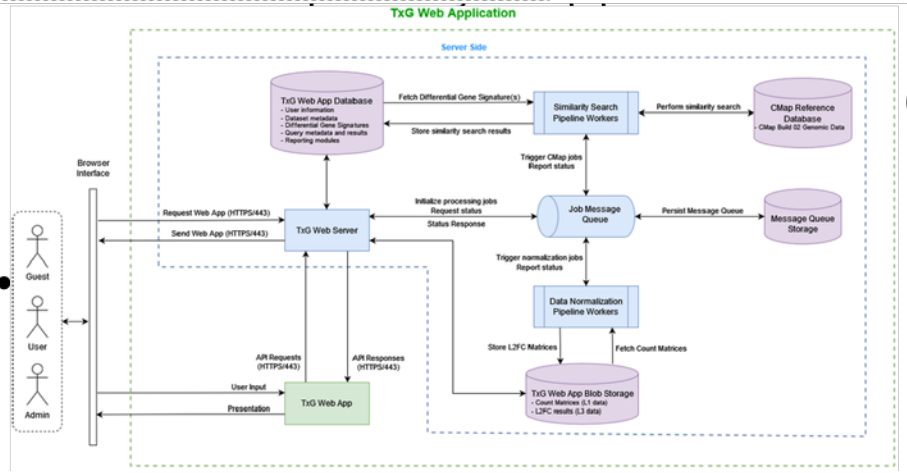
Highest Conc.

Mark Viant & Anthony Reardon

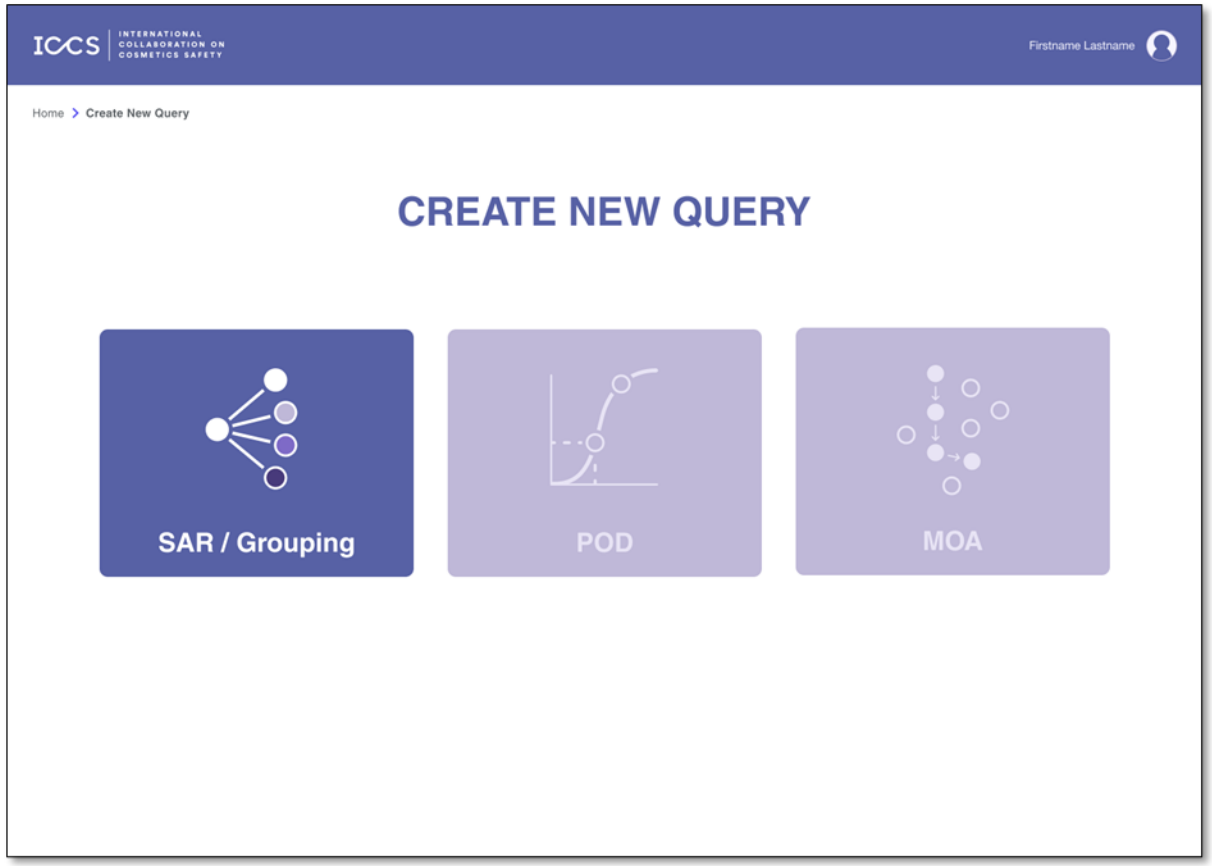
ICCS Toxicogenomics Web Application



User Story: As a user, I want to export a standardized report for a dataset, so that I can submit them to third parties (e.g. regulatory bodies for risk assessments).



data and



Future of the OORF: the “last mile” of NAMs?

- Reporting module for ‘omics biomarkers/pattern matching
  - Outcome from discussion at International Workshop on Genotoxicity Testing (IWGT) in Ottawa (2022), Working group on “TGx-Biomarkers that Predict Genotoxicity”
- OECD Omics website provides a resource for sharing and disseminating the OORF
  - https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/omics-technologies-chemical-testing.html
- Application Reporting Modules (ARMs)
  - Development and trialling of ‘Chemical Grouping’ ARM adopted into WPHA work plan in March 2022
- Revise and update as required
  - It would be valuable to harmonize the format of the OORF into fully machine-readable schema
- Health Canada is aiming for better interoperability with international agencies for consistency in analytical approaches used for transcriptomics
- The OORF contributes guidance and a framework, key components for getting NAMs implemented in practice
  - Case studies, training, and implementation are the other key components for omics use in decision-making
  - A remaining challenge is encouraging regulators to request a filled OORF where omics experiments may have been done (solution: make it easier for people to fill out, e.g., ICCS web application; education on use – that is, teach about modular nature and re-useability of components)

Collaborators and Acknowledgements

University of Ottawa

Dr. Carole Yauk
The Genomics in Regulatory and Applied Toxicology Laboratory

Health Canada, PSD

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Health Canada, MSD

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Maastricht University

Dr. Florian Caiment

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Sam Reed

Scott Fasone

Kelly Semrau

ICCS TxG WG

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Chemicals Management Plan

Regulatory partners - Health Canada

NSERC

Canada Research Chairs Program

Health Canada Santé Canada

GENOMICS R&D INITIATIVE

United States Environmental Protection Agency

STATE OF SCIENCE ON DEVELOPMENT AND USE OF NAMS FOR CHEMICAL SAFETY TESTING
Research Triangle Park, NC

Recent Developments in High-Throughput Toxicokinetics

John Wambaugh, Barbara Wetmore, Caroline Ring, Sarah Davidson-Fritz, Gilberto Padilla Mercado, Meredith Scherer, Sahar Tabatabaei Sadeghi, Colin Thompson, Rachael Cogbill, Risa Sayre, Rogelio Tornero-Velez, Elaina Kenyon, Marina Evans, Kimberly Truong, Taylor Wall, Katie Paul Friedman, Michael Devito, and Russell Thomas

Center for Computational Toxicology and Exposure
U.S. Environmental Protection Agency

November 6, 2024

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Estimating Points of Departure (PODs) using New Approach Methods (NAMs)

- *In vitro-in vivo* extrapolation (IVIVE) allows estimation of chemical-specific Points of Departure (PODs) based on new approach methods (NAMs)

***In Vitro* Measured Bioactive Point of Departure ($POD_{in\ vitro}$)** **µM**

Paul Friedman et al. (2020)

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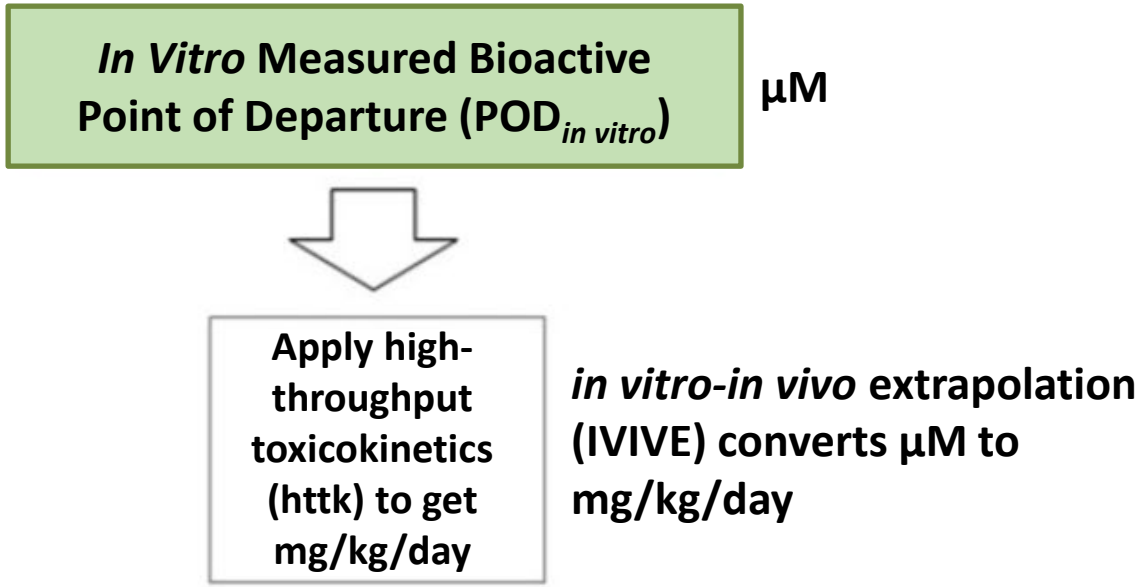

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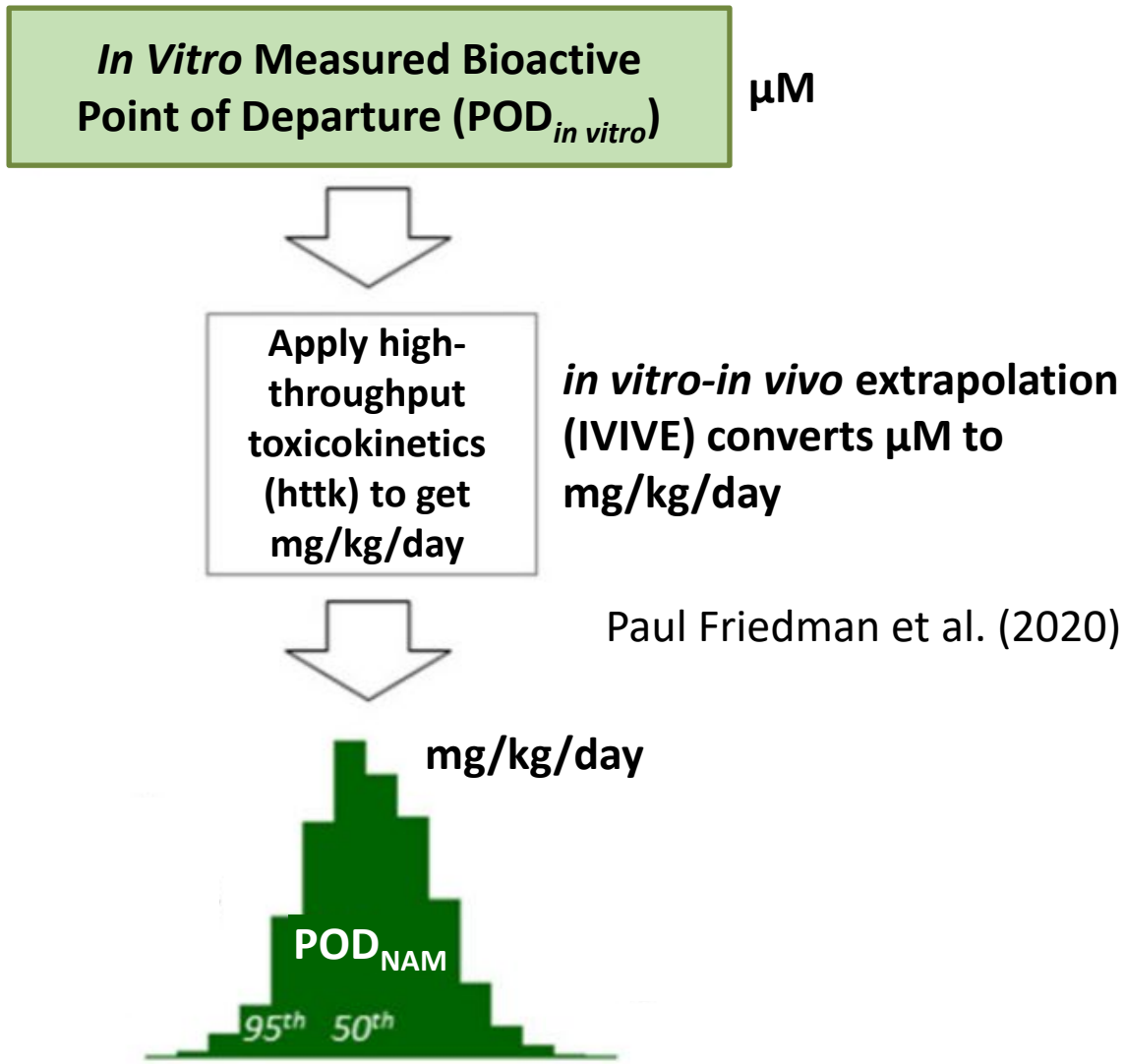


Distribution of POD_{NAM} for a single chemical

Estimating Points of Departure (PODs) using New Approach Methods (NAMs)

- *In vitro-in vivo* extrapolation (IVIVE) allows estimation of chemical-specific Points of Departure (PODs) based on new approach methods (NAMs)

***In Vitro* Measured Bioactive Point of Departure ($POD_{in\ vitro}$)** **µM**

↓

Apply high-throughput toxicokinetics (httk) to get mg/kg/day

***in vitro-in vivo* extrapolation (IVIVE) converts µM to mg/kg/day**

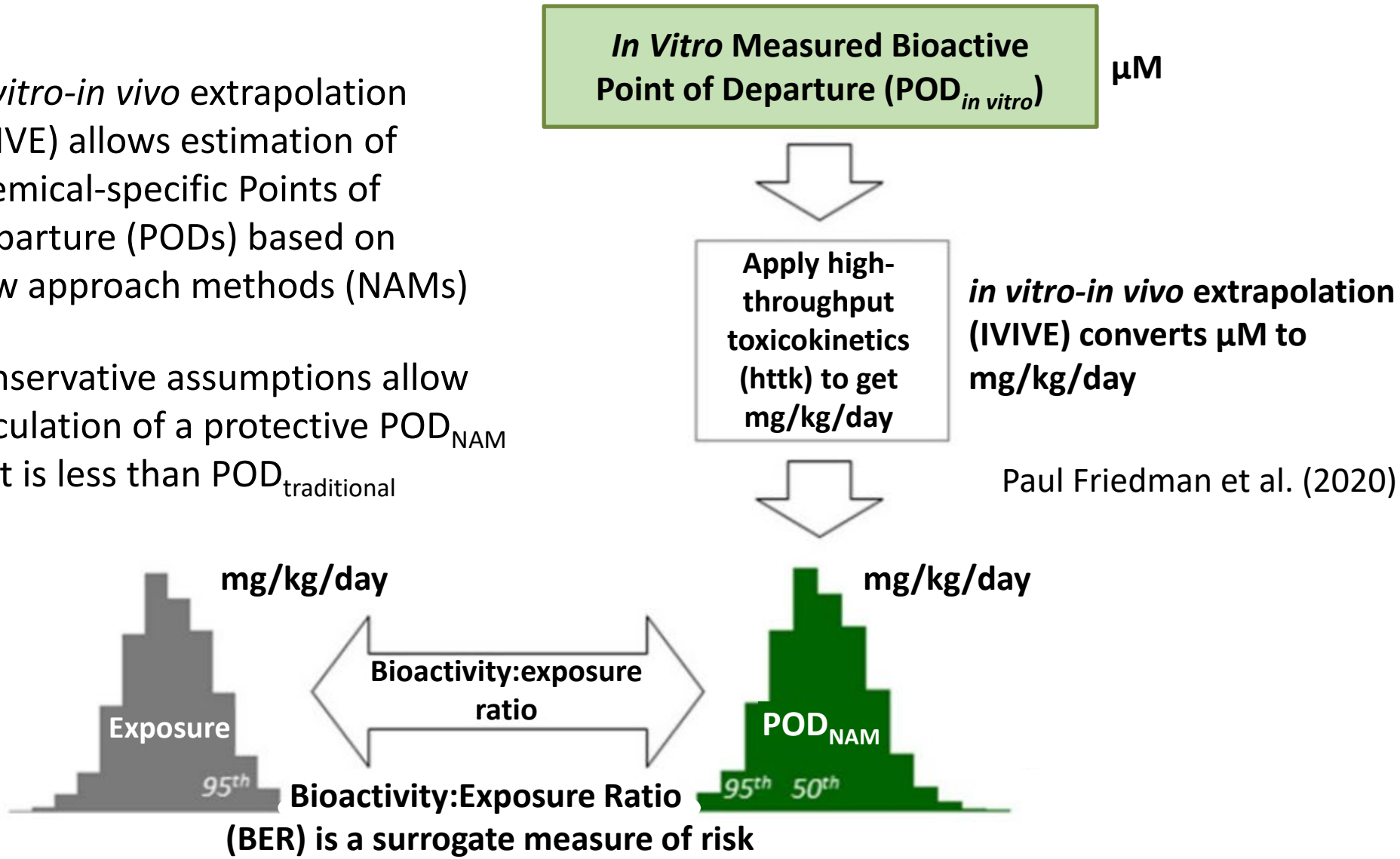
↓

Paul Friedman et al. (2020)

Uncertainty in intake rate for single chemical

Estimating Points of Departure (PODs) using New Approach Methods (NAMs)

- *In vitro-in vivo* extrapolation (IVIVE) allows estimation of chemical-specific Points of Departure (PODs) based on new approach methods (NAMs)
- Conservative assumptions allow calculation of a protective POD_{NAM} that is less than $POD_{traditional}$



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In Vitro Measured Bioactive Point of Departure ($POD_{in\ vitro}$) **µM**

Apply high-throughput toxicokinetics (httk) to get mg/kg/day

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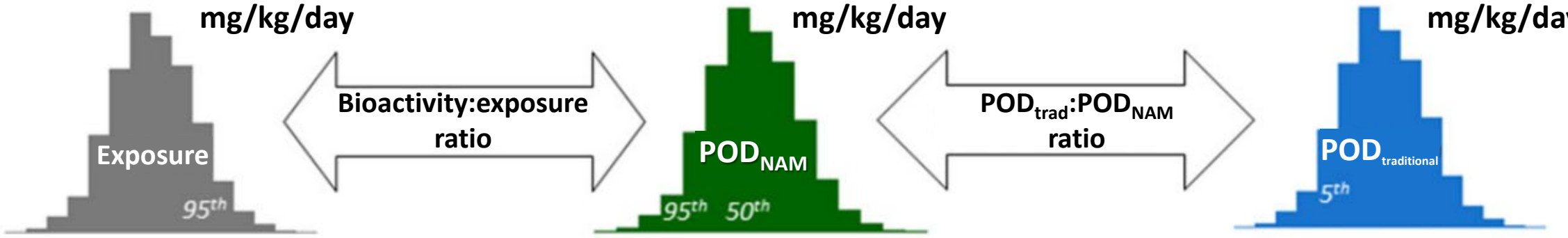
Paul Friedman et al. (2020)

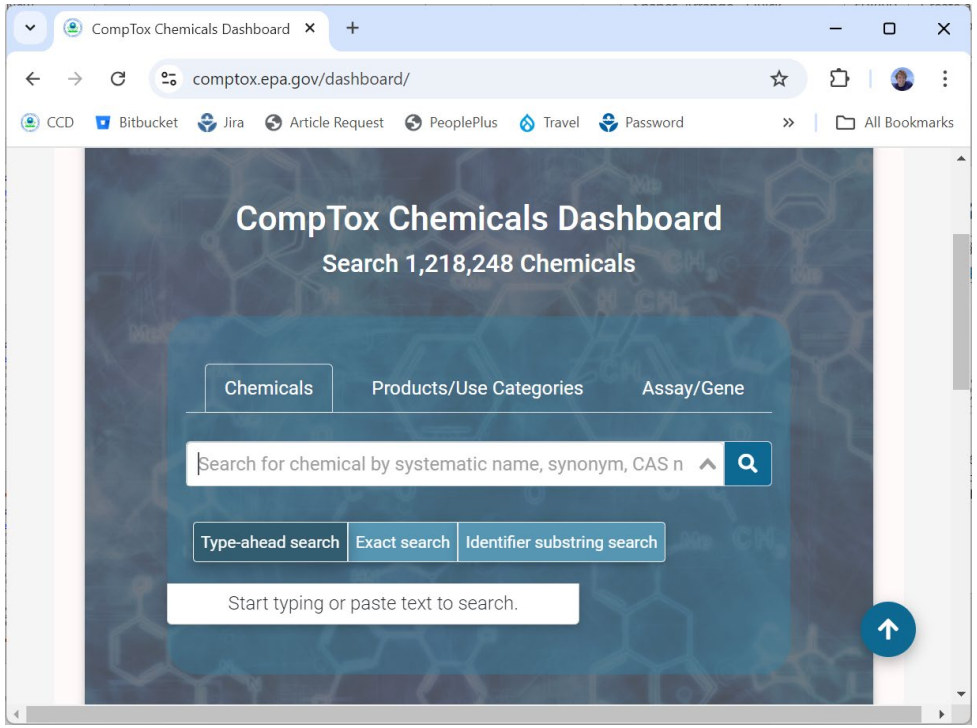


Distribution of $POD_{traditional}$ for a single chemical

Standardized NAM Data and Tools

https://comptox.epa.gov/dashboard

- **Hazard (ToxCast/Tox21):** There are nearly 10,000 chemicals with *in vitro* bioactivity data
- **Exposure:** There are more than 400,000 chemicals with “exposure forecasts” (ExpoCast)
- **Dose-Response:** There are currently 7,569 chemicals with high throughput toxicokinetics (**HTTK**) data/predictions (including C_{ss}, V_d, t_{half})



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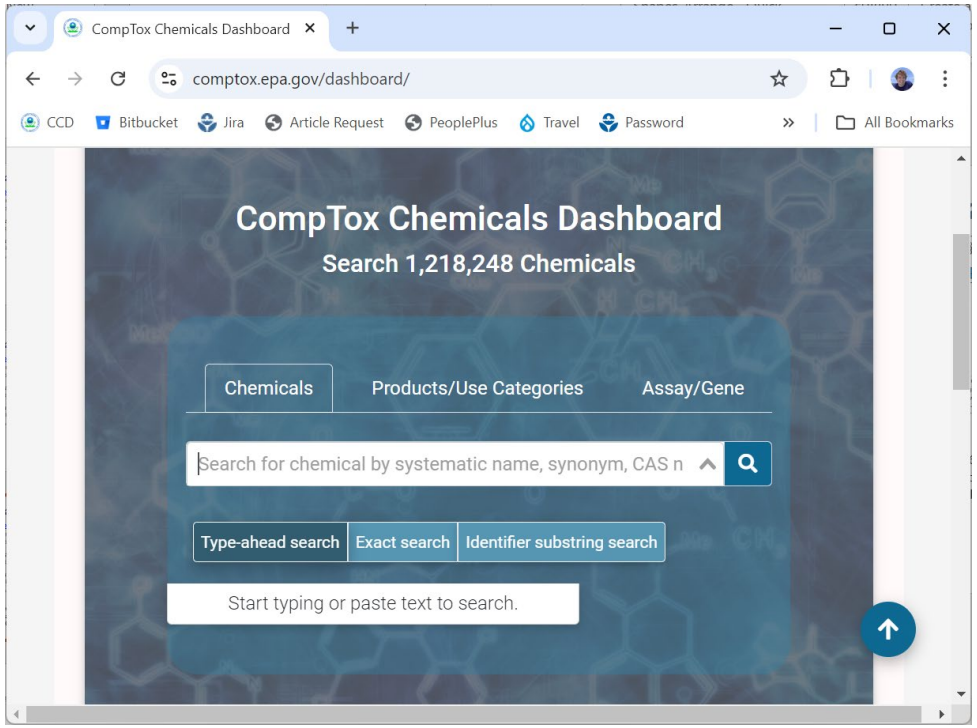
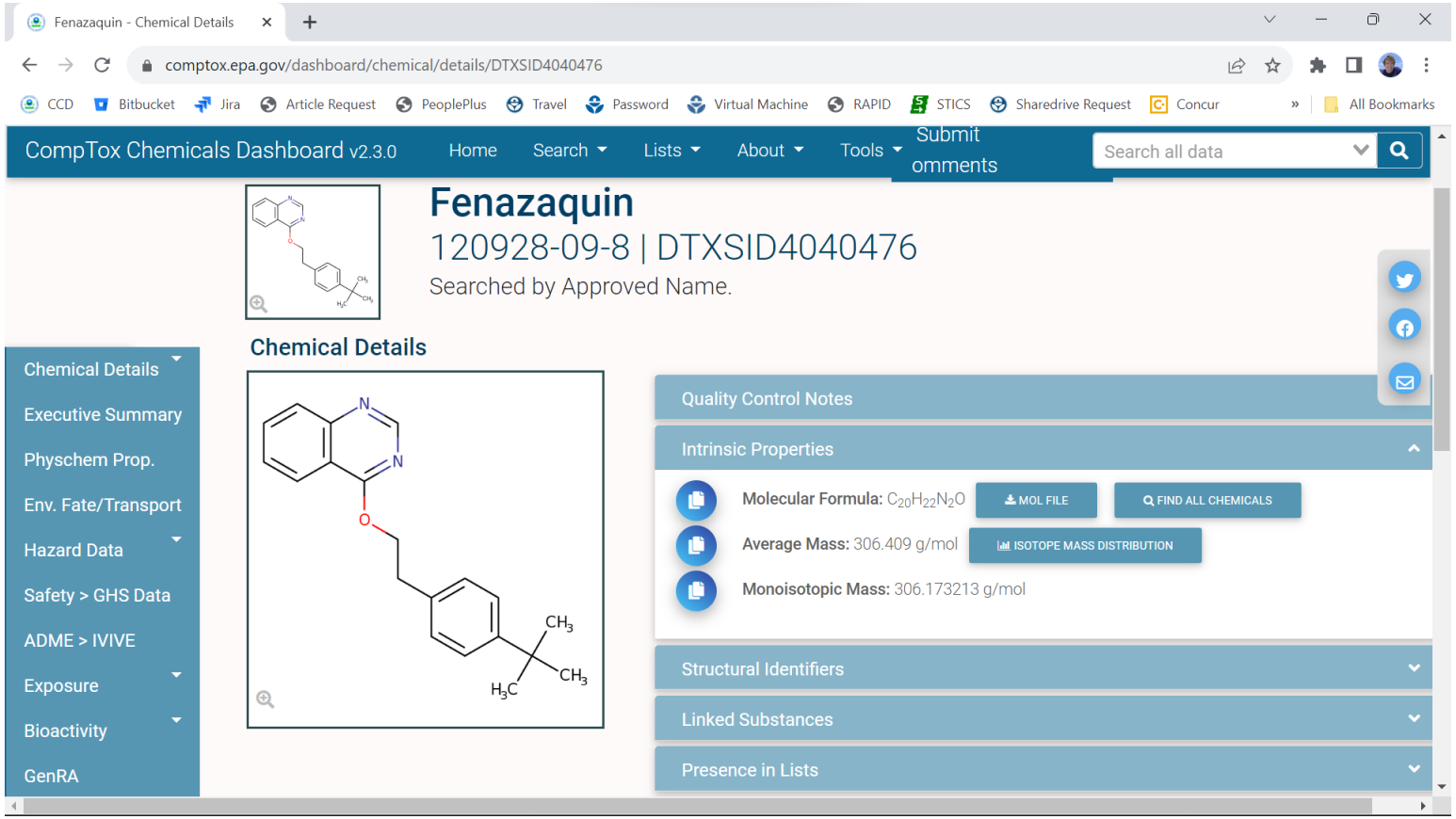


HTTK is the combination of chemical-specific *in vitro* TK data and generic physiologically-based TK models (Breen et al., 2021)

Openly Available TK Information

- EPA's data and tools for HTTK are made available through R package "httk"
- The "httk" tool has been used to calculate key TK information that is available on the CompTox Chemicals Dashboard and elsewhere



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The current HTTK data in CCD is HTTK v2.2.1. Please see the Data Sources table in the Release Notes for more information

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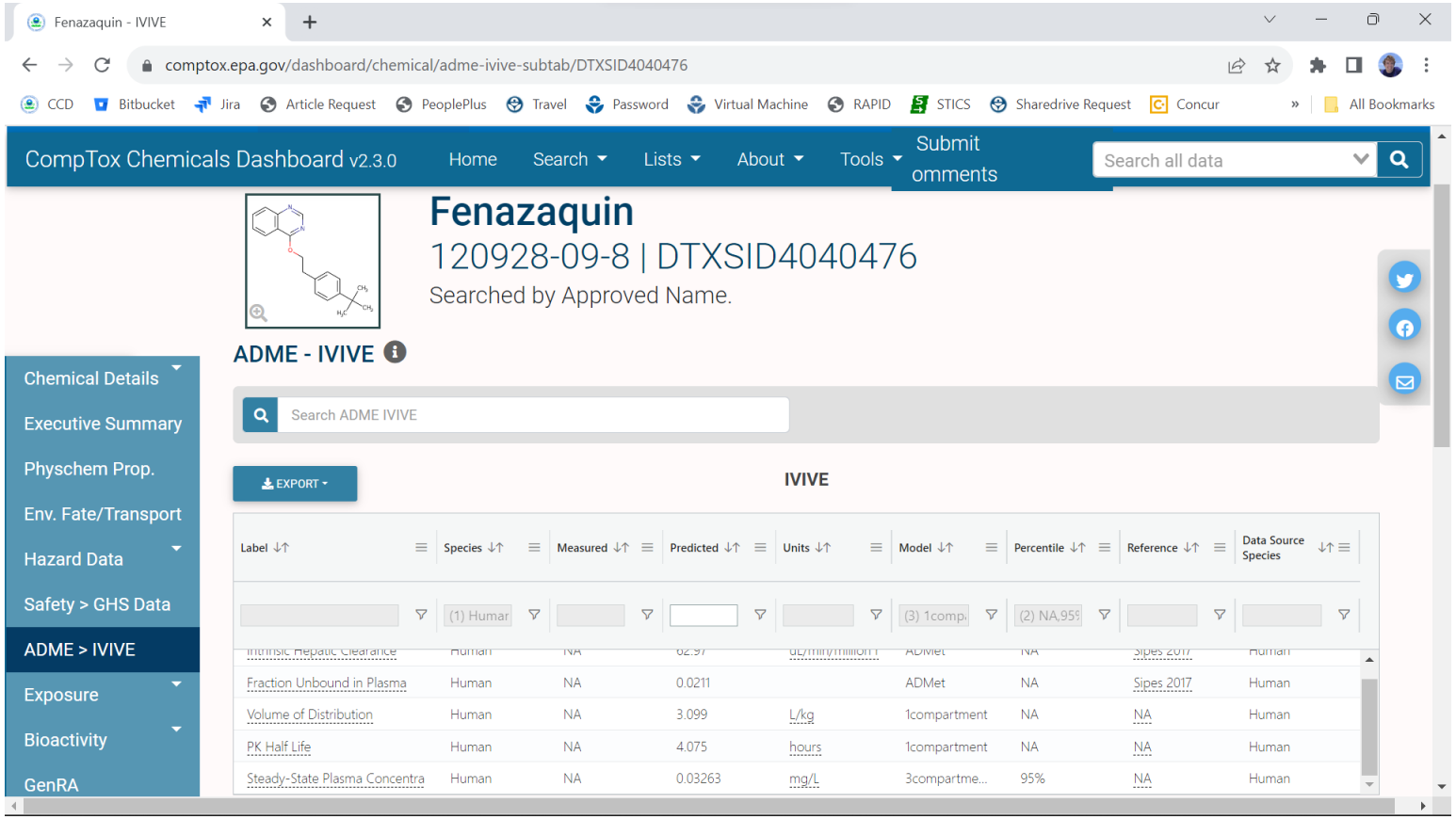

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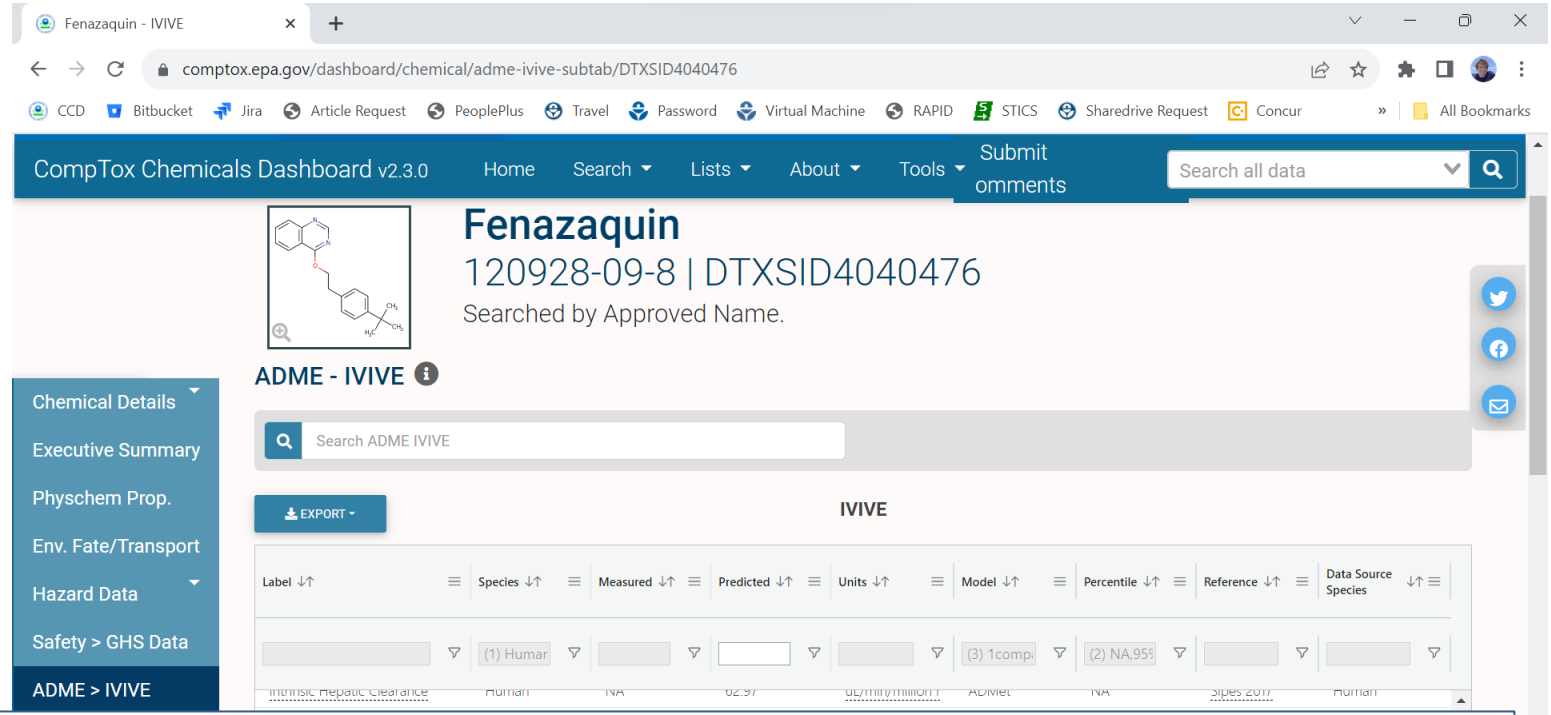

V_d	Volume of Distribution	Human	NA	3.099	L/kg	1compartment	NA	NA	Human
t_{half}	PK Half Life	Human	NA	4.075	hours	1compartment	NA	NA	Human
C_{ss}	Steady-State Plasma Concentra	Human	NA	0.03263	mg/L	3compartme...	95%	NA	Human

https://comptox.epa.gov/dashboard

The current HTTK data in CCD is HTTK v2.2.1. Please see the Data Sources table in the Release Notes for more information

IVIVE by Scaling Factor

- There are many approaches to IVIVE, but we choose a relatively simple one:
- We make various assumptions that allow conversion of an *in vitro* concentration $[\boldsymbol{X}]$ (µM) into an **administered equivalent dose** (AED) with units of mg/kg body weight/day:

$$\mathbf{AED} = \boldsymbol{F_{IVIVE}} \times [\boldsymbol{X}]$$

- **AED** is the **external dose rate** that would be needed to **produce a given steady-state plasma concentration**
- F_{IVIVE} is a scaling factor that varies by chemical

HTTK can predict F_{IVIVE}

IVIVE by Scaling Factor

"httk" predicts IVIVE scaling factors using probabilistic methods that account for human variability and measurement uncertainty

- For a given chemical, $\mathbf{F_{IVIVE} = 1 / C_{ss,95}}$
- $C_{ss,95}$ is the steady-state plasma concentration resulting from a 1 mg/kg/day exposure
- HTTK can predict $C_{ss,95}$ using "reverse dosimetry" IVIVE (Tan et al., 2007), leading to an oral equivalent dose (OED):

$$\mathbf{OED_{95}} = \frac{[\boldsymbol{X}]}{\boldsymbol{C_{ss,95}}}$$

- The "95" refers to the upper 95th percentile – due to human variability and measurement uncertainty there are a range of possible C_{ss} values

Means of Obtaining HTTK Scaling Factors

- EPA's R package "httk" (targeting bioinformatics community) (Pearce et al., 2017) https://CRAN.R-project.org/package=httk
- CompTox Chemicals Dashboard (in use by US EPA) (Williams et al., 2017) https://comptox.epa.gov/dashboard/
- SimCYP SimRFlow Tool (in use by EU-ToxRisk) (Khalidi et al., 2022) https://www.certara.com/software/simcyp-pbpk/
- NICEATM Integrated Chemistry Environment (in use by US NTP) (Bell et al., 2020) https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-ivive/ivive
- TKPlate (in use by EFSA) (Dorne et al., 2023) https://zenodo.org/record/2548850

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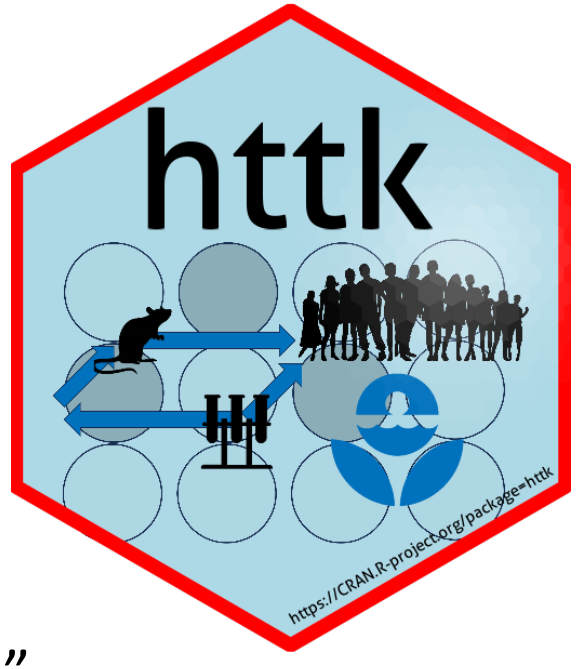
- SimCYP SimRFlow Tool (in use by EU-ToxRisk) (Khalidi et al., 2022) https://www.certara.com/software/simcyp-pbpk/
- NICEATM Integrated Chemistry Environment (in use by US NTP) (Bell et al., 2020) https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-ivive/ivive
- TKPlate (in use by EFSA) (Dorne et al., 2023) https://zenodo.org/record/2548850

All these tools make use of data/models from EPA's open-source "httk" package

Recent Updates to EPA "httk" Package

- Updated "httk-pop" human variability simulator to reflect most recent NHANES cohorts (Breen et al., 2022)
- Developed human gestational exposure model (Kapraun et al., 2022)
- Measured *in vitro* gut permeability data using Caco-2 cell-line for non-pharmaceuticals
  - Values allow prediction of chemicals that are poorly absorbed orally (Honda et al., 2024)
- EPA now analyzing *in vitro* TK data with upcoming tool "invitroTKstats"
  - "invitroTKstats" is a standardized workflow that allows auditable and reproducible analysis
  - Analysis estimates chemical-specific measurement uncertainty



https://CRAN.R-project.org/package=httk

Adjusting for Oral Absorption

- *In vitro* Caco-2 measurements (or QPSRs) characterize absorption from the gut
- Administered equivalent dose (AED) depends on predicted steady-state plasma concentration:

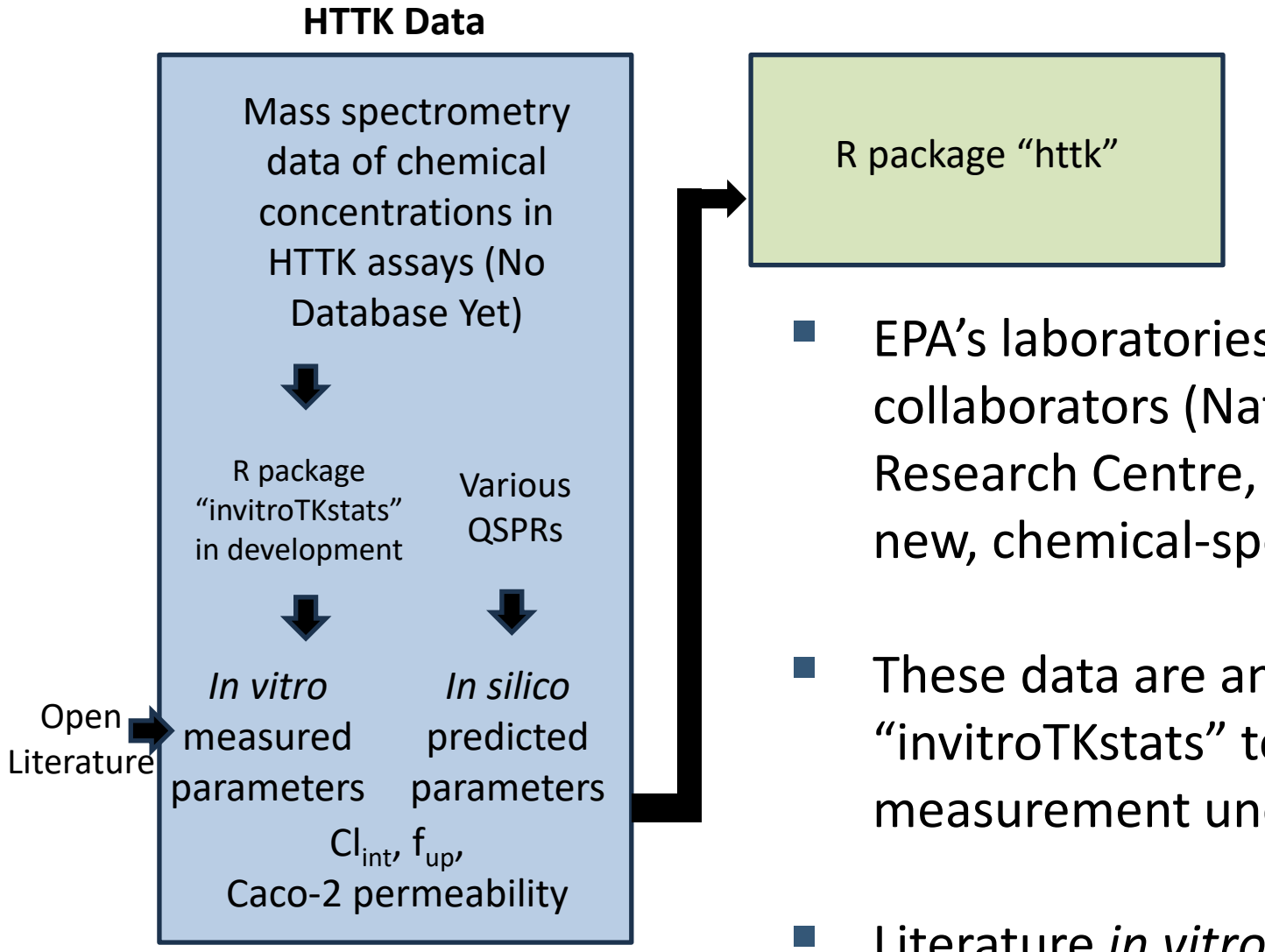
$$\mathbf{OED_{95}} = \frac{[\boldsymbol{X}]}{\boldsymbol{C_{ss,95}}}$$

- Steady-state plasma concentration is proportional to dose and fraction absorbed (F_{abs}):

$$\boldsymbol{C_{ss,95} \sim dose * F_{abs}}$$

- If F_{abs} < 100%, then $C_{ss,95}$ decreases and therefore the necessary AED_{95} increases

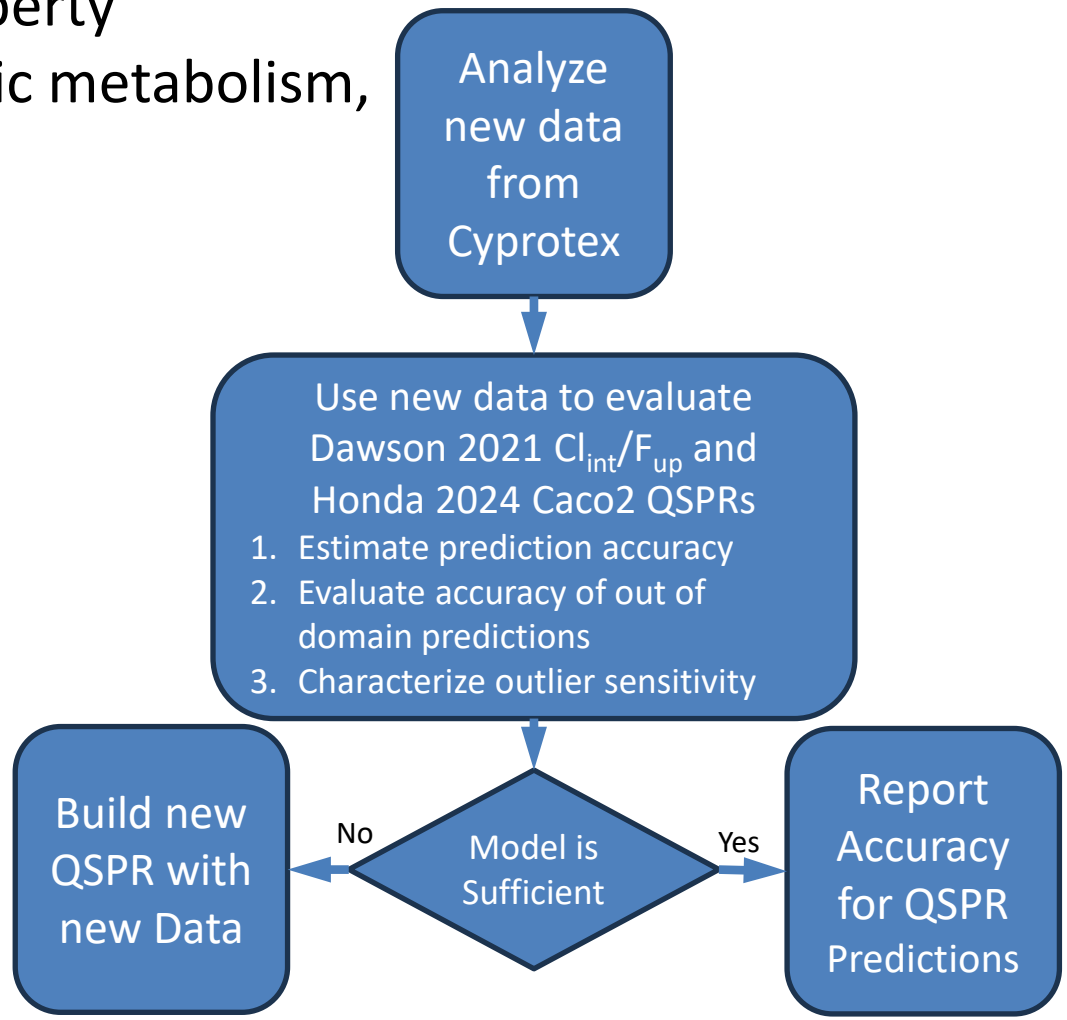
New *In Vitro* TK Measurements



- EPA's laboratories (Wetmore), contractors (Cyprotex), and collaborators (National Toxicology Program, EC Joint Research Centre, Health Canada) continue to generate new, chemical-specific *in vitro* TK measurements
- These data are analyzed with unreleased tool "invitroTKstats" to produce chemical-specific estimates of measurement uncertainty
- Literature *in vitro* TK data curated by ICF

HTTK QSPRs

- Machine-learning based quantitative structure-property relationship (QSPR) models now available for hepatic metabolism, plasma protein binding, and Caco-2 (Dawson et al., 2021, Honda et al., 2024)
- Model domains of applicability indicate chemical properties that are consistent with the training set
- Nearly 10,000 predictions based on HTTK QSPRs available on the CompTox Chemicals Dashboard
- New *in vitro* TK measured data being used to establish accuracy of QSPRs and build new models (Tabatabaei Sadeghi, in preparation)



https://github.com/HumanExposure/Dawson-et-al.-2021_Clint_fup_predictions

HTTK Confidence Assessment

Characterizing Confidence

CvTdb
(TK Concentration vs. time database)

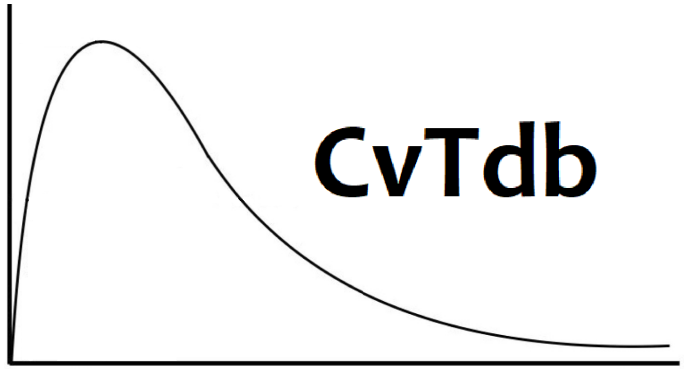
↓

R package "invivoPKfit"

↓

In vivo steady-state concentration (Css), half-life, volume of distribution, days to steady-state

- How well does HTTK predict chemical absorption, distribution, metabolism, and excretion?

