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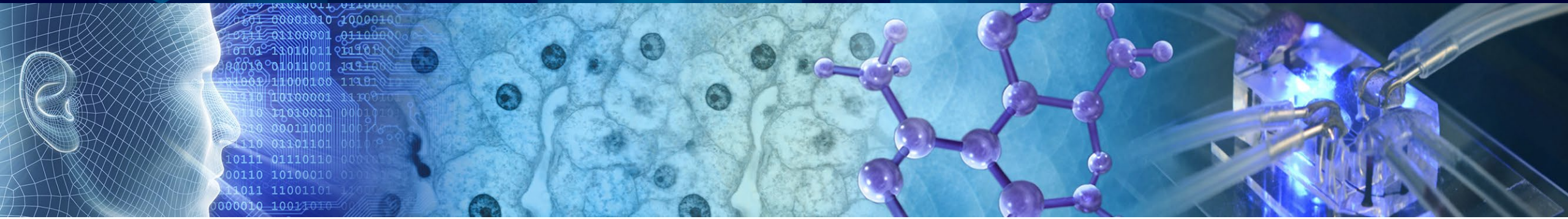

NAMs Activities at NIEHS/DTT and NICEATM

Nicole C. Kleinstreuer, PhD

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

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

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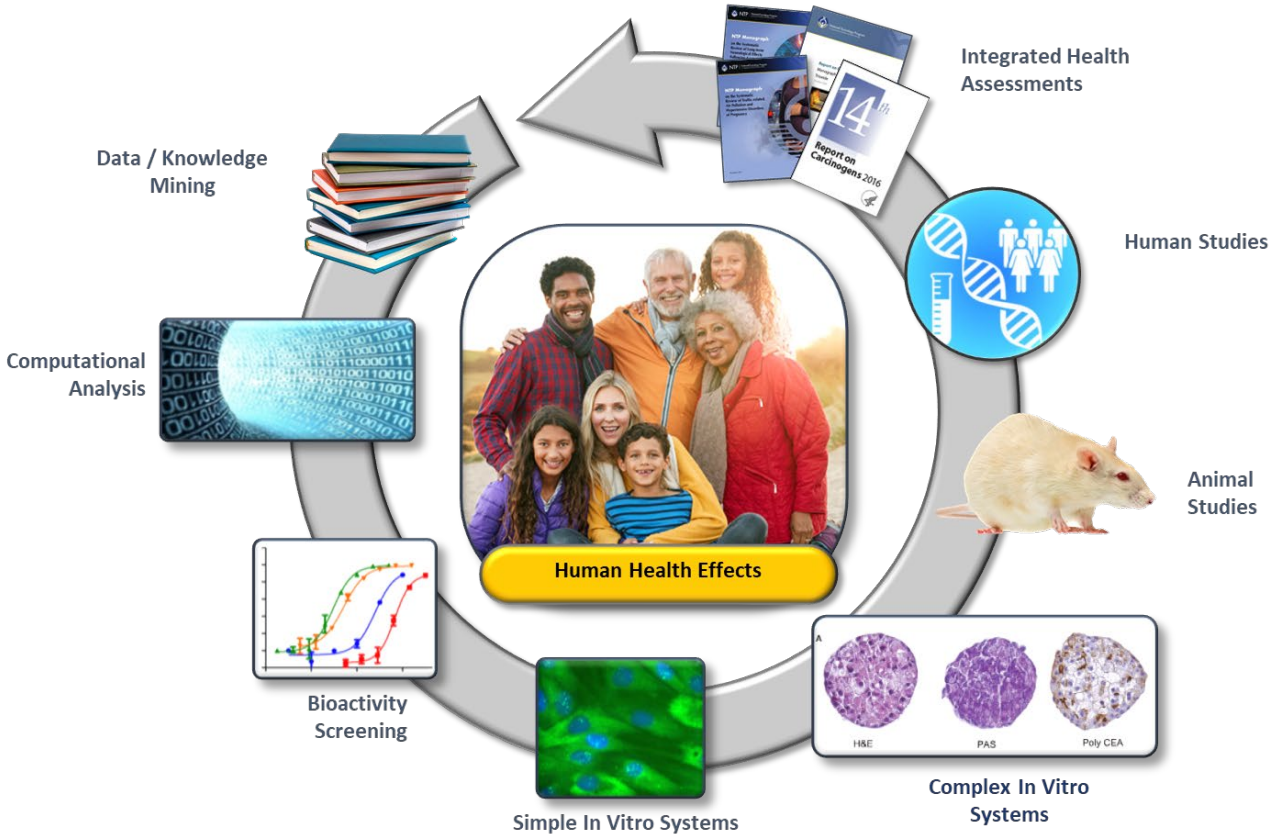
NAMs at NIEHS/DTT

Exposure-based Research Programs

Health Effects Innovation Programs

Responsive Research Programs

Strengthening Capabilities Programs



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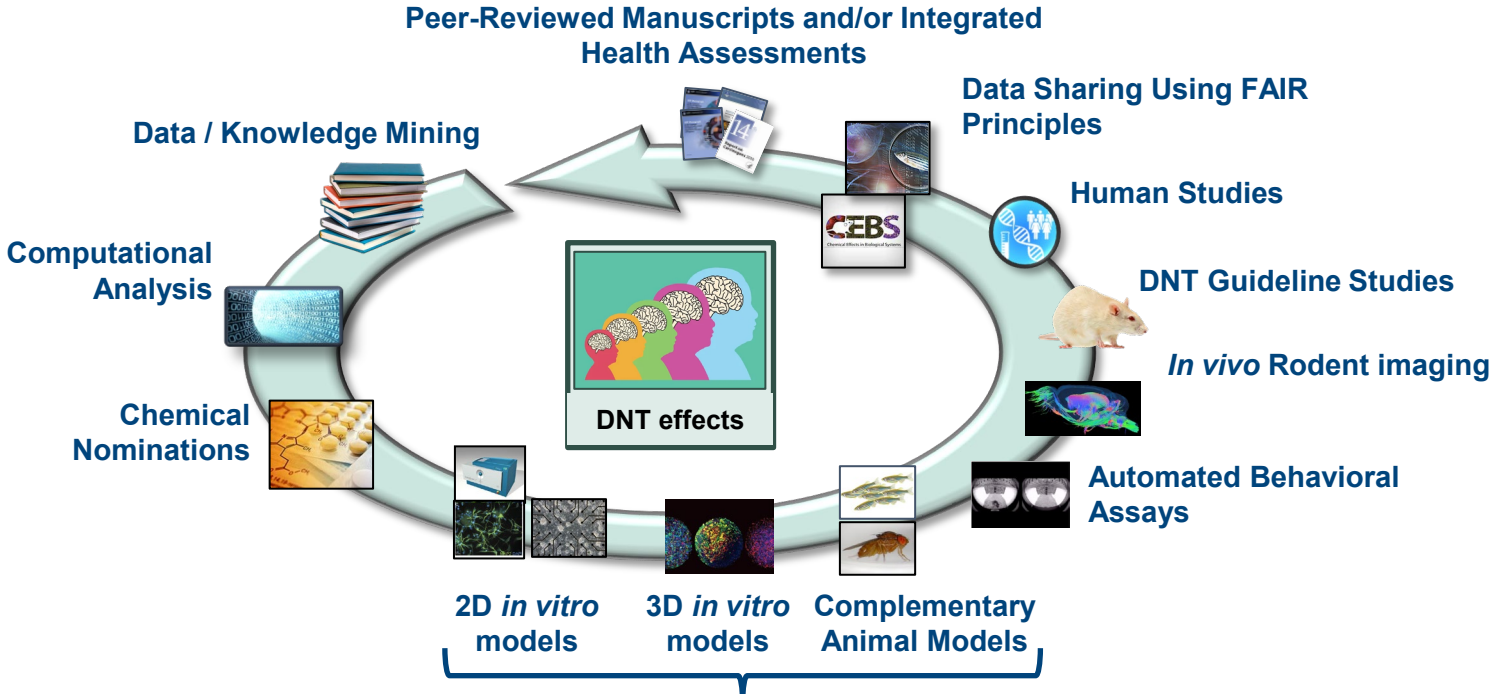
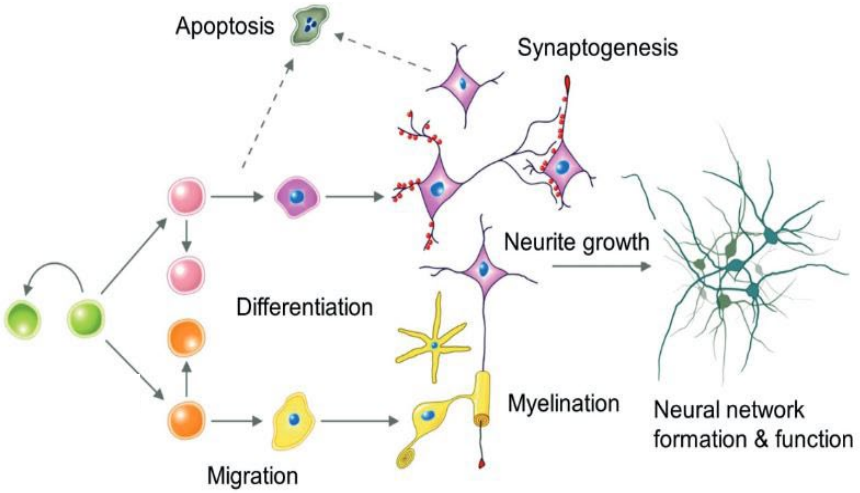
Developmental Neurotoxicity (DNT) Health Effect Innovation (HEI) Program