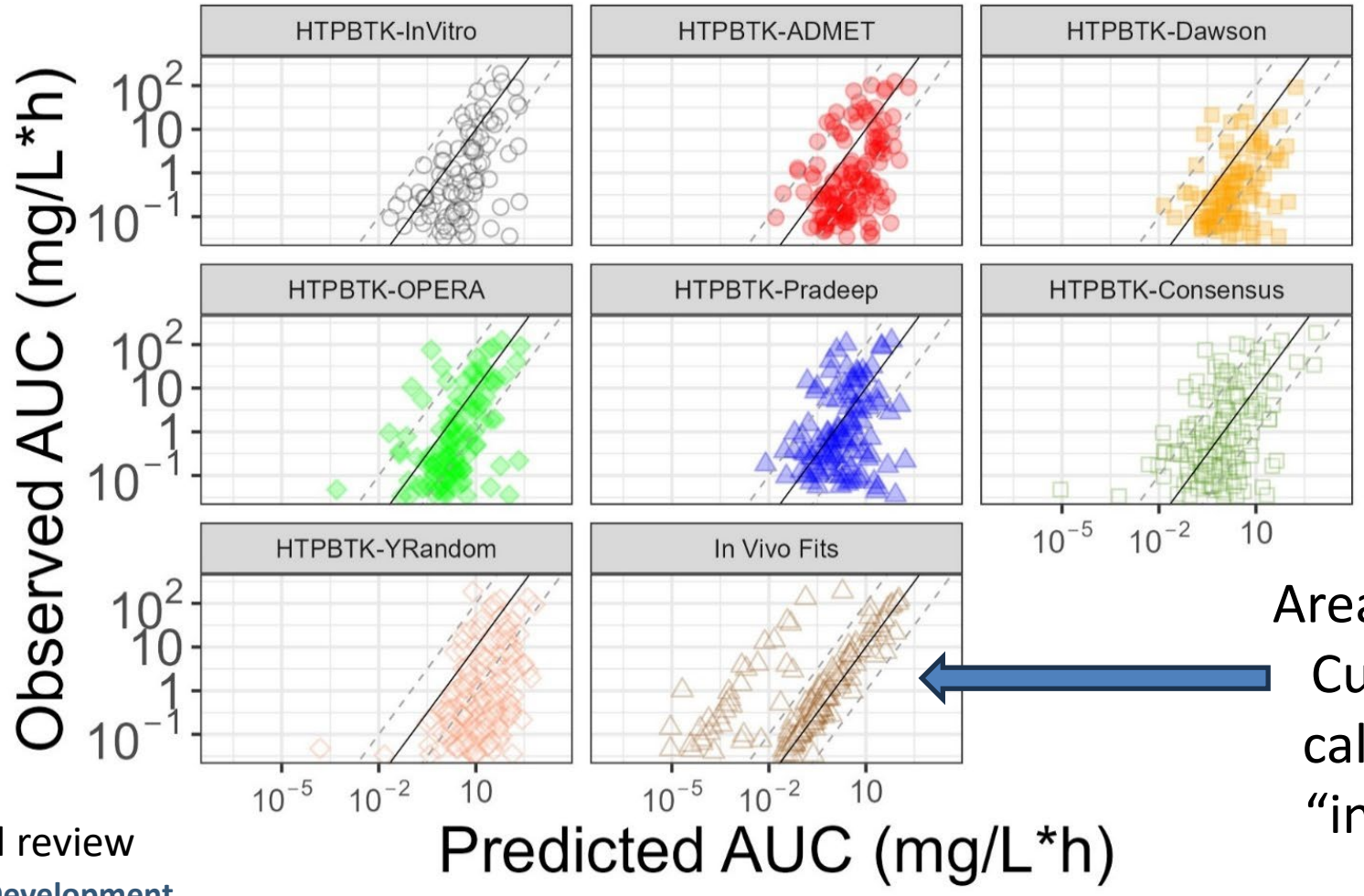


- Toxicokinetic Concentration vs. Time database (CvTdb) contains structured TK data from the peer-reviewed literature (Sayre et al., 2020)
  - EPA actively curating data from publications
  - Collaborators including Showa Pharmaceutical also providing data
- CvTdb data can be analyzed with open-source tool "invivoPKfit" to estimate TK statistics such as half-life and volume of distribution (Padilla Mercado et al., in preparation)

Taylor Wall, Risa Sayre, Caroline Ring, Gilberto Padilla Mercado

HTTK Confidence Assessment

CvTdb combined with "invivoPKfit" allows evaluation of HTTK predictions



Area Under the Curve (AUC) calibrated by "invivoPKfit"

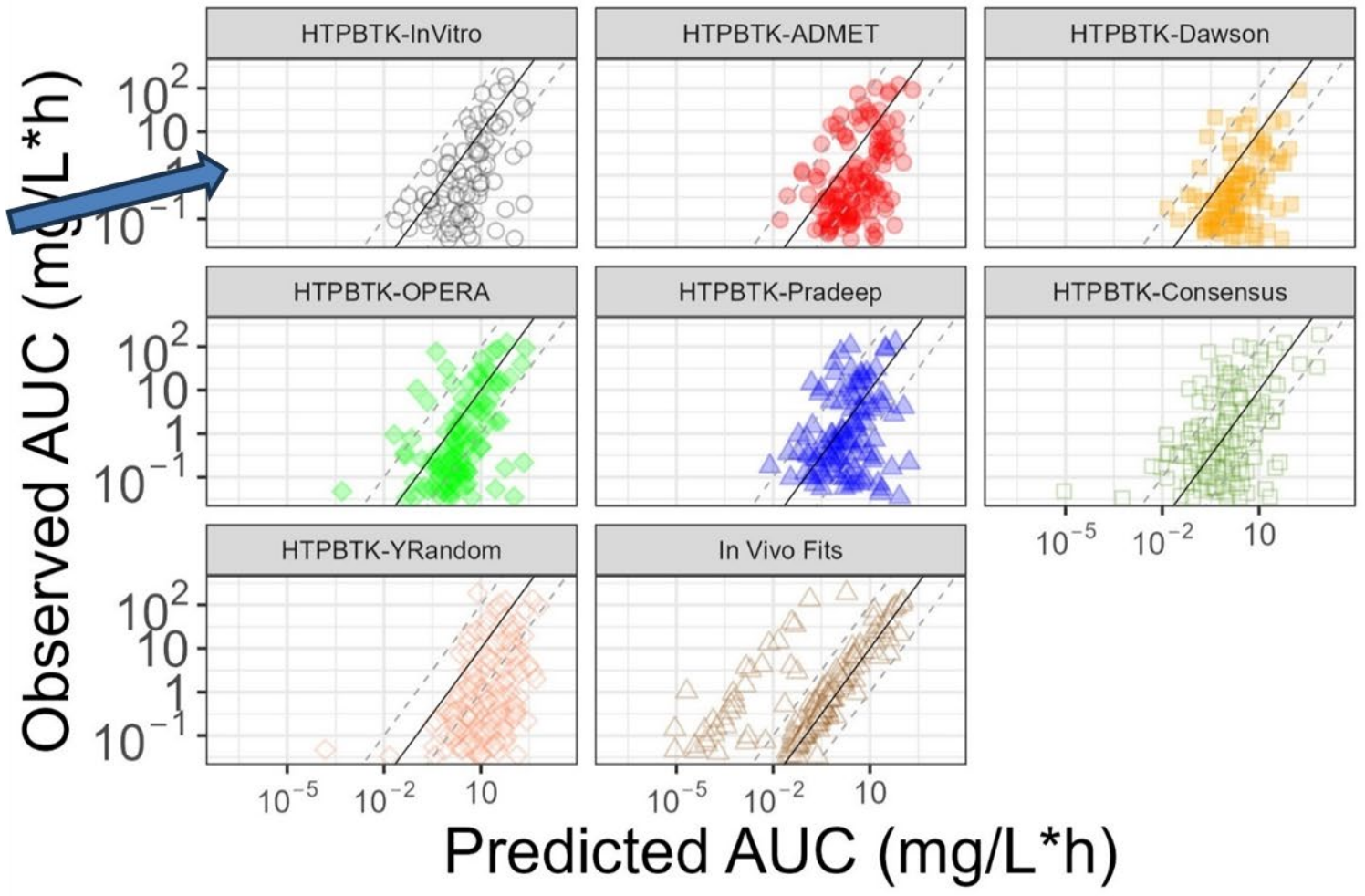
Manuscript under internal review

Gilberto Padilla Mercado, Rogelio Tornero-Velez, Taylor Wall, Risa Sayre, Caroline Ring

HTTK Confidence Assessment

CvTdb combined with "invivoPKfit" allows evaluation of HTTK predictions

Predictions based on *in vitro* TK measurements



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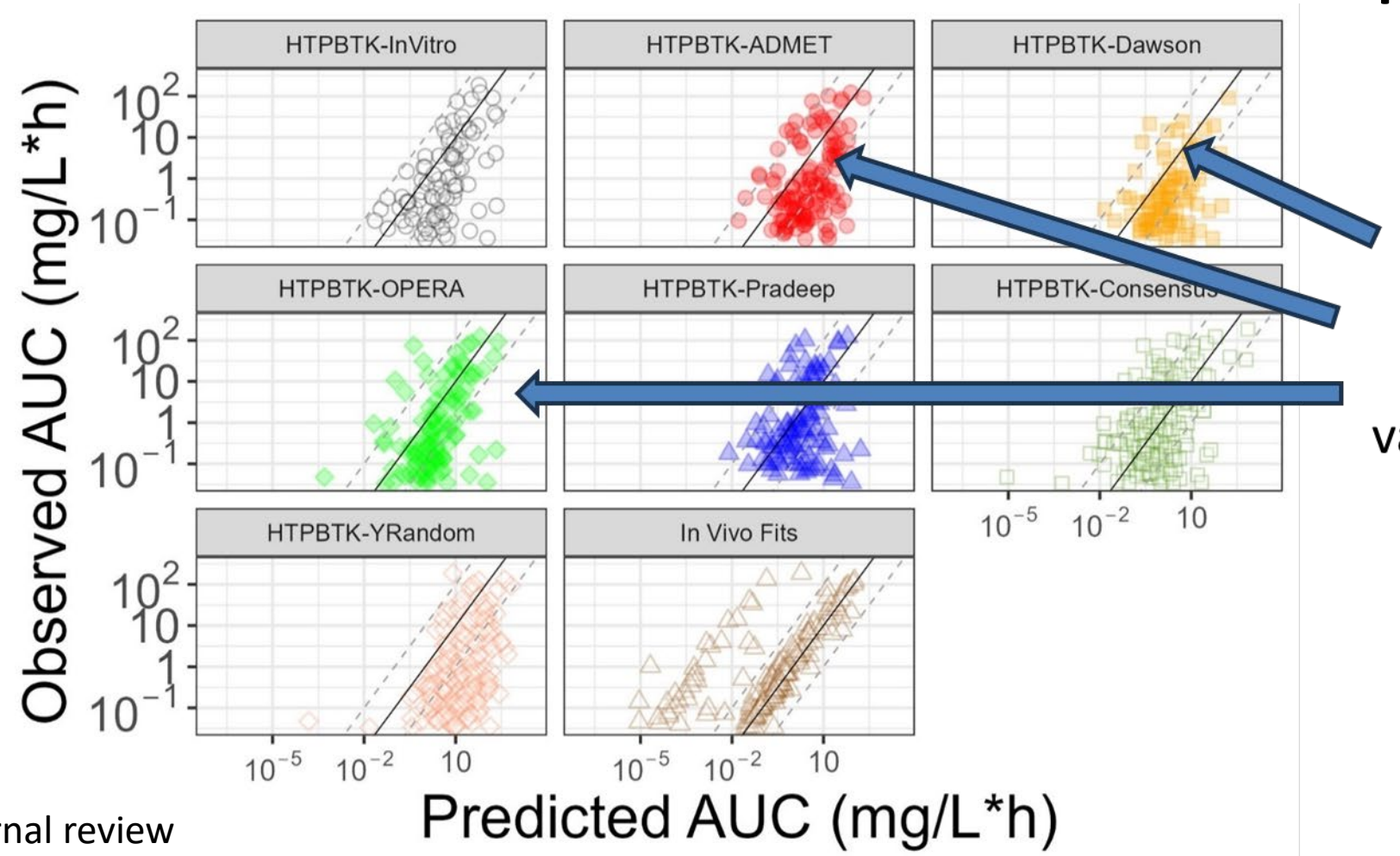

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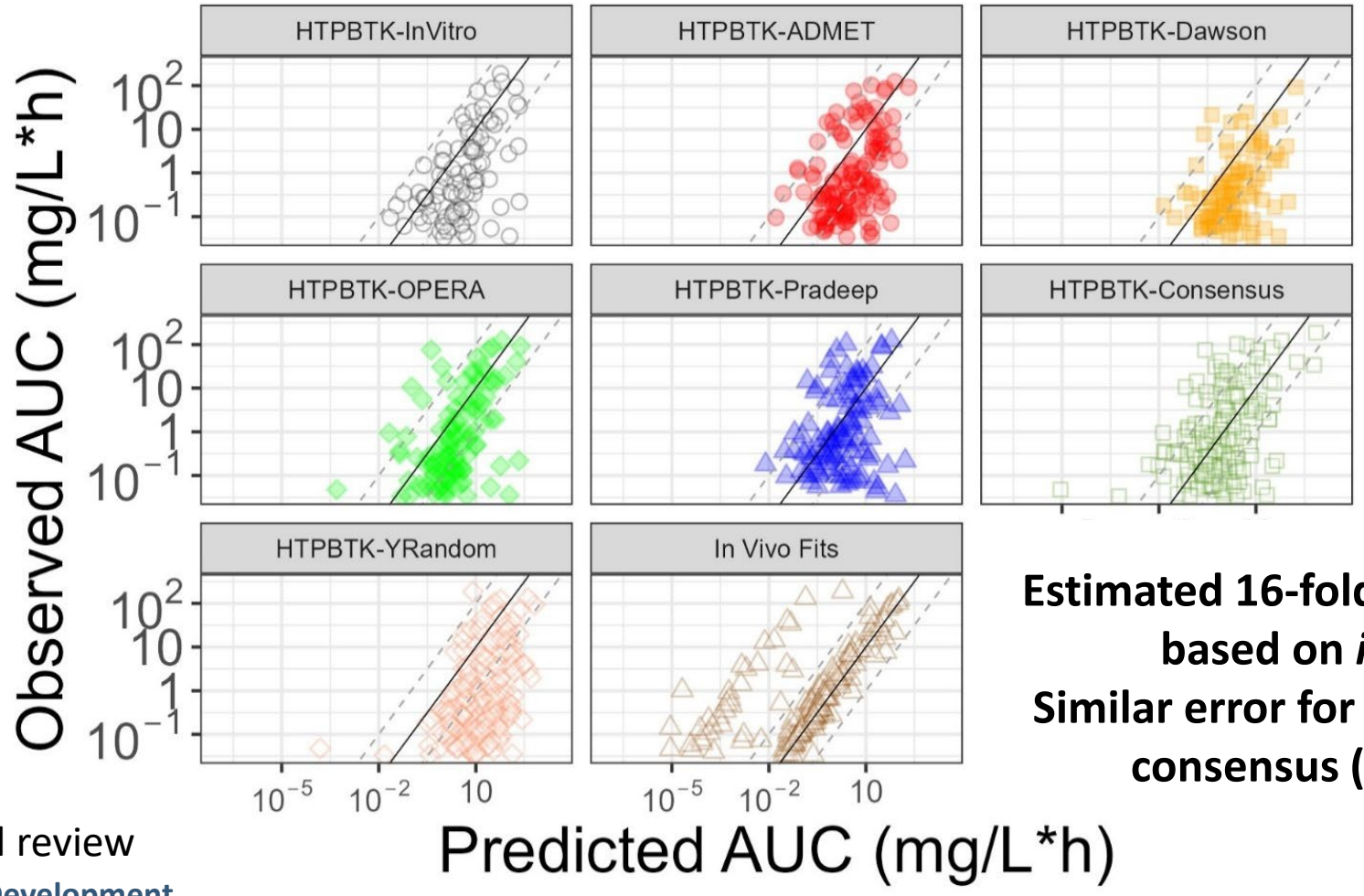


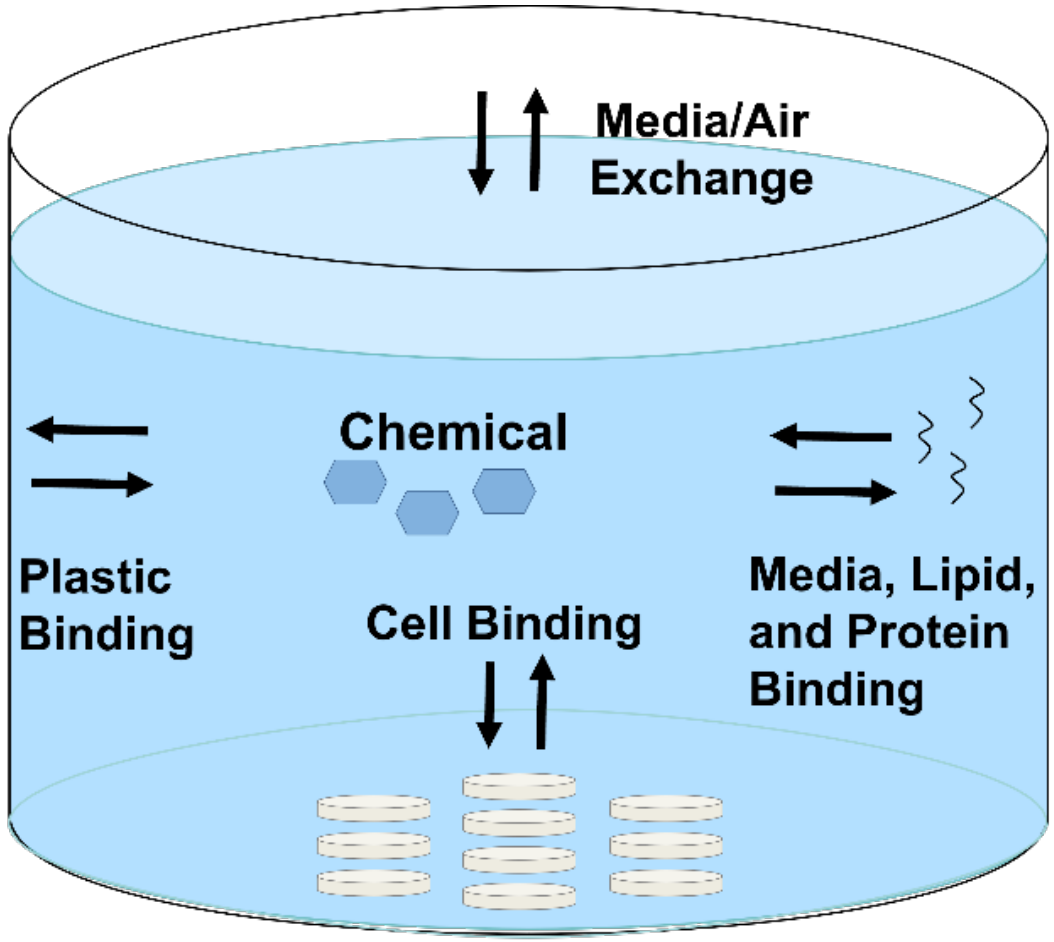
Estimated 16-fold error for predictions based on *in vitro* TK data
Similar error for predictions based on consensus (multiple) QSPRs

Manuscript under internal review

Gilberto Padilla Mercado, Rogelio Tornero-Velez, Taylor Wall, Risa Sayre, Caroline Ring

In Vitro Distribution

- "httk" tool includes modified* Armitage et al. (2014) model for estimating *in vitro* distribution
- Nominal tested concentration does not equal concentration in the cells



*Model has been updated to include ionization and plastic binding

Meredith Scherer, Katie Paul Friedman

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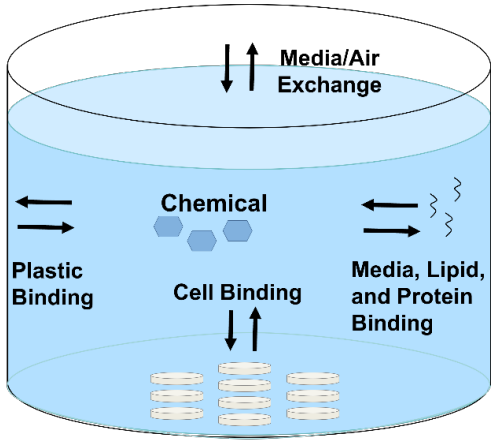

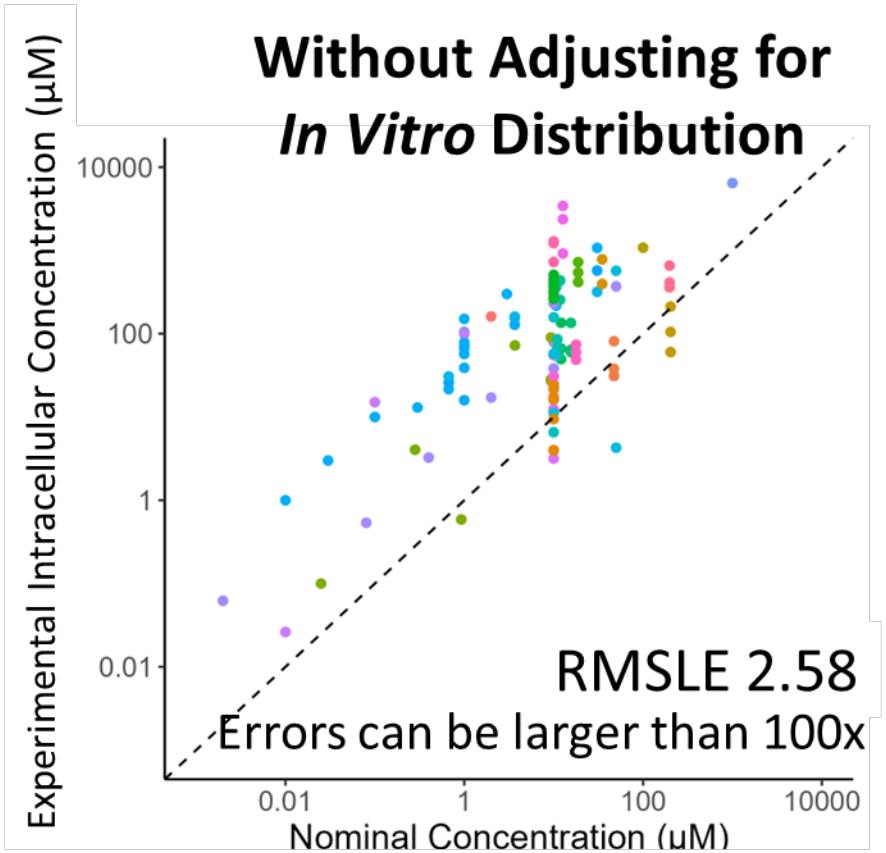

Chemical

- 3-Methylcholanthrene
- Acetaminophen
- Atrazine
- BPA
- Caffeine
- Chenodeoxycholic Acid
- Colchicine
- Cyclosporine A
- Fenarimol
- Flusilazole
- Flutamide
- Genistein
- Ketoconazole
- Methyltosterone
- N-Phenyl-1,4-benzenediamine
- Omeprazole
- PBDE 153
- PBDE 47
- PBDE 99
- Phenobarbitol
- Rifampicin
- Ritonavir
- Rosiglitazone
- Tamoxifen
- Thiacloprid
- Trenbolone
- Triphenyl phosphate
- Warfarin

Meredith Scherer, Katie Paul Friedman

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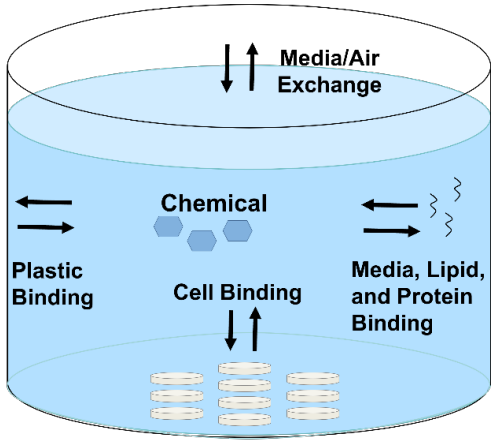
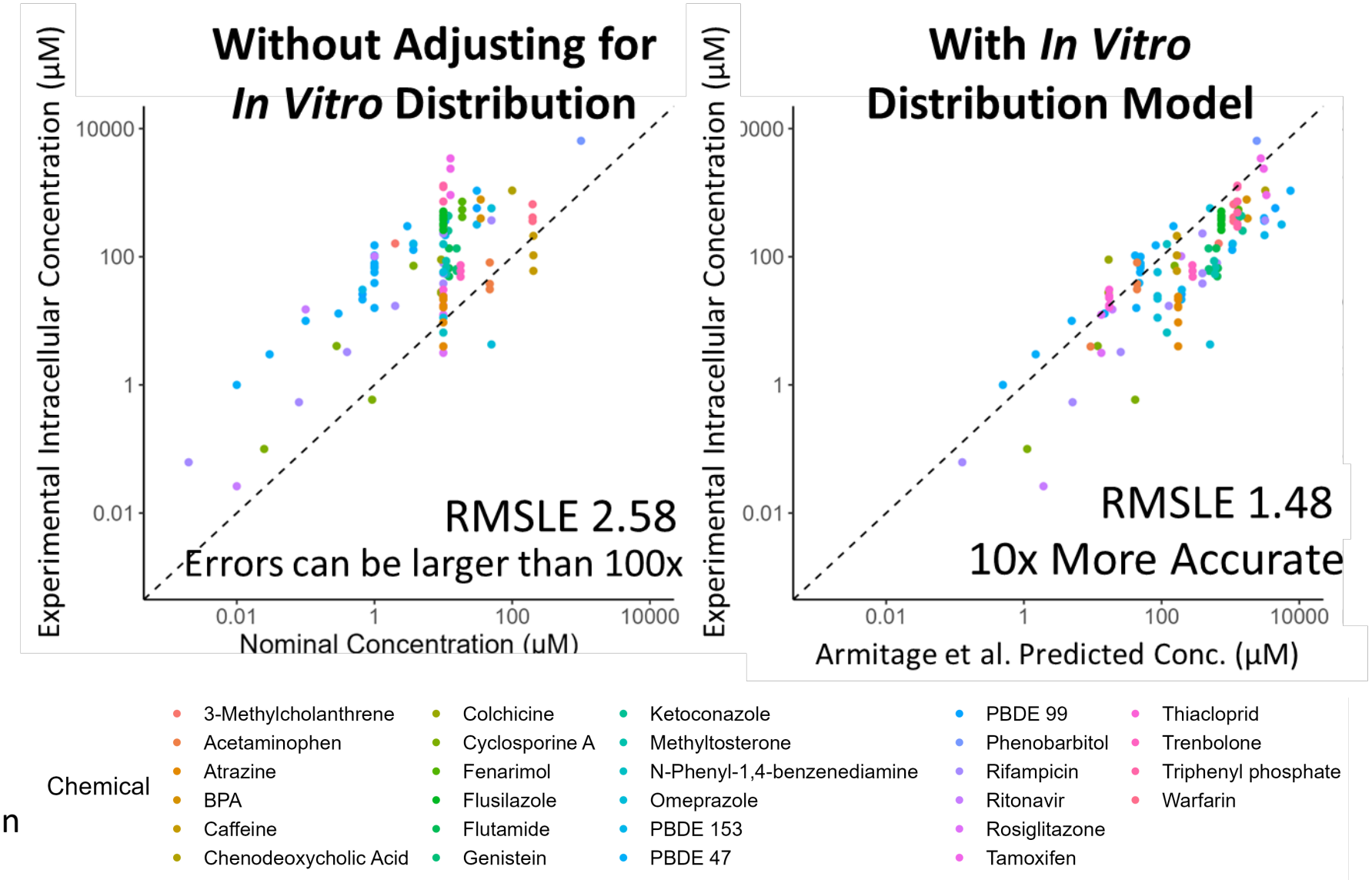




Meredith Scherer, Katie Paul Friedman

New Models for HTTK

- HTTK is the combination of *in vitro* TK data and high throughput physiologically-based toxicokinetic (HT-PBTK) models

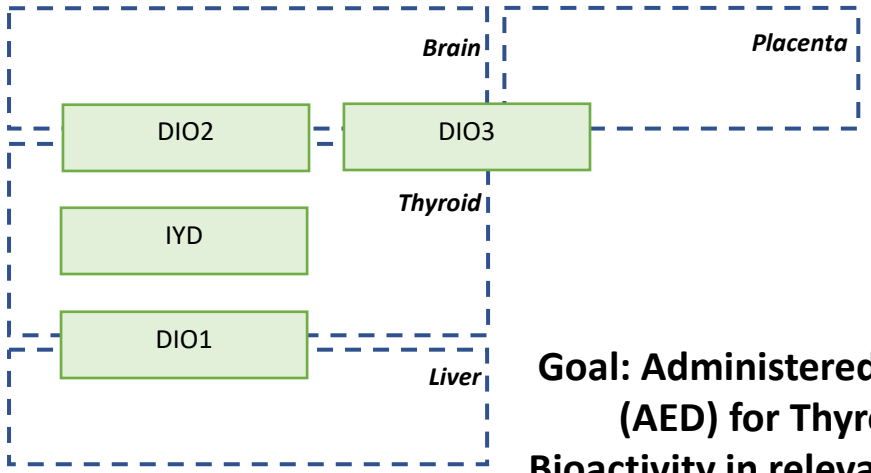
A recent manuscript provides a guide to developing new HT-PBTK models using existing HTTK data and methods (Davidson-Fritz et al., pre-print)

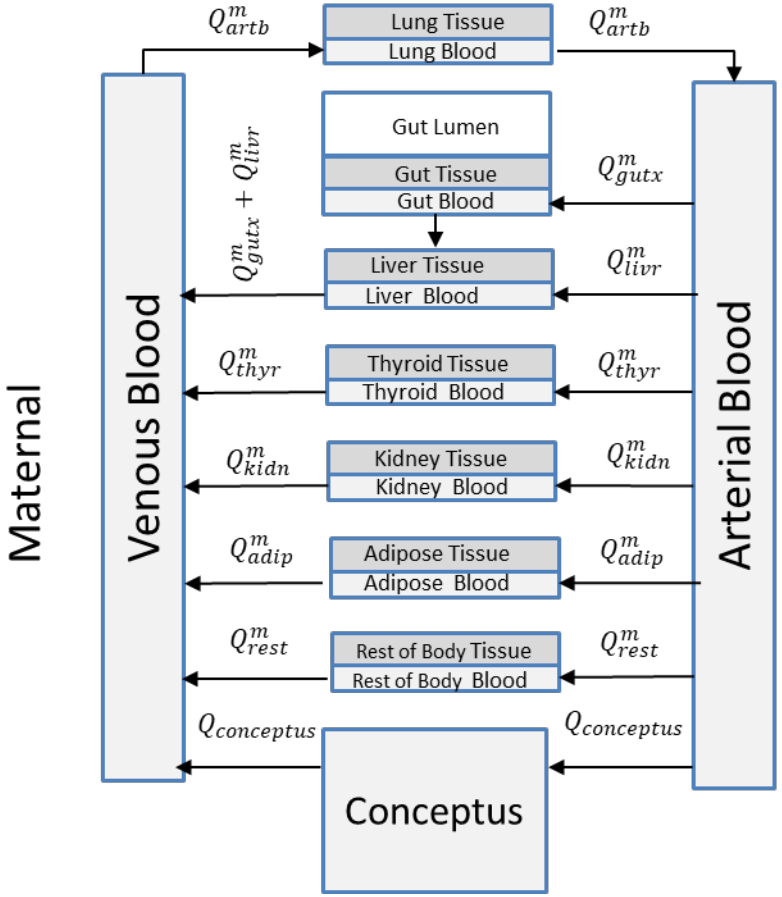
Multiple new models are in development including:

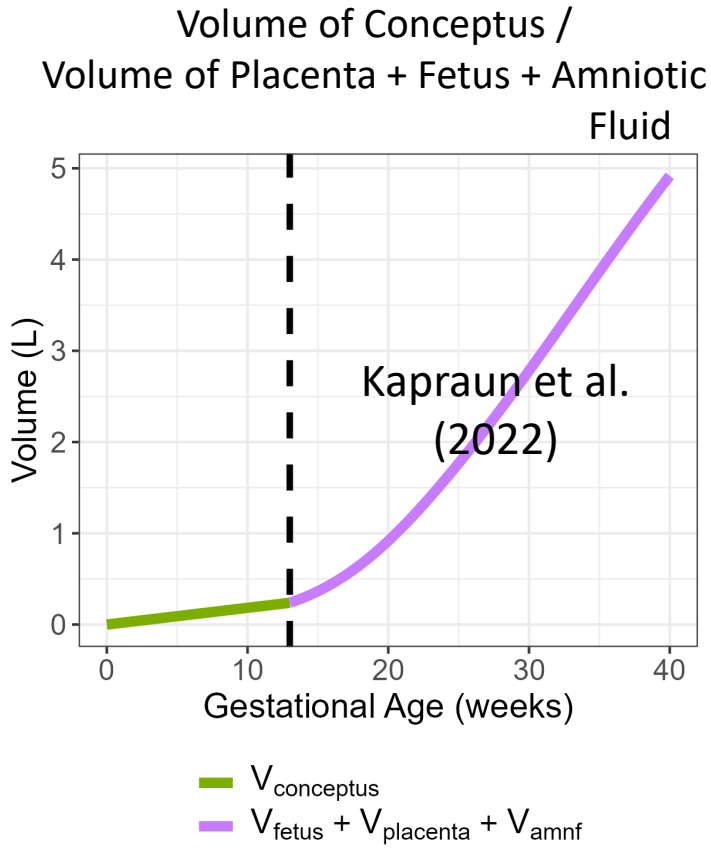
- Full human gestational (Truong and Paul Freidman)
- Chemical mixtures (Schacht and Evans)
- PFAS (Wetmore and Tornero-Velez)
- Dermal Exposure (Meade and Evans)
- Inhalation Steady-State (Ring and Schacht)
- Blood-Brain Barrier (Unnikrishnan, Chang, Sluka, Kreutz, Li…)

Full Human Gestational IVIVE

- Kapraun et al. (2022) model describes human gestation in second and third trimesters
- Truong et al. have developed a new HT-PBTK model for full gestation
- Allows IVIVE for thyroid bioactivity during the human perinatal period







Goal: Administered Equivalent Dose (AED) for Thyroid-Relevant Bioactivity in relevant compartments and lifestages of concern

Kimberly Truong, Dustin Kapraun, Katie Paul Friedman
Manuscript under internal review

HTTK for PFAS

- Typical *in vitro* TK measurements do not capture the role of transporters that may be important for understanding per- and polyfluorinated alkyl substances (PFAS)
- Dawson et al. (2023) machine learning model approximates the impact of transporters on toxicokinetics
- New PFAS-specific *in vitro* TK measurements from Wetmore lab and NTP were recently published (Smeltz, et al. 2023, Kreutz, et al. 2023, Crizer et al. 2024)
- New HT-PBTK models specifically for PFAS allow prediction of TK and IVIVE, including interspecies differences

PFAS	Wallis et al. (2023) Observed Half-Life (Days)	Dawson et al. (2023) Predicted Half-Life (Days)
DTXSID30892354	397-1980	>33
DTXSID90723993	72.9-340	>33
DTXSID50723994	287-1220	>33
DTXSID80515849	8.80-111	>33
DTXSID20892348	13-133	<7

Barbara Wetmore, Rogelio Tornero-Velez, Rachael Cogbill, Michael Devito, Chris Lau

Inhalation IVIVE

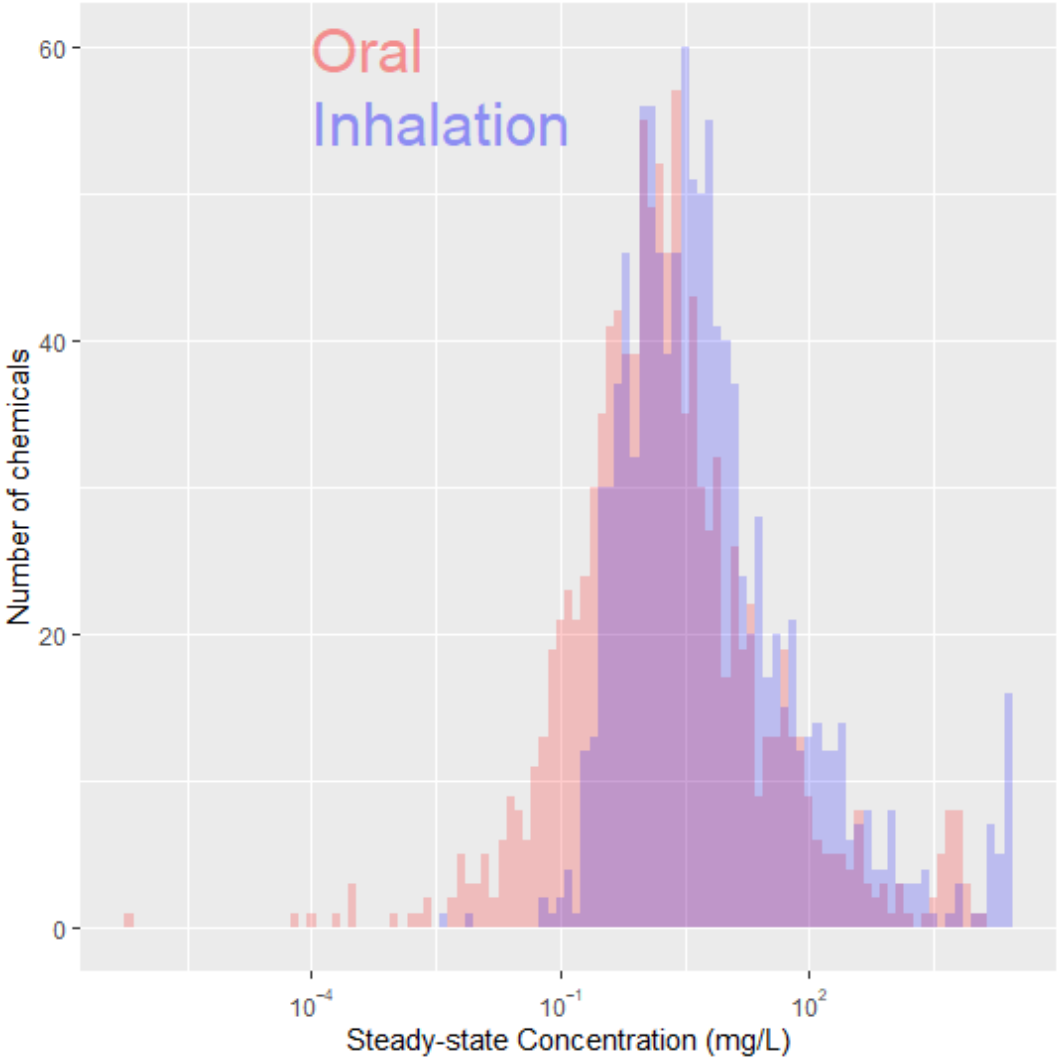
New IVIVE model including inhalation and exhalation allows estimation of inhalation equivalent doses in ppm

Administered Equivalent Dose

$$\mathbf{AED_{95}} = \frac{[\boldsymbol{X}]_{\textit{In Vitro Point of Departure}}}{\boldsymbol{C_{ss,95,unit}}}$$

Inhalation Equivalent Dose

$$\mathbf{IED_{95}} = \frac{[\boldsymbol{X}]_{\textit{In Vitro Point of Departure}}}{\boldsymbol{C_{ss,95,1ppm}}}$$



Celia Schacht, Caroline Ring
Manuscript under internal review

Open-Source Tools

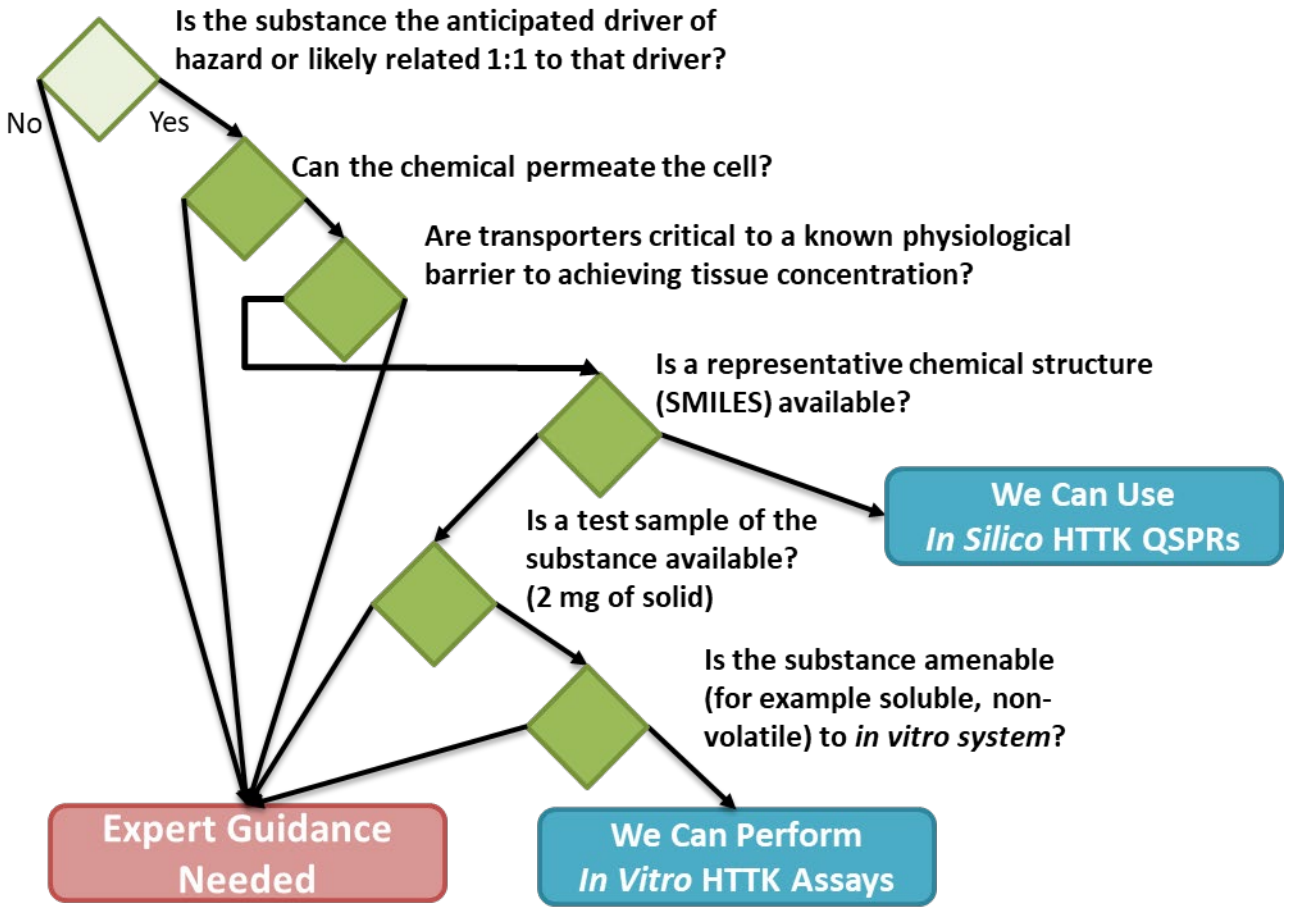
- R package “httk” allows for probabilistic *in vitro-in vivo* extrapolation (IVIVE) and toxicokinetics (TK)
  Pearce et al. (2017)
  - Simulates human variability and propagates measurement uncertainty
  - *In vitro* TK data for >1,000 chemicals and QSPR predictions for ~9,000 more
  - https://CRAN.R-project.org/package=httk

- Toxicokinetic Concentration vs. Time database (CvTdb) provides public, curated data with study annotation
  Sayre et al. (2020)
  - >250 analytes from hundreds of studies
  - https://github.com/USEPA/CompTox-PK-CvTdb

- R package “invivoPKfit” allows for consistent, reproducible TK parameter estimation from CvT data
  Padilla Mercado et al. (2024)
  - https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

- Forthcoming R package “invitroTKstats” allows for transparent estimation of *in vitro* TK parameters
  Wambaugh et al. (2019)
  - Calculates chemical-specific measurement uncertainty

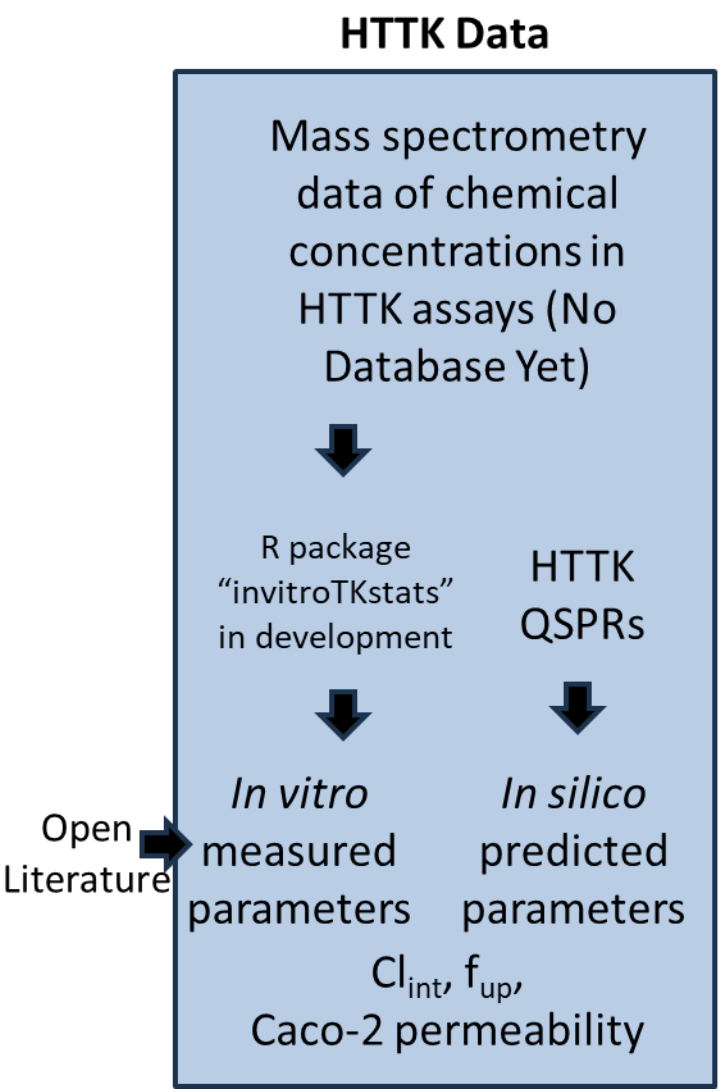
Using HTTK in Decision Making



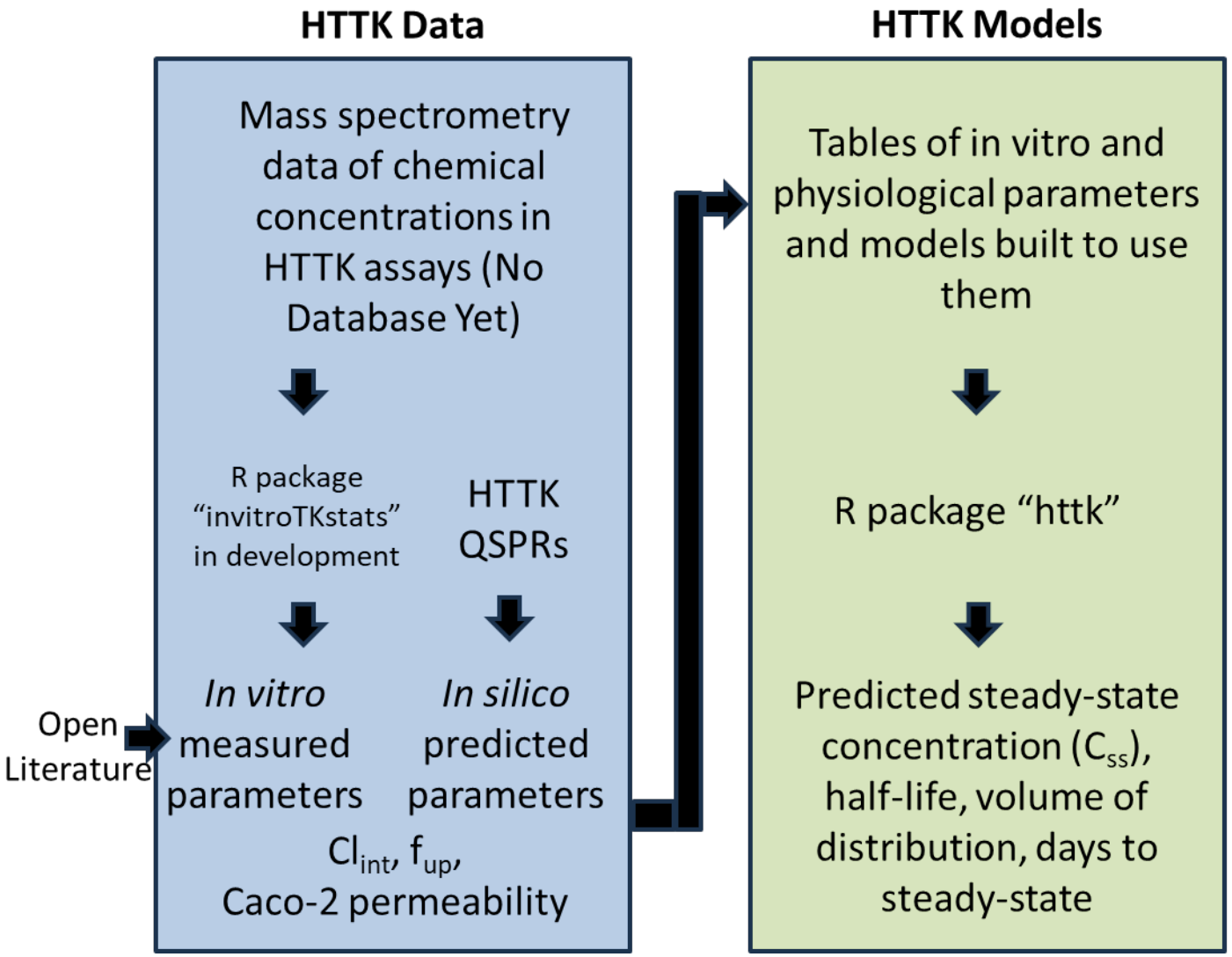
- Accelerating the Pace of Chemical Risk Assessment (APCRA) international government collaboration is developing decision trees to guide consideration of using HTTK in decision making