Ieurobehavior

Stakeholder nominated chemical library

Selection Criteria

- Evidence of DNT *in vivo*
- Known human exposure
- Guideline study complete, lacking *in vitro*
- Suggested by multiple stakeholders
- Incomplete *in vitro* battery data

Phase I: 115 chemicals

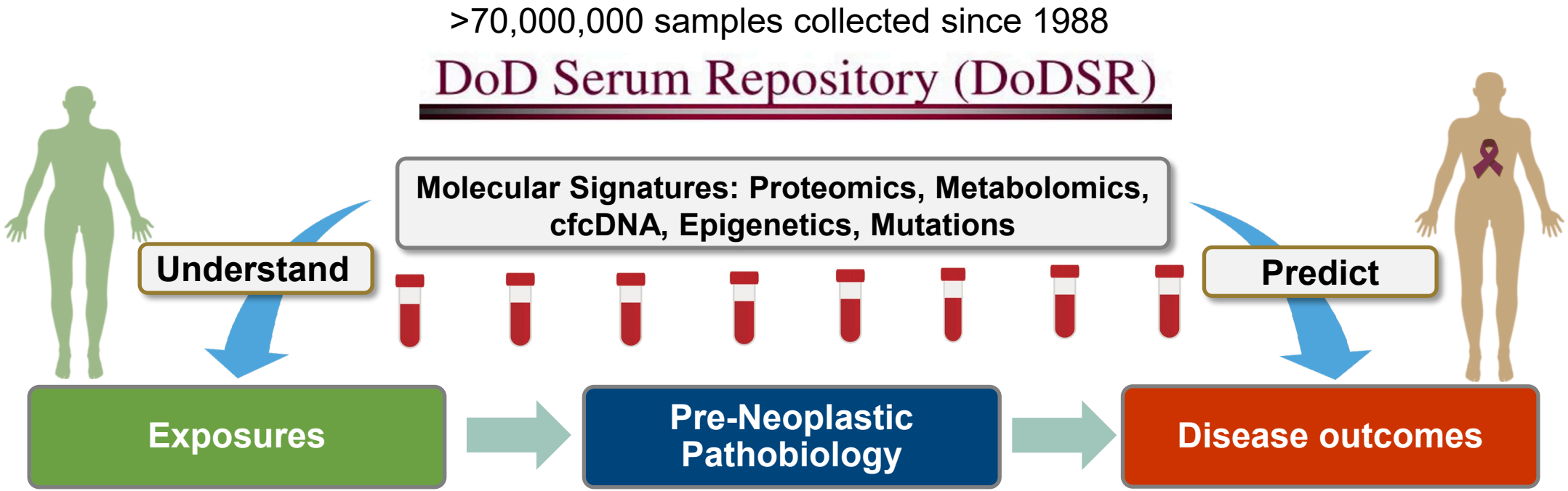
- Testing finalized by contractors
- Data analyses finalized
- Manuscript in preparation

Phase II: ~100 chemicals

- Chemicals have been sent to contractors for testing

Carcinogenesis (Carci)
Health Effect Innovation (HEI) Program

Environmental Exposures and Disease



Translational Human Research in Cancer

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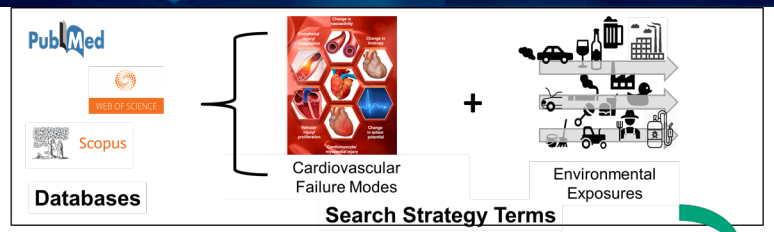
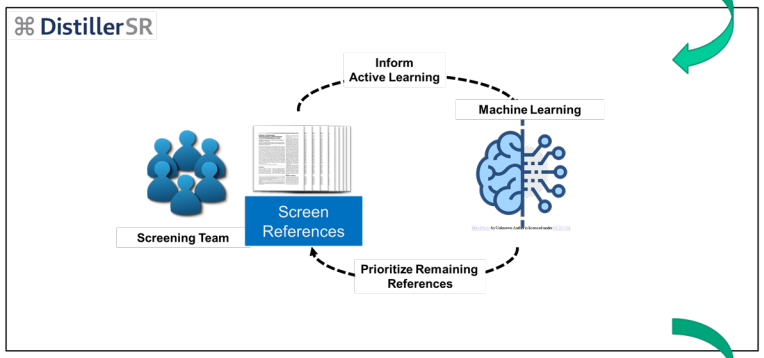
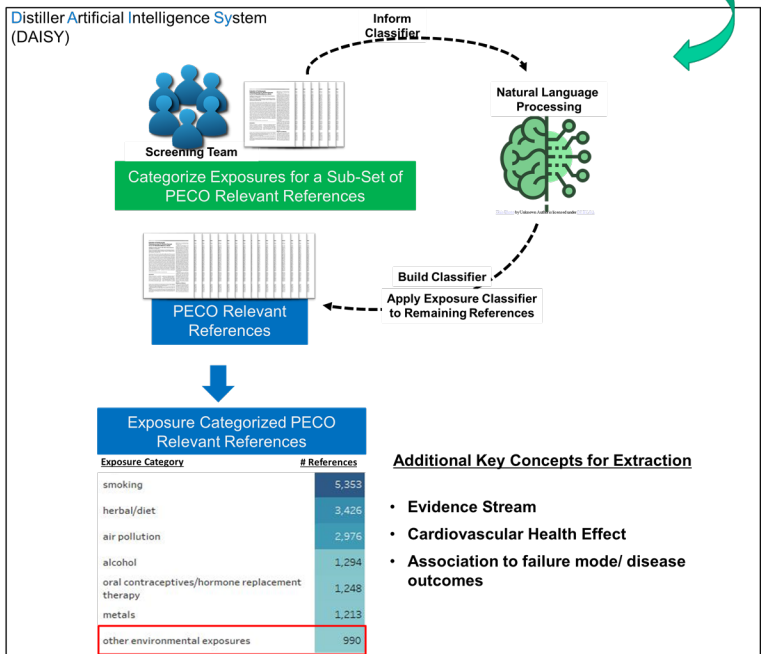
Cardiovascular (CV) Health Effect Innovation (HEI) Program

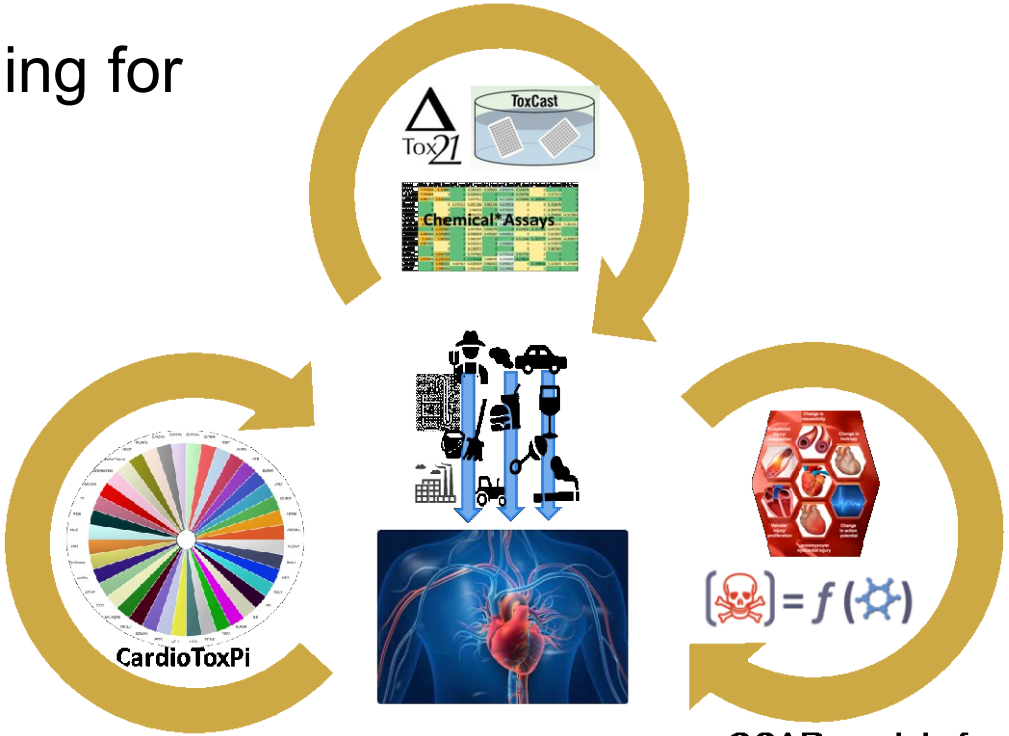






Systematic Evidence Mapping and Computational Modeling for CV Risk

Integrating HTS assay data and exposure



Identification of CV-relevant targets and endpoints

QSAR models for CardioToxPi mechanistic target groupings

Combined Exposures and Mixtures Program

Support for the Botanical Safety Consortium: A public-private partnership aiming to evaluate the suitability of NAM-based toxicity assays for use with complex mixture botanical ingredients