EPA HTTK Research

APCRA workgroup
HTTK in
decision making



EPA HTTK Research



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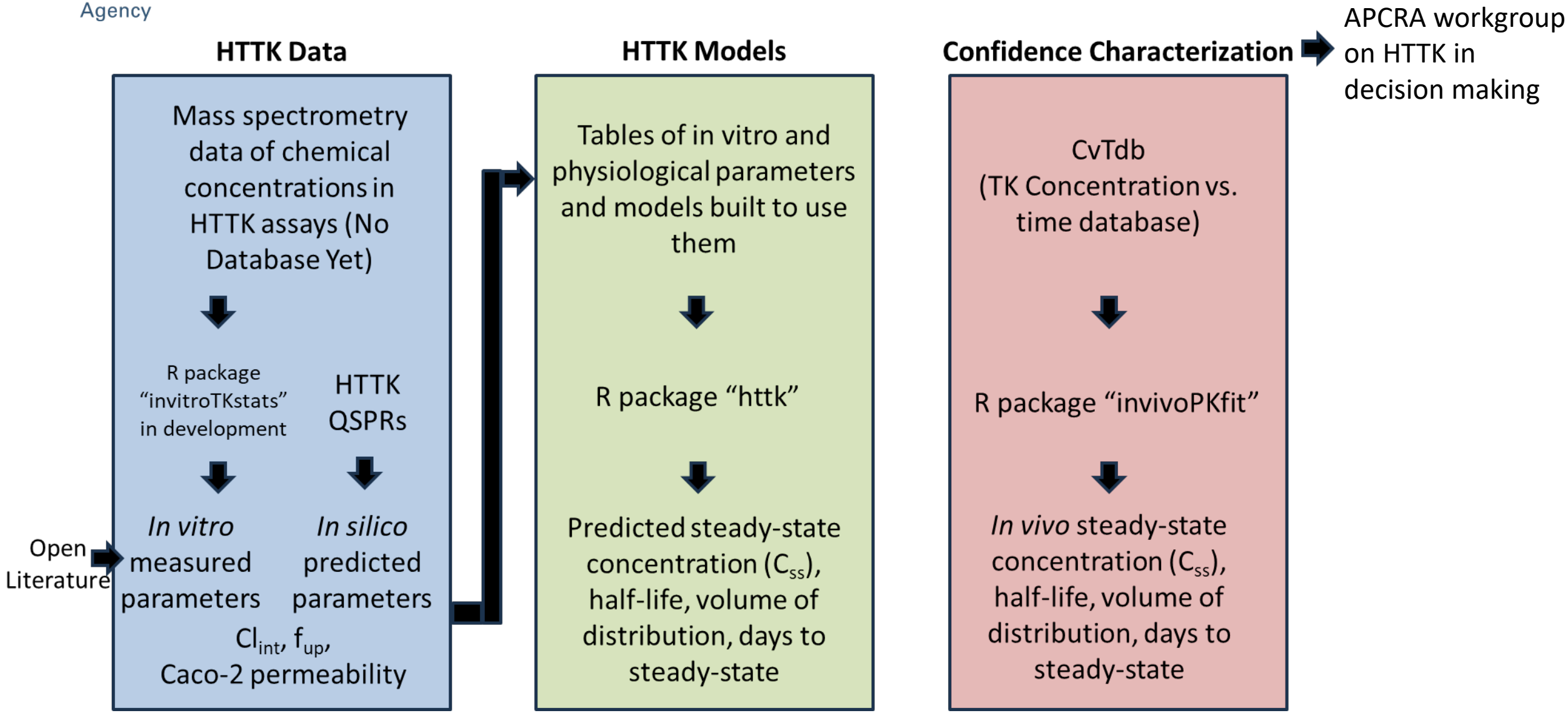
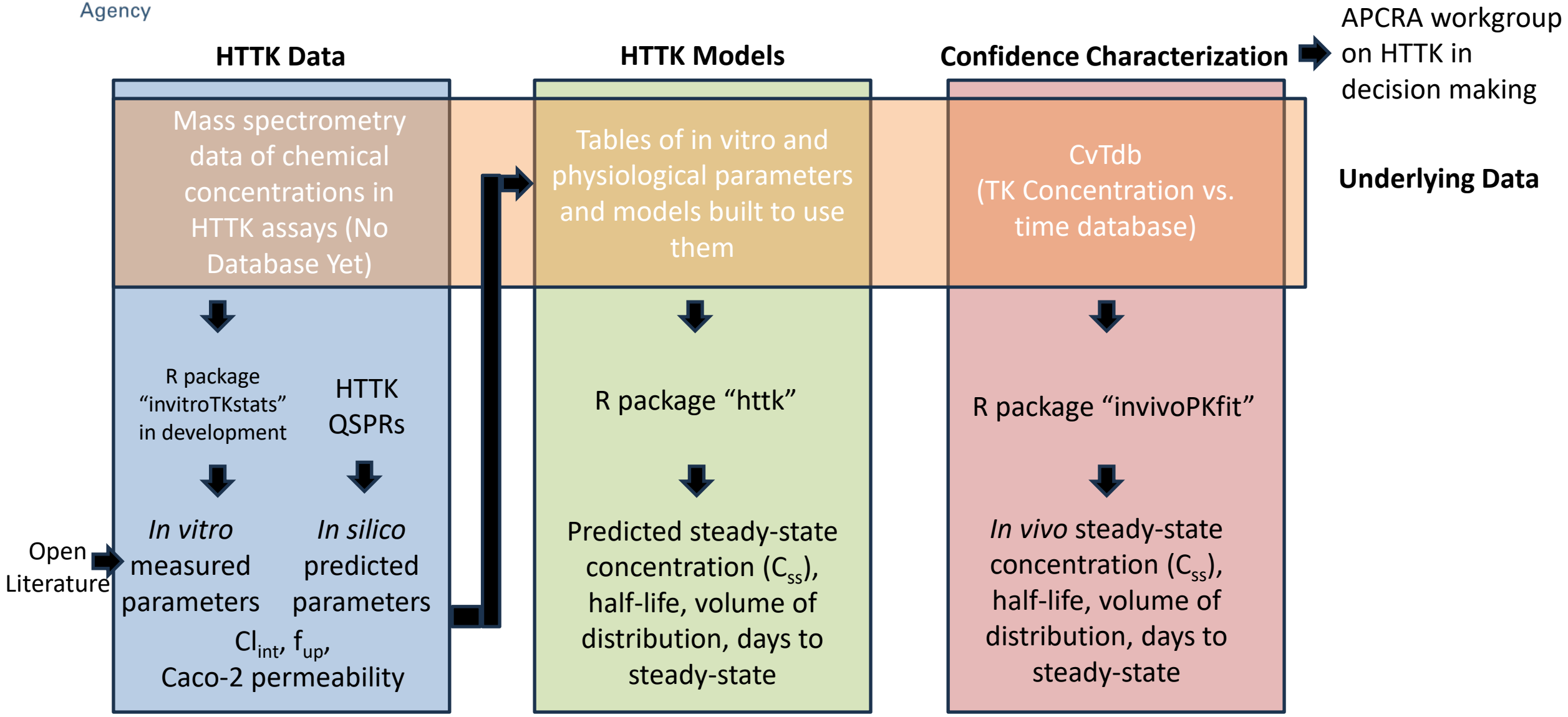
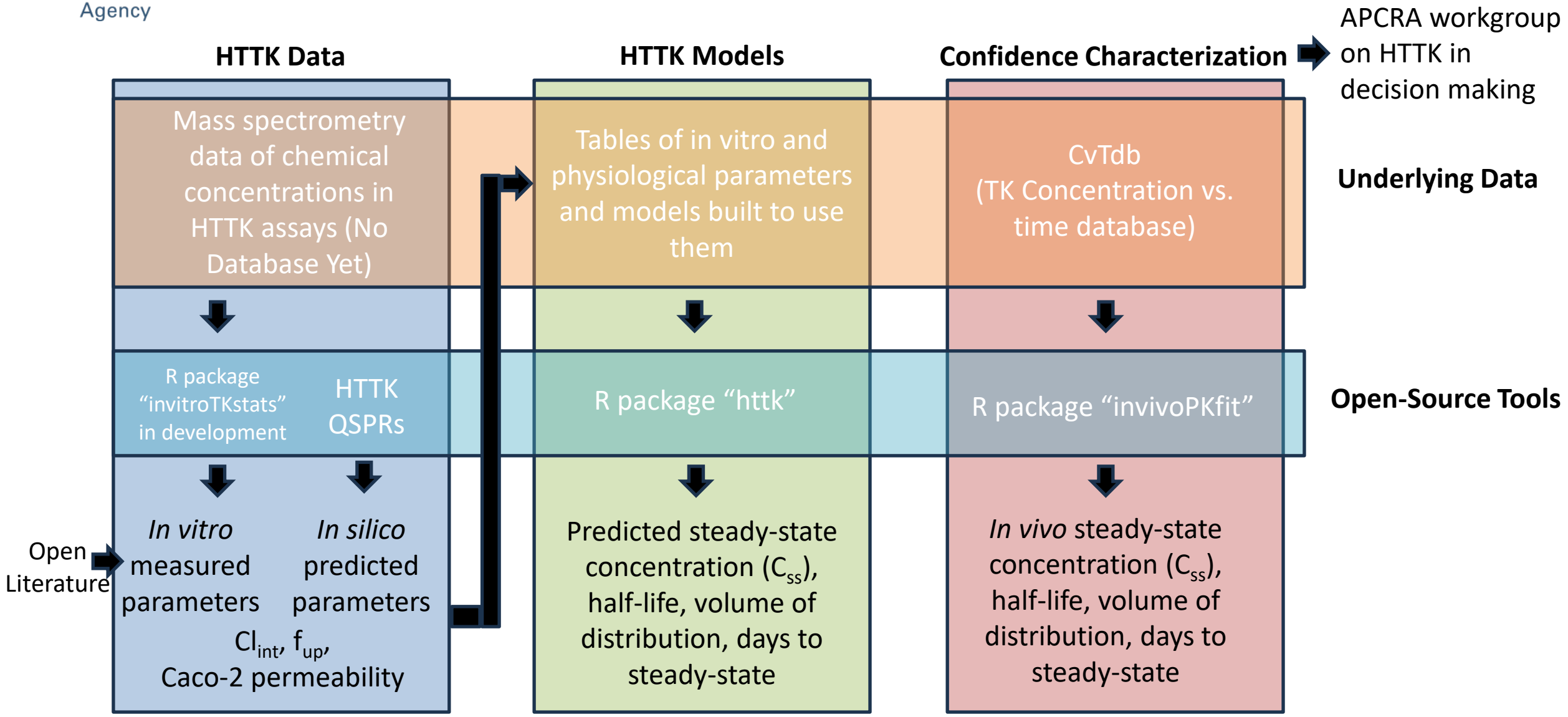


EPA HTTK Research



EPA HTTK Research



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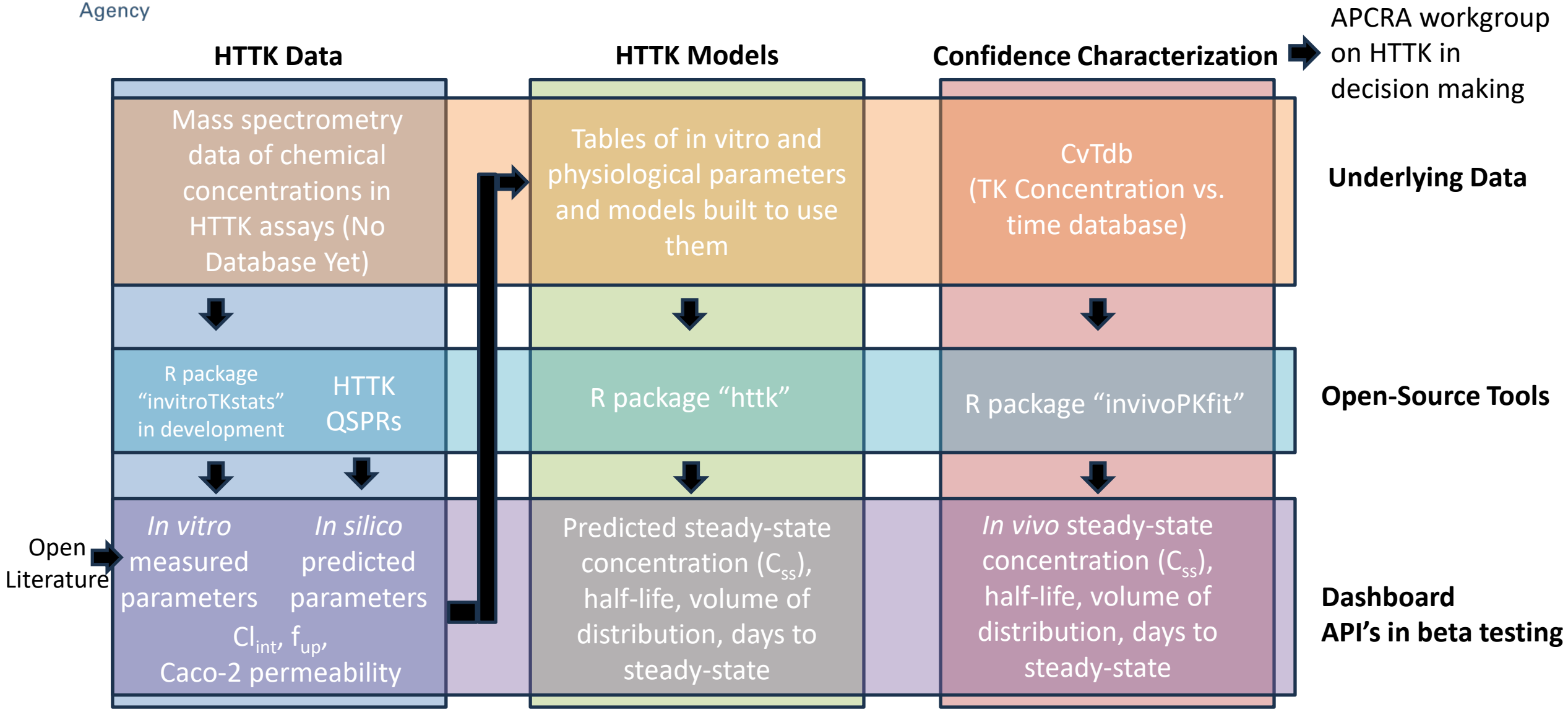
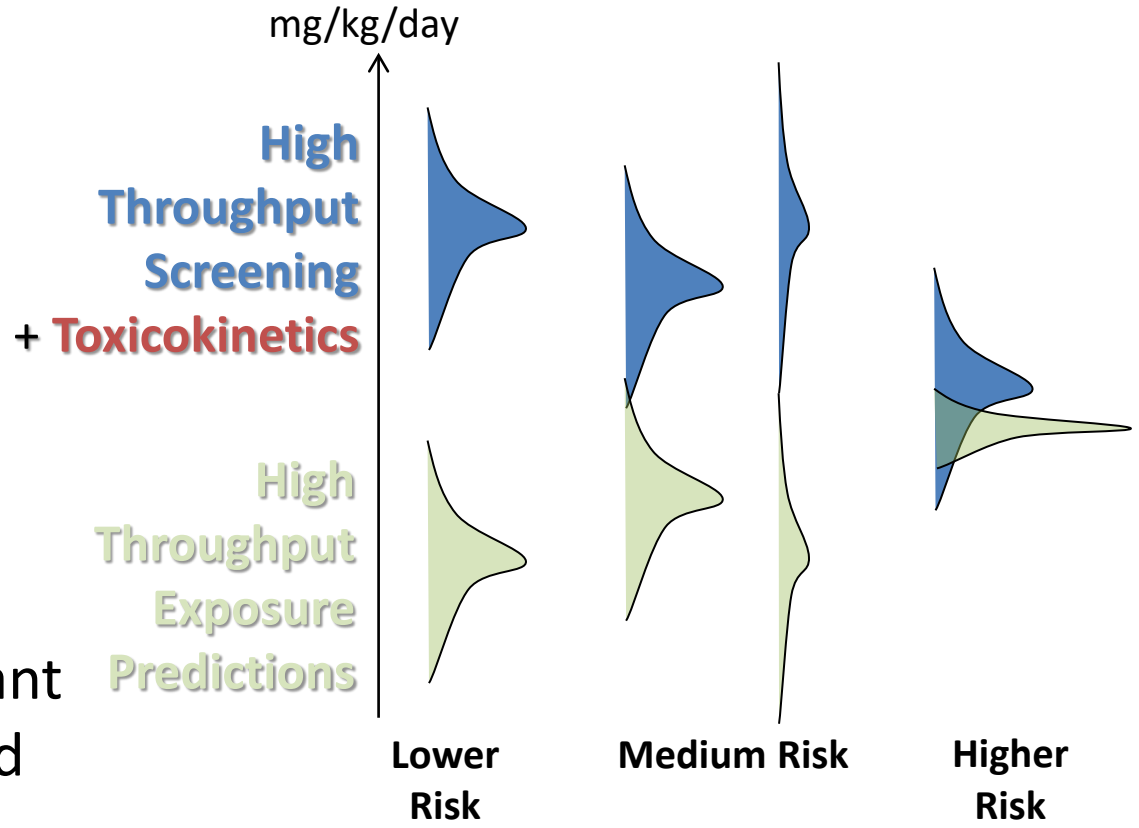
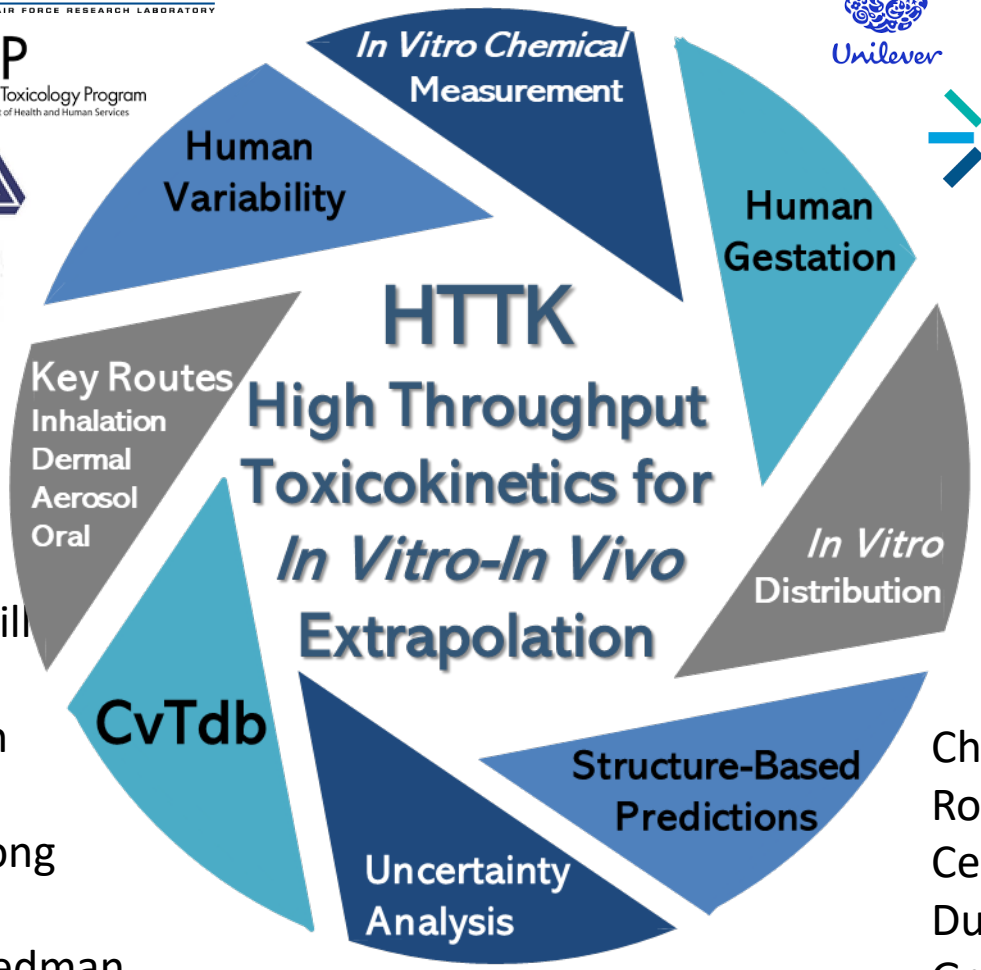


Conclusions

- HTTK is needed to convert from bioactive *in vitro* concentrations to putative dose rates needed to produce those concentrations in the body
- HTTK allows rapid calculations for a variety of scenarios
- HTTK is being expanded to better cover relevant chemicals (volatiles, PFAS) and susceptible and highly exposed populations (pregnancy, occupational)
- HTTK resources are widely available on-line



Please send any questions to:
wambaugh.john@epa.gov



Kristin Isaacs
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David Perez
Katherine Phillips
Gerardo Ruiz Mercado
Jon Sobus
John Wambaugh
Barbara Wetmore

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Caroline Ring
Sarah Davidson-Fritz
Gilberto Padilla Mercado
Meredith Scherer
Sahar Tabatabaei Sadeghi
Risa Sayre
Rogelio Tornero-Velez'
Chris Gonzales
Colin Thompson

Rachael Cogbill
Alex George
Elaina Kenyon
Marina Evans
Kimberly Truong
Taylor Wall
Katie Paul Friedman
Michael Devito
Nisha Sipes
Russell Thomas

Chris Strock
Roger Dinallo
Celia Schacht
Dustin Kapraun
Grace Chappell
Colin Guider
Aswani Unnikrishnan
Marci Smeltz

Xiaoqing Chang
Anna Kreutz
Victoria Hull
James Sluka
Dingsheng Li
Matthew Linakis
Nicole Kleinstreuer

United States Environmental Protection Agency

References

Please send questions and slide requests to wambaugh.john@epa.gov

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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

Translation of *In Vitro* Effect Concentrations: Equilibrium or Kinetic Distribution Models?

Fabian C. Fischer

Cedric Abele, Luise Henneberger, Rita Schlichting, Maria König, Beate Escher, **and many more…**

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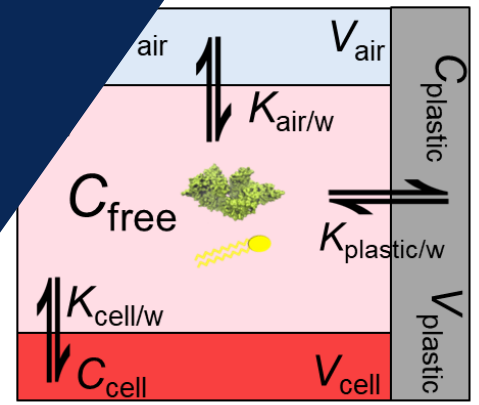
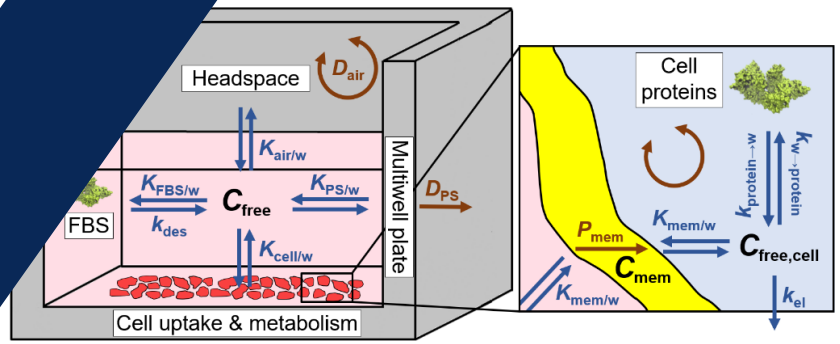
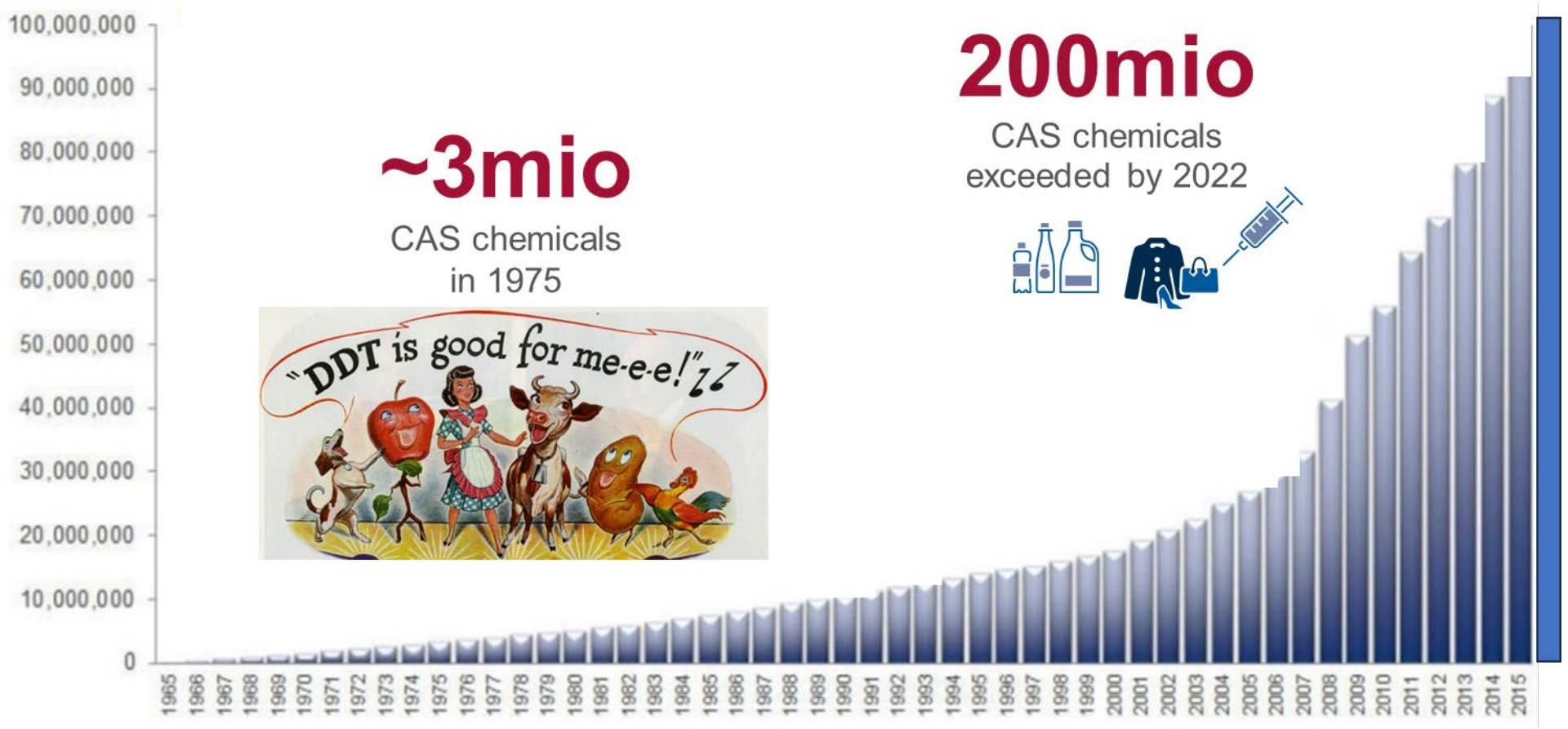




EPA 4th NAMs Conference
November 6th, 2024

The use of anthropogenic organic chemicals has changed over the last decades

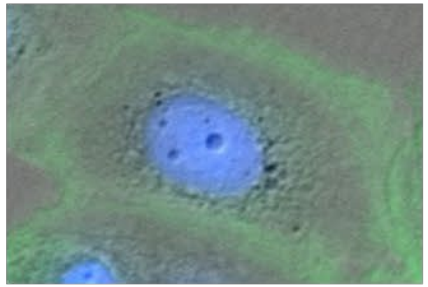
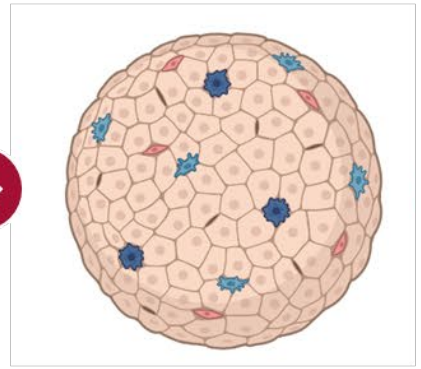
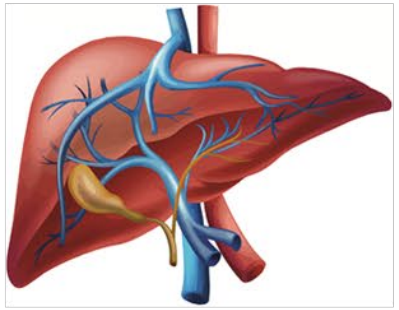
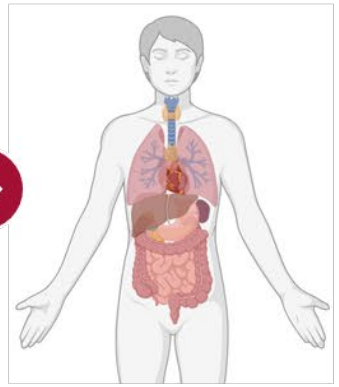


- Not only the number increased, so did the **diversity in chemical structures**!
- Global release into environment: **310 kg** of toxic chemicals **per second**![1]
- **One in every six** children suffers from neurodevelopmental abnormality suspected to be triggered by exposure to environmental chemicals![2]

[1] https://www.worldometers.info/
[2] https://braindrain.dk/

Explore chemical exposures and toxicity based on freely dissolved concentrations

$C_{free} = C_{unbound}$ in pharmaceutical sciences


C_{free} ↕ Tox → C_{free} ↕ Tox → C_{free} ↕ Tox → C_{free} ↕ Tox

Effect extrapolation?

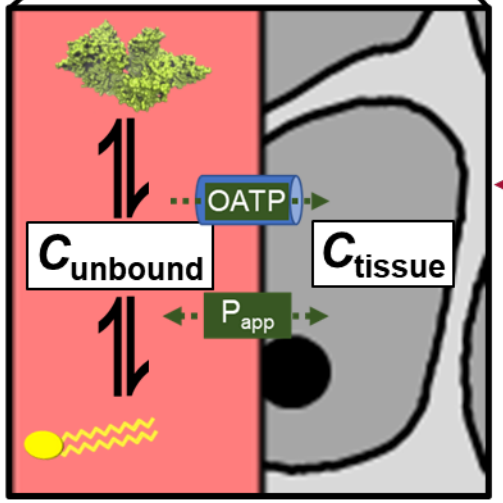
Quantitative in *vitro-in vivo* extrapolation (QIVIVE)

PBTK modeling

Kidneys
Brain
Blood
Liver
Fat

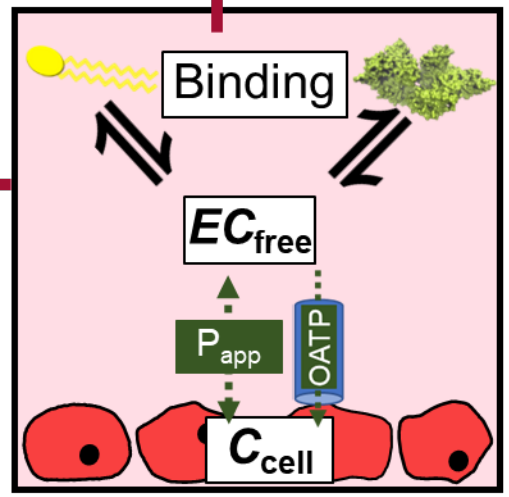
Health effects

Identify vulnerable individuals/groups



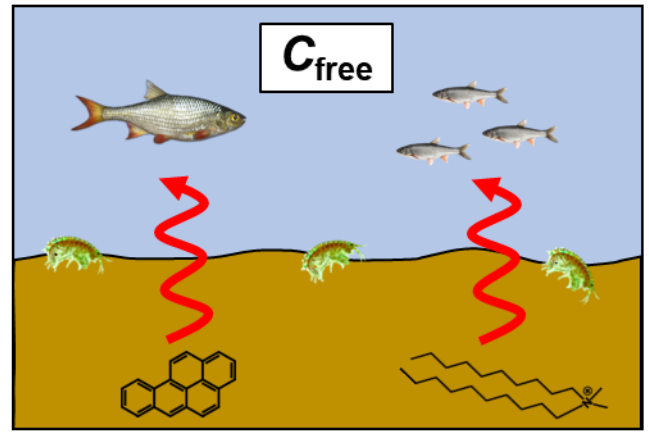
In vivo toxicokinetics to predict effects

QIVIVE
≈
upscale



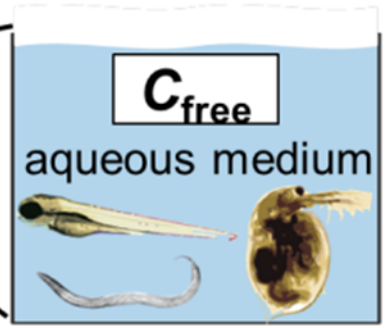
In vitro toxicokinetics & effects

Ecotoxicity testing



Extrapolation

8 well plates
24 well plates
96 well plates



Chemical transport models to investigate the distribution of chemicals in *in vitro* cell assays

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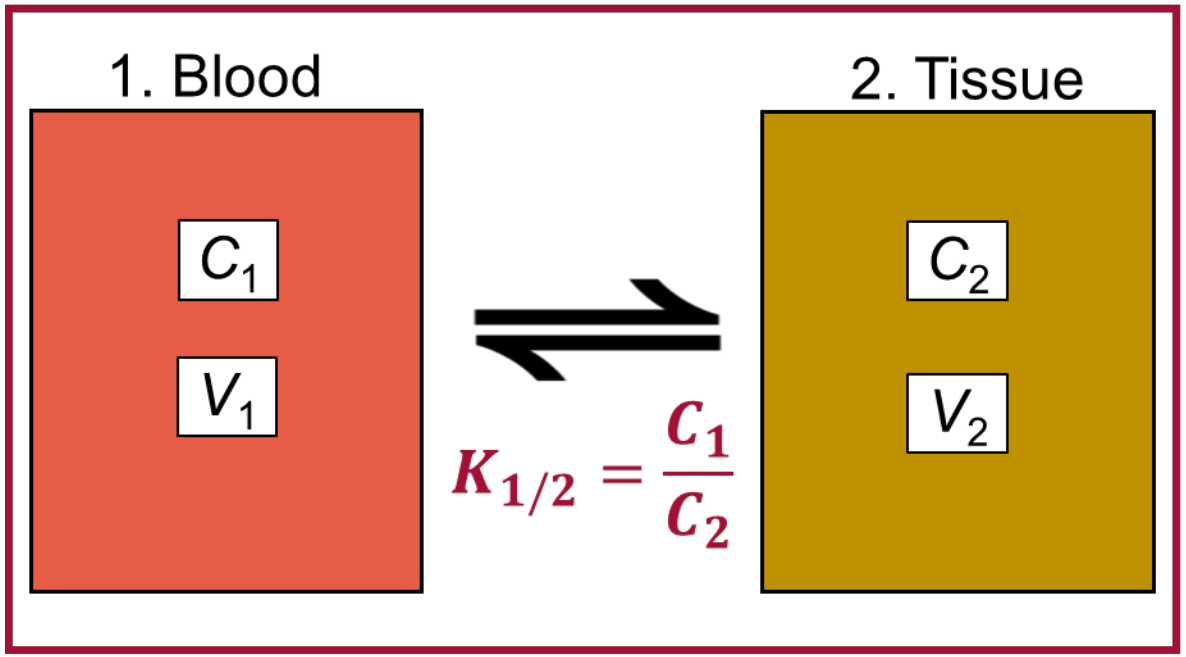
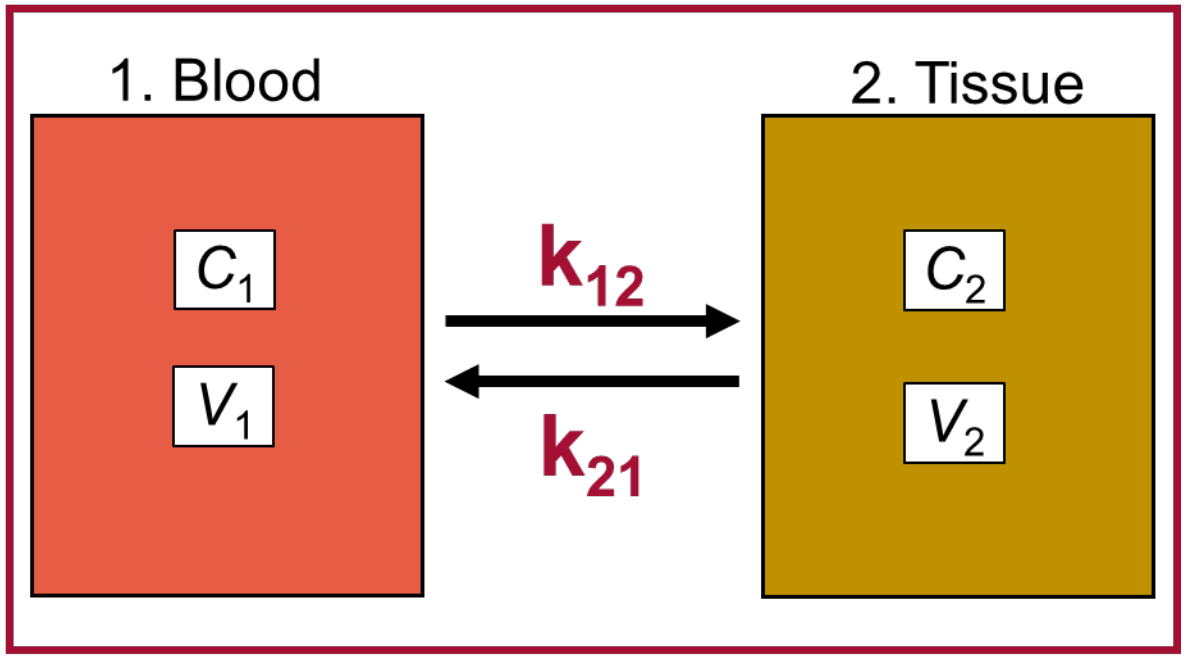
- Can the system be approximated with an equilibrium or kinetic model?
- How much complexity is necessary to predict/explain exposure?

Equilibrium mass balance model



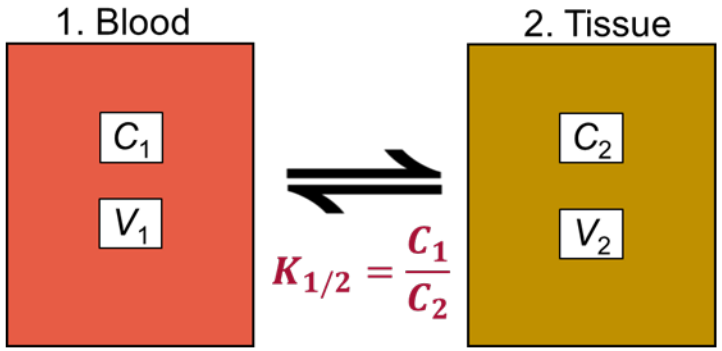
Kinetic model (rate-limited)



Can we simplify?

Computing chemical transport models: The benefit of simplicity

Equilibrium mass balance model



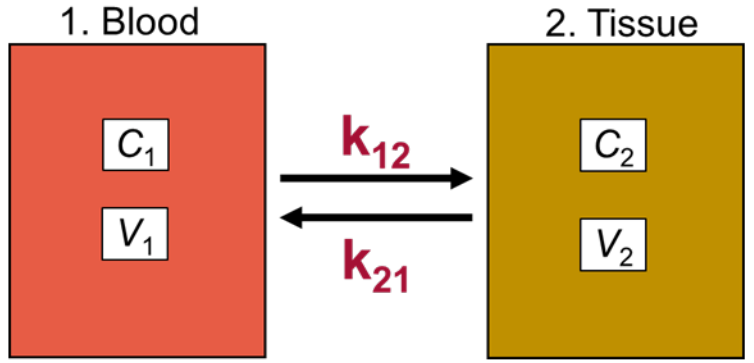
$$K_{2/1} = \frac{1}{K_{1/2}}$$

$$f_1 = \frac{1}{\left(1 + K_{2/1} \cdot \frac{V_2}{V_1}\right)}$$

$$C_1 = \frac{f_1 \cdot n_{\text{total}}}{V_1}$$

Multi-box: $$f_1 = \frac{1}{\left(1 + K_{2/1} \cdot \frac{V_2}{V_1} + K_{\frac{3}{1}} \cdot \frac{V_3}{V_1} + \cdots\right)}$$

Kinetic model (rate-limited)



k = 1/t

$$\frac{dC_1}{dt} = -k_{12} \cdot C_1 + k_{21} \cdot C_2$$

$$C_1(t) = C_1(t_{-1}) - k_{12} \cdot C_1(t_{-1}) + k_{21} \cdot C_2(t_{-1})$$

Multi-box becomes a bit more complex…

Computing chemical transport models: Implementation of numerical models in *R*

Solve the equations in **discrete time steps** – increase the number of time steps (Δt) to increase the model accuracy and to avoid numerical errors

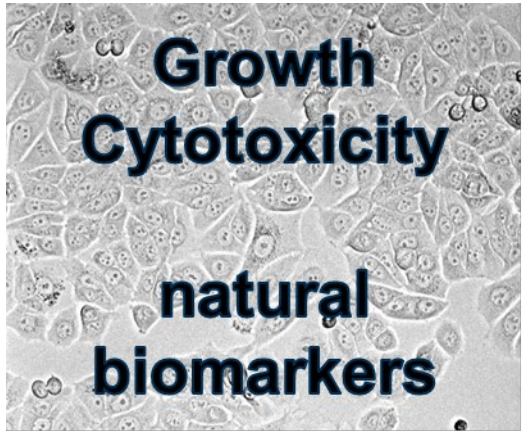
$$C_1(t) = C_1(t_{-1}) - k_{12} \cdot C_1(t_{-1}) \cdot \Delta t + k_{21} \cdot C_2(t_{-1}) \cdot \Delta t$$

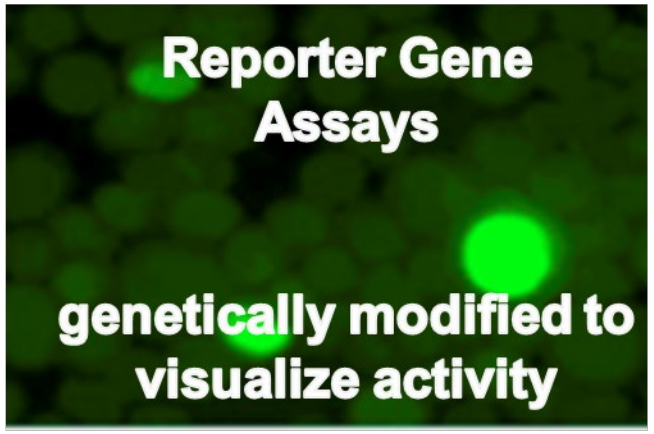
$$C_2(t) = C_2(t_{-1}) + k_{12} \cdot C_1(t_{-1}) \cdot \Delta t - k_{21} \cdot C_2(t_{-1}) \cdot \Delta t$$

```
# Duration of simulation and delta t
days      = 0.01
t_end     = days * 86400          # duration of simulation in seconds
dt        = 0.1                     # time step of iteration

# rate constants (1/s)
k_1_2    = 0.005
k_2_1    = 0.002

# Compartment volumes (cm³)
V_1 = 2
V_2 = 2

# Start concentrations (mol/cm³)
C_1 = 100
C_2 = 0
C_ratio = C_1/C_2
```

```
#### Loop calculation ####
for (t in times) {

  C_1         = C_1
  C_2         = C_2
  C_ratio     = C_ratio

  # Change in concentrations per time point (dt)
  C_1 = C_1 - k_1_2 * C_1 * dt + k_2_1 * C_2 * dt
  C_2 = C_2 + k_1_2 * C_1 * dt - k_2_1 * C_2 * dt

  # Concentration ratio between compartments
  C_ratio = C_2/C_1

if (t %% 60 < dt) {
  C_1_report[i]         = C_1
  C_2_report[i]         = C_2
  C_ratio_report[i]     = C_ratio
  t_report[i] = t/60
  i=i+1
}}
```

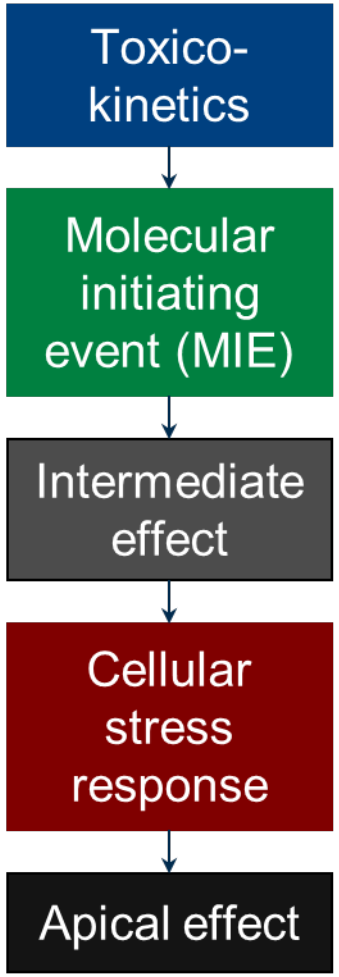
In vitro cell assays to measure toxicokinetics and various toxic endpoints – Translation of effect concentrations



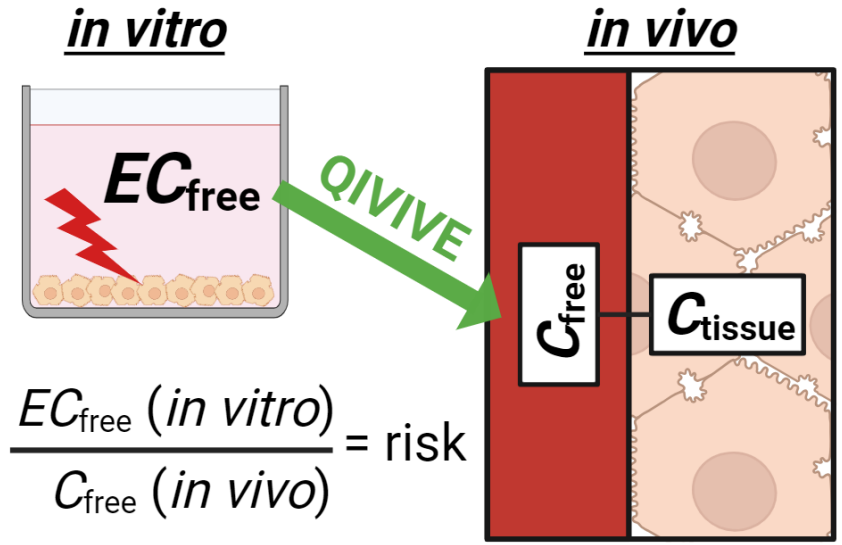


adapted from Escher and Leusch (2012)

- Cost-efficient, reproducible in high-throughput format, classified as non-animal test[1]
- Potential to reveal mode of action (MoA)
- Large databases already exist, e.g., "Toxicology in the 21st Century" (Tox21) program[2]
  → Databases neglect chemical bioavailability



- Quantitative predictive power depends on controlling & assessing **effective concentrations!**
- C_{free} and C_{cell}



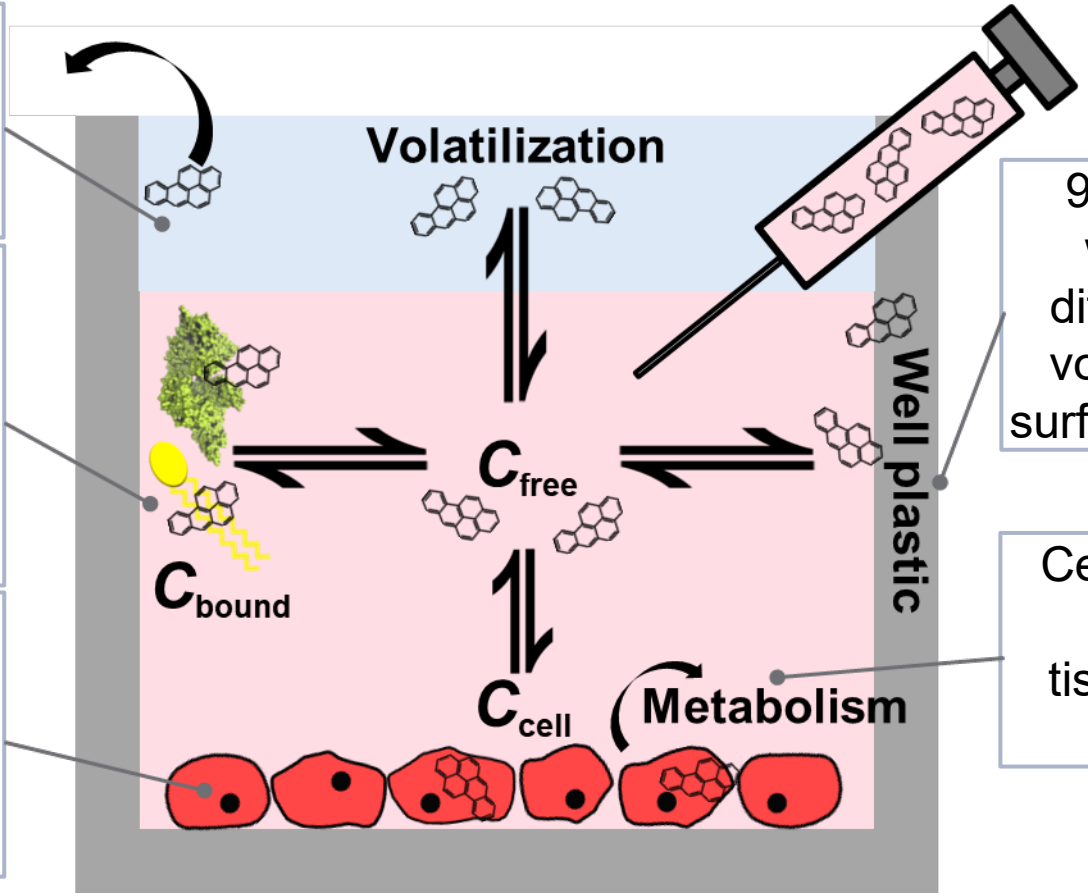
1 Escher, B., and Leusch, F. (2012) ISBN: 9781843393689.
2 Tox21 10K library, U.S. EPA.

Chemical fate in *in vitro* cell-based assays results from various processes

Standard well plates with plastic lids: exchange with outside air possible

Base medium often supplemented with 0.5 - 10% protein- and lipid-rich foetal bovine serum (FBS)

Extend and kinetics of cellular uptake: active and passive transport mechanisms



96-, 384-, 1536-well plates with different medium volume to plastic surface area ratios

Cell lines derived from different tissue: metabolic capacity?

? What level of model complexity do we need to predict exposure (C_{free}, C_{cell}) reasonably accurate?

Equilibrium mass balance model to quantify the effective dose in HTS *in vitro* bioassays

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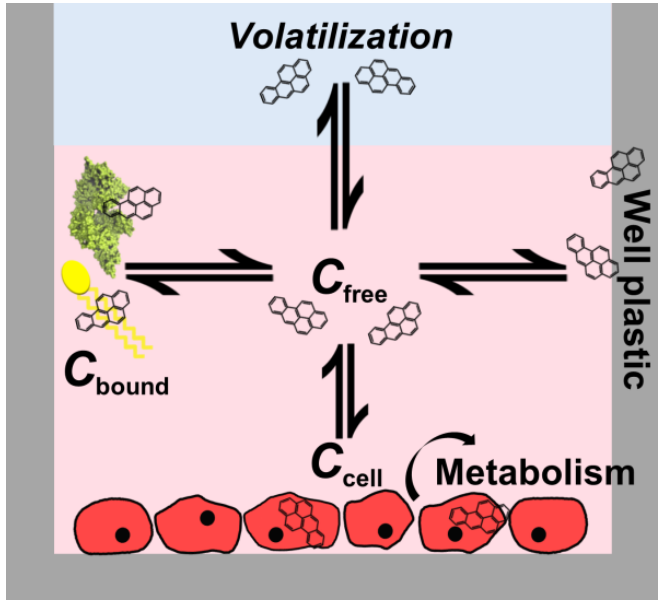
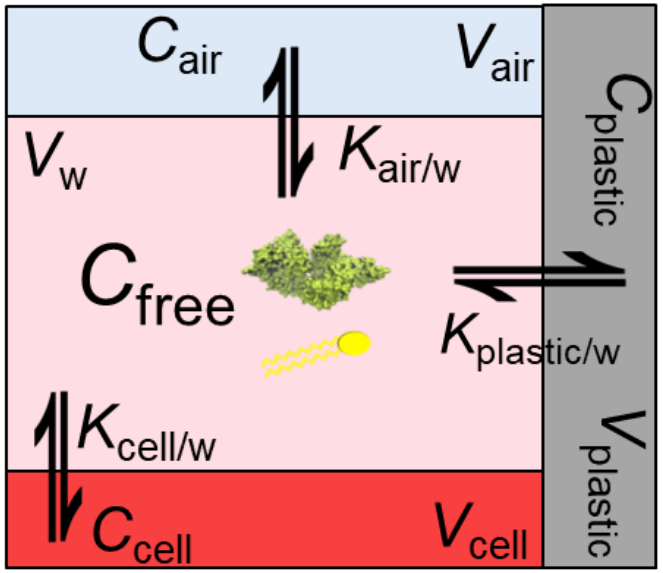
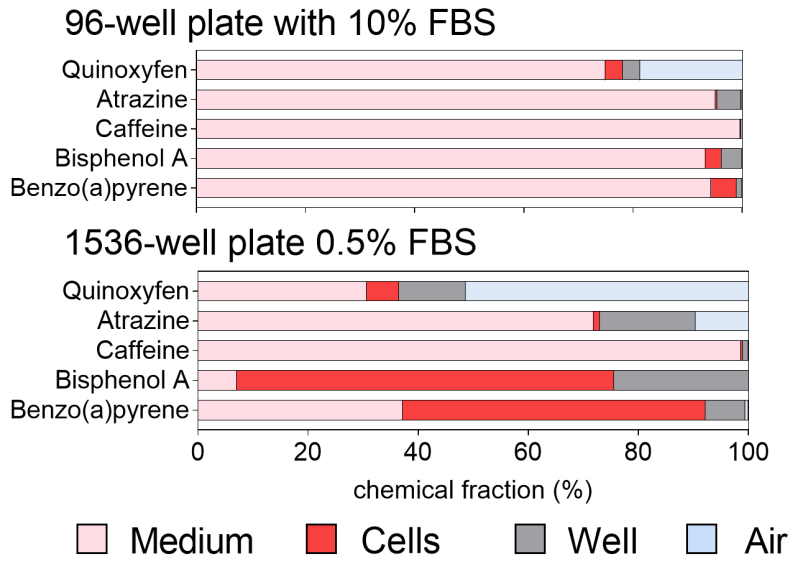
Complex system



Simplified model

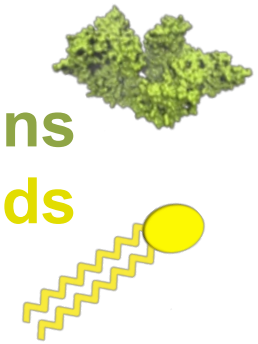


Model simulations



$$f_{free} = \left(1 + K_{cell/w} \cdot \frac{V_{cell}}{V_w} + K_{air/w} \cdot \frac{V_{air}}{V_w} + K_{plastic/w} \cdot \frac{V_{plastic}}{V_w}\right)^{-1}$$

$$C_{free} = C_{nom} \cdot f_{free} \cdot \frac{V_{total}}{V_w}$$



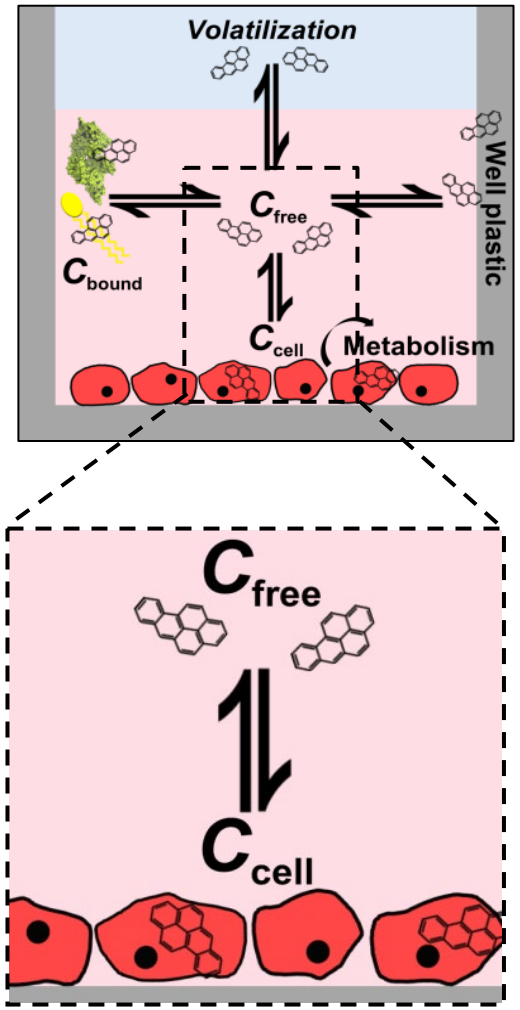
FBS **proteins** and **lipids**

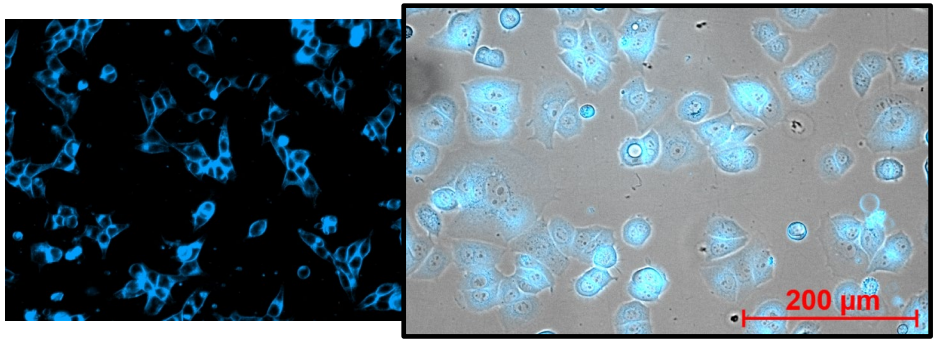
Chemical Research in Toxicology

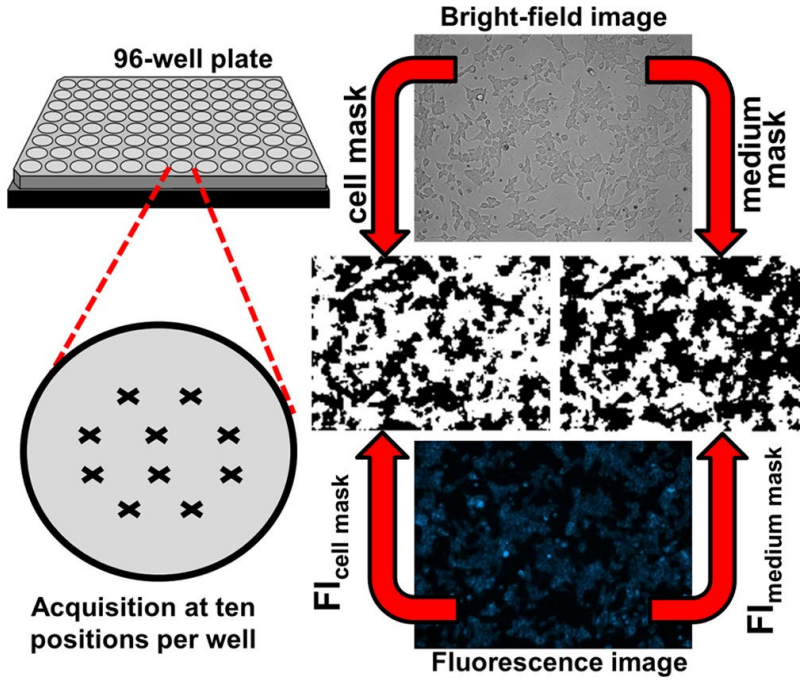
Modeling Exposure in the Tox21 *in Vitro* Bioassays

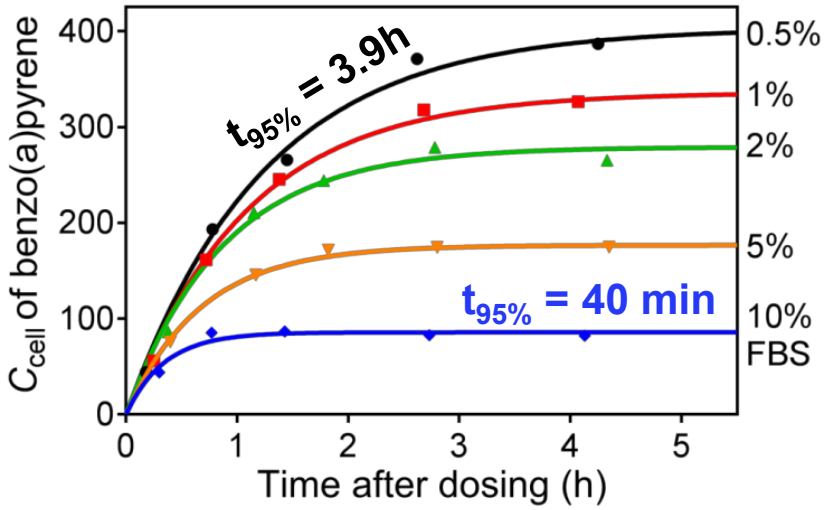
Fabian C. Fischer,*[†] Luise Henneberger,[†] Maria König,[†] Kai Bittermann,[‡] Lukas Linden,[‡] Kai-Uwe Goss,[‡] and Beate I. Escher[†]

Measuring chemical uptake kinetics with fluorescence microscopy

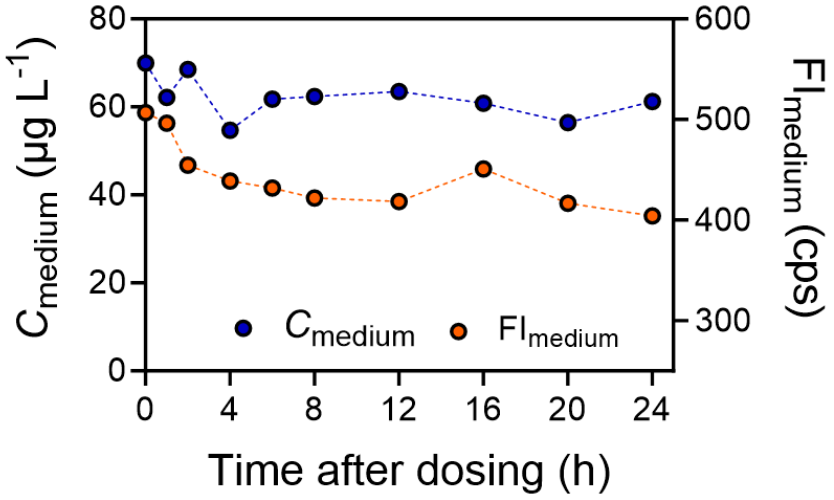








BaP at 10% FBS in HEK293

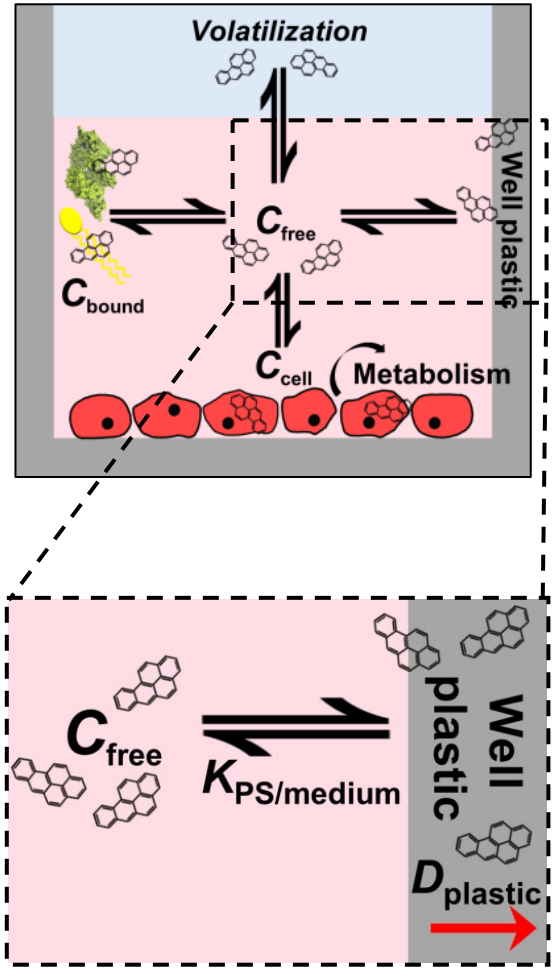


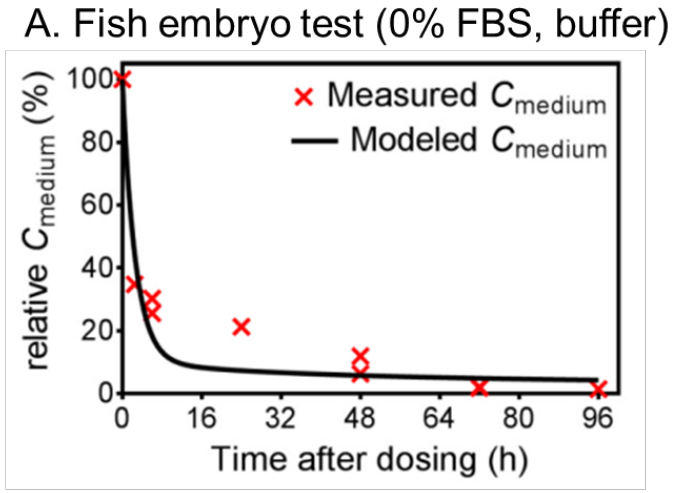
Chemical Research in Toxicology

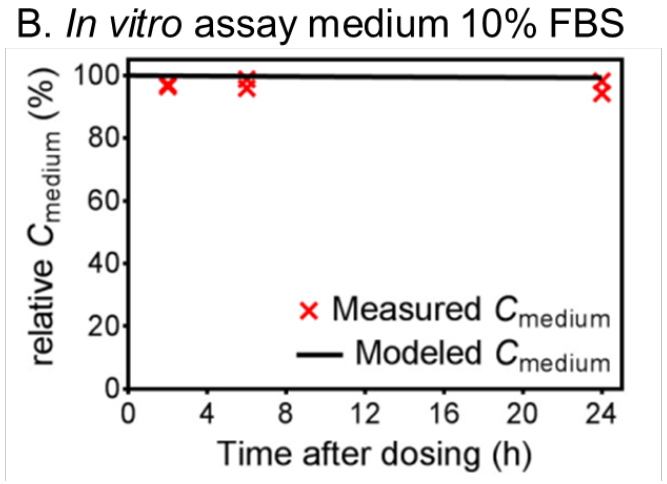
Cellular Uptake Kinetics of Neutral and Charged Chemicals in *in Vitro* Assays Measured by Fluorescence Microscopy

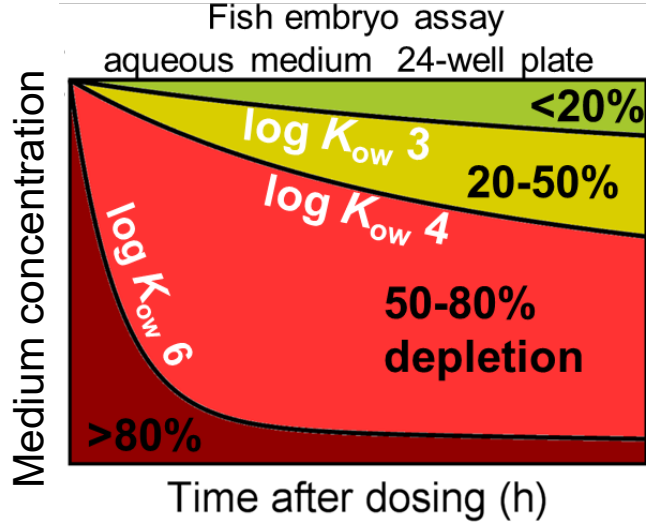
Fabian C. Fischer,[*,†] Cedric Abele,[†] Steven T. J. Droge,[‡] Luise Henneberger,[†] Maria König,[†] Rita Schlichting,[†] Stefan Scholz,[§] and Beate I. Escher[†,∥]

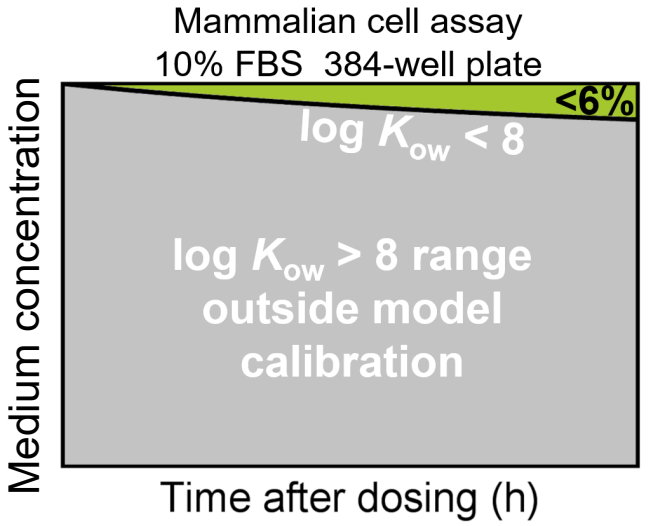
Transport of chemicals into the plastic material of multi-well plates used for bioassays











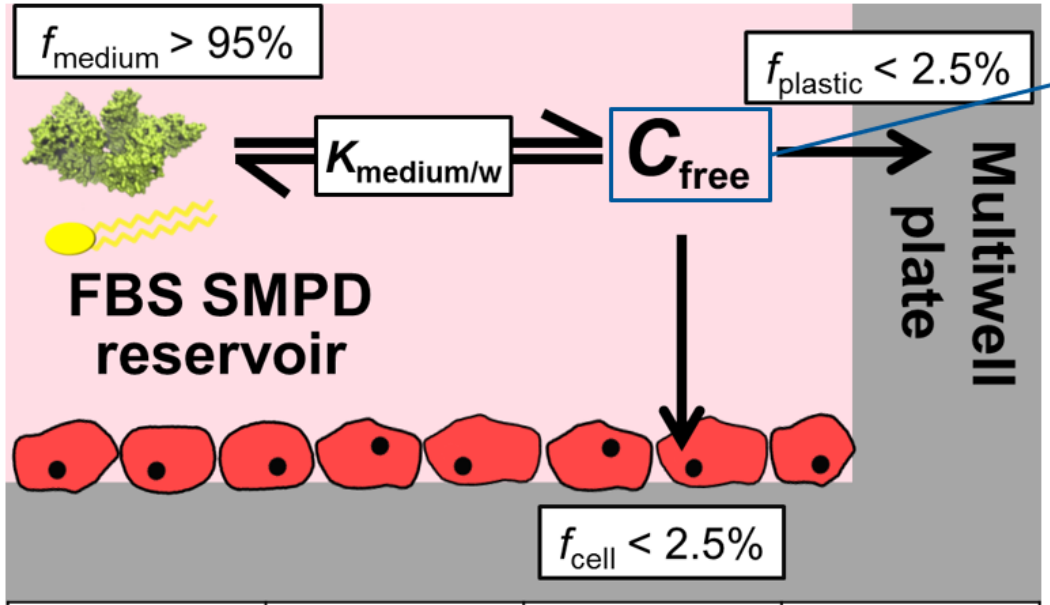
Environmental Science & Technology

Application of Experimental Polystyrene Partition Constants and Diffusion Coefficients to Predict the Sorption of Neutral Organic Chemicals to Multiwell Plates in in Vivo and in Vitro Bioassays

Fabian C. Fischer,[*,†] Olaf A. Cirpka,[‡] Kai-Uwe Goss,[§] Luise Henneberger,[†] and Beate I. Escher[†,‡]

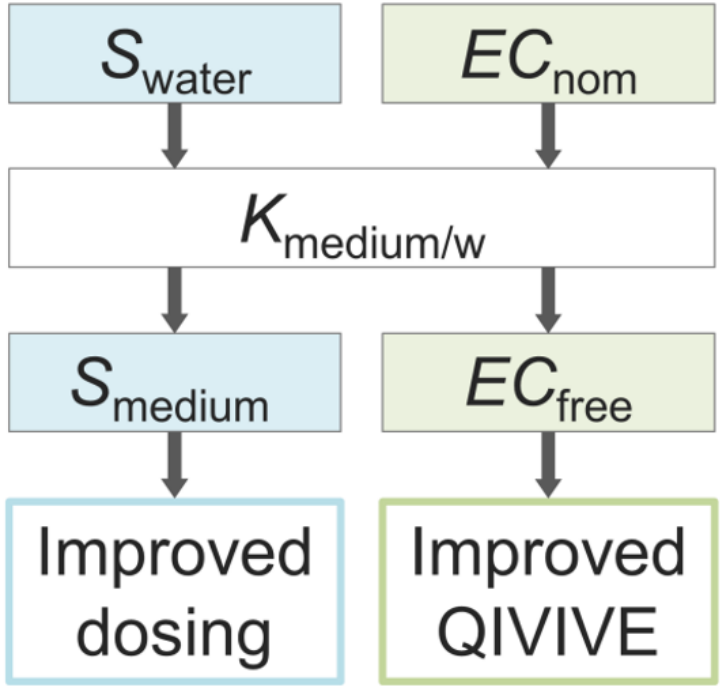
Adjusting the FBS content in the medium to achieve controlled exposure conditions and easier modeling

Equilibrium model sufficiently accurate?



Setup	96-well	384-well	1536-well
V_{medium}	120 µL	40 µL	6 µL
Cell No.	10,000	5,000	2,000
% FBS	≥ 3%	≥ 5%	≥ 10%

if C_{free} remains stable over time by SMPD



Chemical Research in Toxicology

How To Improve the Dosing of Chemicals in High-Throughput *in Vitro* Mammalian Cell Assays

Fabian C. Fischer,[†] Luise Henneberger,[†] Rita Schlichting,[†] and Beate I. Escher*[,†,‡]

Establishment of a quantitative extrapolation framework for risk assessment to protect human health

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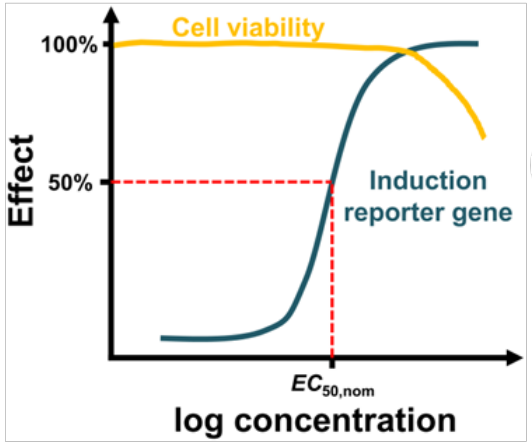
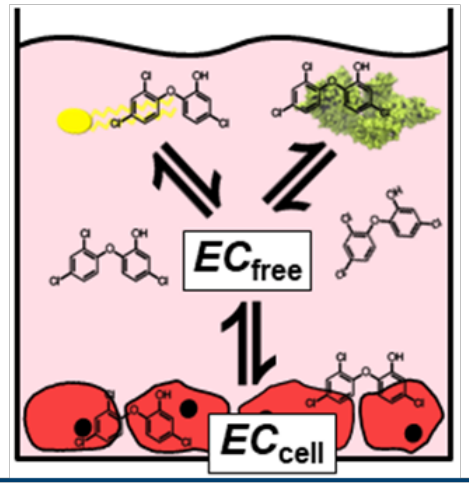
In vitro effect data

Measure specific effect and cytotoxicity

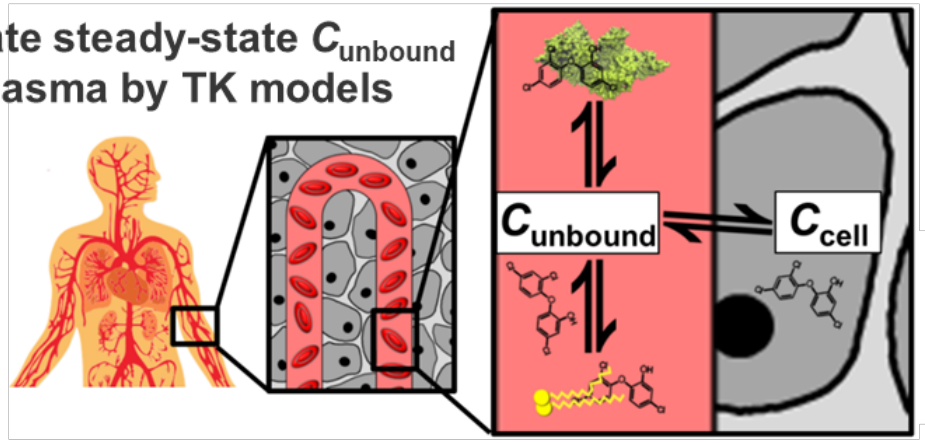


Convert $EC_{50,nom}$ values to $EC_{50,free}$ and $EC_{50,cell}$



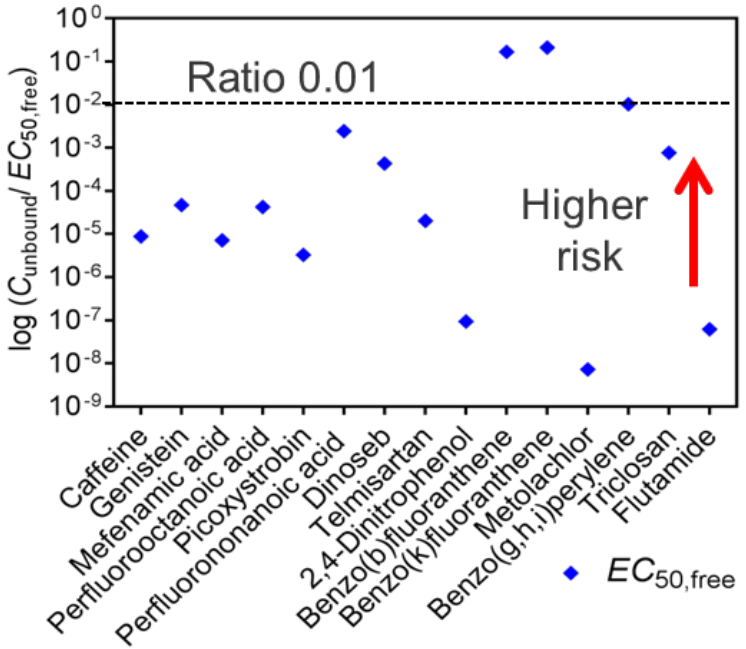
In vivo exposure data

Calculate steady-state $C_{unbound}$ in plasma by TK models



Compare *in vitro* effect to *in vivo* exposure data

Antioxidant response assay (AREc32 assay)



Ratio of $C_{unbound}/EC_{50,free}$ > 0.01 could indicate risk

Cytotoxicity burst – Specificity analysis of *in vitro* HTS effect data using mass balance modeling

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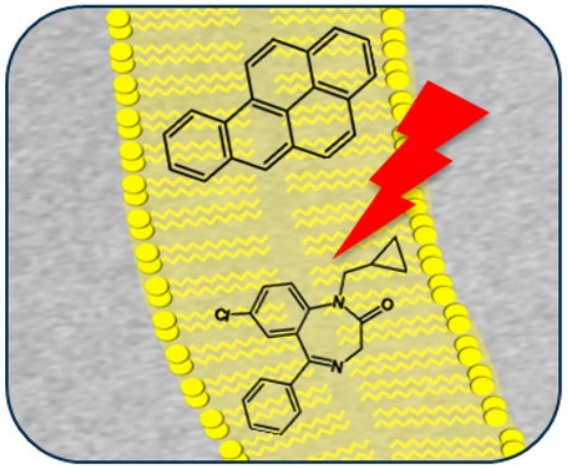
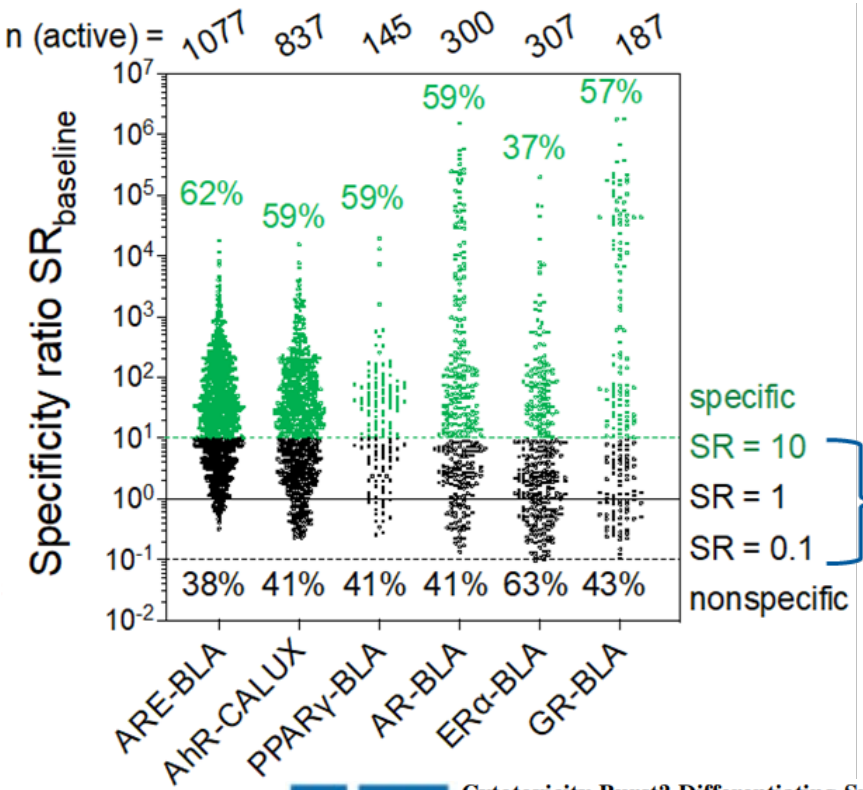
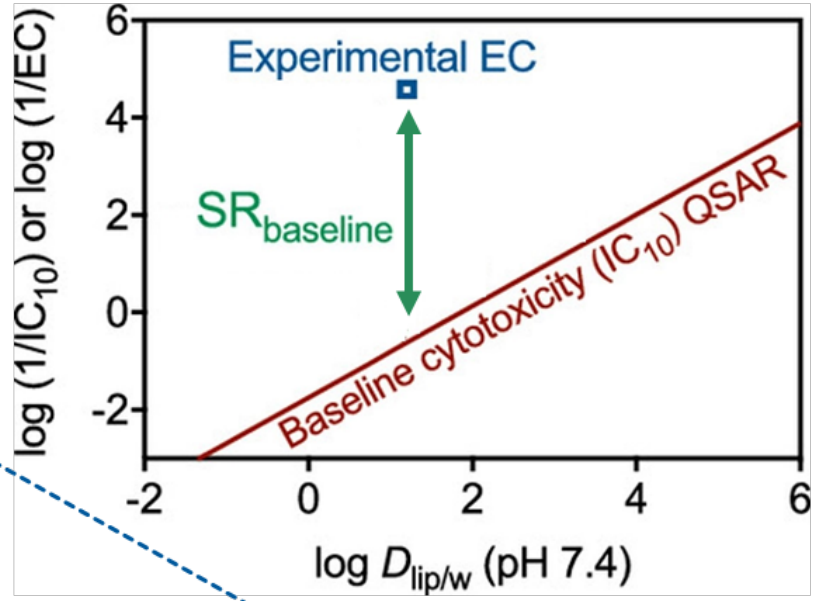
- Automated processing of ~8,000 chemicals with ~380,000 data points in MATLAB
- Specificity analysis of ToxCast/Tox21 effect data to identify highly potent chemicals and *potentially* false-positive effects





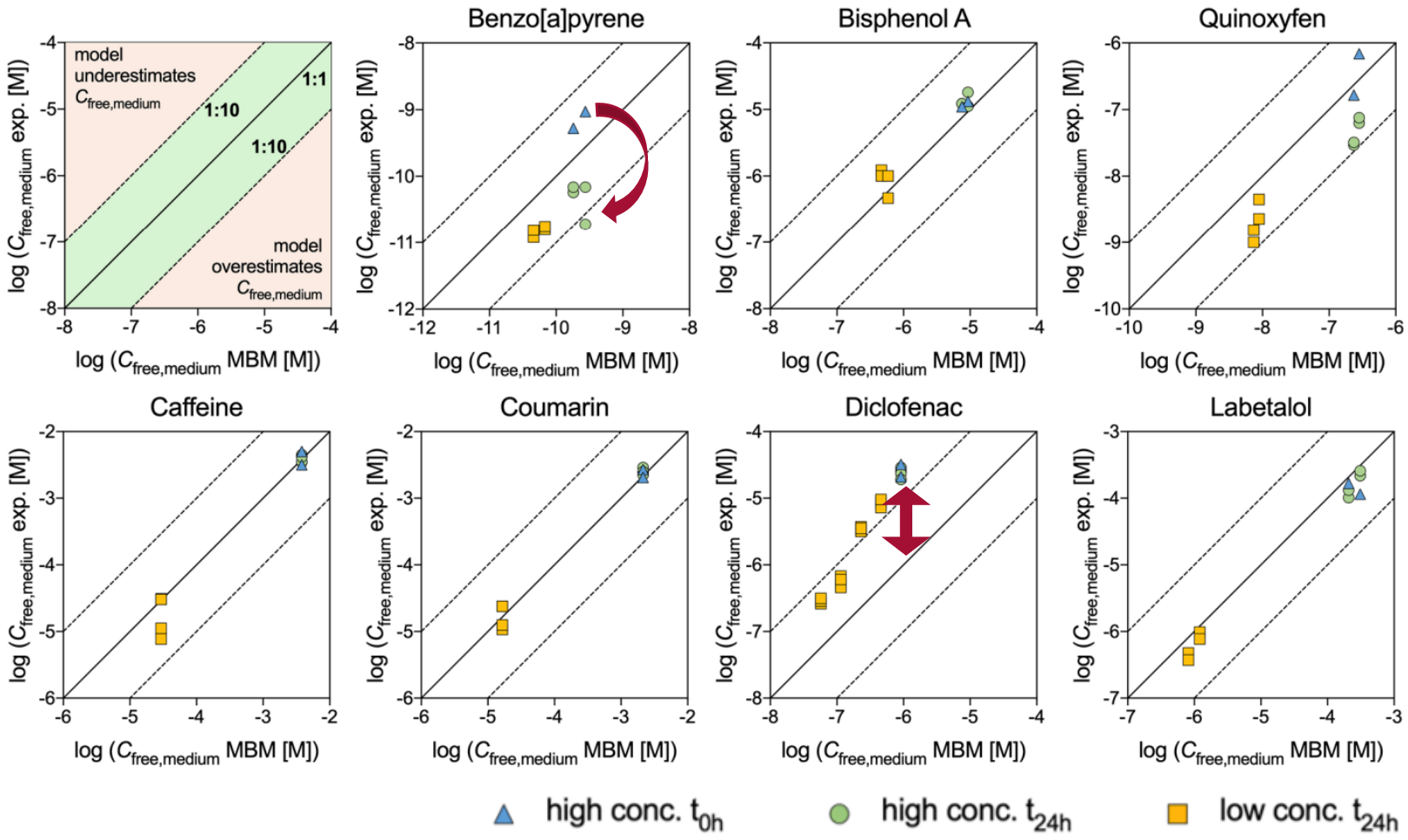


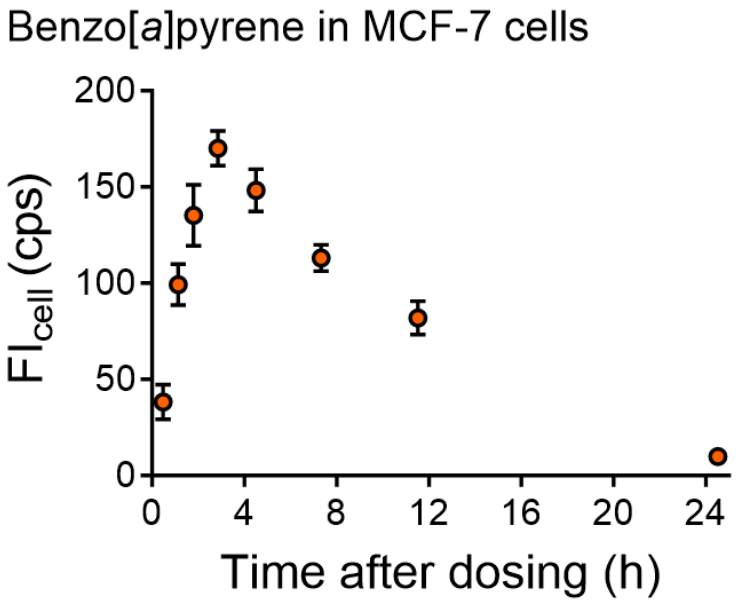
Effect artifacts?

Concept applicable to other modes of action?

ehp
Cytotoxicity Burst? Differentiating Specific from Nonspecific Effects in Tox21 *in Vitro* Reporter Gene Assays
Beate I. Escher,[1,2] Luise Henneberger,[1] Maria König,[1] Rita Schlichting,[1] and Fabian C. Fischer[1†]

Experimental evaluation of model performance: Measuring C_{free} in *in vitro* cell assays



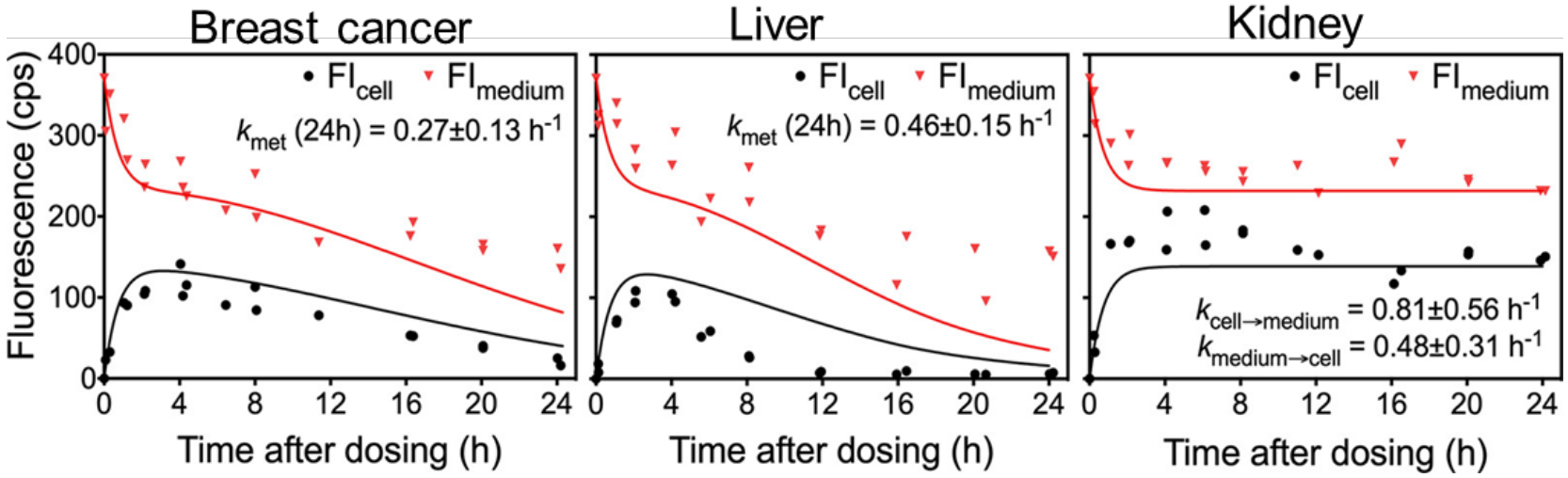


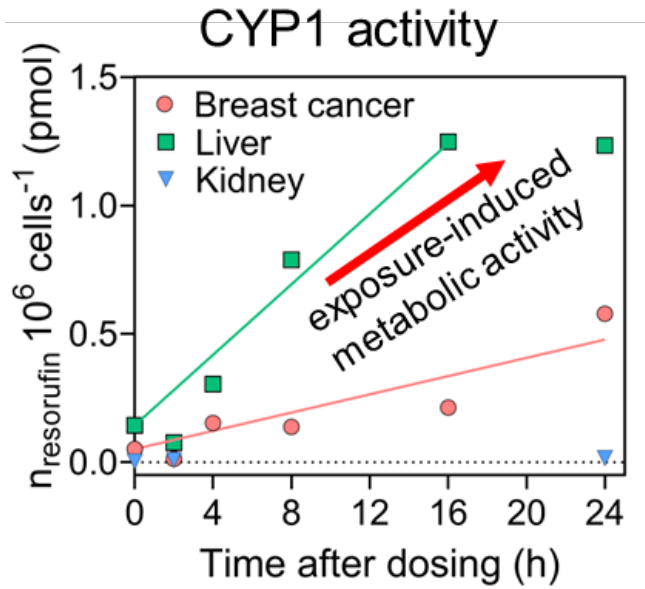
? Metabolism?

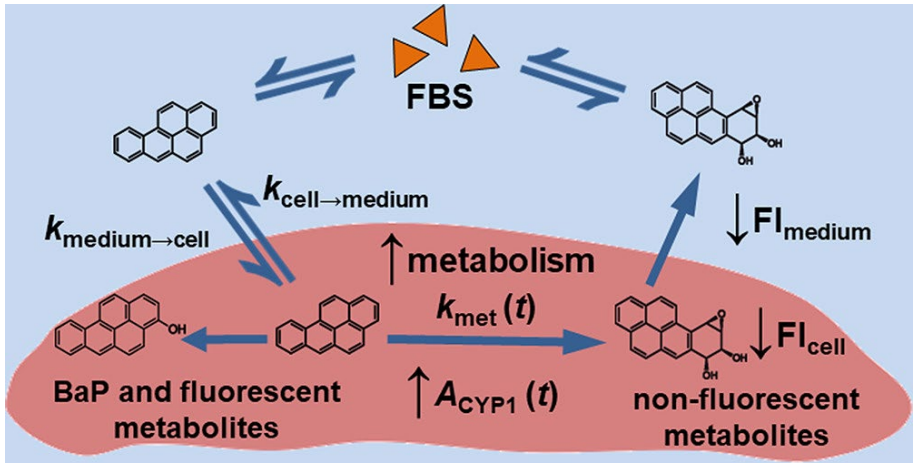
Quantification of freely dissolved effect concentrations in in vitro cell-based bioassays

Luise Henneberger[1] · Marie Mühlenbrink[1] · Maria König[1] · Rita Schlichting[1] · Fabian C. Fischer[1] · Beate I. Escher[1,2]

Characterizing metabolism of *in vitro* cells: Fit experimental data to 2-comp kinetic transport model







$$\frac{dFI_{medium}}{dt} = k_{cell→medium} \cdot FI_{cell}(t) - k_{medium→cell} \cdot FI_{medium}(t)$$

$$\frac{dFI_{cell}}{dt} = k_{medium→cell} \cdot FI_{medium}(t) - (k_{cell→medium} + k_{met}(t)) \cdot FI_{cell}(t)$$

$$k_{met}(t) = k_{met} t \frac{m_{CYP}}{A_{control}}$$

Chemical Research in Toxicology

Cellular Metabolism in High-Throughput *In Vitro* Reporter Gene Assays and Implications for the Quantitative *In Vitro–In Vivo* Extrapolation

Fabian C. Fischer, Cedric Abele, Luise Henneberger, Nils Klüver, Maria König, Marie Mühlenbrink, Rita Schlichting, and Beate I. Escher*

Chemical transport models to investigate the distribution of chemicals in *in vitro* cell assays

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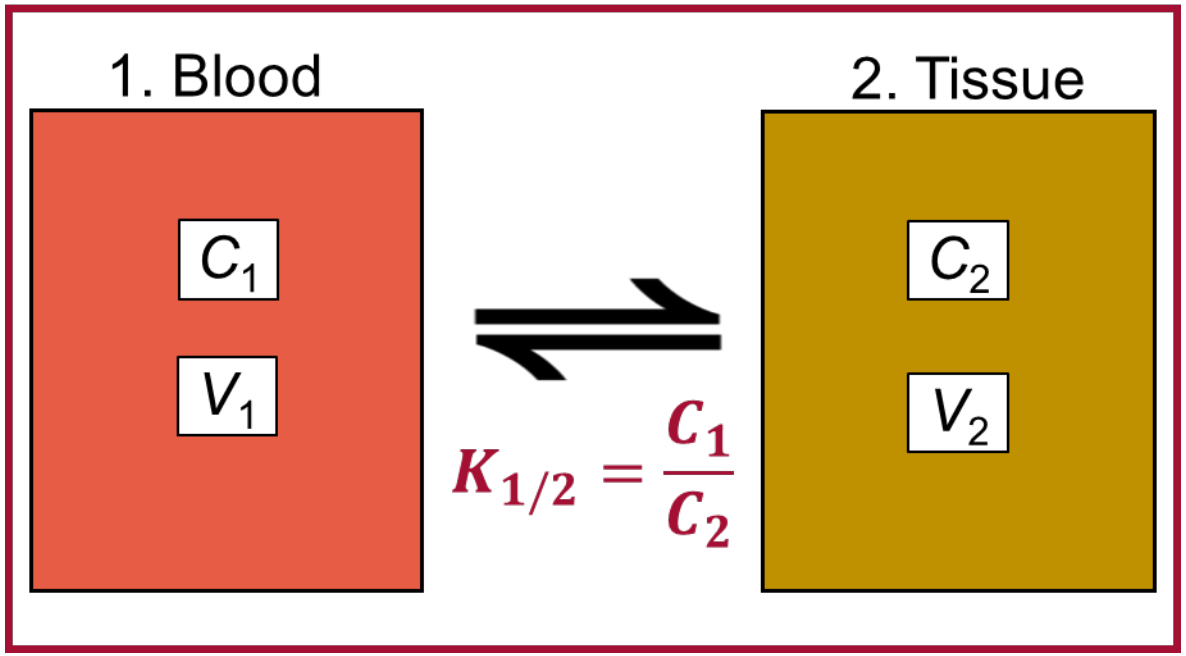
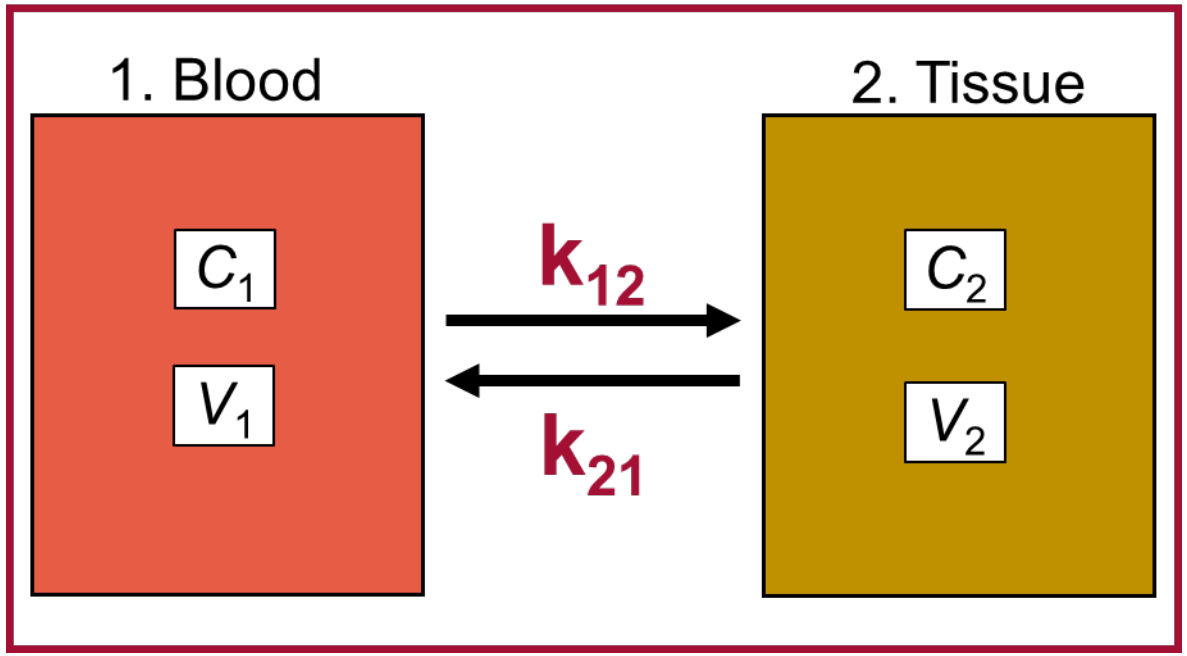
- ? Can the process be approximated with an equilibrium or kinetic model?
- ? How much complexity is necessary to predict/explain exposure?

Equilibrium mass balance model



Kinetic model (rate-limited)

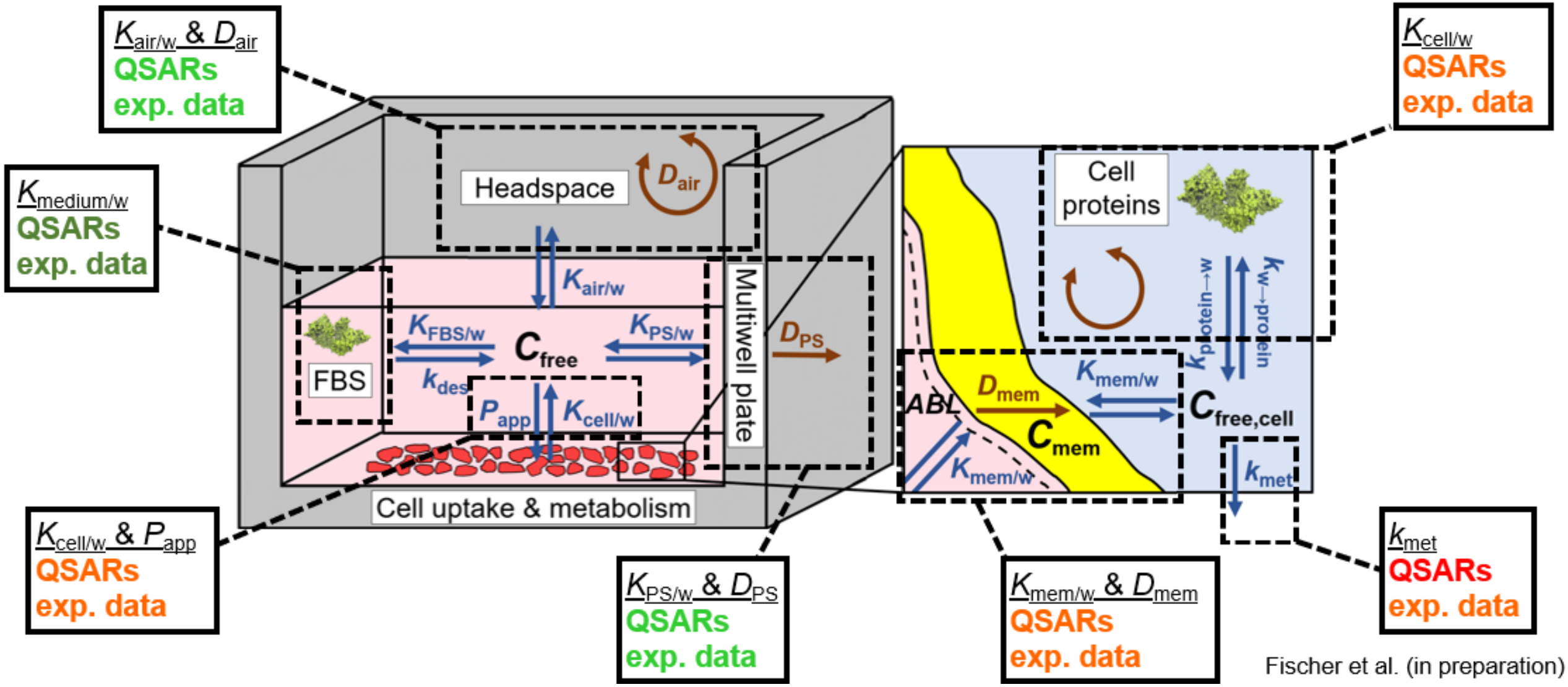


Can we simplify?
For *in vitro* exposure, YES*!