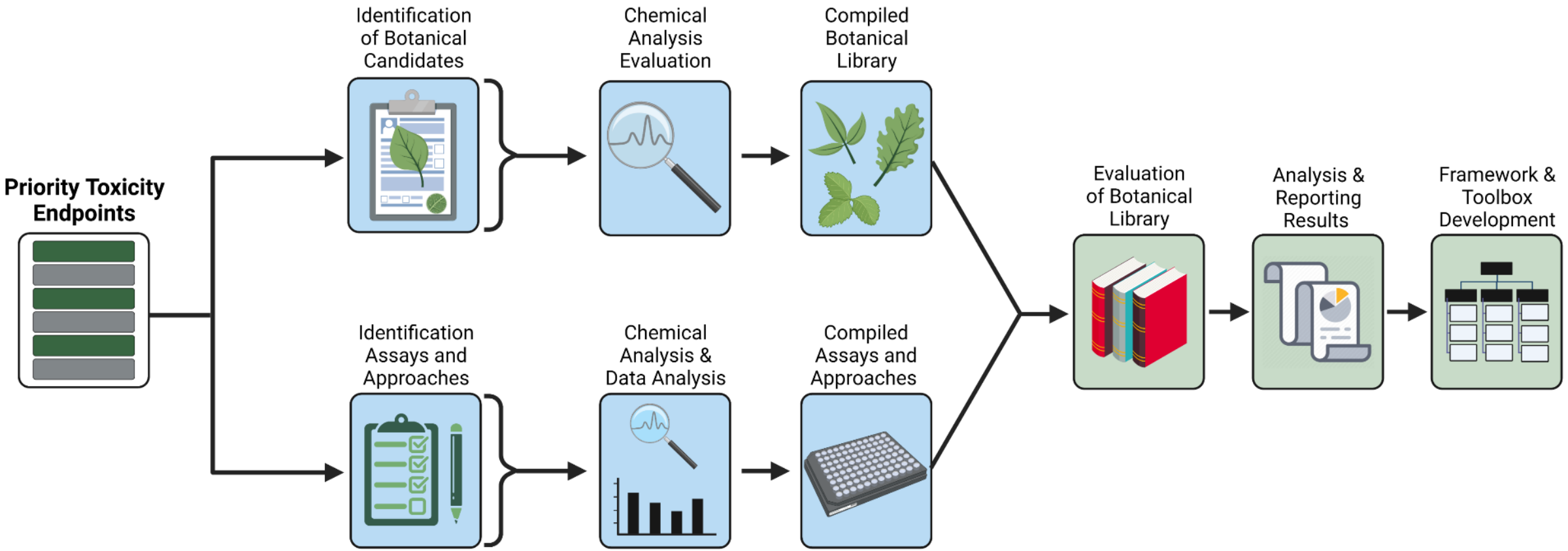


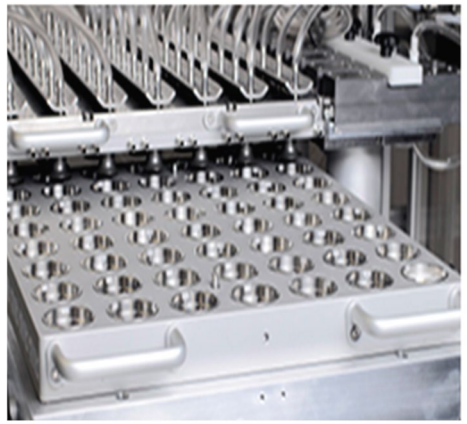
Figure courtesy of HESI

Occupational and Inhalation Exposures (OIE) Program

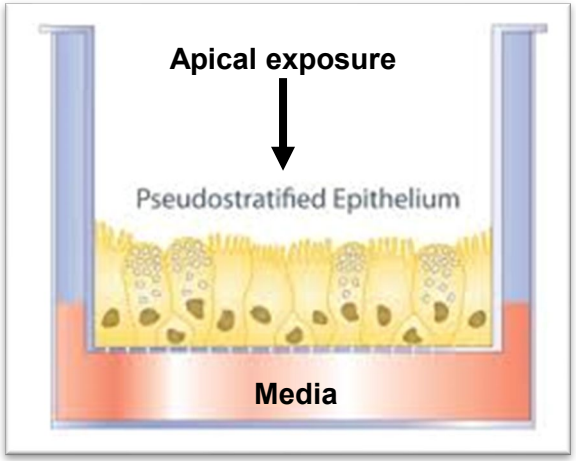
- The OIE Program aims to expand capabilities for predicting adverse health effects
- Currently evaluating novel/alternative technologies (i.e., in vitro models and microphysiological systems) to investigate human-relevant inhalation (respiratory) toxicity
  - ➢ Screening level assessments to predict toxicity, guide additional study design, mechanistic evaluations of MoAs/AOPs

In vitro air-liquid interface (ALI) airway cultures with Vitrocell 48 2.0 plus exposure system

Exposure to vapors, gases, aerosols, or particles (↑ doses & throughput)



www.vitrocell.com



www.atcc.org

Human-derived primary airway cells from tracheobronchial, bronchial, or alveolar compartment (testing of multiple donors and rodent cells also possible)

Proof-of-concept (pilot) study in-progress testing 2,3-pentanedione vapors (Q3/2023)

Consumer Products & Therapeutics Program

- **Clustering and classification workshop:**
  - Convened international experts to discuss methods, their applications guide toxicology research and inform hazard and risk assessment.
  - **Accomplishments:**
    - Defined the similarity concept for supervised and unsupervised approaches
    - Introduced different approaches, corrected 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)

Applications to Investigate Adverse Effects of Chemicals on Human Health and Environment Workshop
October 3-4, 2022 • 8:30 a.m. – 4:00 p.m. EDT

…to be continued

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

- **Systematic Evidence Mapping:**
  - Established evidence-base to inform and direct class-based health-effect evaluations
  - **Key projects:**
    - Health effects of OFRs
    - Personal care products and (1) early puberty and (2) fetal growth
    - Resource for researchers and regulators to search, sort, filter published studies

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Data Standardization Efforts at DTT

Getting to FAIR+ requires standard metadata, terminologies, and quality curation

NIH Office of Data Science Strategy FAIR checklist

- Establishment of a DTT Data Dictionary
  - e.g., populations, treatments, outcomes, endpoints
  - Incorporated into DSMPs
  - In-house expertise with ontologies

- Community is key, both local and global:
  - DTT Knowledge Management Team
  - DTT-EPA collaborations
  - Engagement with ontology communities, e.g.
    - Ontology for Biomedical Investigations (OBI)
    - Adverse Outcome Pathways (AOPs)
  - Engagement with NIEHS grantee communities
  - Environmental Health Language Collaborative

Charles Schmitt (NIEHS/ODS), Jennifer Fostel (NIEHS/DTT)

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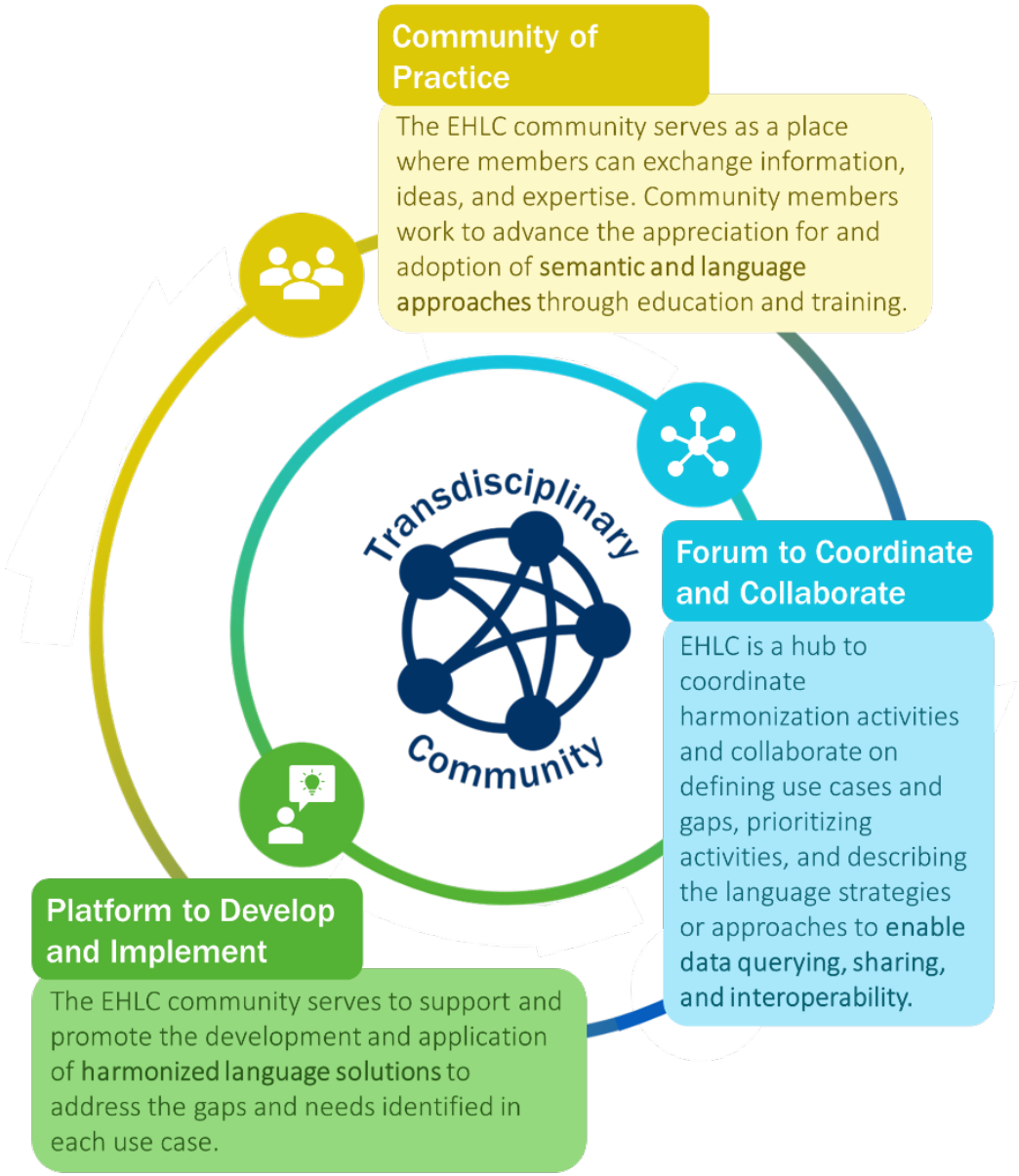
Environmental Health Language Collaborative



EHLC Mission: to advance integrative environmental health research by promoting access, use, and harmonization of data through interoperable terminologies and best practices.