***but there are exceptions**

Work in progress: Implementation of a kinetic transport model for *in vitro* cell-based bioassays

- Limited parameters available to parameterize a comprehensive *in vitro* kinetic model



Fischer et al. (in preparation)

Work in progress: Implementation of a kinetic transport model for *in vitro* cell-based bioassays

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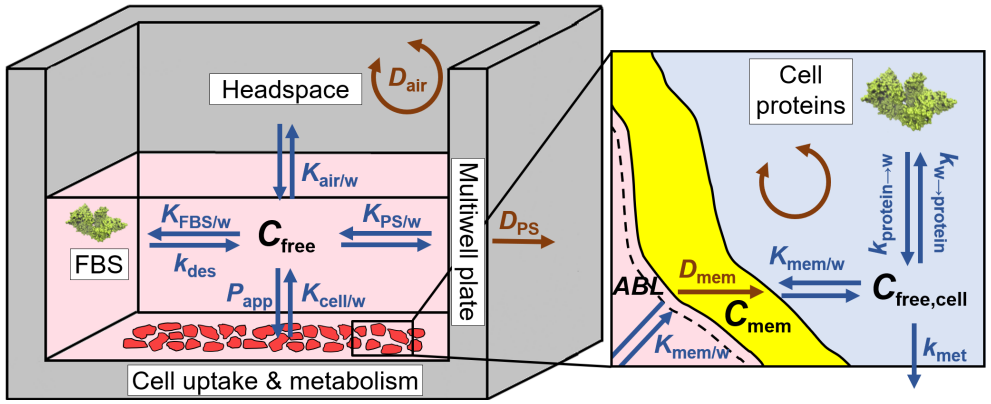
```
#### SECTION 2: DEFINITION OF CHEMICAL PARAMETERS ####
Mw      = 250                          # Molecular weight of chemical
Mw_BSA  = 66500                        # Molecular weight of BSA
D_BSA   = 7.0 * 10^-5 * Mw_BSA^(-0.45)

# Literature partition coefficients
K_alb_w     = 1 * 10^4                 # albumin-water partition coefficient (L_w/L_alb)
K_lip_w     = 1 * 10^4                 # phospholipid-water partition coefficient (L_w/L_lip)
K_well_w    = 1 * 10^4                 # well plastic-water partition coefficient (L_w/L_well)
K_aw        = 5 * 10^-7                # air-water partition coefficient (L_w/L_air)

# Kinetic parameters
D_water     = 9.9 * 10^-5 * Mw^(-0.45)                          # Diffusion coefficient of chemical in water (cm2/s)
D_ABL       = D_water + K_alb_w * V_prot_medium * D_BSA          # Diffusion coefficient of chemical in ABL (cm2/s)
D_mem       = 10^(-5.13-0.453*log10(Mw))                        # Diffusion coefficient of chemical in membrane (cm2/s)
P_app       = 1 / ((x_mem/(D_mem * K_lip_w))+(x_w/D_ABL))       # Apparent permeability of chemical (cell uptake) (cm/s)
D_well      = 1 * 10^-12                                        # Diffusion coefficient of chemical in well plastic (cm2/s)
k_met       = 0.01                                              # Elimination rate constant (cell metabolism) (1/s)
k_abiot     = 1 * 10^-5                                         # Abiotic degradation rate constant in medium (1/s)
```

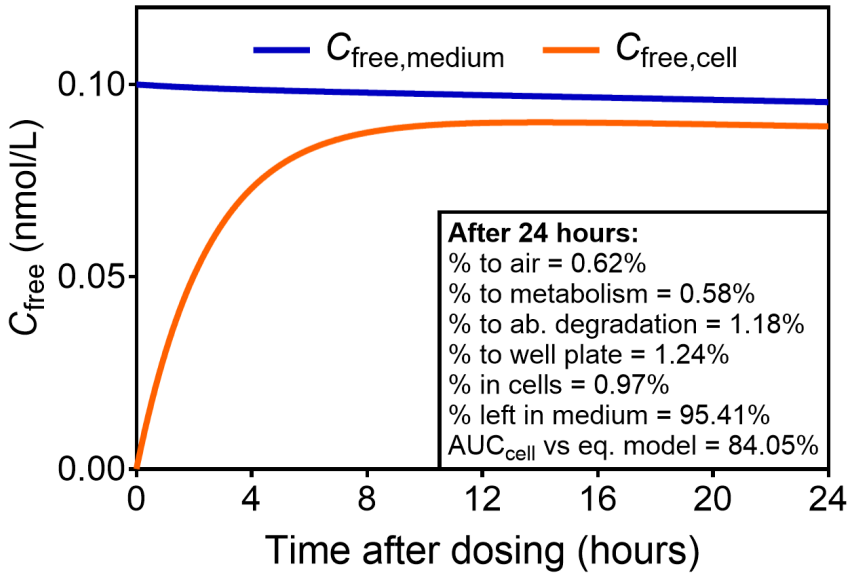


Fischer et al. (in preparation)

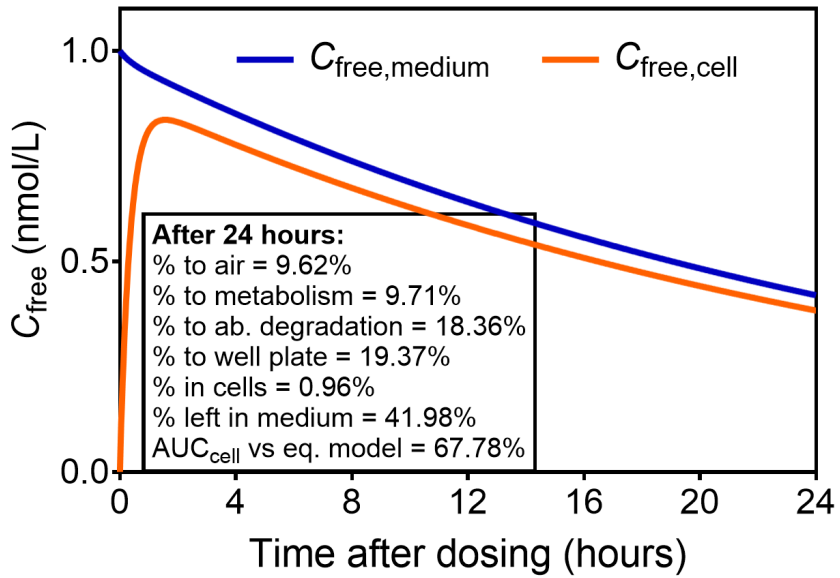
➢ 96-well plate, 120 µL OptiMEM + 10% FBS

➢ 0.5% - 10% FBS

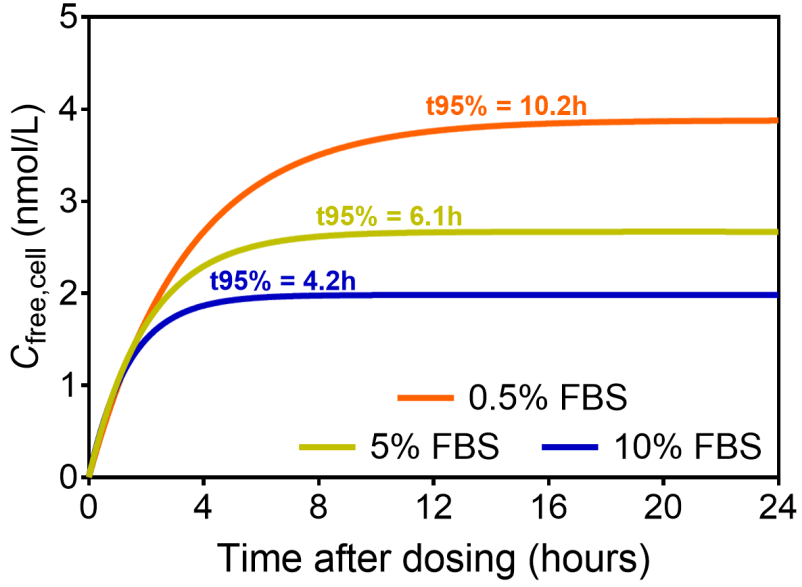
A. $K_{medium/w}$ = 10,000



B. $K_{medium/w}$ = 1,000



C. BaP at different FBS



Acknowledgments

Harvard John A. Paulson School of Engineering and Applied Sciences

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Collaborators and Colleagues

THE UNIVERSITY OF RHODE ISLAND Graduate School of Oceanography

UFZ

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Funding

STEEP
Sources, Transport, Exposure & Effects of PFAS
Nr.: P42ES027706

NIH

ATSDR Agency for Toxic Substances and Disease Registry
Nr.: 1U01TS000313-01

Thank you for your attention! Questions?

Impact of Chemical-PDMS Interactions on IVIVE from Microphysiological Systems

PI: M. Shane Hutson

Co-Investigators:

Lisa J. McCawley

Dmitry Markov

Students

Nate Hermann

Richard Ficek

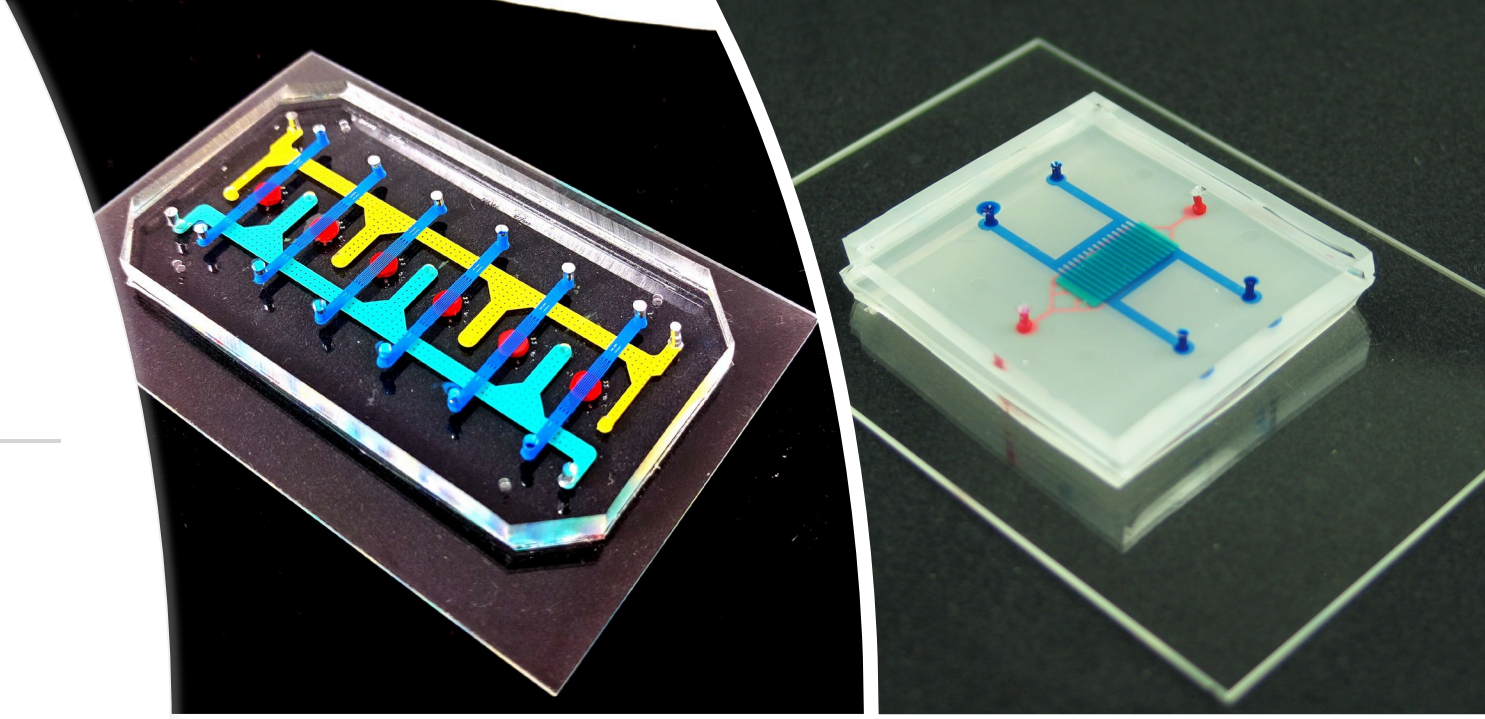
Kazi Tasneem (Genentech)

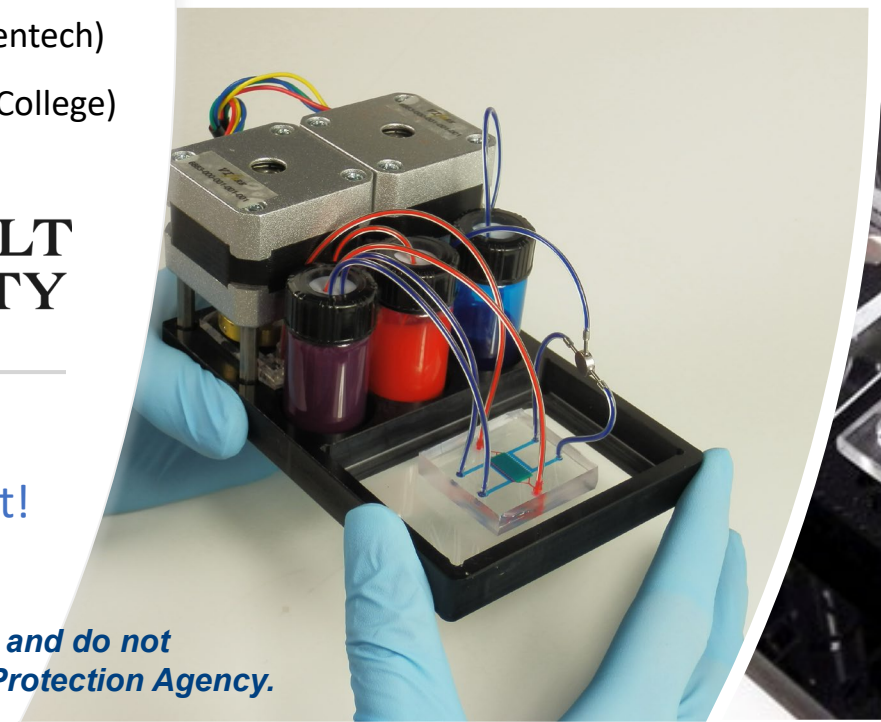
Alex Auner (Boston College)

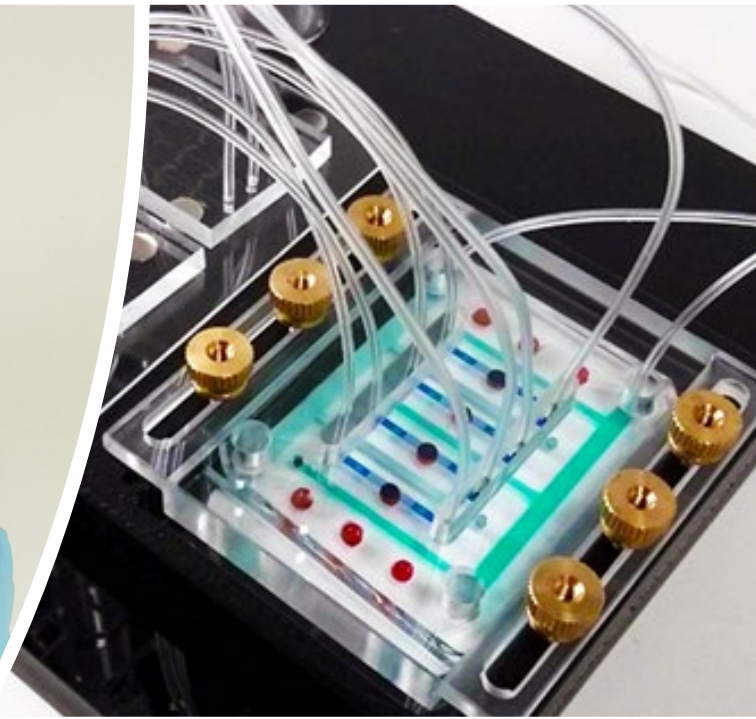
QA Manager

Phillip Fryman

VANDERBILT UNIVERSITY



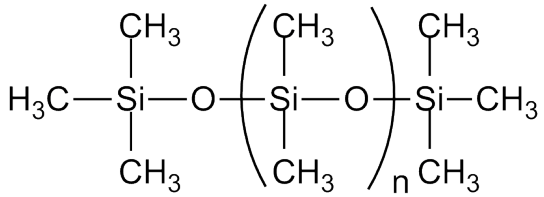




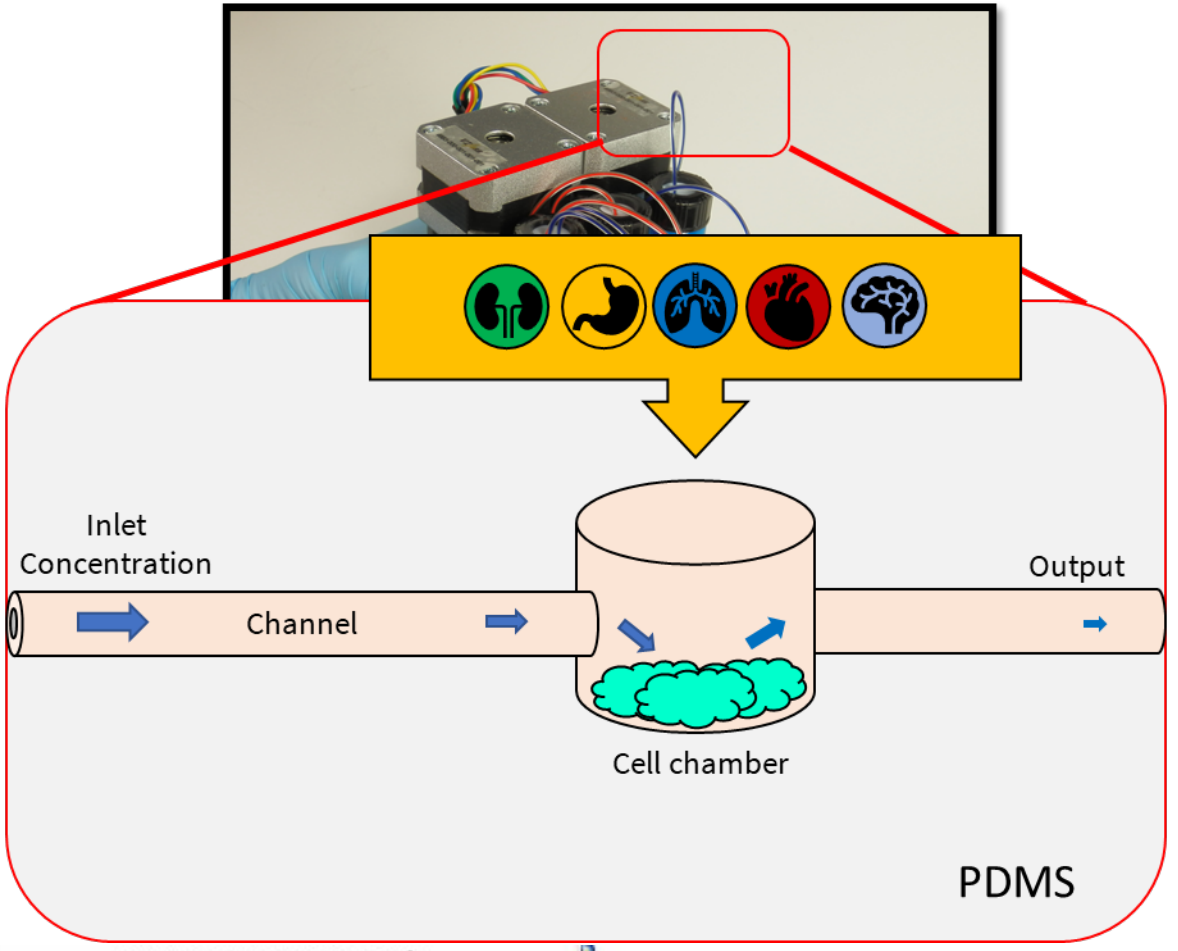
Thanks to U.S. EPA for funding support!

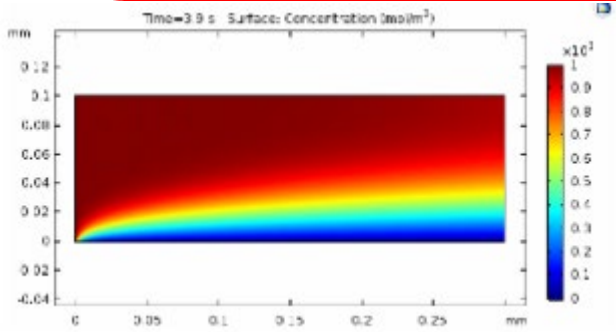
Microphysiological Systems (Organ-on-Chip Devices)

- A New Approach Method (NAM) to fill the bridge between cell-based assays and animal trials.
  - Better representation of human response
  - Useful for high throughput testing
  - Addresses ethical concerns
- Typically made of polydimethylsiloxane (PDMS):
  - Inexpensive
  - Flexible
  - Transparent
  - Gas permeable



- **But PDMS tends to sequester hydrophobic compounds.**
- Need to avoid, mitigate, or measure and model.





Talk Outline

1. Measure chemical-PDMS interaction and transport parameters.
   a. Partition coefficients: $k = C_{PDMS}/C_{sol}$
   b. Diffusion coefficients in PDMS: D_P
   c. Evaluate correlations with chemical properties

2. Be wary of read-across methods.

3. Validate multiphysics models for in-device toxicokinetics.

4. Investigate the effectiveness of mitigation strategies for reducing chemical sequestration in microfluidic devices:
   a. SEBS co-polymers as PDMS alternatives; and
   b. media with carrier proteins.

Talk Outline

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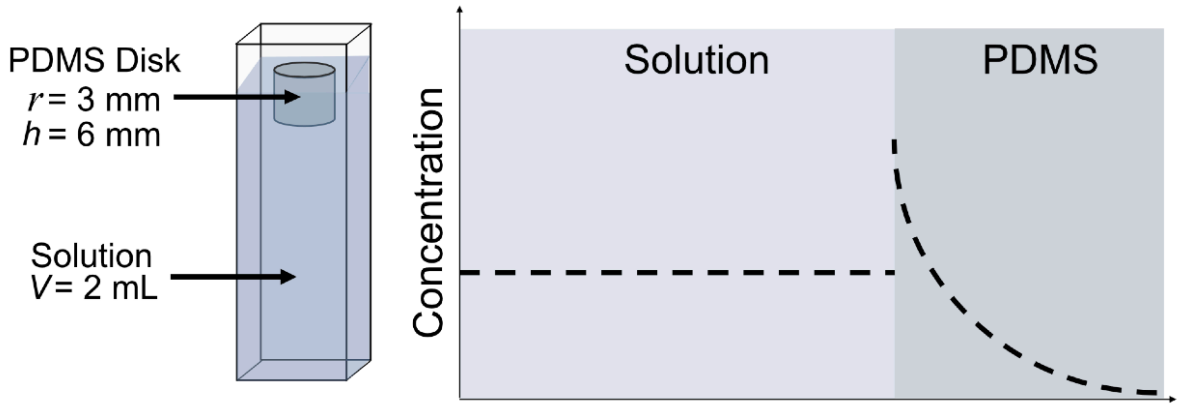
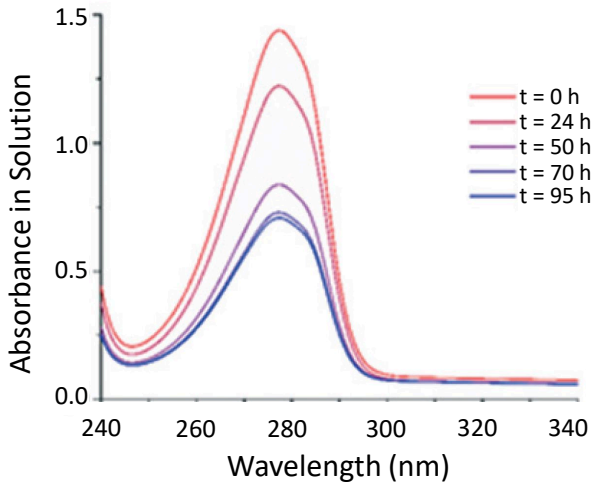
Measuring Chemical-PDMS Interaction and Transport Parameters

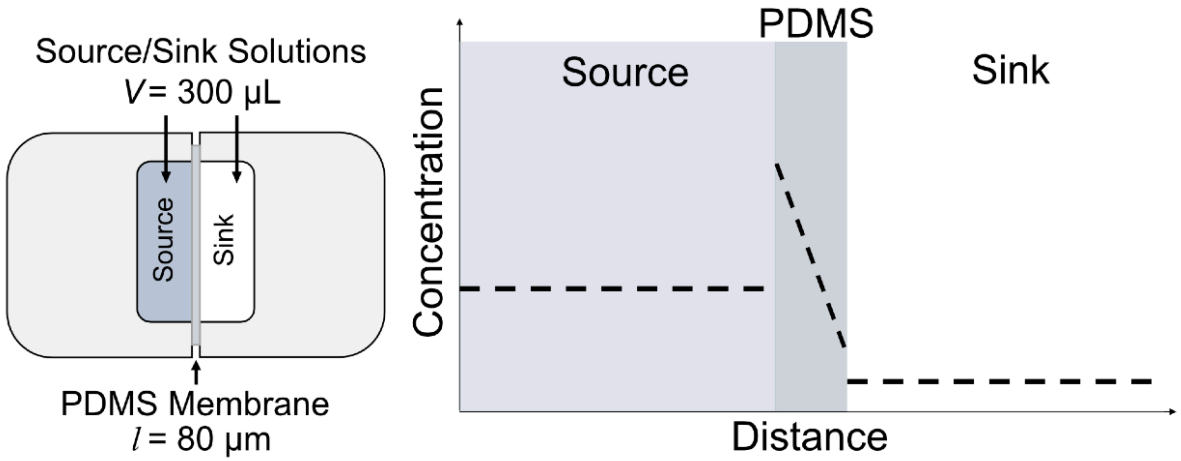
Disk Soak Experiments

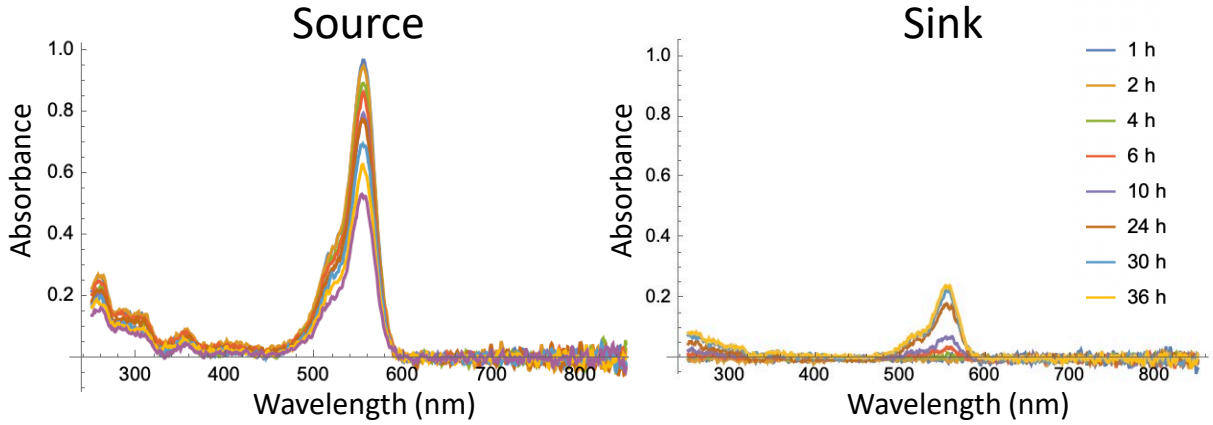




Auner et al, *Lab on a Chip* 2019
Hermann et al, *bioRxiv*, 2024

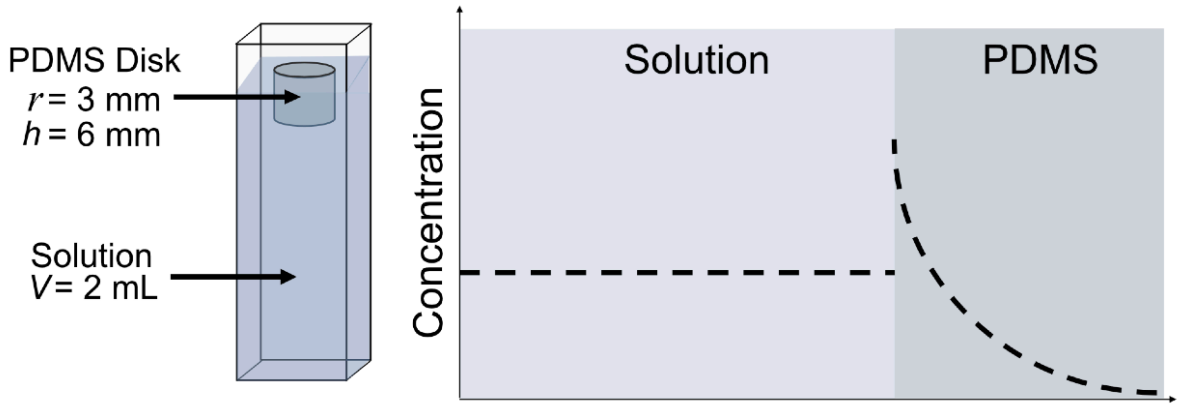
Membrane Experiments



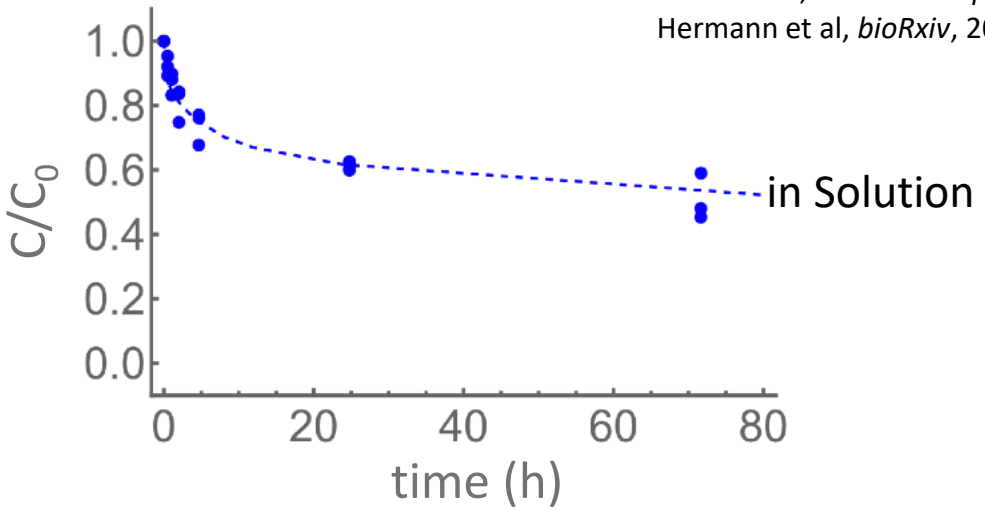


Measuring Chemical-PDMS Interaction and Transport Parameters

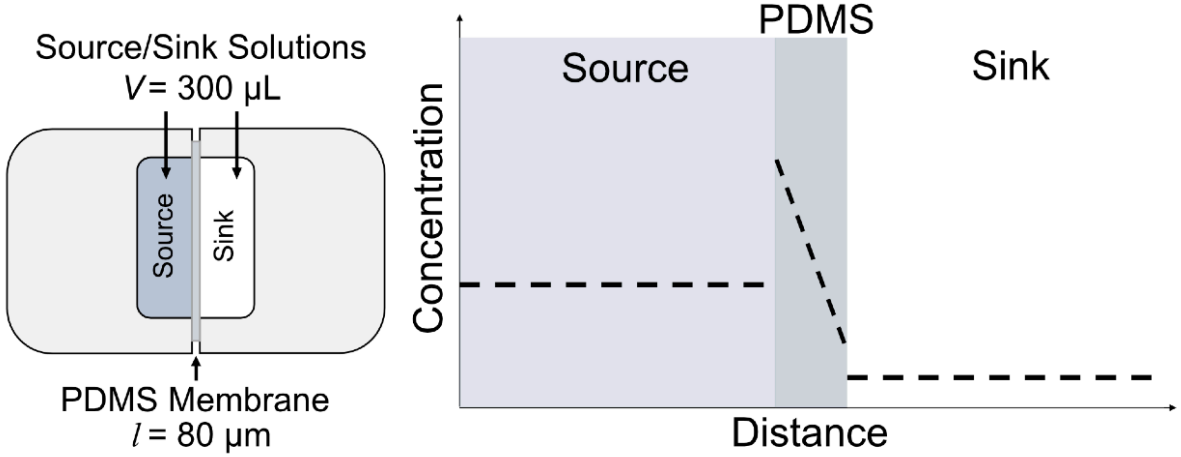
Disk Soak Experiments

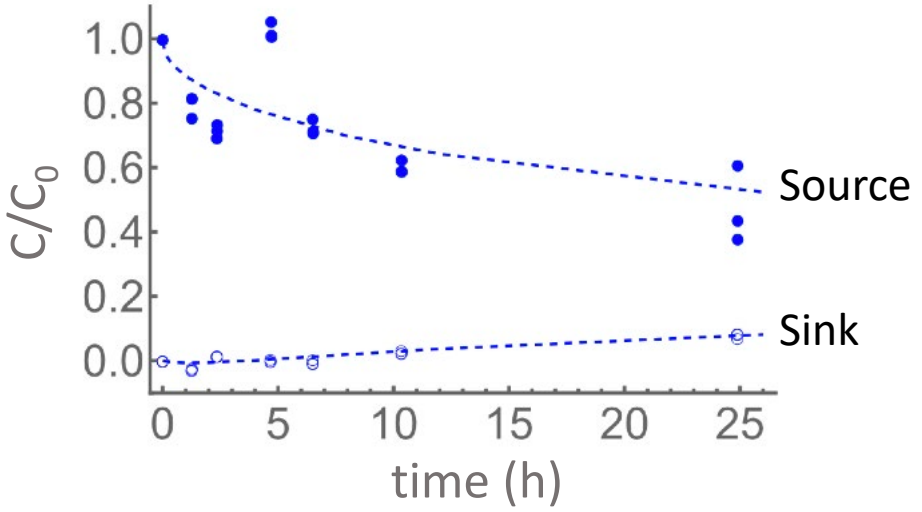


Auner et al, *Lab on a Chip* 2019
Hermann et al, *bioRxiv*, 2024



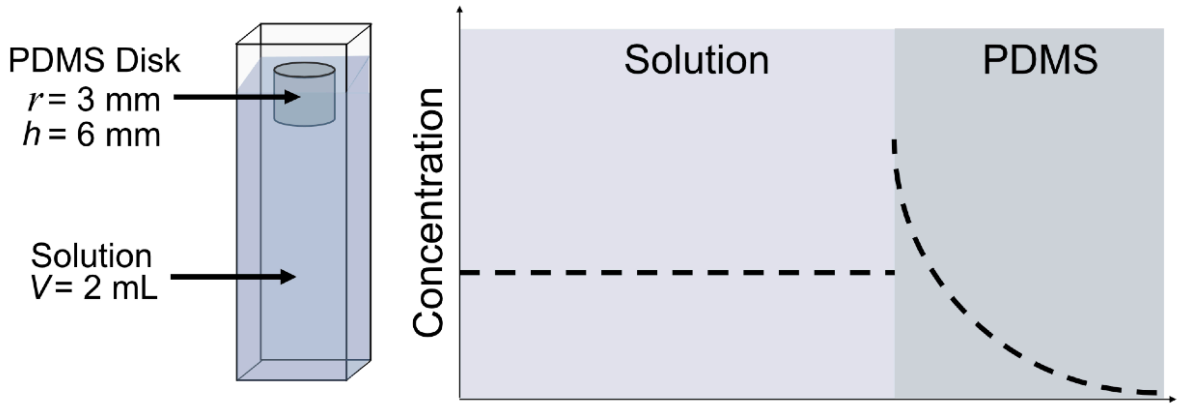
Membrane Experiments





Measuring Chemical-PDMS Interaction and Transport Parameters

Disk Soak Experiments



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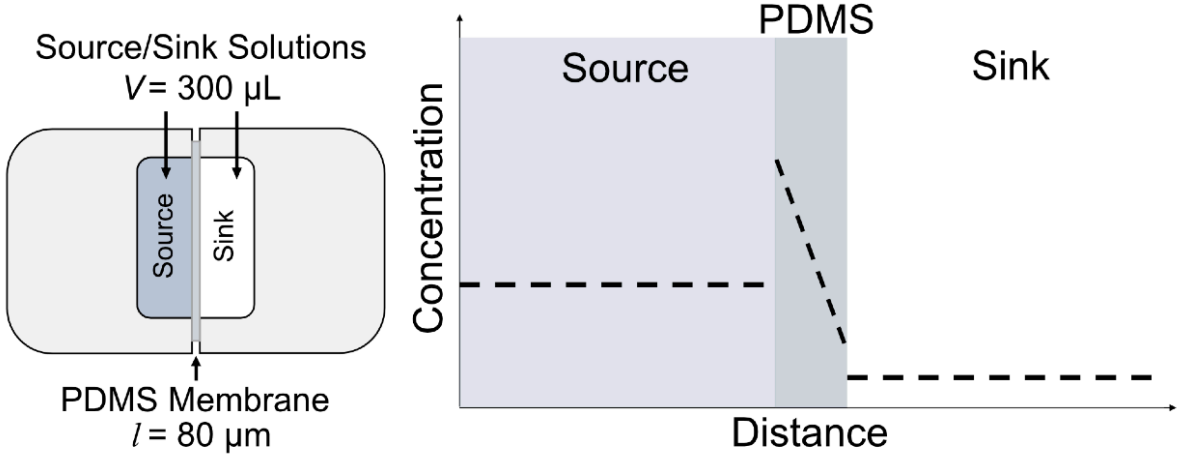

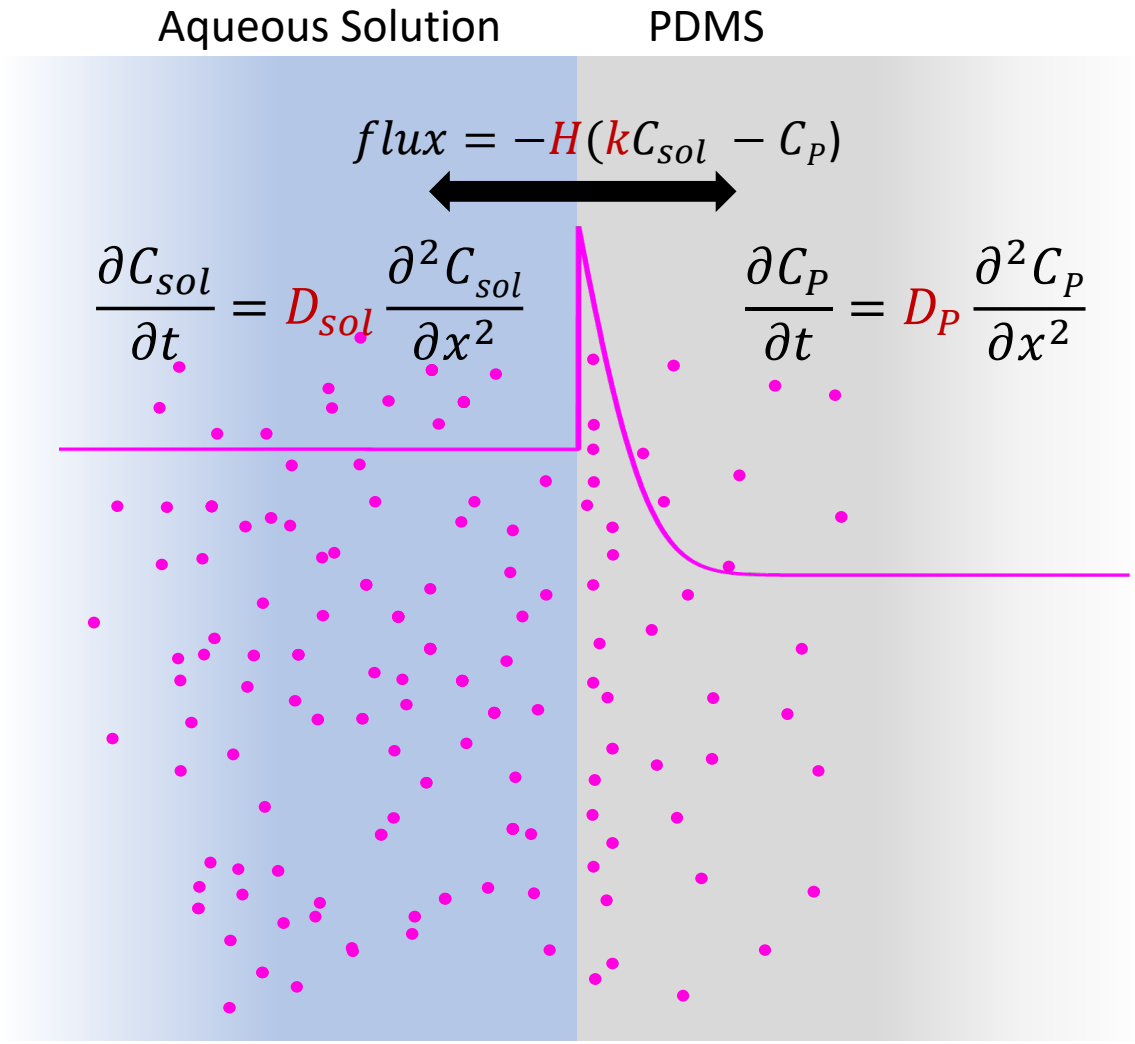

Data fit to a 4-parameter partition-diffusion model

Test set of 10 chemicals

Table 1 CAS numbers, relevance, and selected properties for chemicals tested. Chemical properties sourced from PubChem.[34]

Chemical name	CAS number	Relevance	logP	H-Bond Donors	Molar Mass (amu)
Rhodamine 6G	989-38-8	Fluorescent dye	6.4	2	479.0
Benzo[a]pyrene	50-32-8	PAH/Combustion byproduct	6.1	0	252.3
Chlorpyrifos	2921-88-2	Organophosphate pesticide	5.0	0	350.6
Fluorescein-5-isothiocyanate	3326-32-7	Fluorescent dye	4.8	2	389.4
Parathion	56-38-2	Organophosphate pesticide	3.8	0	291.3
Amodiaquine	82-46-0	Pharmaceutical	3.7	2	355.9
Fluorescein	2321-07-5	Fluorescent dye	3.4	2	332.3
Indole	120-72-9	Indole	2.1	1	117.2
Paraoxon	311-45-5	Organophosphate pesticide metabolite	2.0	0	275.2
Rhodamine B	81-88-9	Fluorescent dye	1.9	1	479.0

Test set of 10 chemicals

7 were sufficiently soluble to perform experiments directly in phosphate-buffered saline

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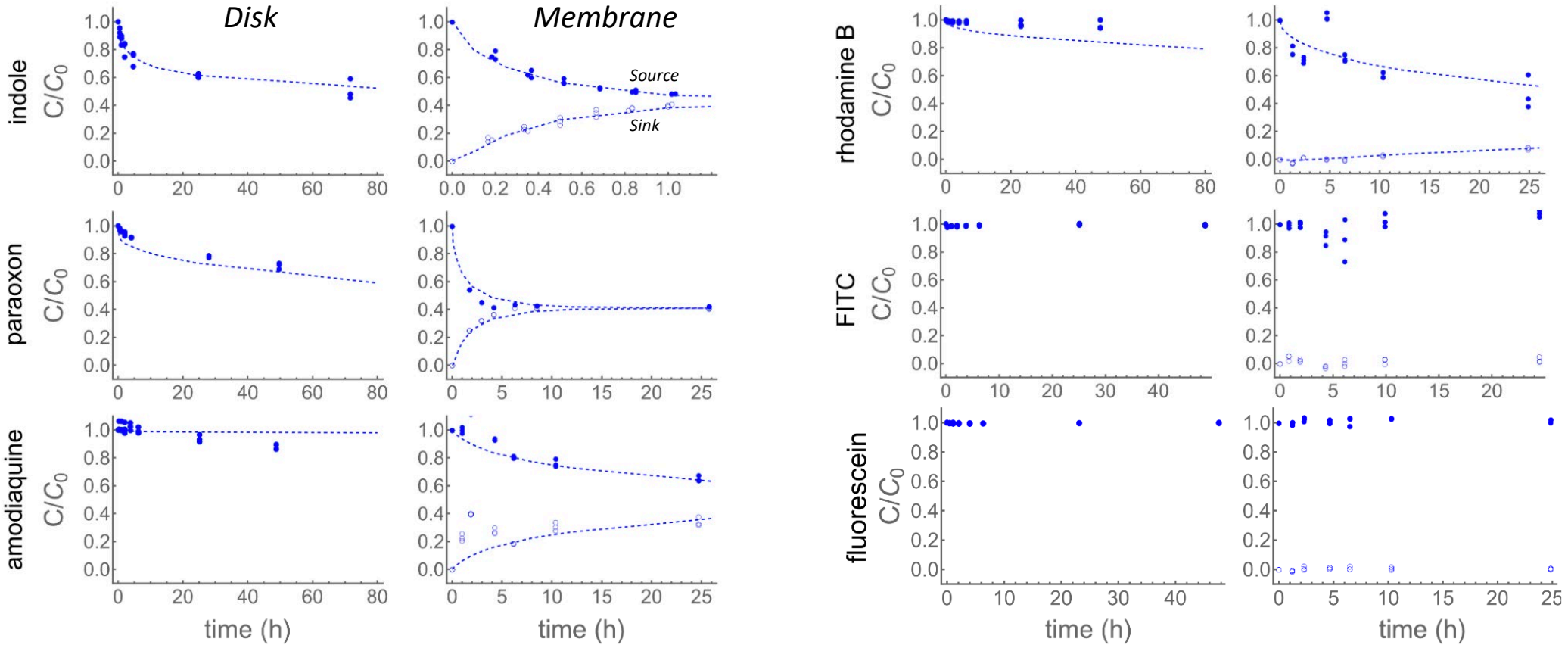

Test set of 10 chemicals

3 required the addition of DMSO as a cosolvent

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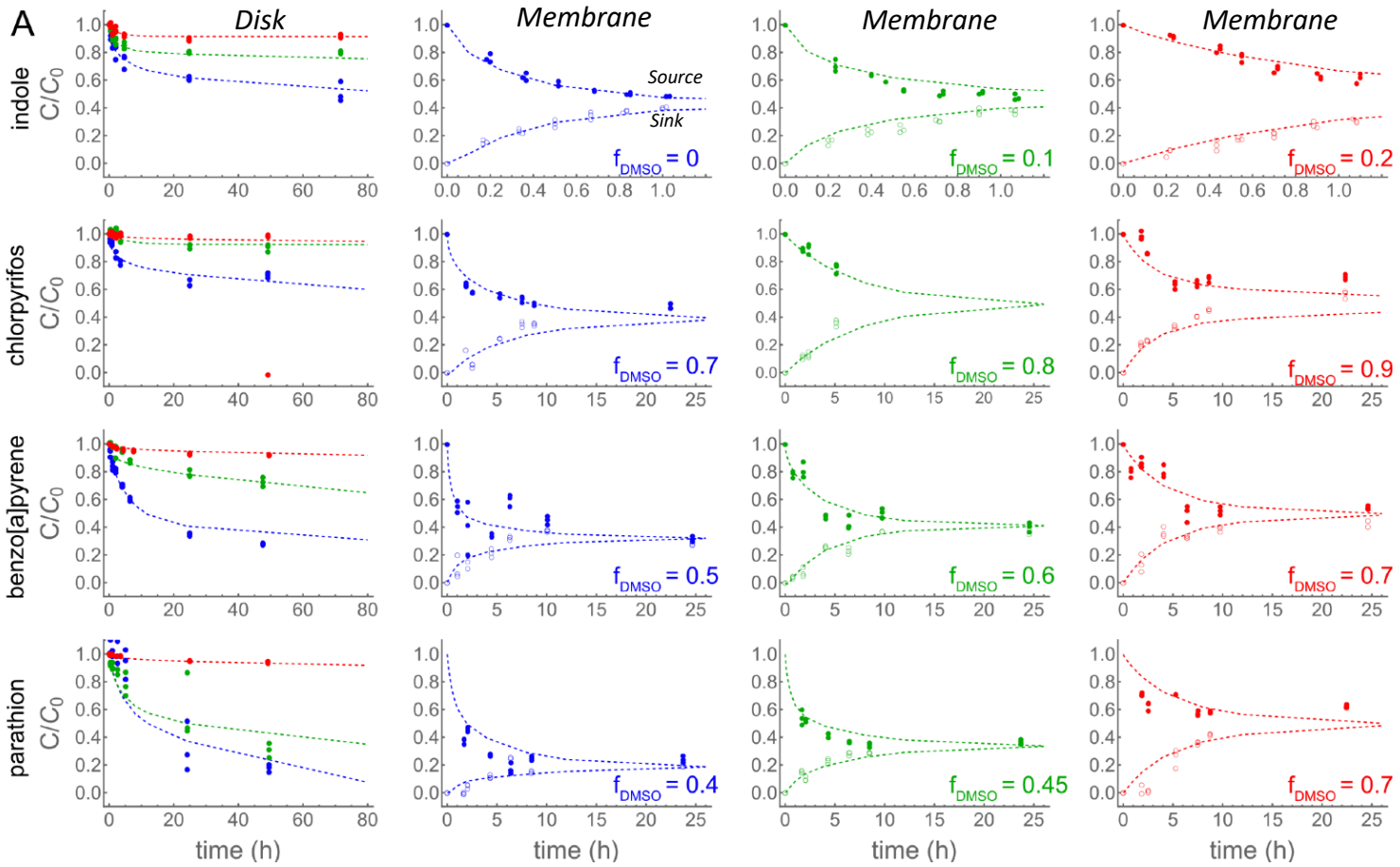