Use Case	Champion
1. What data exists for a given chemical/endpoint/exposure scenario?	Michelle Angrish, EPA Shannon Bell, RTI
2. What are the biological processes and biomarkers associated with exposure and how do they relate to the potential for an adverse outcome associated with a given exposure	Steve Edwards, RTI Chirag Patel, Harvard University
3. How do we combine individual-level data from multiple independent studies to understand how exposures X+Y impact health outcome Z?	Jeanette Stingone, Columbia University

Contact: Stephanie Holmgren (Holmgren@niehs.nih.gov)

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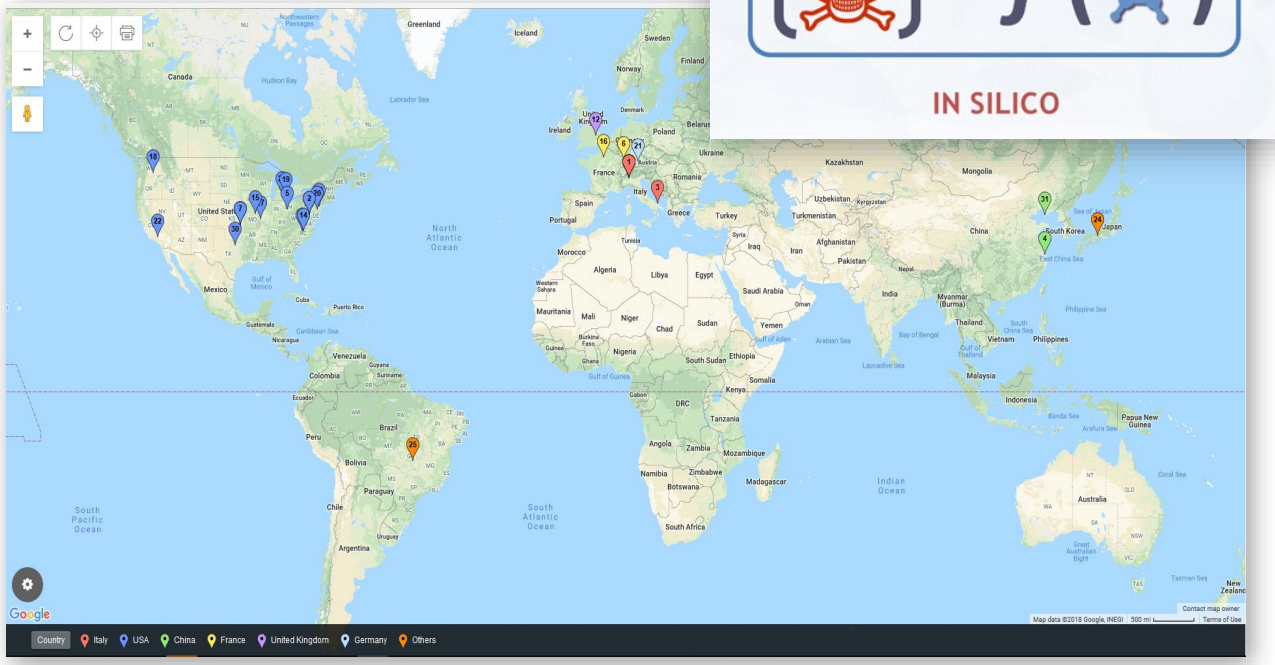
Ongoing NICEATM and ICCVAM Projects

- 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

https://ntp.niehs.nih.gov/go/2021iccvamreport

Subscribe to NICEATM News email list
https://ntp.niehs.nih.gov/go/niceatm

Global Crowdsourcing Predictive Models



- 35 Groups: academia, industry, govt
- Curate reference data to train & test models: >10k chemicals
- Use molecular structure and chemical properties to predict toxicity (e.g. endocrine disruption, acute systemic effects)
- Combine best models together into “ensemble” approaches
- Create open access AI/ML modeling suite

OPERA
OPEn (q)saR App

https://github.com/NIEHS/OPERA

Integrated Chemical Environment

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

Kleinstreuer et al. Comp Tox (2018); Mansouri et al. J Cheminform (2018), Env Health Persp (2020, 2022)

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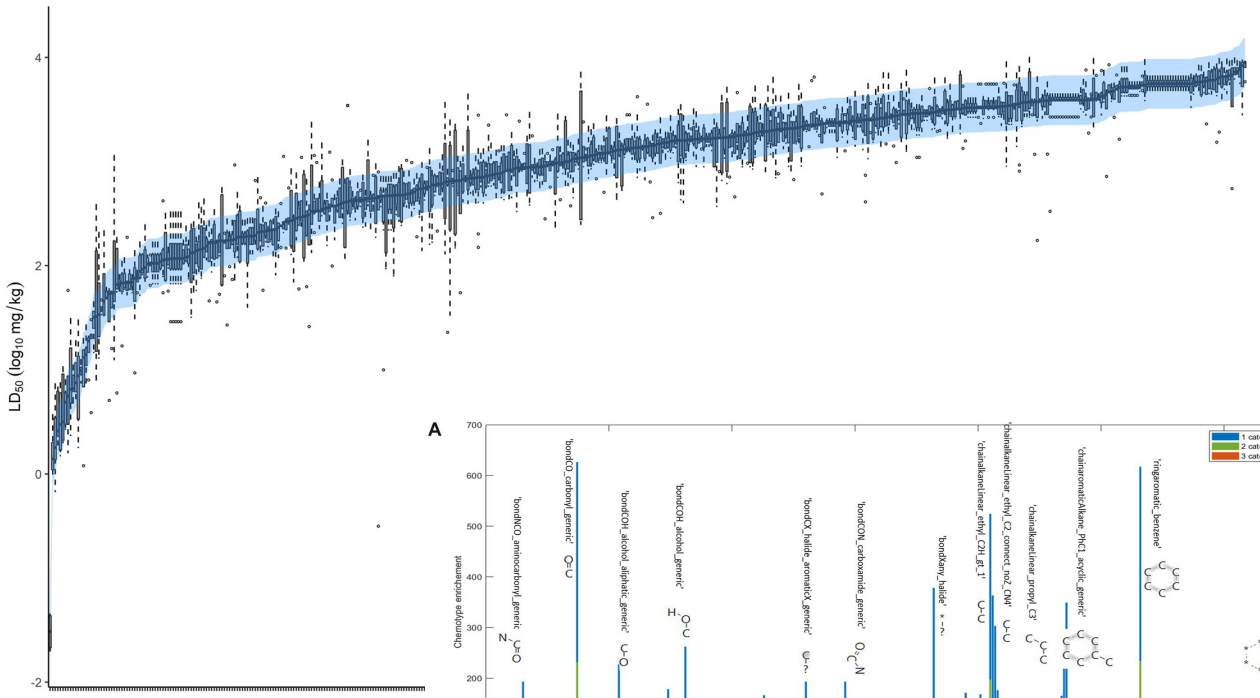
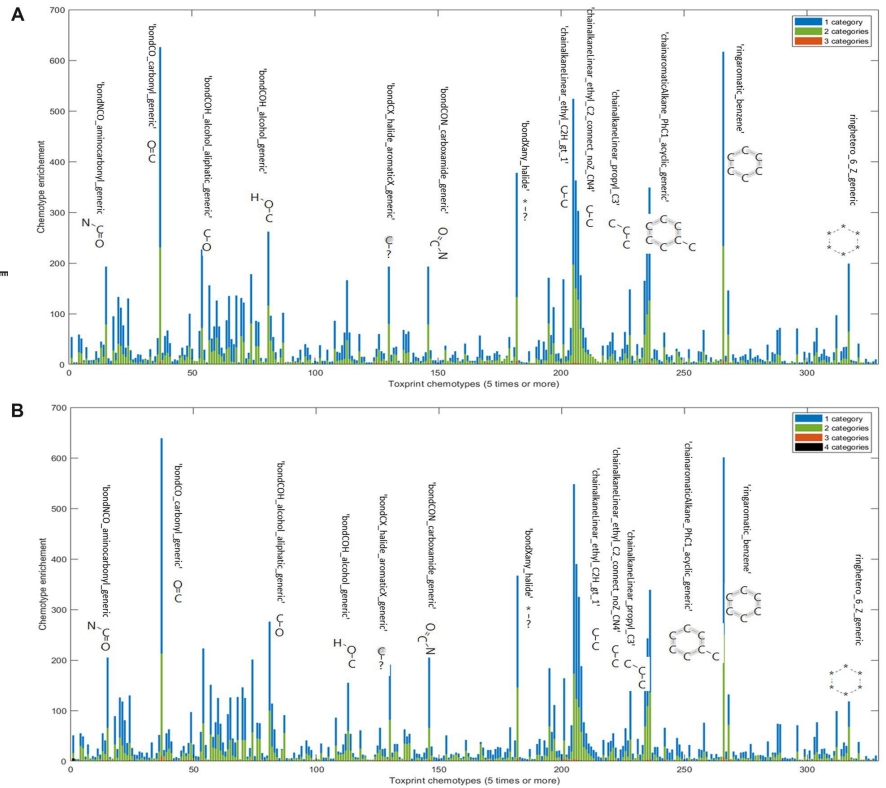
Characterizing Variability and Applying to Model Evaluation