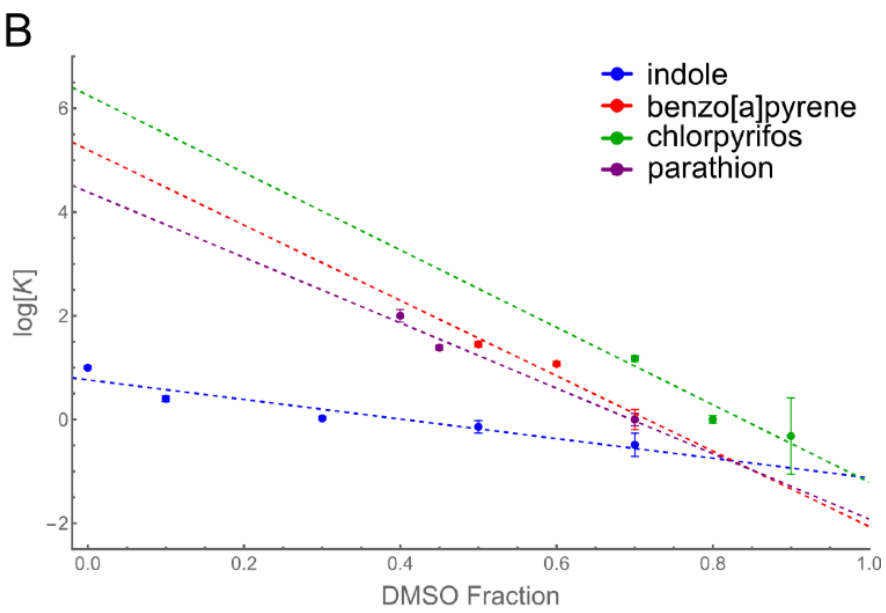

Log-linear relationship between partition coefficient (*k*) and cosolvent fraction

Test set of 10 chemicals

3 required the addition of DMSO as a cosolvent

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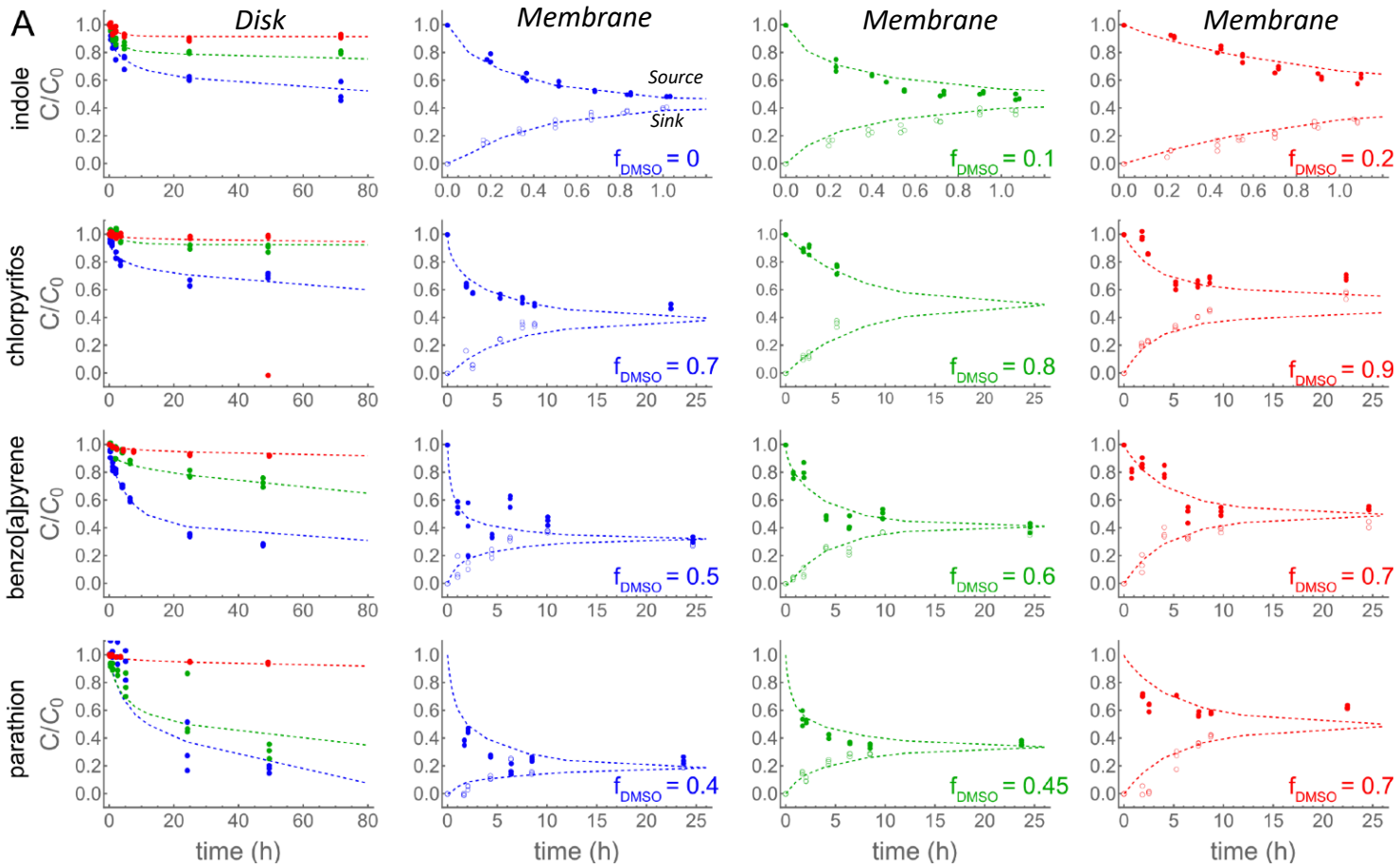
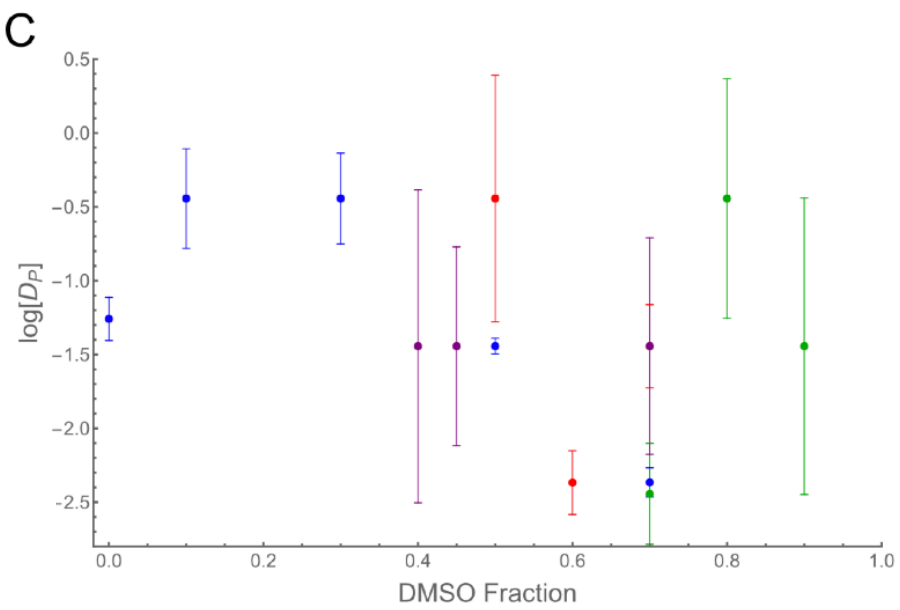


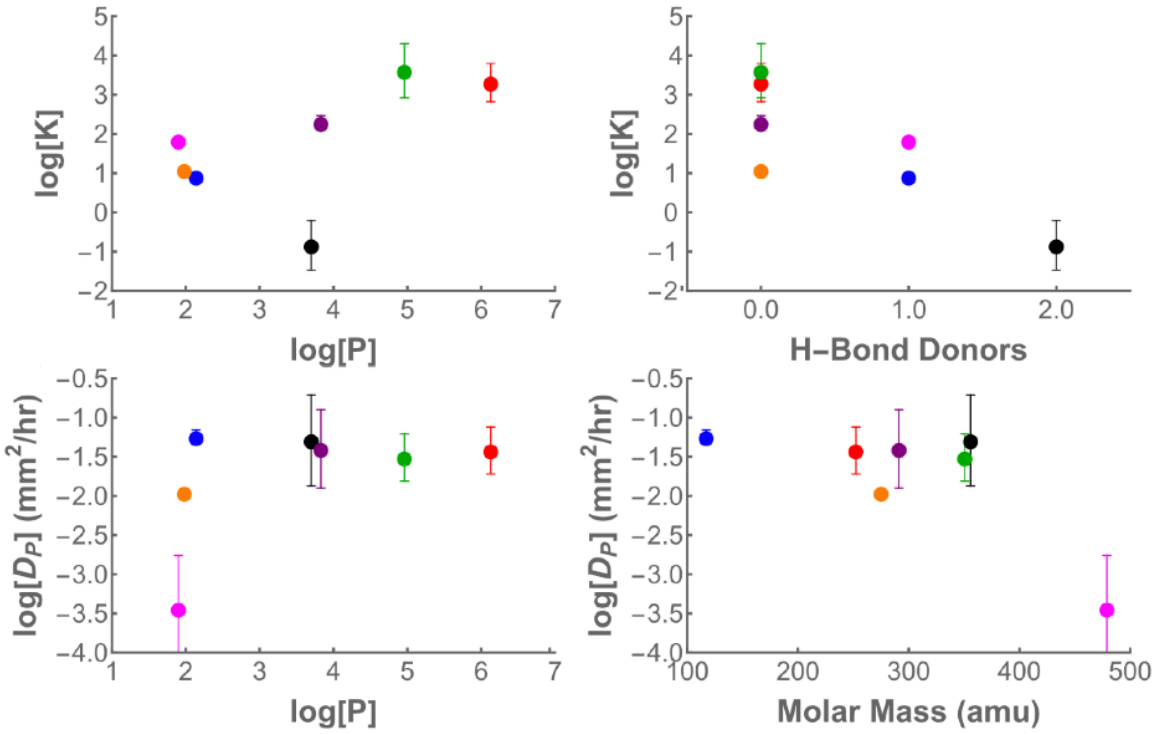


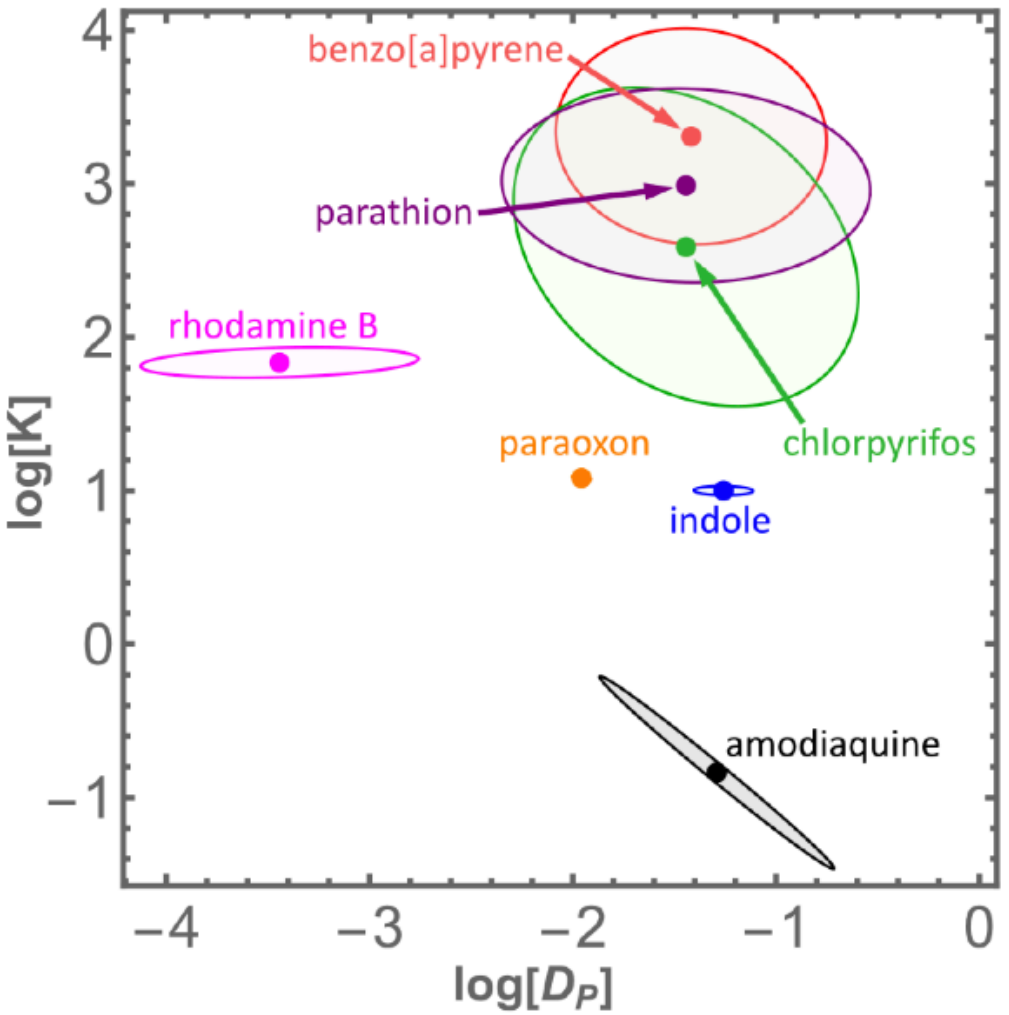
No clear relationship between diffusion constant in PDMS (D_P) and cosolvent fraction

The best-fit chemical-PDMS interaction parameters span several orders of magnitude

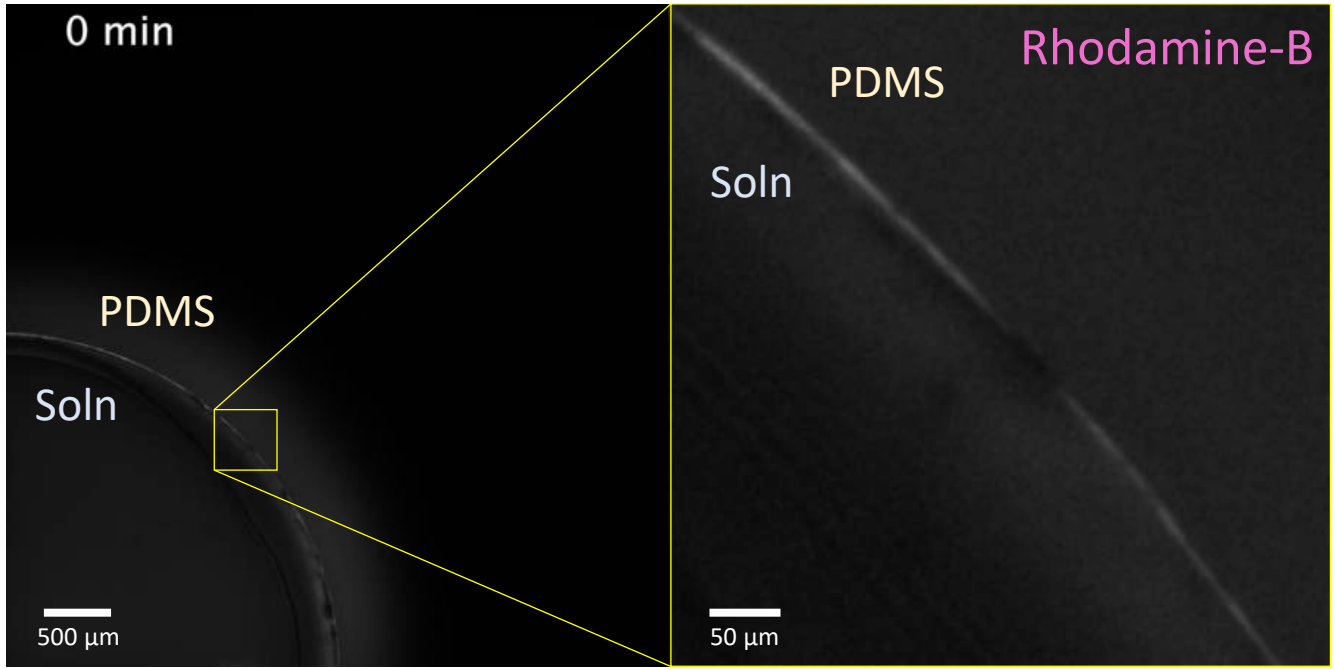
Table 2 Best-fit parameter values for the partition-diffusion model

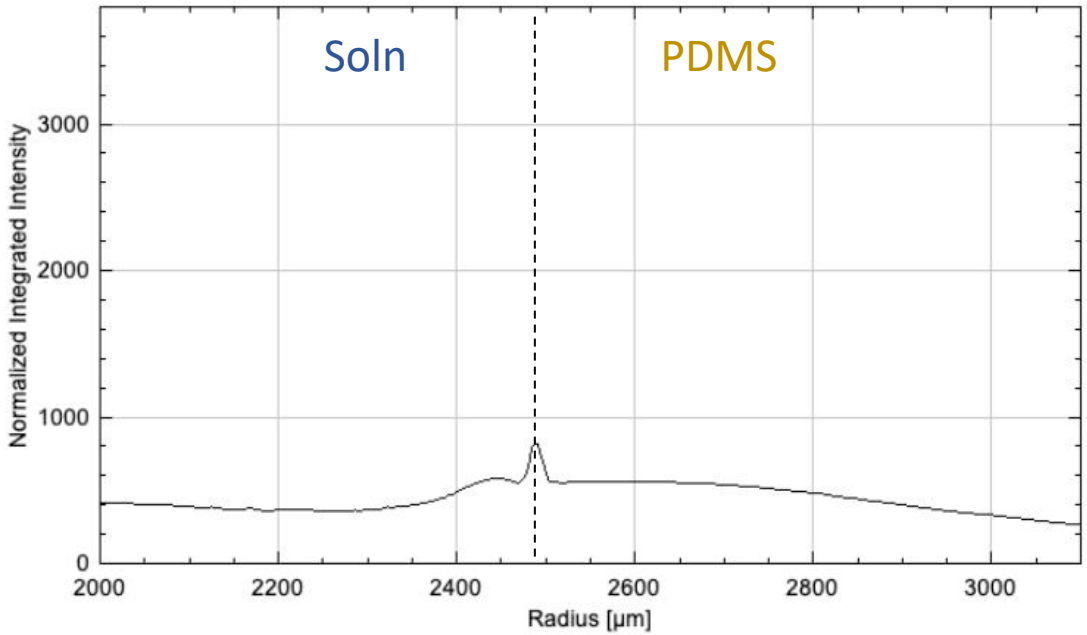
Chemical	$\log K_{PW}$	$\log K_{PD}$	$\log D_P$ ($mm^2\ h^{-1}$)
Chlorpyrifos	6.25 ± 1.98	-1.21 ± 0.53	-1.51 ± 0.30
Benzo[a]pyrene	5.20 ± 1.22	-2.06 ± 0.82	-1.42 ± 0.30
Parathion	4.39 ± 0.50	-1.92 ± 0.47	-1.40 ± 0.50
Rhodamine B	1.83 ± 0.10	–	-3.44 ± 0.68
Paraoxon	1.08 ± 0.03	–	-1.96 ± 0.05
Indole	0.91 ± 0.03	-1.12 ± 0.26	-1.25 ± 0.09
Amodiaquine	-0.84 ± 0.63	–	-1.29 ± 0.58





Validate D_P by visualizing diffusion into the PDMS bulk

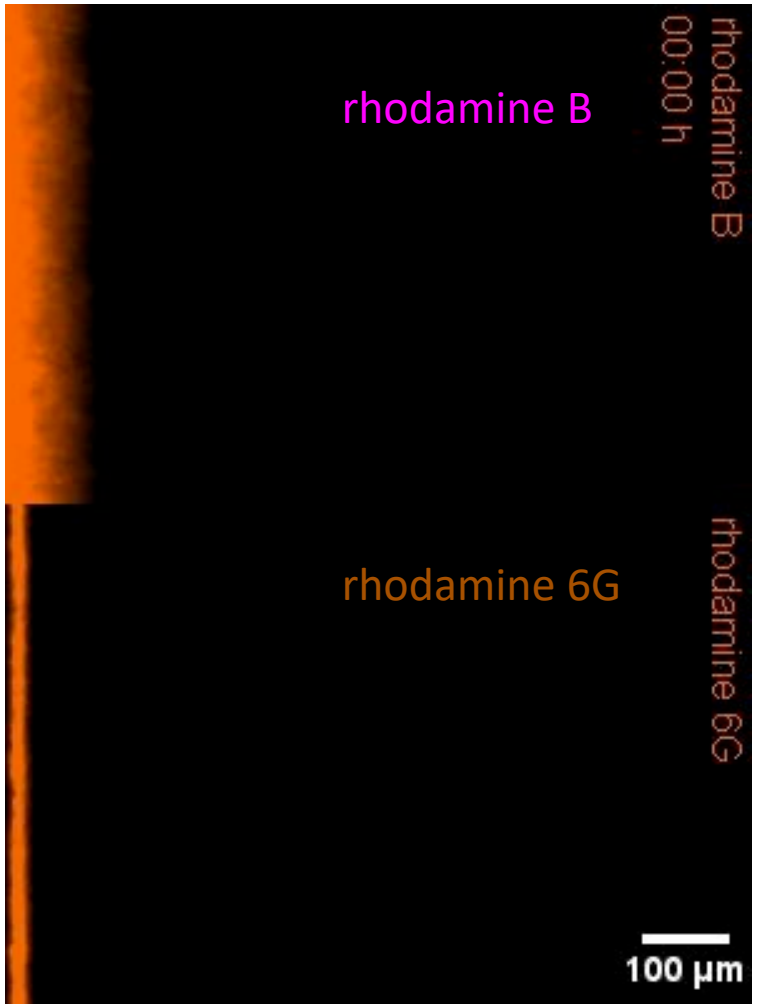


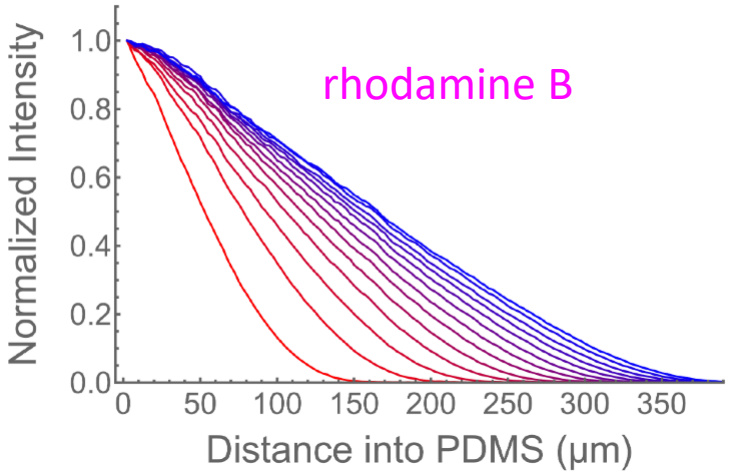


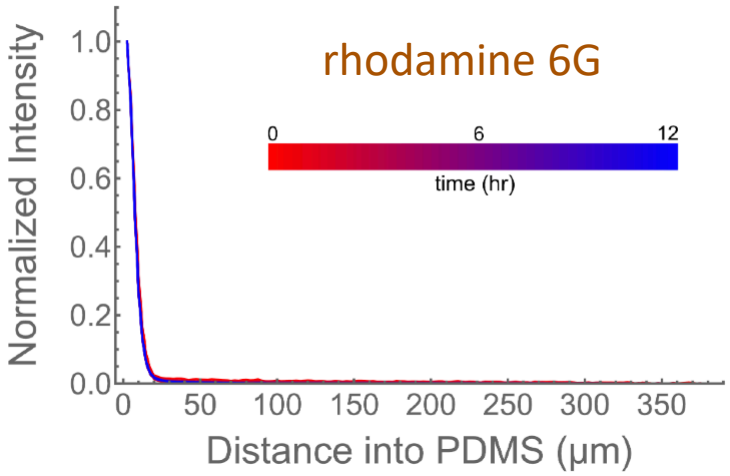
Spreads ~30 µm into PDMS within 3 hours: $D_P \sim \frac{l^2}{t} \sim$ 300 µm²/h ~ 3 x 10^{-4} mm²/h

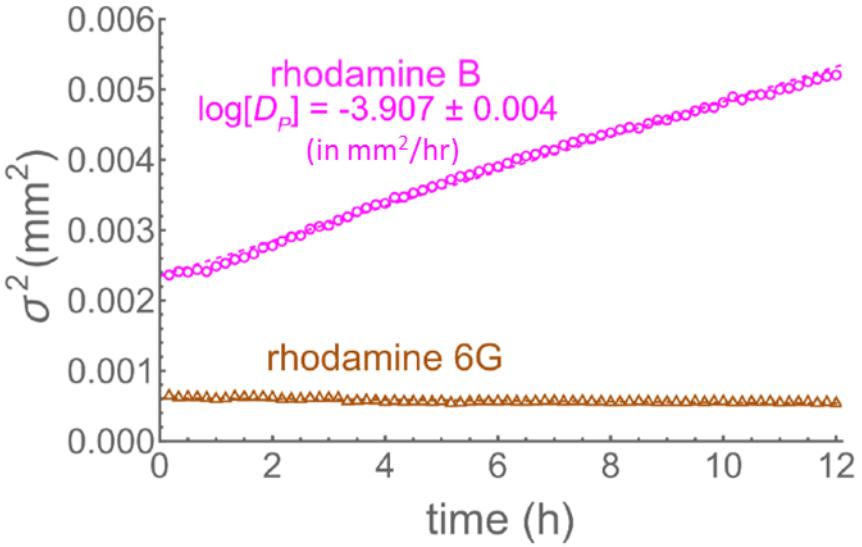
$\log[D_P] \sim$ -3.5

Validate D_P by visualizing diffusion into the PDMS bulk





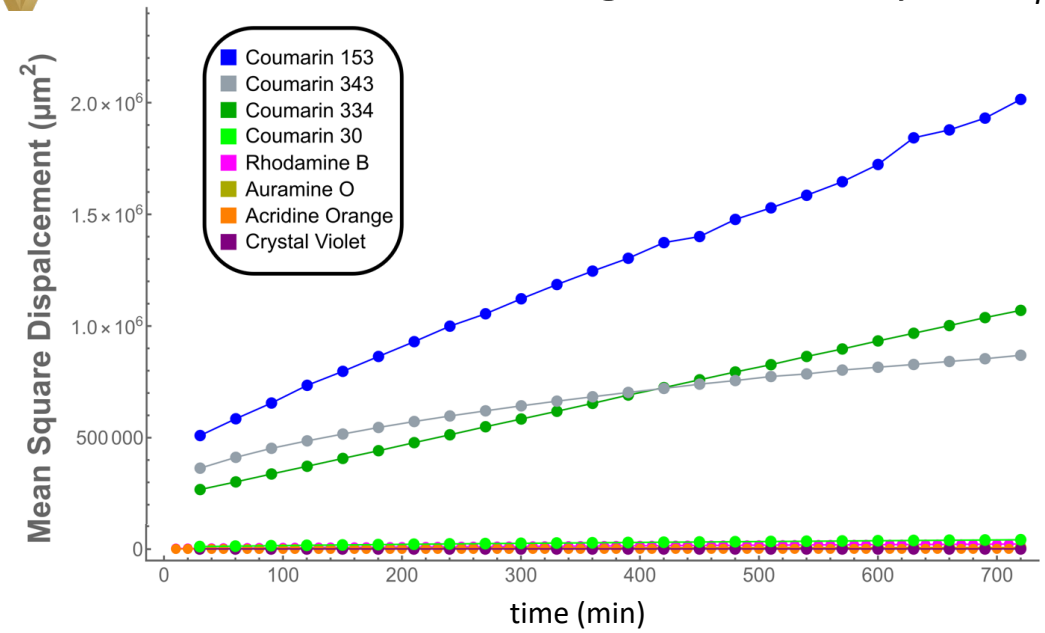


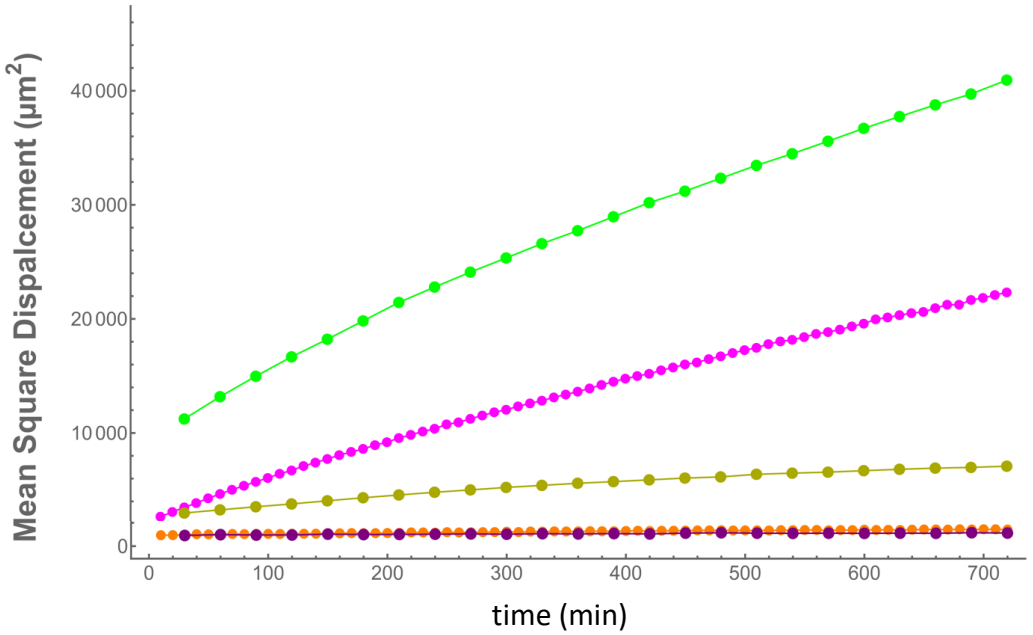


Result from disk/membrane experiments for rhodamine B:

$\log[D_P] = -3.44 \pm 0.68$ (in mm^2/hr)

Tracking additional dyes: D_P can vary over 4 orders of magnitude





Chemical name	$\log D_P$ (in mm^2/hr)
Coumarin 153	-1.192
Coumarin 334	-1.461
Coumarin 343	-1.562
Coumarin 30	-2.838
Rhodamine B	-3.042
Auramine O	-3.683
Acridine Orange	-4.536
Crystal Violet	-4.912

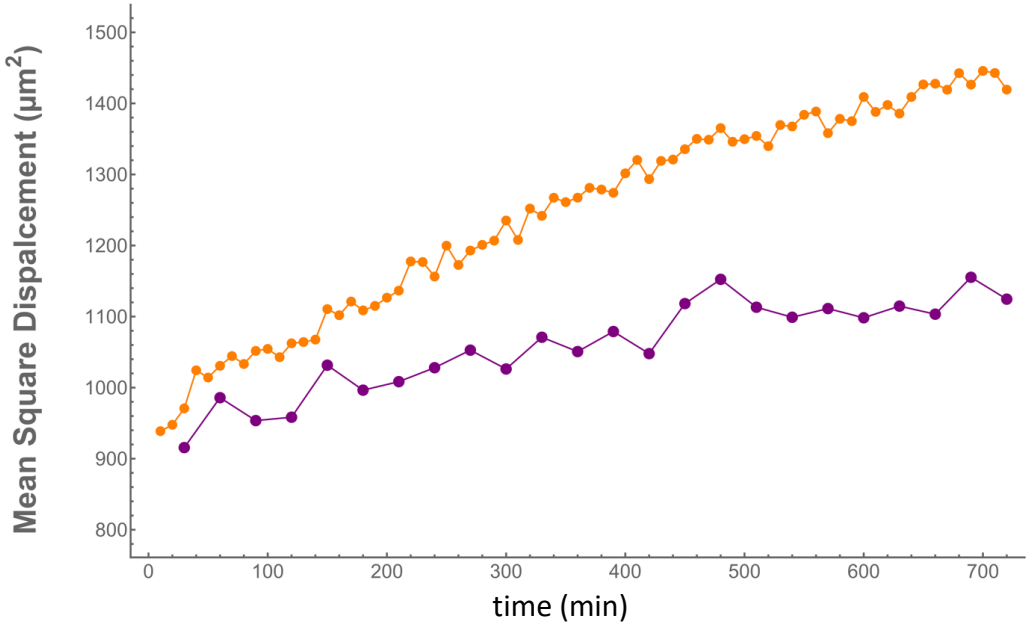
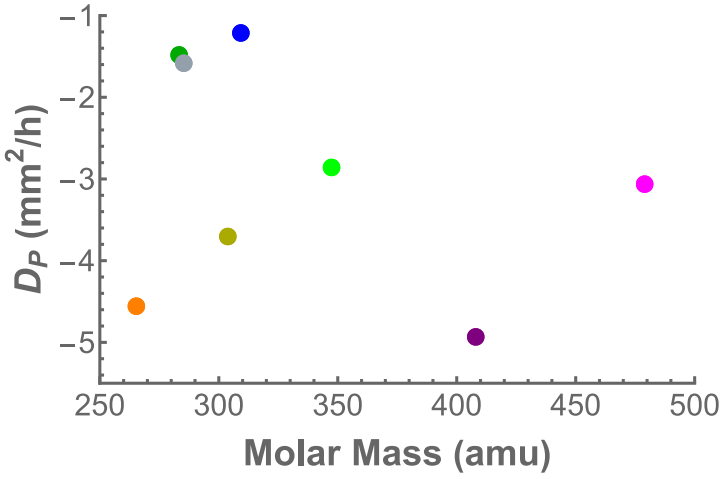
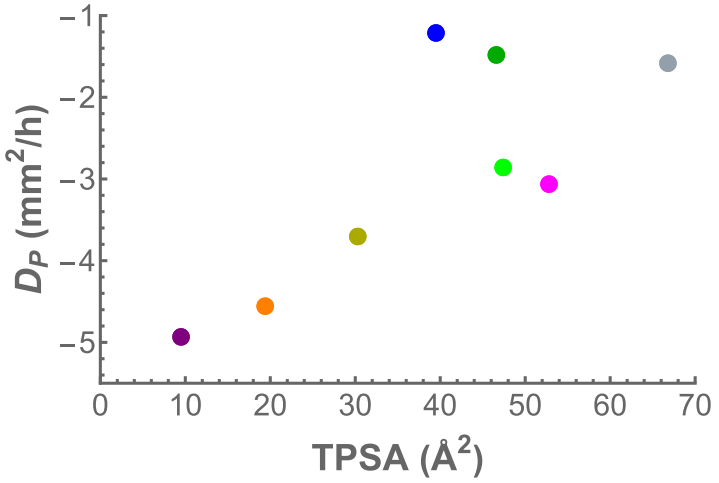


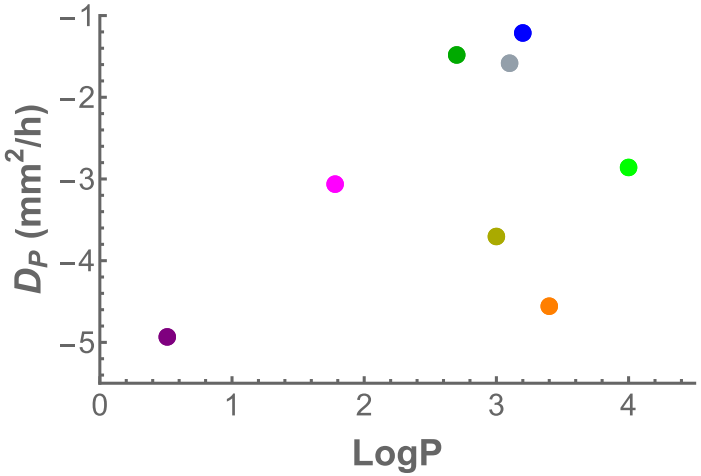
Table 1: Fluorescent dyes and selected properties. (*) Calculated using RDKit [13]. All other properties sourced from PubChem [14]

Chemical name	logP	Mass (amu)	H-Bond Donors	H-Bond Acceptors	TPSA (Å^2)
Rhodamine 6G	6.4	479.0	2	5	61.5
X-34	5.9	402.4	4	6	115
Eosin B Diphenol	5.6	580.1	2	9	168
Fluorescein-5-isothiocyanate	4.8	389.4	2	7	120
Coumarin 30	4.0	347.4	0	4	47.4
Auramine O	3.7*	303.8	2	3	30.3
Fluorescein	3.4	332.3	2	5	76
Acridine Orange	3.4	265.35	0	3	19.4
Coumarin 153	3.2	309.28	0	6	29.5
Coumarin 343	3.1	285.29	1	5	66.8
Coumarin 334	2.7	283.32	0	4	46.6
Rhodamine B	1.9	479.0	1	5	52.8
Crystal Violet	1.5*	408.0	0	3	9.5
Merocyanine 540	0.7*	569.7	0	8	151

Dyes without a color-code did not diffuse into PDMS (but some bound to surface)







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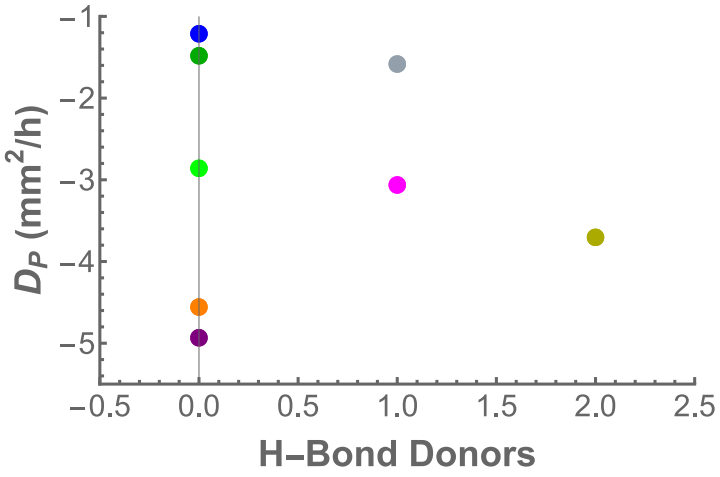
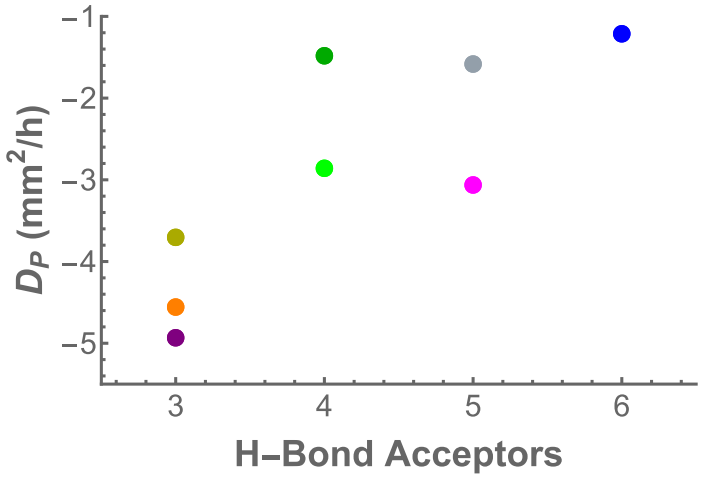
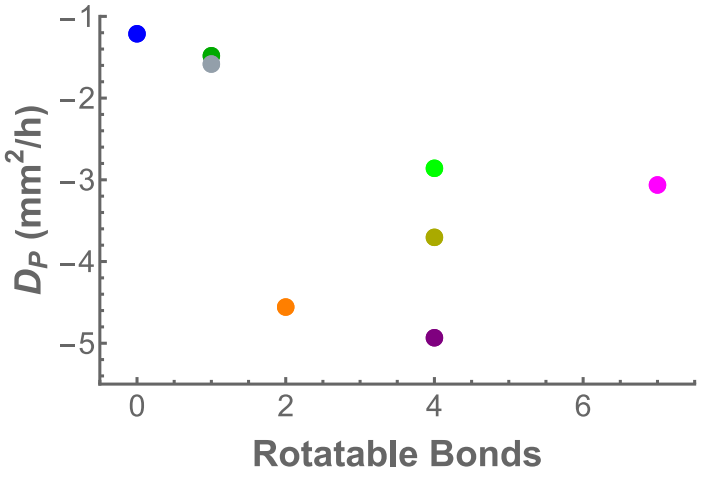






Talk Outline

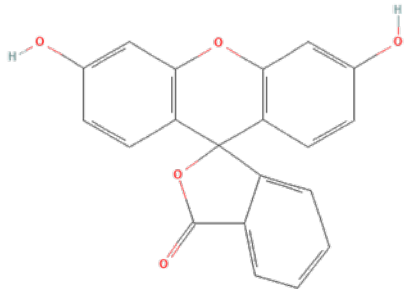
1. Measure chemical-PDMS interaction and transport parameters.
   a. Partition coefficients: $k = C_{PDMS}/C_{sol}$
   b. Diffusion coefficients in PDMS: D_P
   c. Evaluate correlations with chemical properties

2. Be wary of read-across methods.

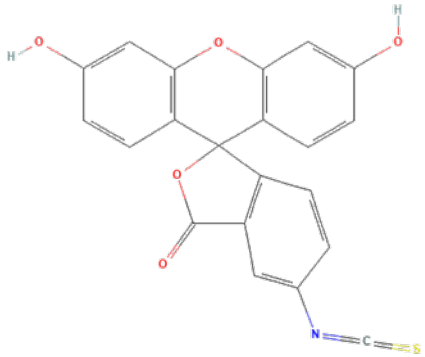
3. Validate multiphysics models for in-device toxicokinetics.

4. Investigate the effectiveness of mitigation strategies for reducing chemical sequestration in microfluidic devices:
   a. SEBS co-polymers as PDMS alternatives; and
   b. media with carrier proteins.

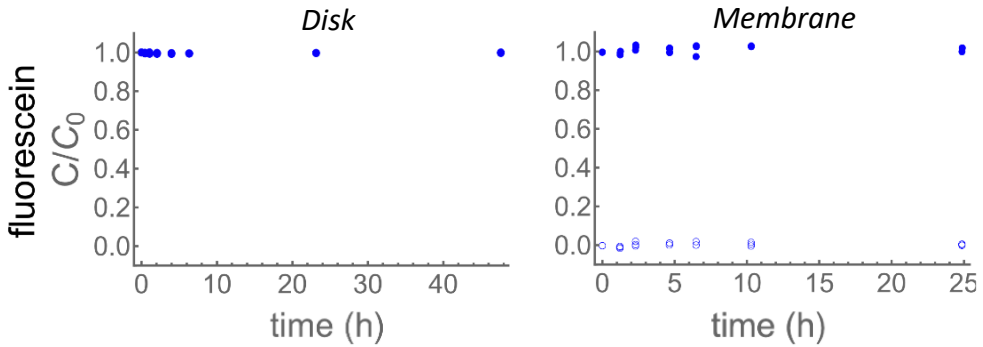
Some chemically similar pairs do have similar PDMS interactions.

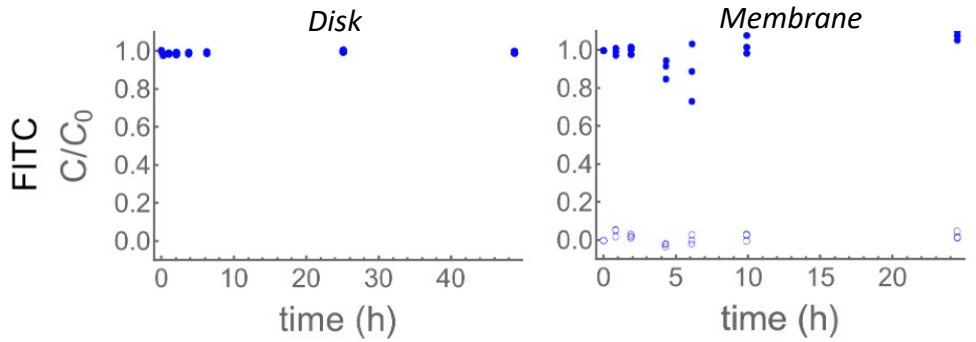


Fluorescein
MW = 332.3 amu
LogP = 3.4
H-Bond Donors = 2
TPSA = 76 Å



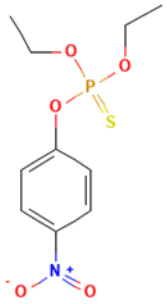
Fluorescein-5-isothiocyanate (FITC)
MW = 389.4 amu
LogP = 4.8
H-Bond Donors = 2
TPSA = 120 Å



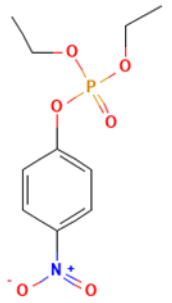


Both are quite hydrophobic,
but neither interacts measurably with PDMS.

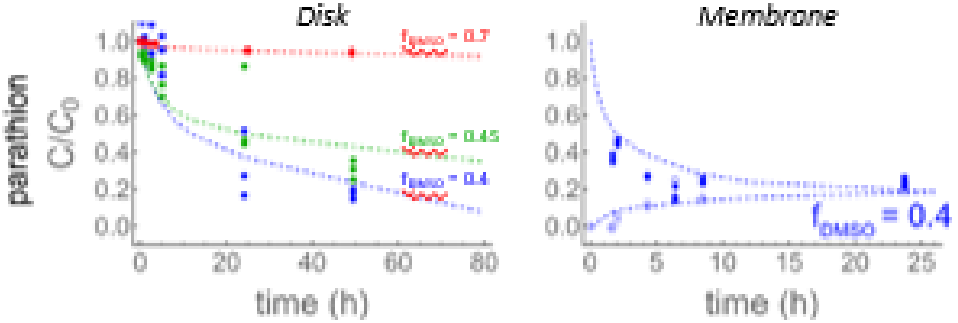
Similar chemicals can interact with PDMS quite differently!



Parathion
MW = 291.3 amu
LogP = 3.83
H-Bond Donors = 0
TPSA = 106.0 Å



Paraoxon
MW = 275.2 amu
LogP = 1.98
H-Bond Donors = 0
TPSA = 90.6 Å



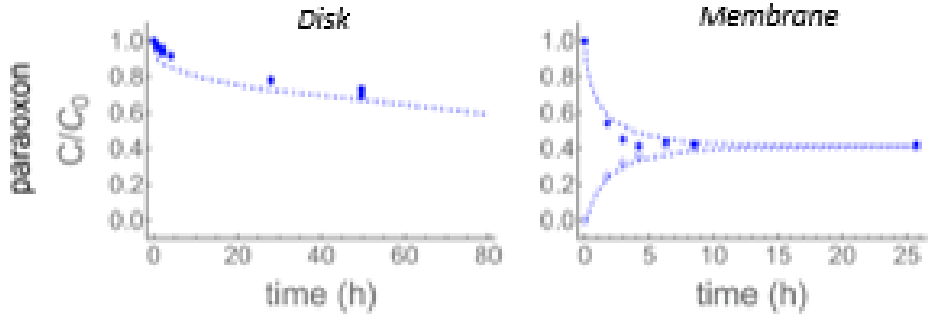
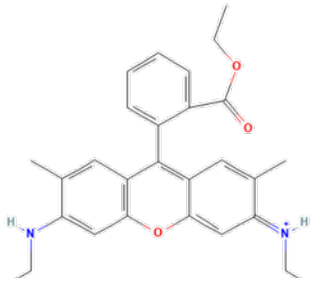


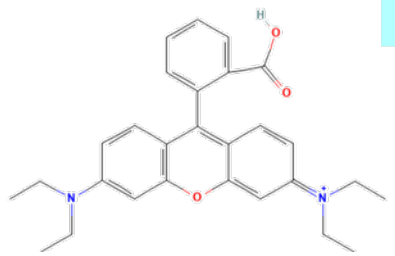
Table 2 Best-fit parameter values for the partition-diffusion model

Chemical	$\log K_{PW}$	$\log K_{PD}$	$\log D_P$ ($mm^2\ h^{-1}$)
Chlorpyrifos	6.25 ± 1.98	-1.21 ± 0.53	-1.51 ± 0.30
Benzo[a]pyrene	5.20 ± 1.22	-2.06 ± 0.82	-1.42 ± 0.30
Parathion	4.39 ± 0.50	-1.92 ± 0.47	-1.40 ± 0.50
Rhodamine B	1.83 ± 0.10	–	-3.44 ± 0.68
Paraoxon	1.08 ± 0.03	–	-1.96 ± 0.05
Indole	0.91 ± 0.03	-1.12 ± 0.26	-1.25 ± 0.09
Amodiaquine	-0.84 ± 0.63	–	-1.29 ± 0.58

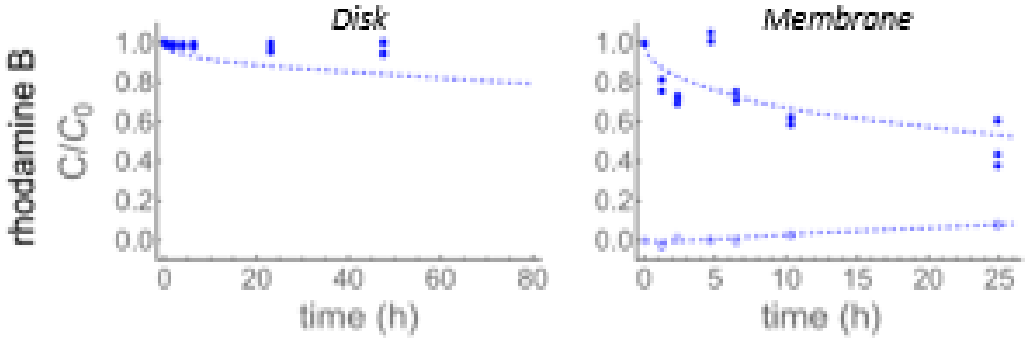
Similar chemicals can interact with PDMS quite differently!

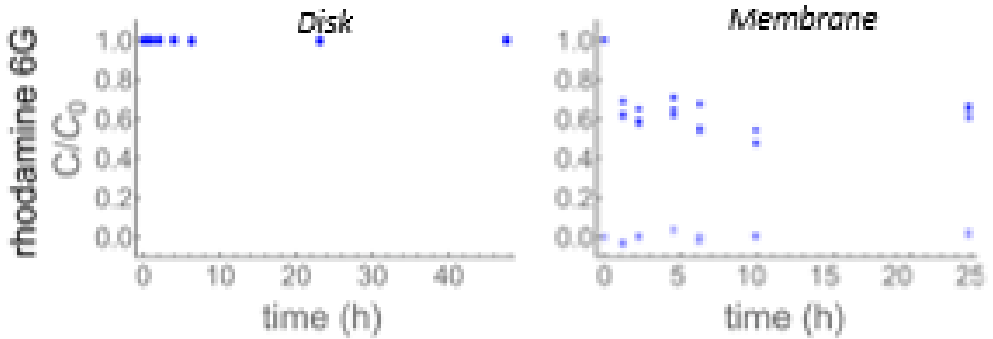


Rhodamine B
MW = 479.0 amu
LogP = 1.95
H-Bond Donors = 2
TPSA = 59.9 Å



Rhodamine 6G
MW = 479.0 amu
LogP = 6.4
H-Bond Donors = 2
TPSA = 61.5 Å





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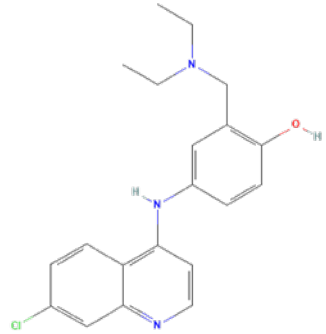
Paraoxon	1.08 ± 0.03	–	-1.96 ± 0.05
Indole	0.91 ± 0.03	-1.12 ± 0.26	-1.25 ± 0.09
Amodiaquine	-0.84 ± 0.63	–	-1.29 ± 0.58

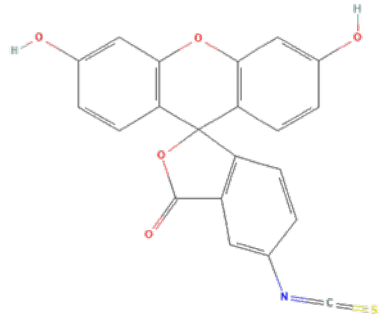
Rhodamine 6G binds to PDMS surfaces, but does not diffuse into PDMS bulk.