Analyzing sources of variability in acute oral toxicity data & quantifying 95% confidence interval

Collaborative Acute Toxicity Modeling Suite (CATMoS) Performance

	Very Toxic		Non-Toxic		EPA		GHS	
	Train	Eval	Train	Eval	Train	Eval	Train	Eval
Sensitivity	0.87	0.70	0.88	0.67	0.81	0.62	0.80	0.58
Specificity	0.99	0.97	0.97	0.90	0.92	0.86	0.95	0.90
Balanced Accuracy	0.93	0.84	0.92	0.78	0.87	0.74	0.88	0.74
***In vivo* Balanced Accuracy**	0.81		0.89		0.82		0.79	

	LD50 values		LD50 values
	Train	Eval	***In Vivo***
R2	0.85	0.65	0.80
RMSE	0.30	0.49	0.42

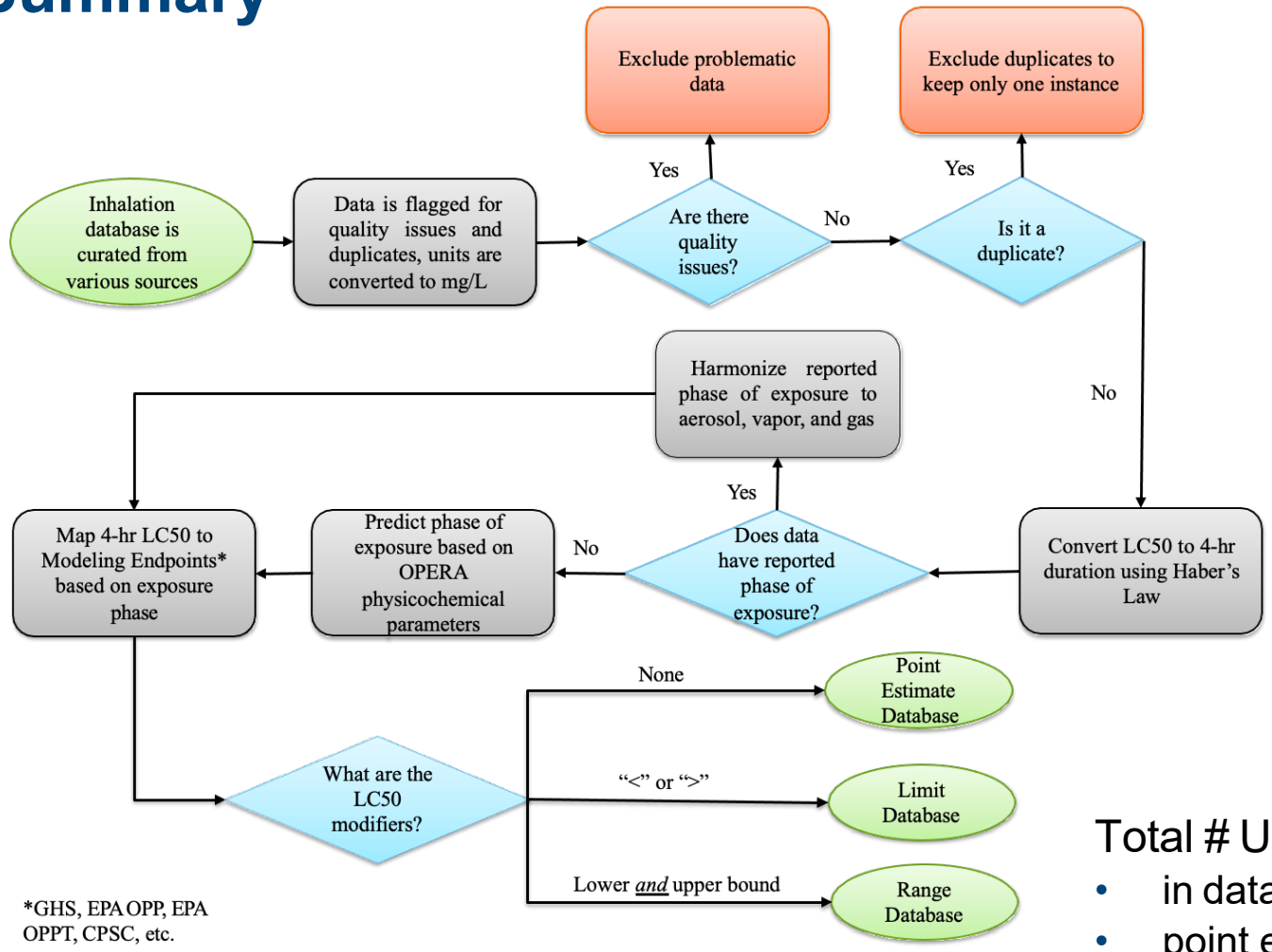
CATMoS QSAR predictions perform just as well as replicate *in vivo* data at predicting oral acute toxicity outcome

Karmaus et al. Toxicol Sci. 2022; Mansouri et al. EHP 2021

Extending to Acute Inhalation Toxicity

Inventory Sources and Summary

- **ECHA REACH Database**
  - Data Rows: 3016
  - Unique Substances: 611
- **ChemIDplus**
  - Data Rows: 2036
  - Unique Substances: 1249
- **Department of Defense**
  - Reports: 22
  - Unique Substances: 13
- **EPA AEGL**
  - Data Rows: 1682
  - Unique Substances: 271
- **NIOSH Pocket Guide**
  - Data Rows: 136
  - Unique Substances: 649



Total # Unique Chemicals
- in database: **1025**
- point estimate data: **780**
- limit data: **312**
- range data: **45**

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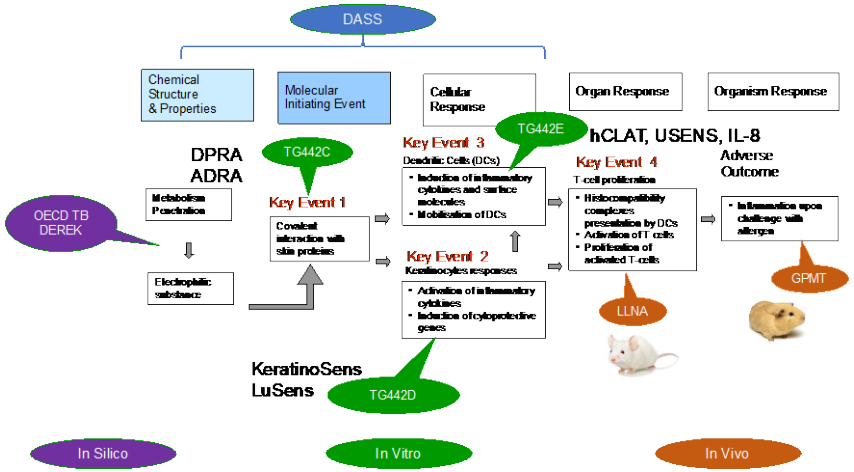
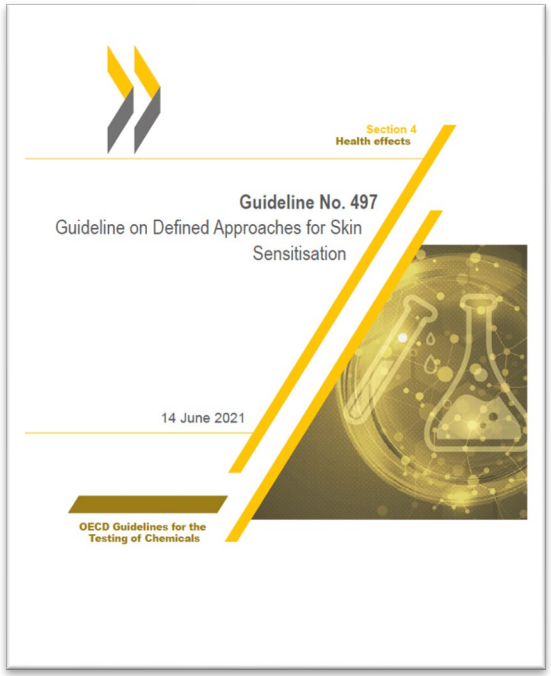
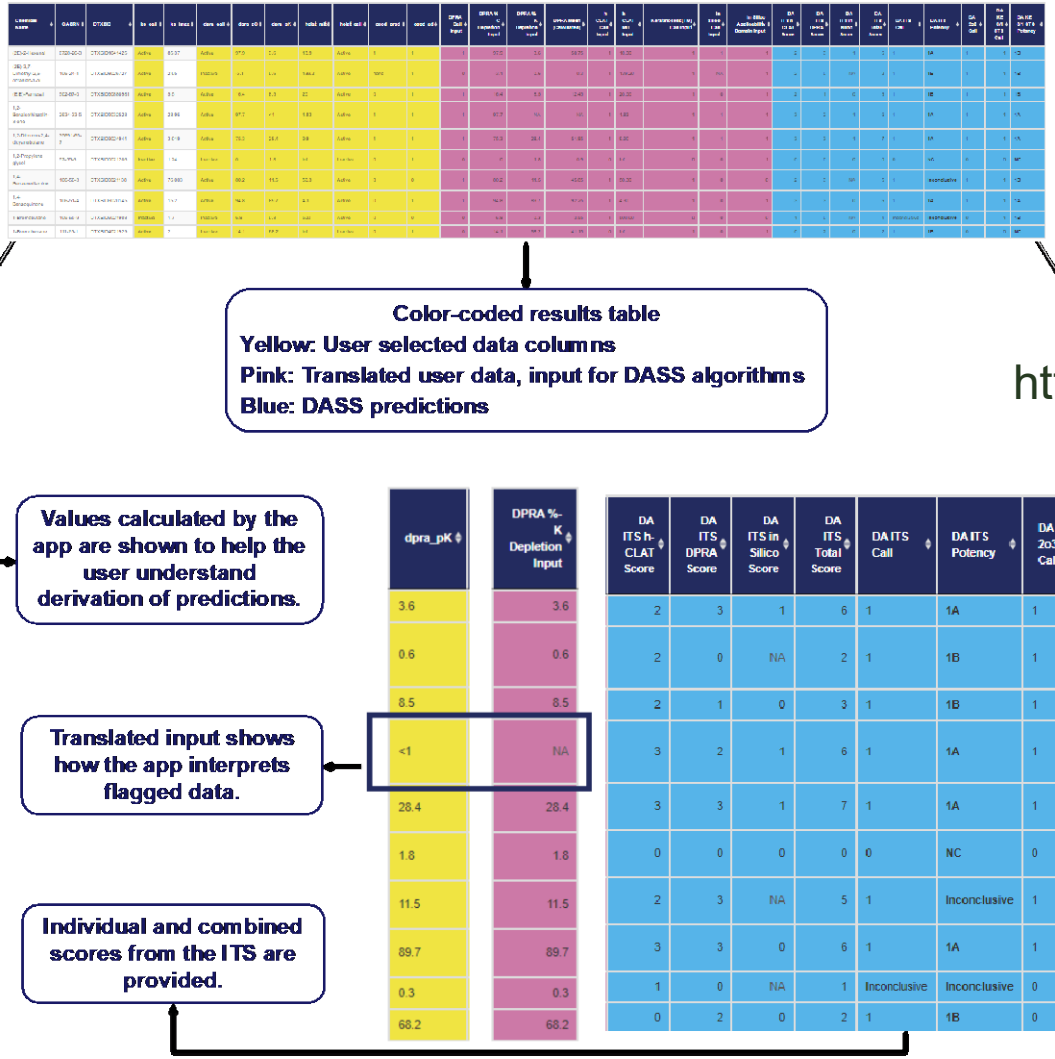
User Friendly DASS App