Similar chemicals can interact with PDMS quite differently!

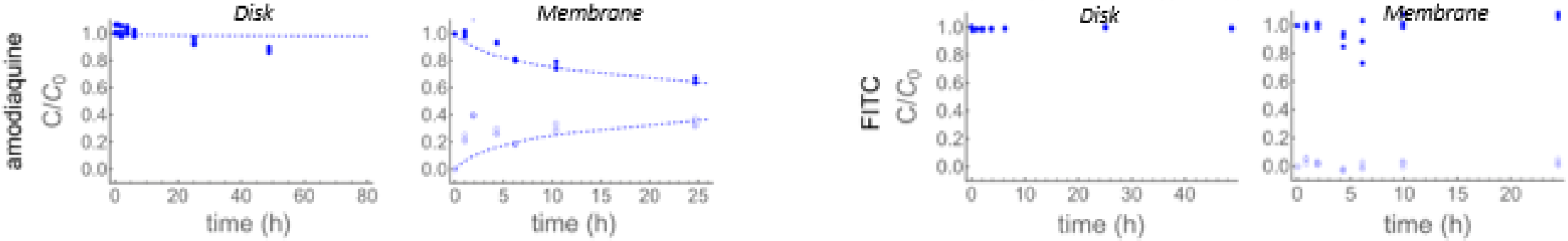


Amodiaquine
MW = 355.9 amu
LogP = 3.7
H-Bond Donors = 2
TPSA = 48.4 Å

Read-across used in
Grant et al, *Lab on a Chip*, 2021



FITC
MW = 389.4 amu
LogP = 4.8
H-Bond Donors = 2
TPSA = 120 Å



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Amodiaquine	-0.84 ± 0.63	–	-1.29 ± 0.58

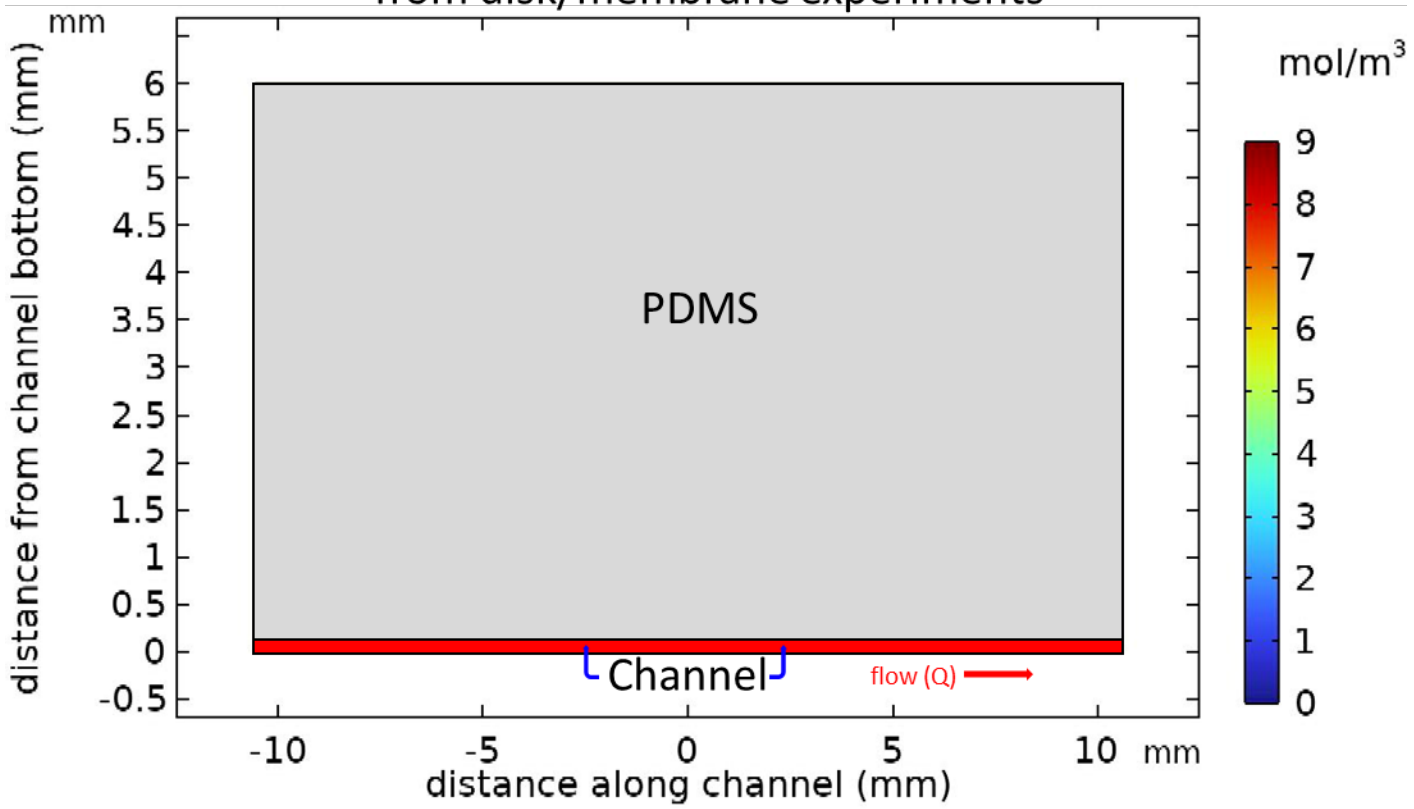
FITC does not measurably bind to, partition into or diffuse through PDMS.

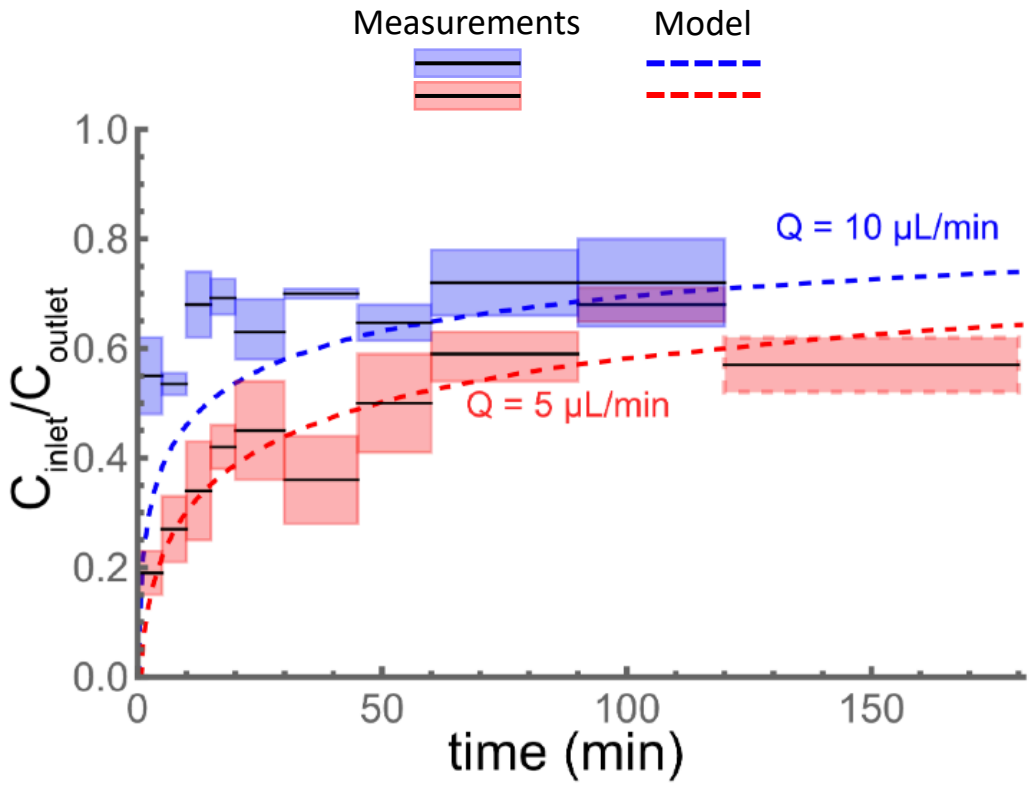
Talk Outline

1. Measure chemical-PDMS interaction and transport parameters.
   a. Partition coefficients: $k = C_{PDMS}/C_{sol}$
   b. Diffusion coefficients in PDMS: D_P
   c. Evaluate correlations with chemical properties
2. Be wary of read-across methods.
3. Validate multiphysics models for in-device toxicokinetics.
4. Investigate the effectiveness of mitigation strategies for reducing chemical sequestration in microfluidic devices:
   a. SEBS co-polymers as PDMS alternatives; and
   b. media with carrier proteins.

Modelling chemical distribution during flow through a microfluidic channel

COMSOL model based on parameters from disk/membrane experiments





Talk Outline

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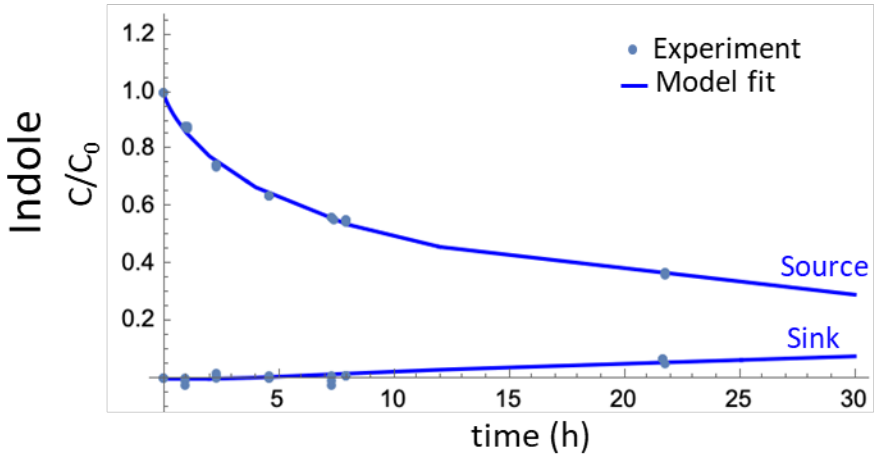
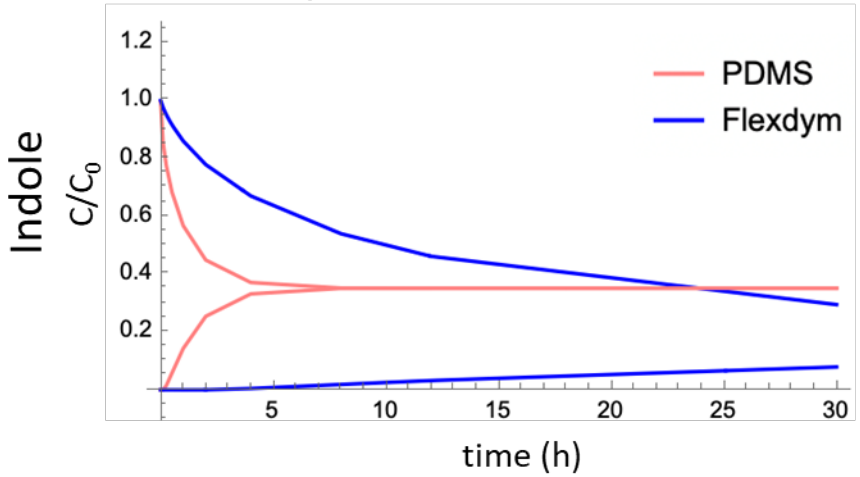
Chemicals still partition into Flexdym™ (SEBS), but diffuse through it more slowly.

Indole diffusing into and across a 250-µm thick Flexdym™ membrane



Compared to predictions for 250-µm thick PDMS membrane

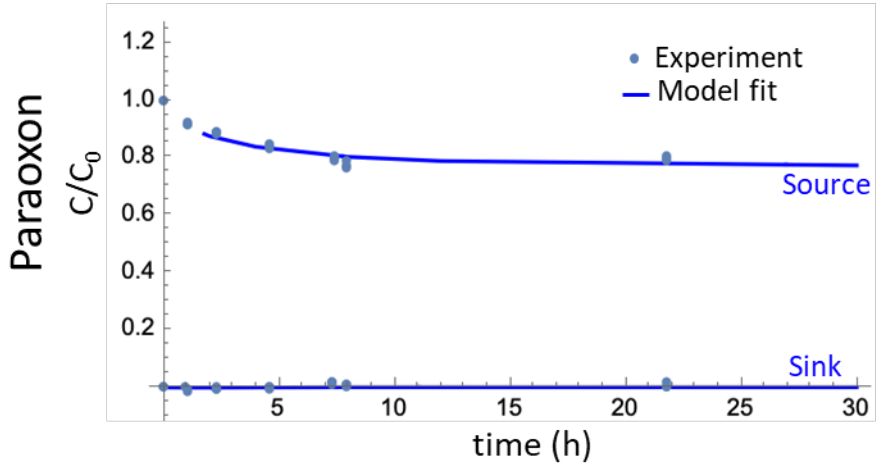


Indole's interaction with	log[k]	log[D_P] in mm²/h
Flexdym™	1.60 ± 0.03	-2.57 ± 0.11
PDMS	0.91 ± 0.03	-1.25 ± 0.09

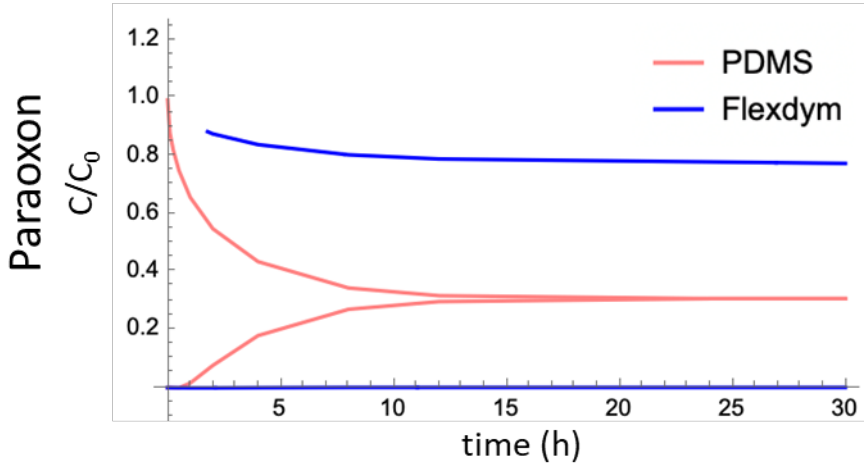
Greater partition coefficient for Flexdym™, but with slower diffusion.

Chemicals still partition into Flexdym™ (SEBS), but diffuse through it more slowly.

Paraoxon diffusing into and across a 250-µm thick Flexdym™ membrane



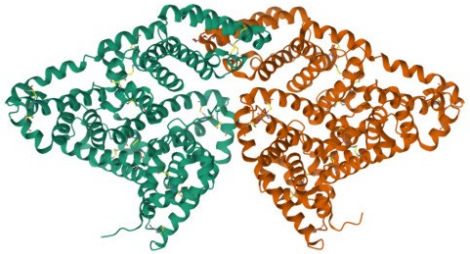
Compared to predictions for 250-µm thick PDMS membrane



Paraoxon's interaction with	log[k]	log[D_P] in mm²/h
Flexdym™	1.19 ± 0.36	-6 ± 19
PDMS	1.08 ± 0.03	-1.96 ± 0.05

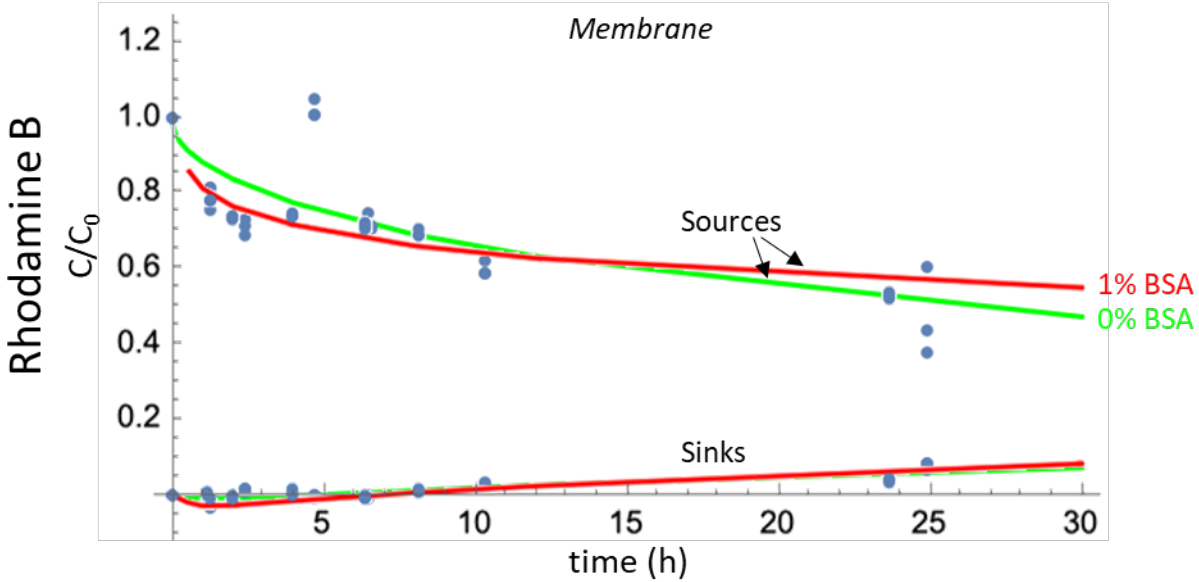
Similar partition coefficient for Flexdym™, but with no detectable diffusion.

Adding BSA to media can reduce partitioning into PDMS



Bovine serum albumin
PDB DOI: https://doi.org/10.2210/pdb4F5S/pdb

Mol Wt = 66,433 Da ⟶ 1% w/v = 15 mM



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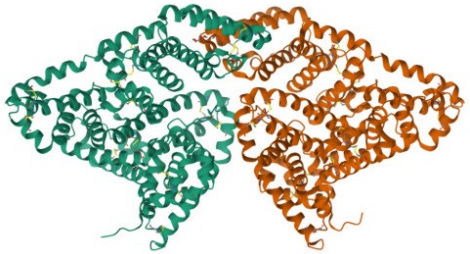

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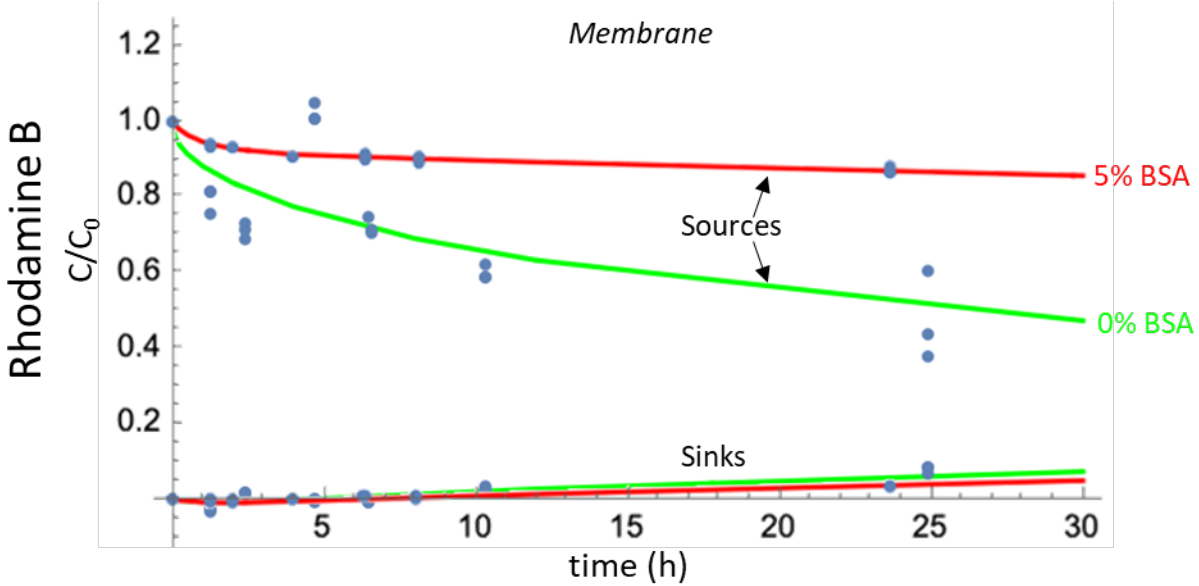

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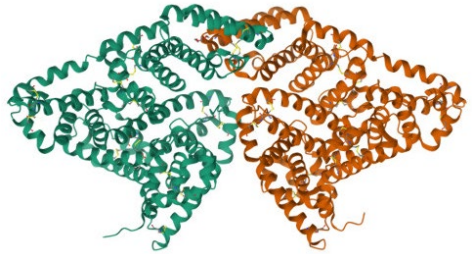

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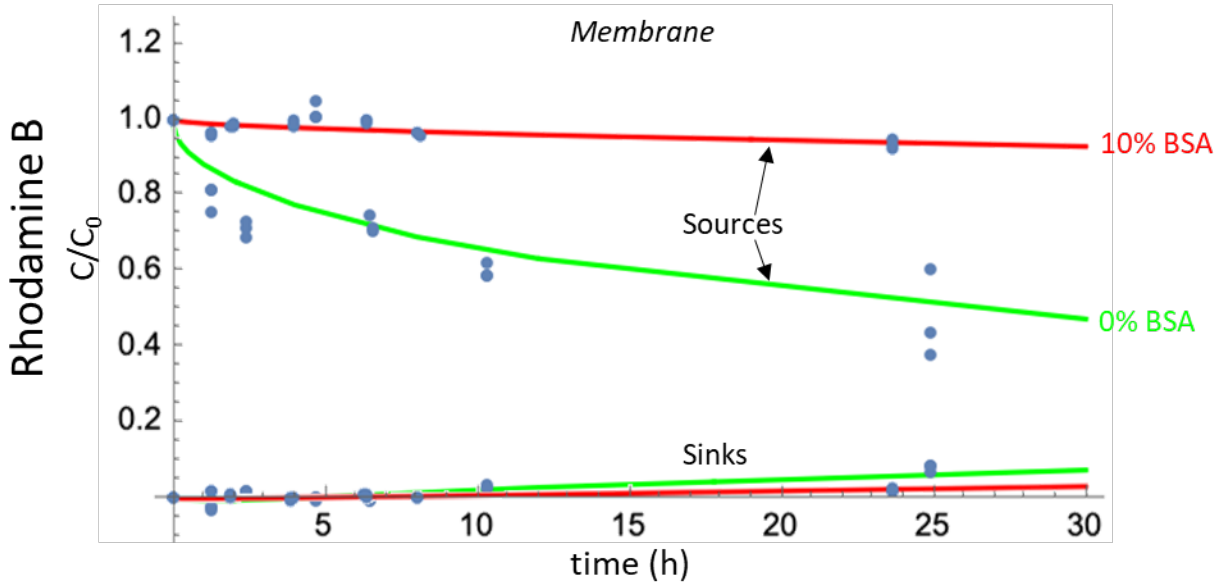
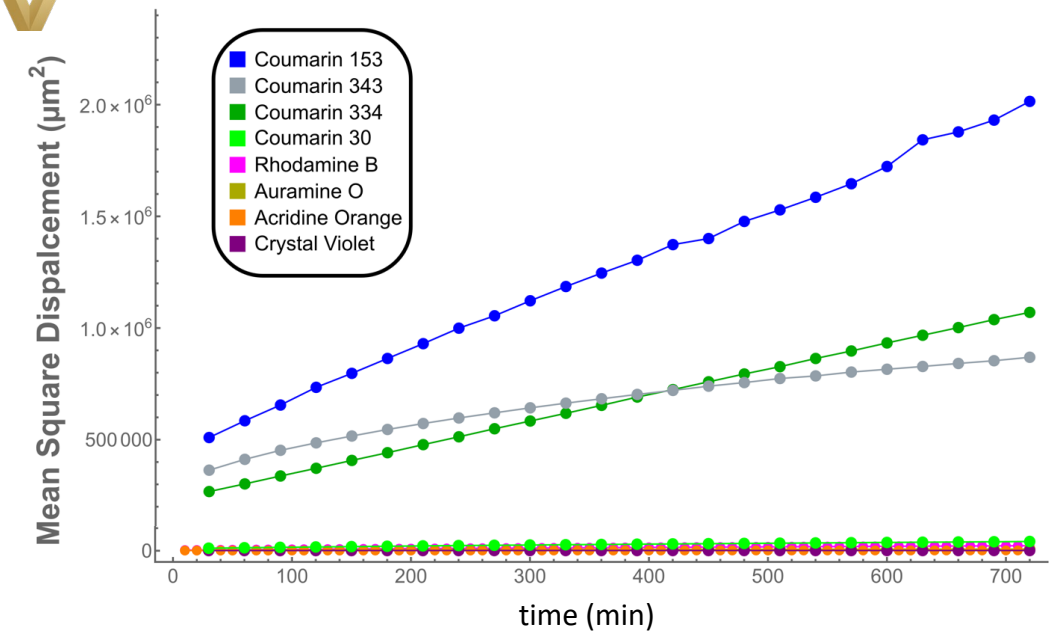
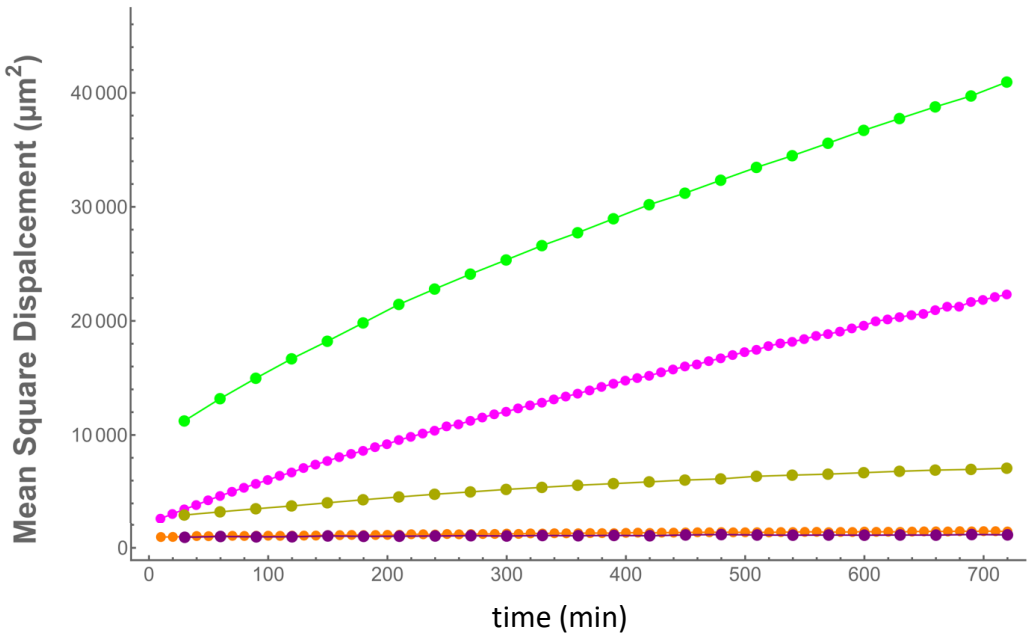


Summary

- Disk-soak and membrane-diffusion experiments are working well.
  - Partition and diffusion coefficients (k and D_P) can be extracted by fitting data to numerical solutions of PDEs.
  - Diffusion coefficients are confirmed by direct observation of dyes.
- PDMS-interaction properties show the same trends noted in Auner et al: hydrophobicity is important, and yet some chemicals with very high LogP have surprisingly weak interactions – **be wary of simple read-across methods!**
- Multiphysics models based on measured k and D_P values do a good job of predicting chemical distributions under flow within a microfluidic device – **measure and model is a viable strategy!**
- In terms of mitigation, substituting SEBS co-polymer reduces but does not eliminate diffusion through the polymer. Including a carrier protein like BSA in the culture medium can reduce chemical partitioning into PDMS.

EXTRAS – ANOMALOUS DIFFUSION





Chemical name	$\log D_P$	$\mathrm{AIC_P}$	$\log D_\alpha$	α	AIC_α
Coumarin 153	-1.192	130.7	-1.124	0.975	127.6
Coumarin 334	-1.461	68.5	-1.505	1.017	48.5
Coumarin 343	-1.562	198.5	-0.625	0.651	113.1
Coumarin 30	-2.838	106.6	-2.288	0.795	20.8
Rhodamine B	-3.042	392.2	-2.621	0.841	133.2
Auramine O	-3.683	61.6	-3.070	0.772	24.3
Acridine Orange	-4.536	215.2	-3.359	0.562	7.4
Crystal Violet	-4.912	5.5	-3.890	0.540	-19.9

D_P in $\mathrm{mm^2/hr}$; D_α in $\mathrm{mm^2/hr^\alpha}$

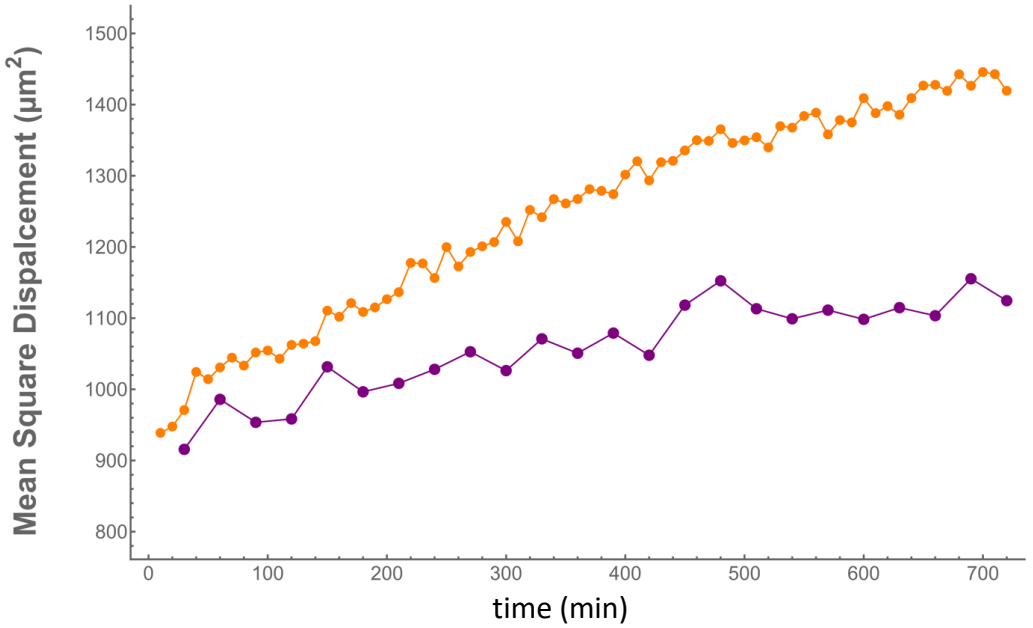
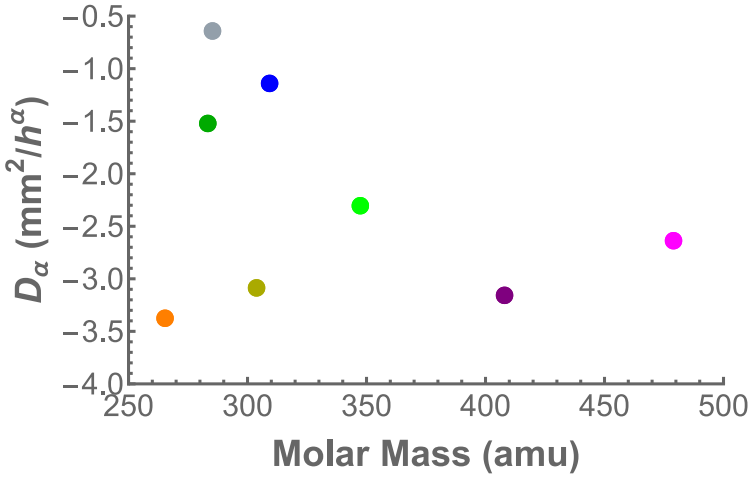
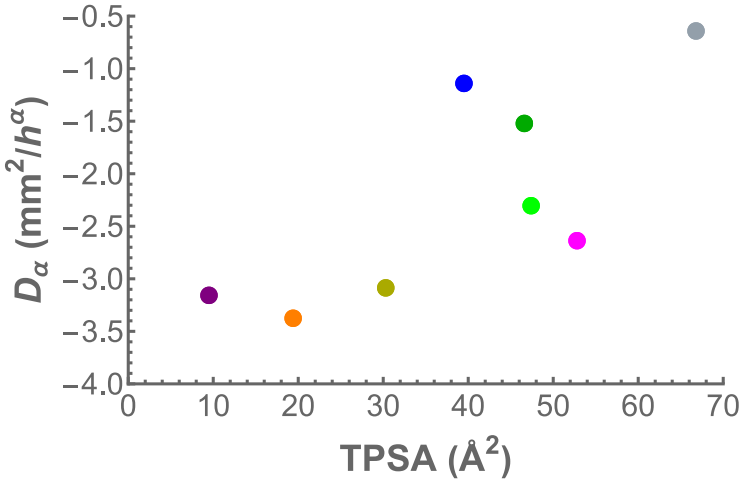


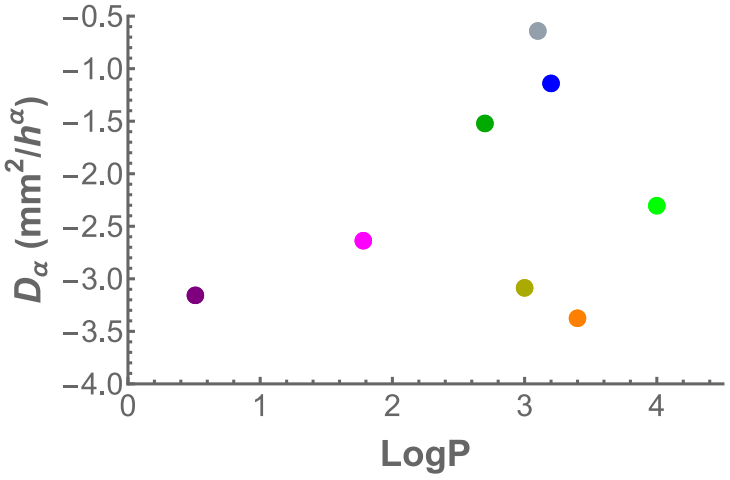
Table 1: Fluorescent dyes and selected properties. (*) Calculated using RDKit [13]. All other properties sourced from PubChem [14]

Chemical name	logP	Mass (amu)	H-Bond Donors	H-Bond Acceptors	TPSA (Å^2)
Rhodamine 6G	6.4	479.0	2	5	61.5
X-34	5.9	402.4	4	6	115
Eosin B Diphenol	5.6	580.1	2	9	168
Fluorescein-5-isothiocyanate	4.8	389.4	2	7	120
Coumarin 30	4.0	347.4	0	4	47.4
Auramine O	3.7*	303.8	2	3	30.3
Fluorescein	3.4	332.3	2	5	76
Acridine Orange	3.4	265.35	0	3	19.4
Coumarin 153	3.2	309.28	0	6	29.5
Coumarin 343	3.1	285.29	1	5	66.8
Coumarin 334	2.7	283.32	0	4	46.6
Rhodamine B	1.9	479.0	1	5	52.8
Crystal Violet	1.5*	408.0	0	3	9.5
Merocyanine 540	0.7*	569.7	0	8	151

Dyes without a color-code did not diffuse into PDMS (but some bound to surface)







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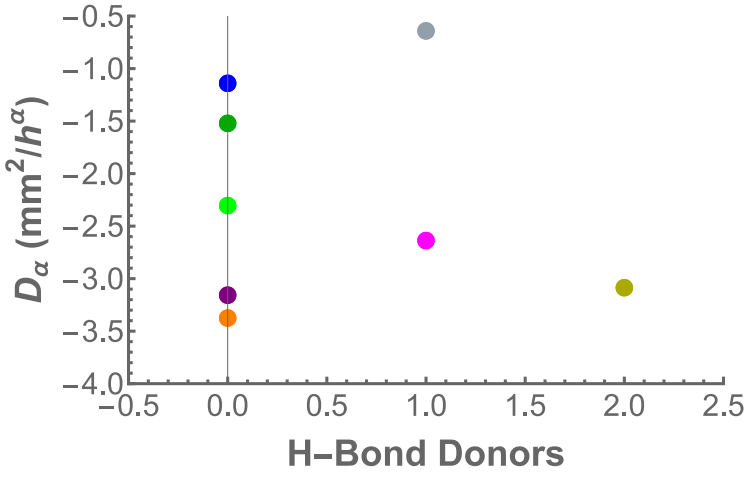
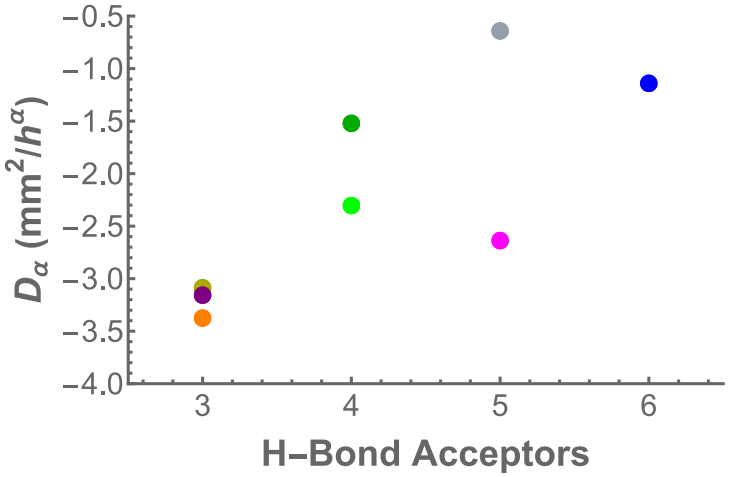




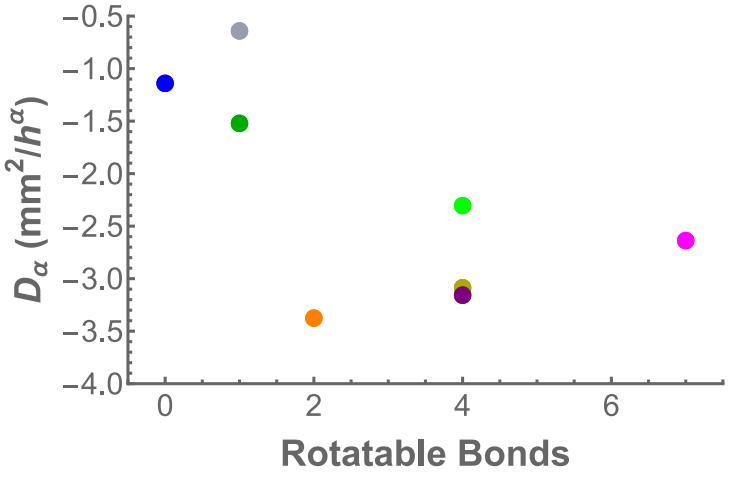
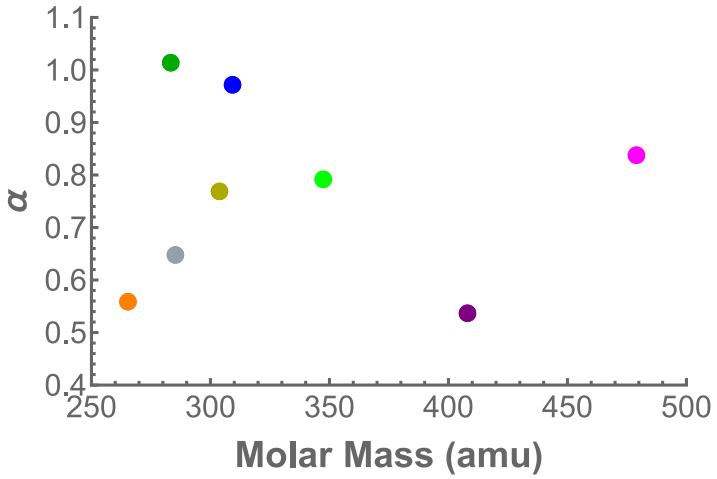
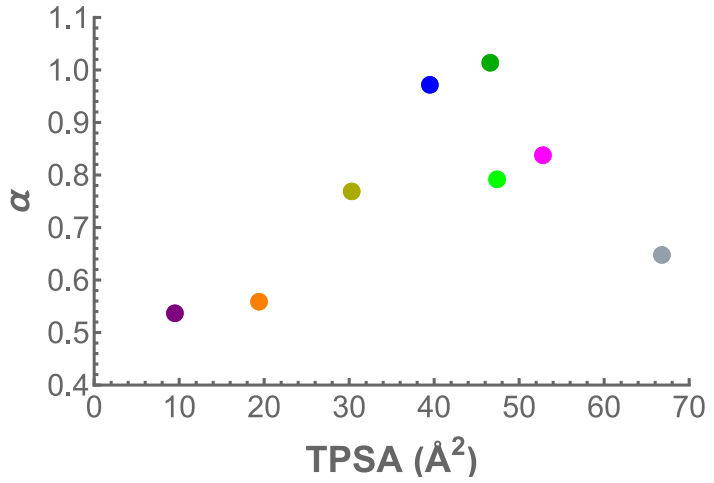


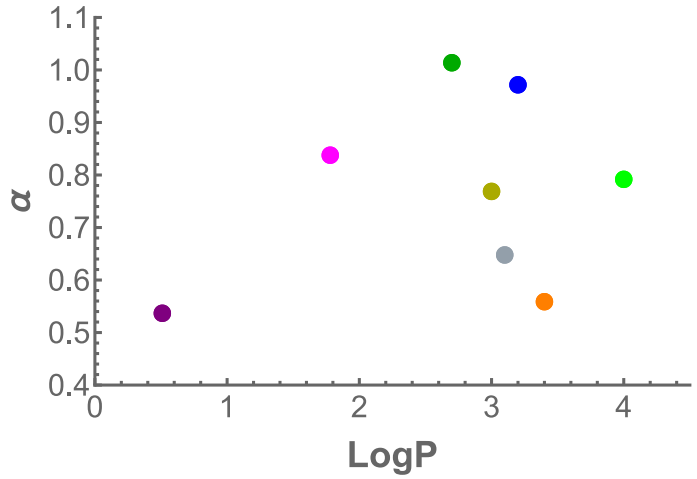
Table 1: Fluorescent dyes and selected properties. (*) Calculated using RDKit [13]. All other properties sourced from PubChem [14]

Chemical name	logP	Mass (amu)	H-Bond Donors	H-Bond Acceptors	TPSA (Å^2)
Rhodamine 6G	6.4	479.0	2	5	61.5
X-34	5.9	402.4	4	6	115
Eosin B Diphenol	5.6	580.1	2	9	168
Fluorescein-5-isothiocyanate	4.8	389.4	2	7	120
Coumarin 30	4.0	347.4	0	4	47.4
Auramine O	3.7*	303.8	2	3	30.3
Fluorescein	3.4	332.3	2	5	76
Acridine Orange	3.4	265.35	0	3	19.4
Coumarin 153	3.2	309.28	0	6	29.5
Coumarin 343	3.1	285.29	1	5	66.8
Coumarin 334	2.7	283.32	0	4	46.6
Rhodamine B	1.9	479.0	1	5	52.8
Crystal Violet	1.5*	408.0	0	3	9.5
Merocyanine 540	0.7*	569.7	0	8	151

Dyes without a color-code did not diffuse into PDMS (but some bound to surface)







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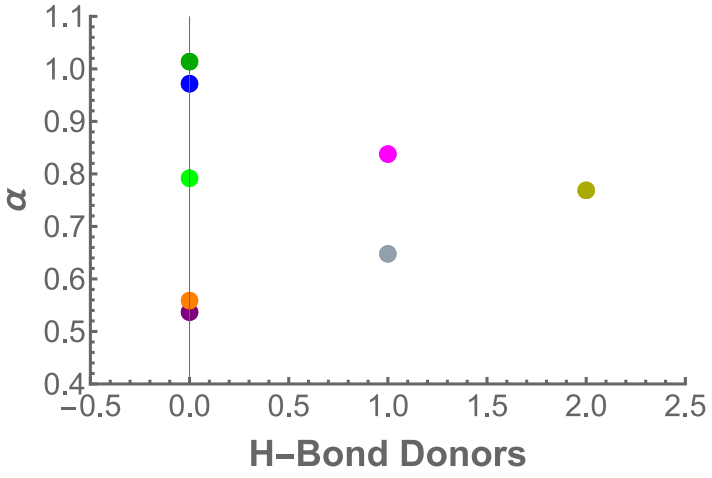
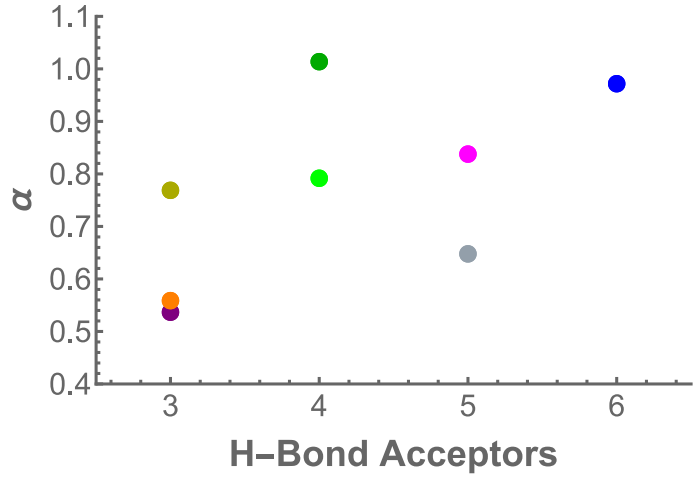
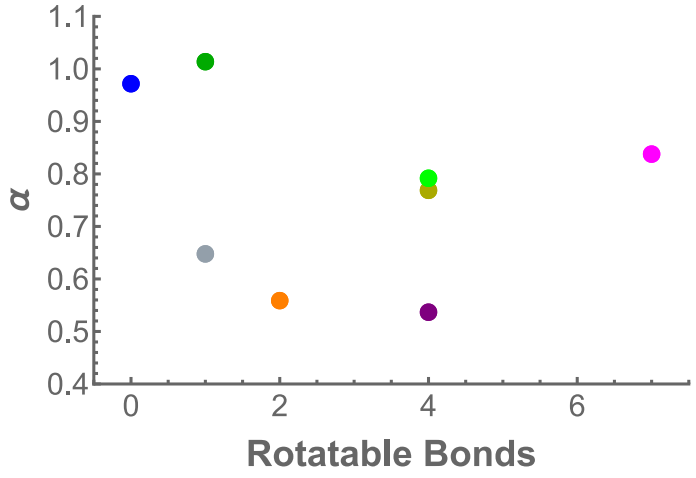






EXISTING OECD GUIDANCE ON TOXICOKINETICS, IN VITRO-TO-IN VIVO EXTRAPOLATION, AND EXPLORING FUTURE OPPORTUNITIES

Dr. Alicia Paini

Senior Scientific Officer

Scientific Committee (SC)

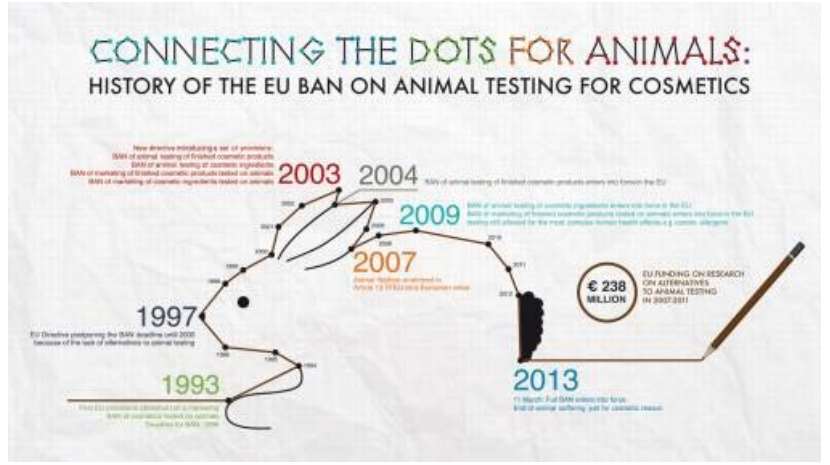
Methodology and Scientific Support (MESE)

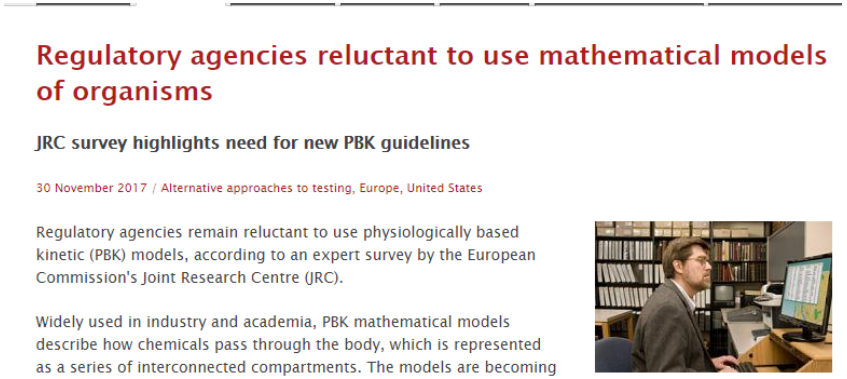
EUROPEAN FOOD SAFETY AUTHORITY

U.S. EPA 4th NAMs Conference

BACKGROUND

- Chemical Risk Assessment can and should be based on non-animal data!
- This implies the need to use alternatives such as in vitro and in silico methods (New approach methodologies, NAMs)!
- Especially to interpret and use in vitro toxicity data in combination with biokinetic data!
- Biokinetic (ADME) data can be generated by in silico and in vitro models!
- PBK modeling is the way to accurately integrate and use in vitro data for the design of experiments and extrapolate in vitro effect data to in vivo for safety assessment by setting Point of Departure (PoD).





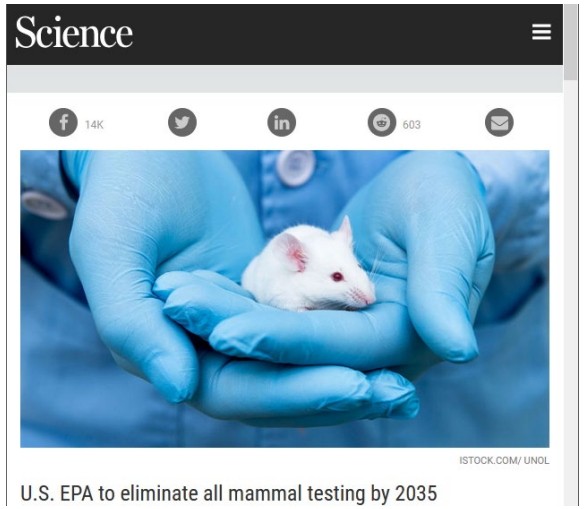
Regulatory agencies reluctant to use mathematical models of organisms

JRC survey highlights need for new PBK guidelines

30 November 2017 / Alternative approaches to testing, Europe, United States

Regulatory agencies remain reluctant to use physiologically based kinetic (PBK) models, according to an expert survey by the European Commission's Joint Research Centre (JRC).

Widely used in industry and academia, PBK mathematical models describe how chemicals pass through the body, which is represented as a series of interconnected compartments. The models are becoming



AN INTERNATIONAL EFFORT TO PROMOTE THE REGULATORY USE OF PHYSIOLOGICALLY BASED KINETIC (PBK) MODELS!

IPCS
INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY
WHO
IPCS Harmonization Project

Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment

efsa
European Food Safety Authority

EFSA Journal 2014;12(3):3589

SCIENTIFIC OPINION

Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

U.S. Environmental Protection Agency (2006)

Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment

U.S. Food & Drug Administration (2018)

Physiologically Based Pharmacokinetic Analyses — Format and Content

Guidance for Industry

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 December 2018
EMA/CHMP/458101/2016
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation

Test No. 417: Toxicokinetics

This Test Guideline describes in vivo studies that provide information on mass balance, absorption, bioavailability, tissue distribution, metabolism, excretion, and basic toxicokinetic parameters [e.g. AUC], as well as supplemental approaches that may provide useful information on toxicokinetics. Information from toxicokinetic studies helps to relate concentration or dose to the observed toxicity and to understand its mechanism of toxicity. The test substance ("unlabelled" or "radiolabelled" forms) is normally administered by an oral route, but other routes of administration may be applicable. Single dose administration of the substance (preferably a minimum of two dose levels) may be adequate, but repeated dose may be needed in some circumstances. Toxicokinetic studies should preferably be carried out in the same species as that used in other toxicological studies performed with the substance (normally the rat, a minimum of 4 animals of one sex for each dose). Initial estimation of absorption can be achieved by mass balance determination, but further investigations such as intravenous (IV) administration and biliary excretion studies might be

OECD Template #56: Biotransformation and kinetics *(Version [9.6]-[August 2024])*

Template #56: Biotransformation and kinetics *(Version [9.6]-[August 2024])*

The following table gives a detailed description of the type of information prompted for by the data entry fields.

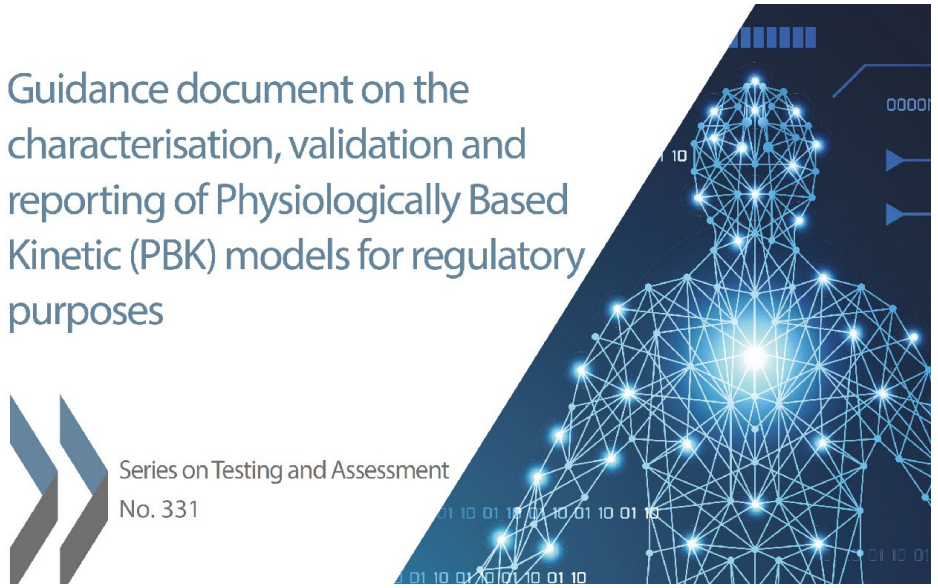
Line no.	Field name	Field type Display type	Picklist Freetext template	Help text	Remarks Guidance Cross-reference
1.	Administrative data	Header 1			
2.		Confidentiality			
3.	Endpoi				

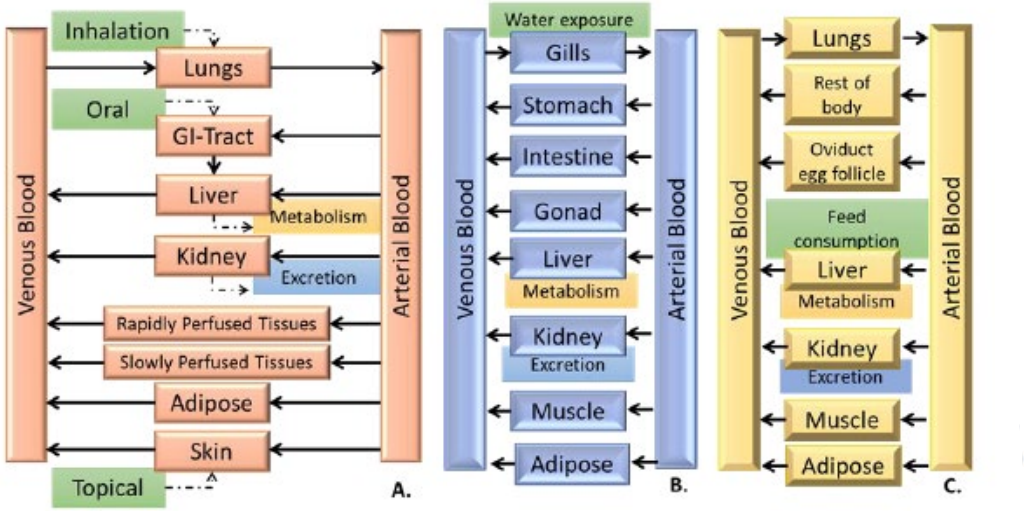
Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes

Series on Testing and Assessment
No. 331

OECD GD 331 GUIDANCE ON CHARACTERIZATION, VALIDATION AND REPORTING OF PBK MODELS FOR REGULATORY PURPOSES

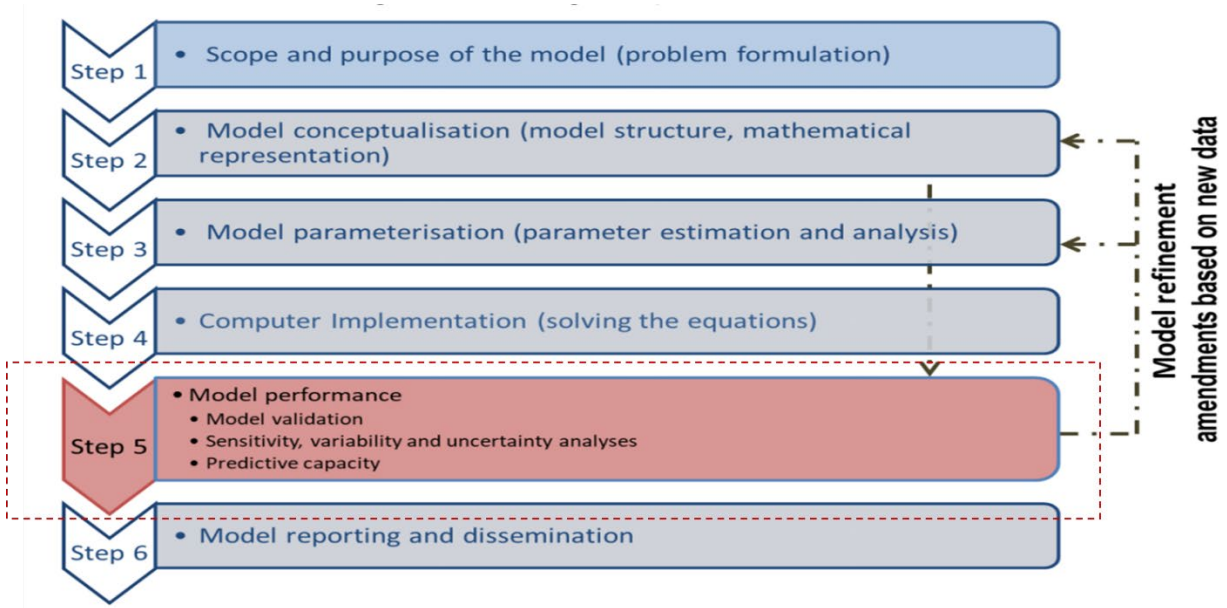
- The GD provides contextual information on the scientific process of model characterization and evaluation, but not a technical guidance on model development or applications
- The GD is not prescriptive guidance on model acceptance; the level of confidence required for a model should depend on the regulatory context of use
- The GD is applicable to most chemicals and all species, provided that appropriate methods/data exit to parameterize a model
- The GD is a living document





GD 331 ELEMENTS

1. Provide a **scientific workflow** for characterizing and validating PBK models, with emphasis on models that are constructed without using *in vivo* data
2. Provide knowledge sources on *in vitro* and *in silico* methods that can be used to generate model parameters

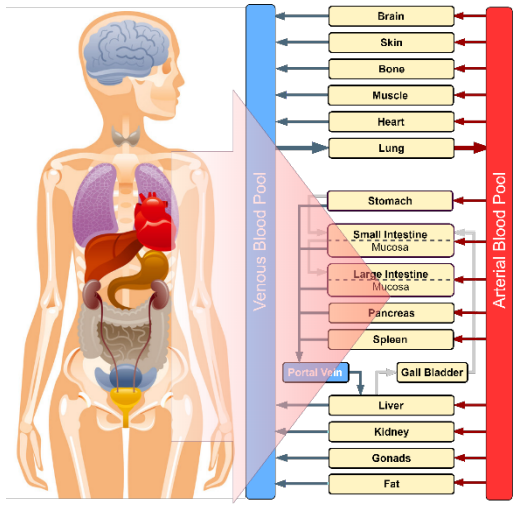


MODEL PARAMETRISATION STEP 3

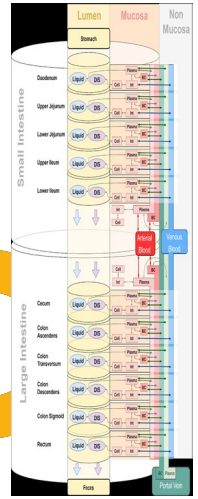
Input parameters

In vitro ADME models

Absorption (P_{app})
Distribution (P_t, fu, P_{app})
Metabolism (V_{max}, Km, CL_{int})
Excretion (P_{ba}, Km, V_{max})



Before using an in vitro parameter, one must up-scale the value - in vitro to in vivo using scaling factors.

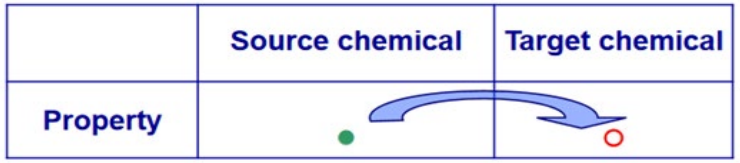


For each of the ADME parameter, the OECD GD reports pointers for the modeler and assessor.

MODEL PERFORMANCE STEP 5

1. Model Validation
2. Sensitivity, Uncertainty Analysis
3. Predictive capacity

Assessment of model predictive capacity by using a read-across approach



Identify an analogue with biokinetic data and /or has an already available PBK model to fill data gaps for a target chemical in the context of a chemical safety assessment. Problem formulation is informed by the intended application of the PBK model, including exposure scenario (see Table 2.1).

Characterisation of target chemicals
Characterisation of analogues

Obtain or predict chemical properties (e.g. using QSARs) relevant to PBK models (e.g. Log P, water solubility, pka, plasma protein binding, clearance, trasporter affinity, metabolic profile and/or other ADME-related properties).

1. Identification of analogues

Identify potential analogues using appropriate similarity metrics (e.g. fingerprints or other related properties, physicochemical, ADME relevant properties). Selection based on scoring system and the information prerequisites.

Information prerequisites (for analogue)

1. Biological basis (ADME/MoA)
2. Biokinetic parameters (e.g. metabolite data)
3. PBK model *(model equations / code)
4. Case specific considerations**

Criterion 1. Chemicals with biokinetic data and similar ADME properties
Criterion 2. Chemicals with valid PBK model

Consult literature and available model databases to identify chemicals with:
1) whole organism biokinetic data (concentration–time curves and related dose metrics such as AUC) for the specific route of exposure and with similar ADME properties.
2) available biotransformation rate parameters (Cl, Vmax, Km)
3) a valid PBK model (predictions were compared to *in vivo* data)

2. Selection and justification of analogues

Shortlist analogues for which biokinetic information is available for the specific route of exposure.
In case there are multiple analogues with biokinetic data, shortlist analogues based on those with most similar ADME properties. Metabolic transformation may need to be known or plausible metabolites predicted where relevant to the biological activity. Provide rationale for selection of analogues.

Similarity criteria to identify more analogues, if needed

Optimise analogue selection based on predictivity and sensitivity analysis, if needed

4. Reporting

Use the PBK model reporting template to describe the model (see Chapter 3). The predictive performance of the model (in predicting the dose-metric of interest for the target) will be justified by the ability of the model to predict (qualitatively or quantitatively) the relevant dose metrics for one or more analogues.

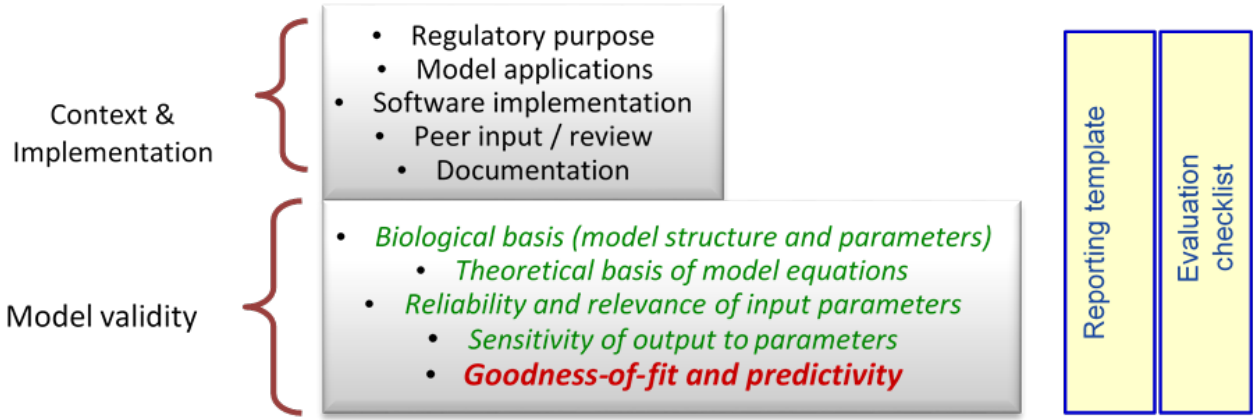
3. Use of analogues in PBK modelling

Use ADME/biokinetic data and PBK model from source analogue(s) to evaluate the new PBK model applicable to target analogue(s). Follow PBK modelling workflow in Chapter 2, iteratively repeat process as necessary (using sensitivity analysis, measures of predictivity etc) to optimise analogue selection and reduce uncertainty in the PBK model.

*Ideally an existing PBK model should be available; where this is absent but there are sufficient data for the analogue, from which a PBK model could be generated, then this is a possibility
**Phenotypes, population variability, lifestyle, genomics etc.

GD 331 ELEMENTS

3. Provide an assessment framework for evaluating PBK models for intended purposes
4. Provide a template for documenting PBK models
5. Provide a checklist to support the evaluation of PBK model applicability according to context of use.



PBK Model Reporting Template sections
A. Name of model
B. Model developer and contact details
C. Summary of model characterisation, development, validation, and regulatory applicability
D. Model characterisation (modelling workflow)
Step 1 – Scope and purpose of the model (problem formulation)
Step 2 – Model conceptualisation (model structure, mathematical representation
Step 3. Model parameterisation (parameter estimation and analysis)
Step 4 – Computer implementation (solving the equations)
Step 5 – Model Performance
Step 6 – Model Documentation
E. Identification of uncertainties
• model structure
• input parameters
• model output
• other uncertainties
F. Model implementation details
• software (version no)
• availability of code
• software verification / qualification
G. Peer engagement (input/review)
H. Parameter tables
I. References and background information
• publications
• links to other resources

PBK Model Reporting Template

Part II Checklist for model evaluation

PBK Model Evaluation Checklist	Checklist assessment	Comments
Name of the PBK model (as in the reporting template)	Generic PBK model for farm animal species	
Model developer and contact details	(1) Leonie Lautz, (1) Jan Hendriks, (1) Ad Ragas, (2) Jean Lou Dorne 1) Radboud University, Nijmegen, Netherlands; 2) European Food Safety Authority, Parma, Italy	
Name of person reviewing and contact details	A Paini	
Date of checklist assessment	10/01/2020	
A. Context/Implementation		
A.1. Regulatory Purpose		
1. What is the acceptable degree of confidence/uncertainty (e.g. high, medium or low) for the envisaged application (e.g. priority setting, screening, full assessment?)	high	
2. Is the degree of confidence/uncertainty in application of the PBK model for the envisaged purpose greater or less than that for other assessment options (e.g. reliance on PBK model and in vitro data vs. no experimental data)?	high	
A.2. Documentation		
3. Is the model documentation adequate, i.e. does it address the essential content of model reporting template, including the following:	YES	
• Clear indication of the chemical, or chemicals, to which the model is applicable?	YES	
• Is the model being applied for the same scientific purpose as it was developed, or has it been repurposed somehow?	YES	
• Model assumptions?	YES	
• Graphical representation of the proposed mode of action, if known?	NO	
• Graphical representation of the conceptual model?	YES	
• Supporting tabulation for parameters (names, meanings, values, mean and standard deviations, units and sources)?	YES	
• Relevance and reliability of model parameters?	YES	
• Uncertainty and sensitivity analysis?	YES	
• Mathematical equations?	YES	
• PBK model code?	YES	Reported in the peer reviewed articles and EFSA

Checklist for Evaluation of Model Applicability

TAKE HOME MSG PART 1

- Models are **designed for purpose**; required confidence varies as a function of the application
- Important Aspects of PBK Model Description and Assessment
  - Quality of the source data
  - Model characterization and implementation
  - Model uncertainty and sensitivity
  - Extent of critical input/prior acceptance
- Best Practice in Developing and Assessing PBK Models to Support Regulatory Application
  - Collaboration with regulatory authorities in development
    - Design for purpose addressing aspects important for the regulatory community
  - Transparency in documentation

OECD DNT-IVB Recommendations

Leads: EFSA and US EPA

Still Missing?

Regulatory and technical experts contributed

>1000

Comments addressed

81

Compounds tested in all the assays of the DNT-IVB

1

OECD Document with Initial Recommendations


OECD

Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2023)13 | 1

English - Or. English

3 November 2023

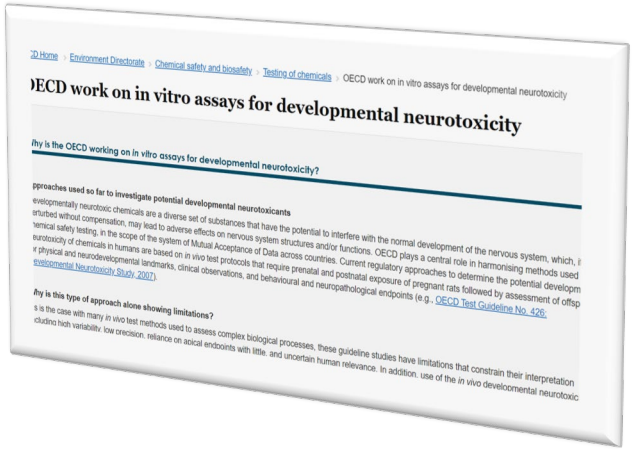
Unclassified

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 9 October 2023

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

Series on Testing and Assessment
No. 377


OECD work on in vitro assays for developmental neurotoxicity

Why is the OECD working on in vitro assays for developmental neurotoxicity?

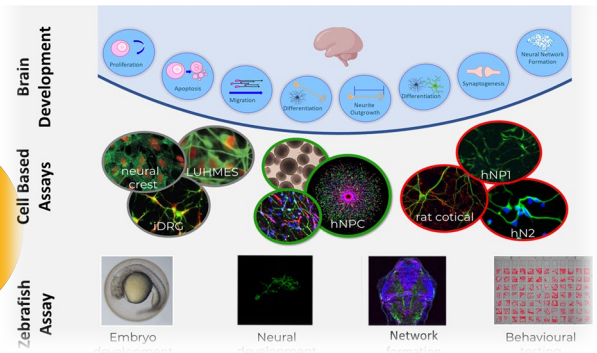
Approaches used so far to investigate potential developmental neurotoxicants

developmentally neurotoxic chemicals are a diverse set of substances that have the potential to interfere with the normal development of the nervous system, which, i
erturbed without compensation, may lead to adverse effects on nervous system structures and/or functions. OECD plays a central role in harmonising methods used
hemical safety testing, in the scope of the system of Mutual Acceptance of Data across countries. Current regulatory approaches to determine the potential developm
eurotoxicity of chemicals in humans are based on *in vivo* test protocols that require prenatal and postnatal exposure of pregnant rats followed by assessment of offsp
r physical and neurodevelopmental landmarks, clinical observations, and behavioural and neuropathological endpoints (e.g., OECD Test Guideline No. 426: evelopmental Neurotoxicity Study, 2007).

Why is this type of approach alone showing limitations?

s is the case with many *in vivo* test methods used to assess complex biological processes, these guideline studies have limitations that constrain their interpretation
cluding high variability, low precision, reliance on apical endpoints with little, and uncertain human relevance. In addition, use of the *in vivo* developmental neurotoxic

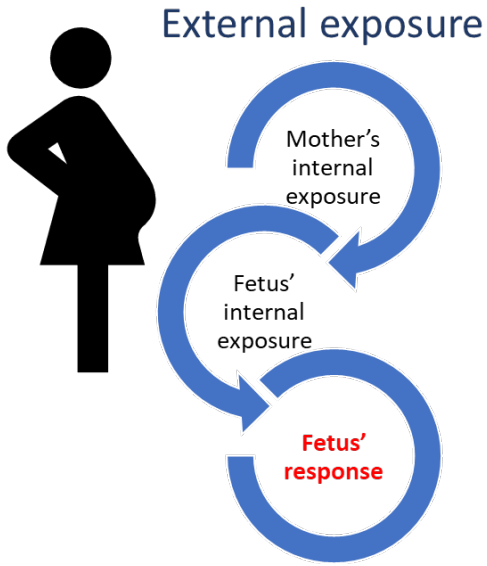
In vitro testing for DNT



https://www.oecd.org/env/ehs/testing/developmental-neurotoxicity.htm

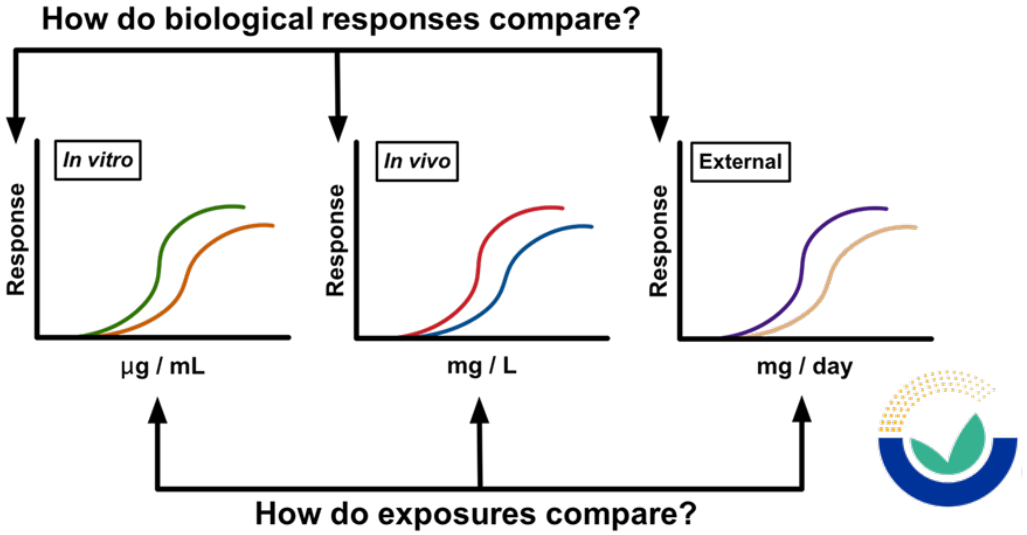
OECD WORKING GROUP 2022

- Exposure
- Kinetics



- How can an in vitro concentration associated with bioactivity be converted to an external exposure level?

- How can a point of departure (PoD) from DNT-IVB be derived, and how can its corresponding in vivo tissue or plasma/blood concentration be determined?



PRINCIPLES OF QUANTITATIVE IN VITRO TO IN VIVO EXTRAPOLATION (QIVIVE) APPLYING PBK MODELLING FOR DNT IVB

The **aim** of this document is to provide an overview of the principles of QIVIVE through the application of PBK modelling to facilitate the incorporation of data from the Developmental Neurotoxicity In Vitro Battery (**DNT IVB**) into chemical hazard characterization and human health risk assessments.

This document **should be read in conjunction** with the Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Battery (IVB).

This **document is not an** exhaustive technical guidance for conducting QIVIVE in regulatory applications.

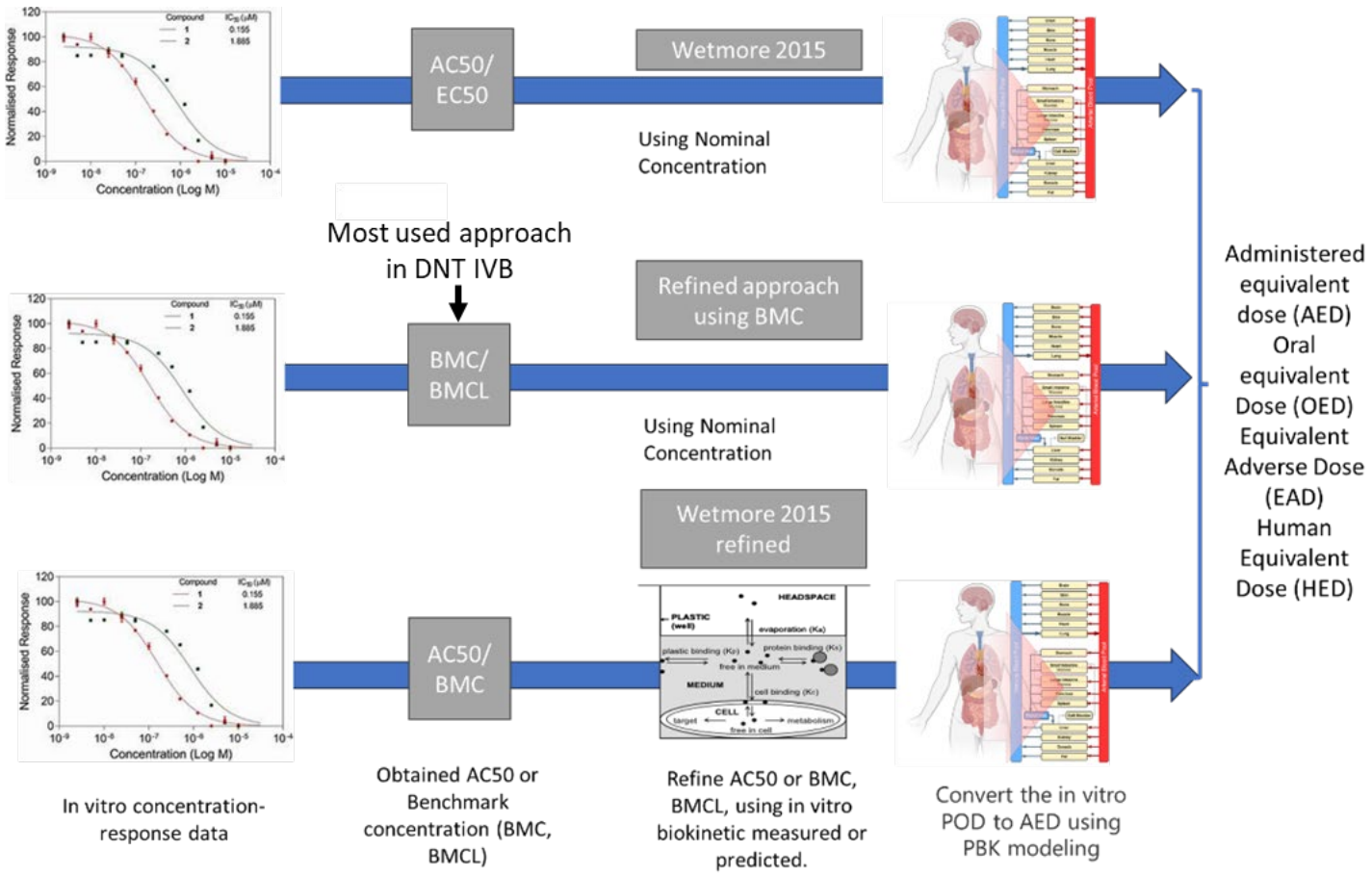
In Draft

QIVIVE Definition

The term 'QIVIVE' describes the process of converting an *in vitro* concentration associated with a specific bioactivity to an external dose (Chang et al., 2022a).

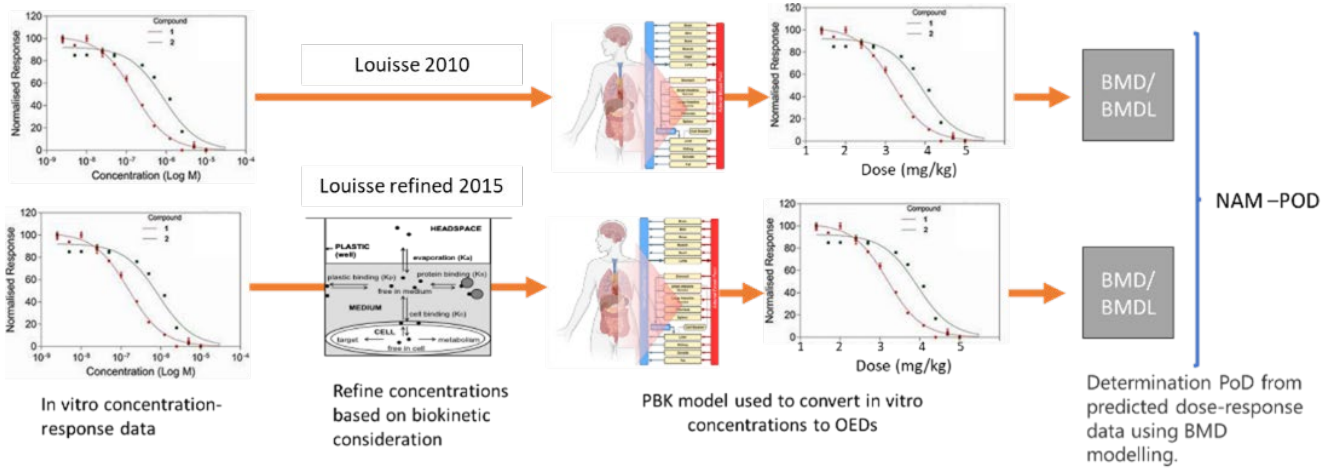
https://www.mdpi.com/2305-6304/10/5/232

QUANTITATIVE IN VITRO TO IN VIVO EXTRAPOLATION (QIVIVE)

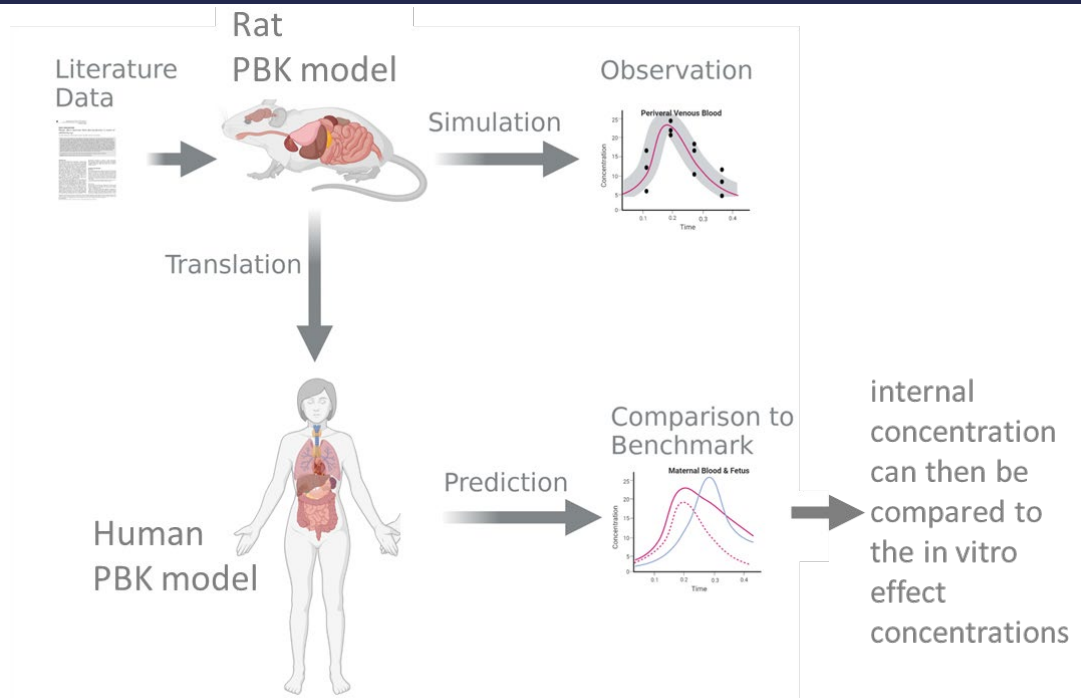


Schematic presentation of a QIVIVE approach in which an in vitro POD is translated to an external dose using PBK modelling. As highlighted in the published Initial Recommendations on Evaluation of Data from the DNT IVB, to date, results from the DNT IVB are expressed as in vitro benchmark concentrations (BMC).

QUANTITATIVE IN VITRO TO IN VIVO EXTRAPOLATION (QIVIVE)



Schematic presentation of a QIVIVE approach in which in vitro concentration-response data are translated into in vivo dose-response data using PBK modelling, from which an external POD can be derived.



In forward dosimetry, a PBK model is used to predict the internal concentrations resulting from a specific or a range of external doses. The predicted internal concentration can then be compared to the in vitro effect concentrations (Maass et al., 2023).

WHAT IS NEEDED – PART I


OECD
Organisation for Economic Co-operation and Development
ENV/CBC/MONO(2023)13 | 1
English - Or. English
3 November 2023
Unclassified
ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE
Cancels & replaces the same document of 9 October 2023
Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery
Series on Testing and Assessment
No. 377

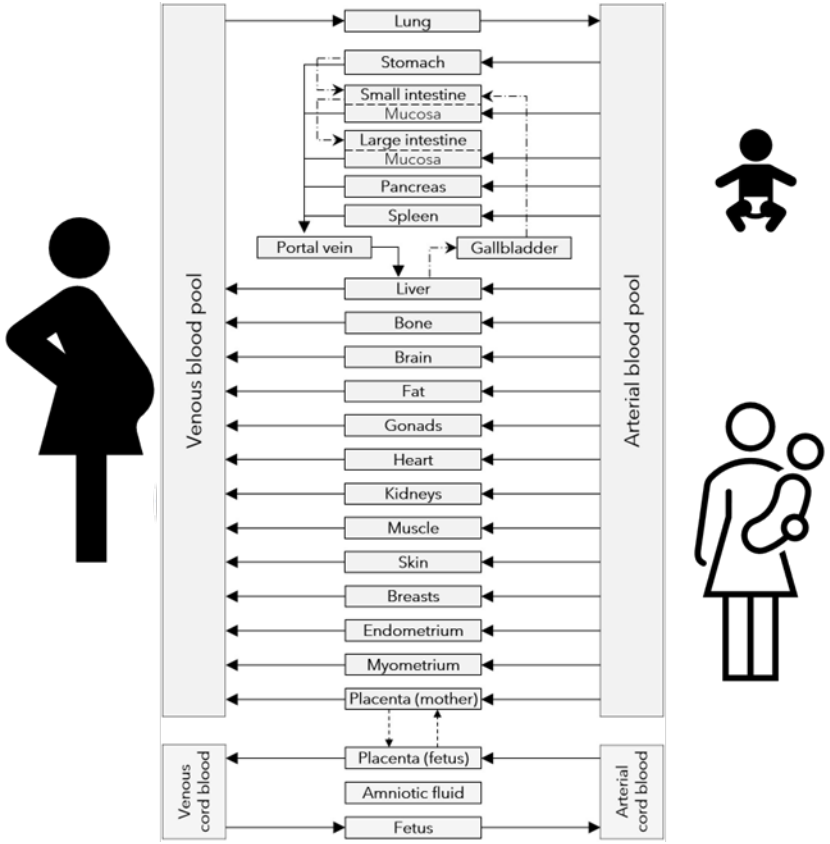
- BMC or in vitro concentration response curve
- In vitro nominal to free concentration

Assays Currently in the DNT IVB mapped with information relevant for the QIVIVE approach.

Test Method (Assay)	Test System (Cell culture)	Assay Duration/ Chemical exposure	DNT Endpoint	Viability/Cytotoxicity Endpoint	Single/repeated exposure	Experimental set up (Medium composition, e.g., % FBS, lipid %, protein concentration) for in vitro distribution models	Experimental set up (Medium volume/plate format (12, 24, 48, 96)/plate material For in vitro distribution models	Is the plate coated?	Life stage - developmental period (prenatal, postnatal, 1st, 2nd, or 3rd trimester)
Proliferation									
NPC1	human NPC grown as proliferating 3D neurospheres	72 h / 72 h	neurosphere area, BrdU incorporation in dividing cells	Resazurin reduction /LDH release	single exposure over 3 days	DMEM (#31966-021, Thermo Fisher, United States) and Hams F12 (#31765-027, Thermo Fisher, United States) in a)	100 µl/ 96-well plate / PS (Polystyrene), #351177 (Falcon)	Yes – poly-(2-hydroxyethyl methacrylate)	Prenatal, gestational week (GW) 16-18

WHAT IS NEEDED – PART II

- PBK model structure



Tiered PBK modelling framework

Tier 0

High throughput adult or juvenile model to predict maternal plasma or juvenile plasma concentration

Tier 1

Whole-body adult or juvenile model to predict maternal plasma or juvenile plasma concentration

Appropriate when maternal plasma concentration is likely to be higher than or equal to plasma/brain concentration in the fetus or nursing infants

Tier 2

Gestational model to predict fetus plasma concentration

Appropriate when plasma concentration in the fetus is expected to be higher than brain concentration in the fetus and maternal plasma concentration

Lactational-infant model to predict plasma concentration in nursing infants

Appropriate when plasma concentration in the nursing infants is expected to be higher than brain concentration in the nursing infants and maternal plasma concentration

Tier 3

Whole-body gestational-fetus model to predict fetus brain concentration

Appropriate when brain concentration in fetus is expected to be higher than maternal and fetus plasma concentration

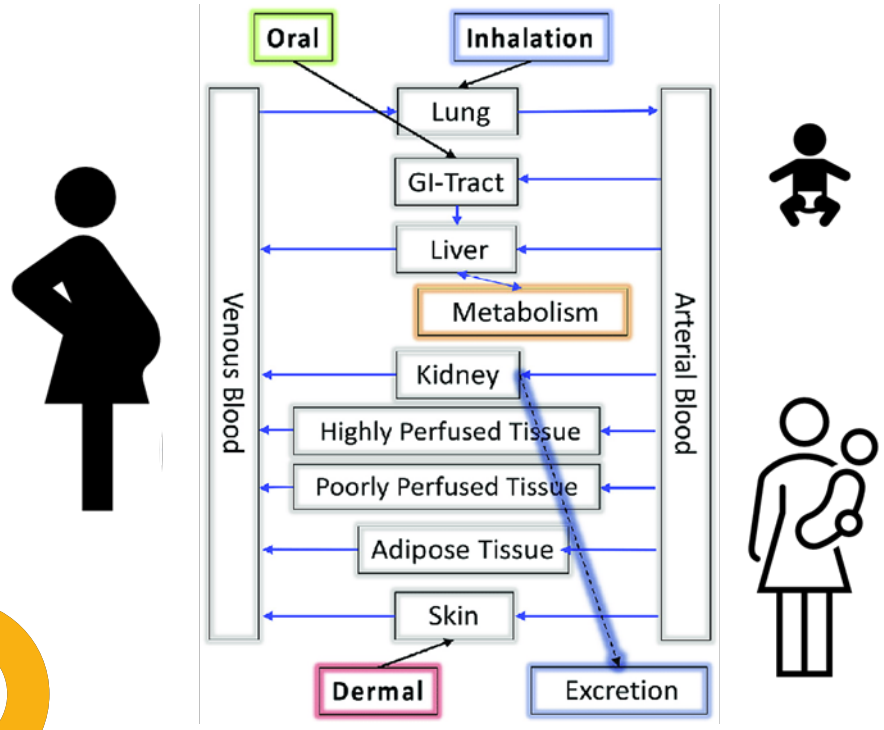
Lactational-infant model to predict brain concentration in nursing infants

Appropriate when brain concentration in the nursing infants is expected to be higher than plasma concentration in the nursing infants and mother

Dallman et al., 2017
https://github.com/Open-Systems-Pharmacology/Pregnancy-Models

WHAT IS NEEDED – PART II

- PBK model structure
- Input data for the model



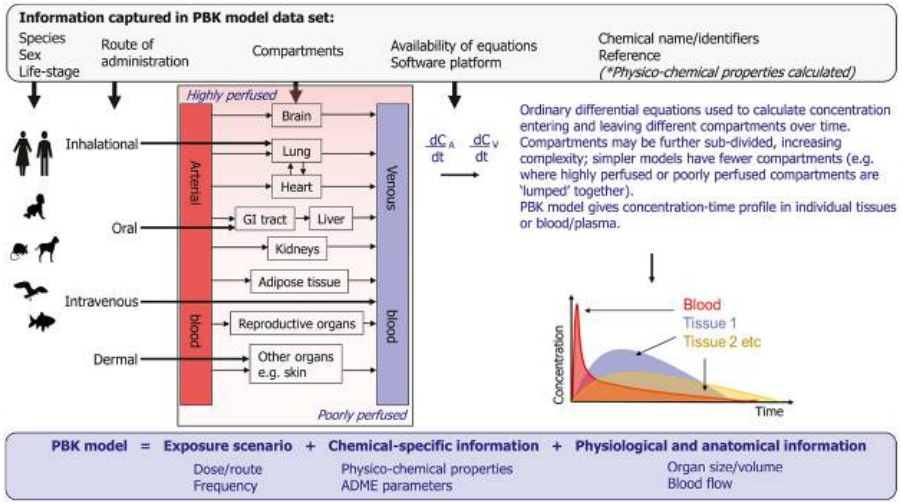
- Physiology – Gestational, I, II, III or Infant + lactation.
- Biochemical – Fub, permeability, BBB, placenta, clearance., metabolism
- Physico chemical – Log Kow, Pka and MW …

The essential set of parameters required for a model should adequately depict the physiology of the target species and the ADME of a chemical for the intended purpose, such as absorption rates, **plasma protein binding, and clearance**. Detailed recommendations for parameterizing a PBK model with in vitro and in silico approaches (OECD 331, 2021), or with in vivo data, such as those outlined in OECD TG417 (2010), can be found in published guidance documents (EPA 2006, WHO 2010).

DATABASE OF AVAILABLE P & L PBK MODELS

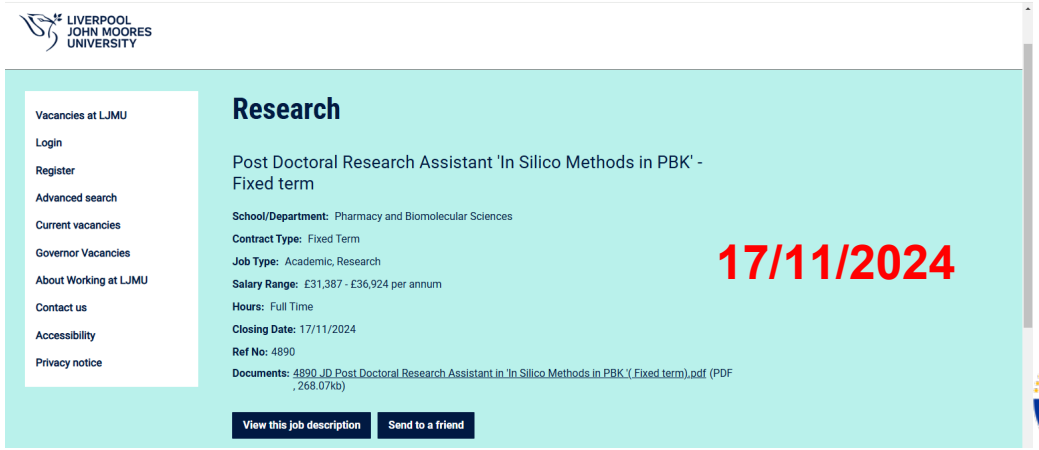
Dr. Pavani Gonnabathula

Building on the Thompson PBK Database



Extracted in excel:

- Information on the paper
- Information on the chemical
- Chemicals identifiers
- Information on the PBK model (Pregnancy/Lactation)
- Information on PBK model parametrisation
- Information on how the model was validated



https://jobs.ljmu.ac.uk/vacancies/9797-491997-Research%20jobs.html

Thompson CV, Firman JW, […] **Madden JC.** A Systematic Review of Published Physiologically-based Kinetic Models and an Assessment of their Chemical Space Coverage. Altern Lab Anim. 2021 Sep;49(5):197-208. doi: 10.1177/02611929211060264.

TAKE HOME MSG PART II

- Provide principles and information to perform QIVIVE for OECD DNT in vitro test battery!
- QIVIVE approaches are presented!
- PBK model framework is proposed!
- PBK model availability is provided!

The document should be ready by end of the year

Overall, there is a need for a general QIVIVE guidance!!!

WHAT NEXT?

STILL TO BE ACCEPTED BY OECD WP !!!

Working Party on Hazard Assessment (WPHA)

Project Title

Update of the OECD 331 Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes

Submitted by:

USEPA/EFSA

Date of Submission to the Secretariat:

December 1, 2024

Details of Lead Country(ies)/Organisation

Country /Organisation:	European Commission United States
Agency/ministry/Other:	European Food Safety Authority US Environmental Protection Agency
Phone:	Alicia Paini: +39 0521 036172 Cecilia Tan:
Email:	alicia.paini@efsa.europa.eu Tan.Cecilia@epa.gov

OECD TEST GUIDELINES PROGRAMME

Standard Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to:
Anne Gourmelon (anne.gourmelon@oecd.org)
and Lesley Smith (Lesley.smith@oecd.org)

PROJECT TITLE

Development of Test Guidelines for Measuring Human Hepatic Clearance and Plasma Protein Binding Using In Vitro Methods and Integration in a Defined Approach for Toxicokinetic Characteristics

SUBMITTED BY (Country / European Commission / Secretariat)

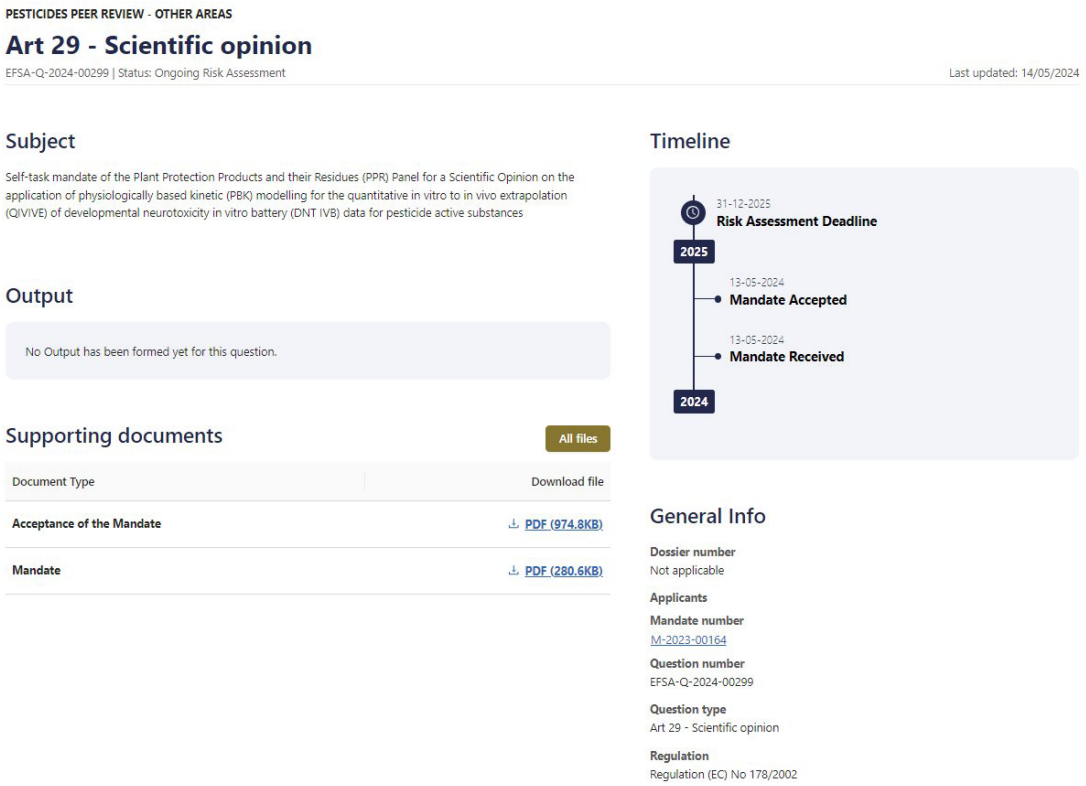
European Commission and United States

DATE OF SUBMISSION TO THE SECRETARIAT

24 October 2024

Working Group of National Co-ordinators of the TGs programme (WNT)

WHAT NEXT? @EFSA PPR WG QIVIVE 4 DNT IVB



PESTICIDES PEER REVIEW - OTHER AREAS

Art 29 - Scientific opinion

EFSA-Q-2024-00299 | Status: Ongoing Risk Assessment — Last updated: 14/05/2024

Subject

Self-task mandate of the Plant Protection Products and their Residues (PPR) Panel for a Scientific Opinion on the application of physiologically based kinetic (PBK) modelling for the quantitative in vitro to in vivo extrapolation (QIVIVE) of developmental neurotoxicity in vitro battery (DNT IVB) data for pesticide active substances

Output

No Output has been formed yet for this question.

Supporting documents — All files

Document Type	Download file
Acceptance of the Mandate	PDF (974.8KB)
Mandate	PDF (280.6KB)

Timeline

2025
- 31-12-2025 — Risk Assessment Deadline

2024
- 13-05-2024 — Mandate Accepted
- 13-05-2024 — Mandate Received

General Info

Dossier number
Not applicable

Applicants

Mandate number
M-2023-00164

Question number
EFSA-Q-2024-00299

Question type
Art 29 - Scientific opinion

Regulation
Regulation (EC) No 178/2002

https://open.efsa.europa.eu/questions/EFSA-Q-2024-00299

Self-task mandate of the Plant Protection Products and their Residues (PPR) Panel for a Scientific Opinion on the application of physiologically based kinetic (PBK) modelling for the quantitative in vitro to in vivo extrapolation (QIVIVE) of developmental neurotoxicity in vitro battery (DNT IVB) data for pesticide active substances.

Kick – off July 2024

ACKNOWLEDGEMENTS

Cecilia Tan (US EPA, US)
Magda Sachana (OECD, F)
Jochem Louisse (EFSA, IT)

OECD GD 331 drafting Team !!!
OECD QIVIVE4DNTIVB drafting Team !!!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THANK YOU!