Access the DASS App
https://ntp.niehs.nih.gov/go/952311

To et al. 2023 in prep

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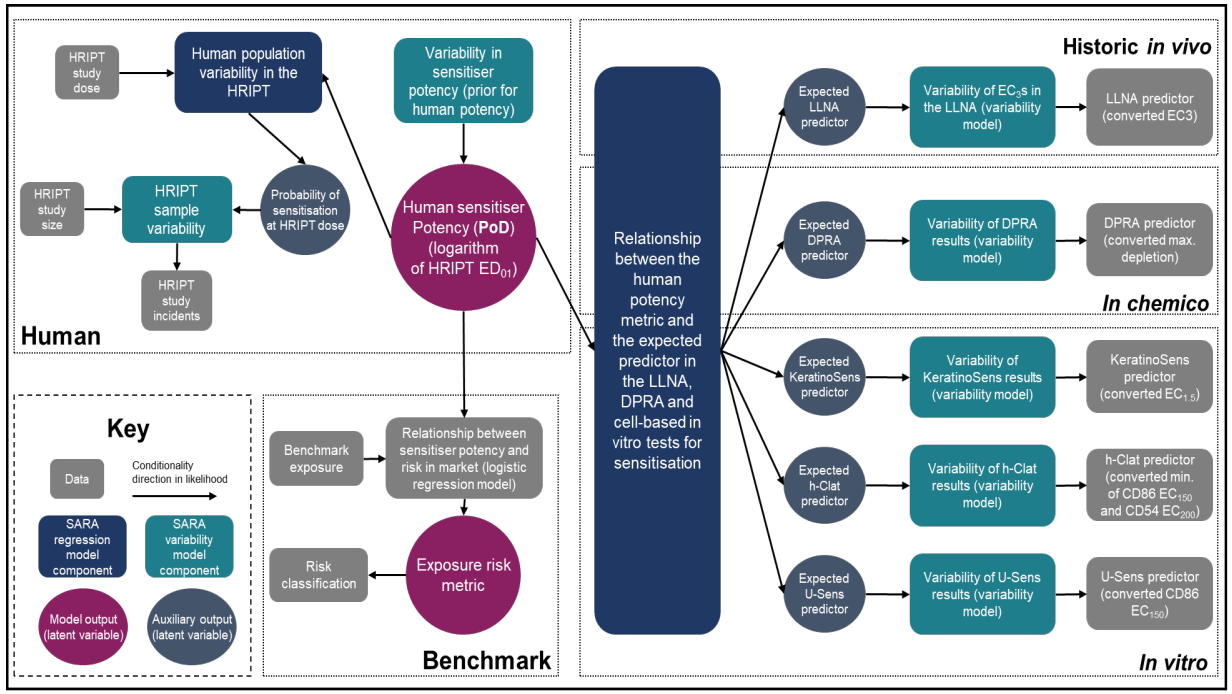
The Skin Allergy Risk Assessment (SARA) Model

Unilever

- Developed by Unilever as a defined approach for skin allergy risk assessment, expanded using data from OECD DASS project and beyond.
- A Bayesian statistical model which infers a human-relevant metric of sensitiser potency (termed ED_{01}), the dose with a 1% chance of human skin sensitisation.
- Accounts for variability of the input data and explicitly quantifies uncertainty.
- Utilises any combination of human repeat insult patch test (HRIPT), LLNA, direct peptide reactivity assay (DPRA), KeratinoSens™, h-CLAT, U-SENS™ data.
- The SARA-ICE Model was designed to be used within an NGRA Framework for decision making.
- On OECD workplan for TG497 evaluation.



SARA Model overview: Reynolds et al 2022

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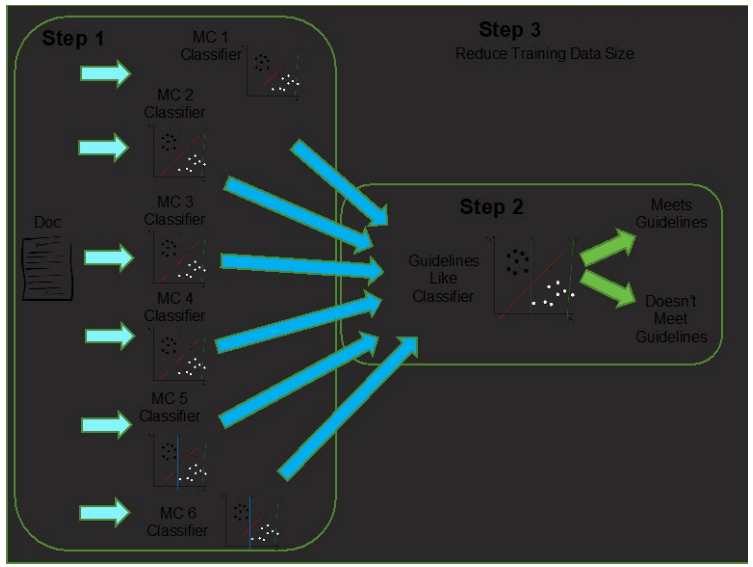
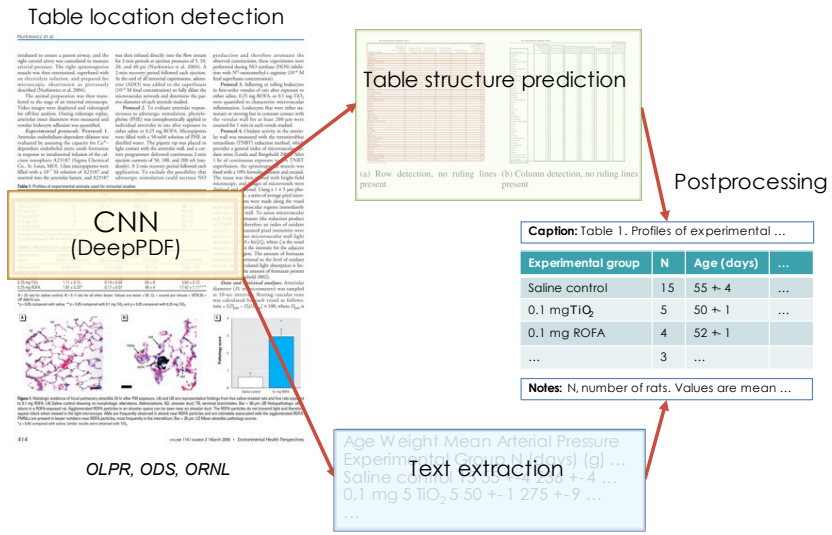
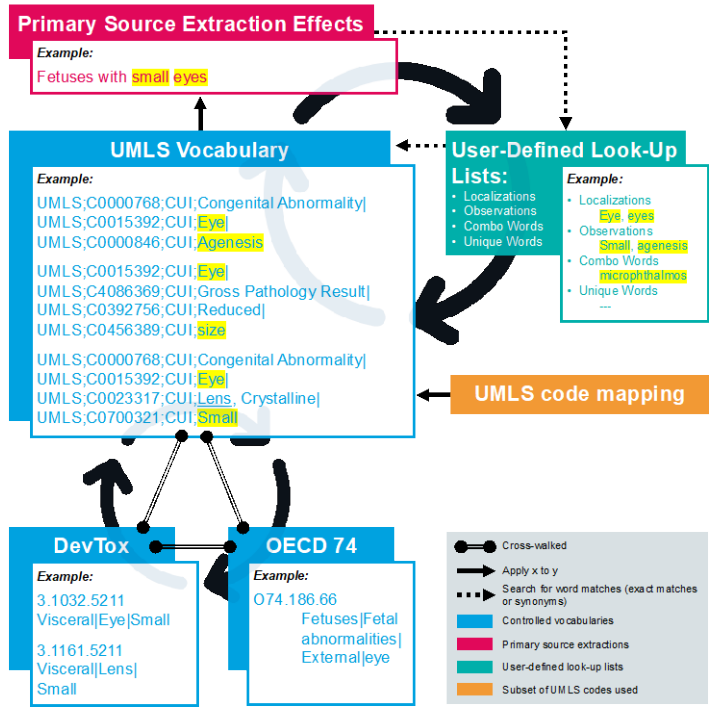
Machine Automating Study Data Curation

Identification → Extraction → Annotation



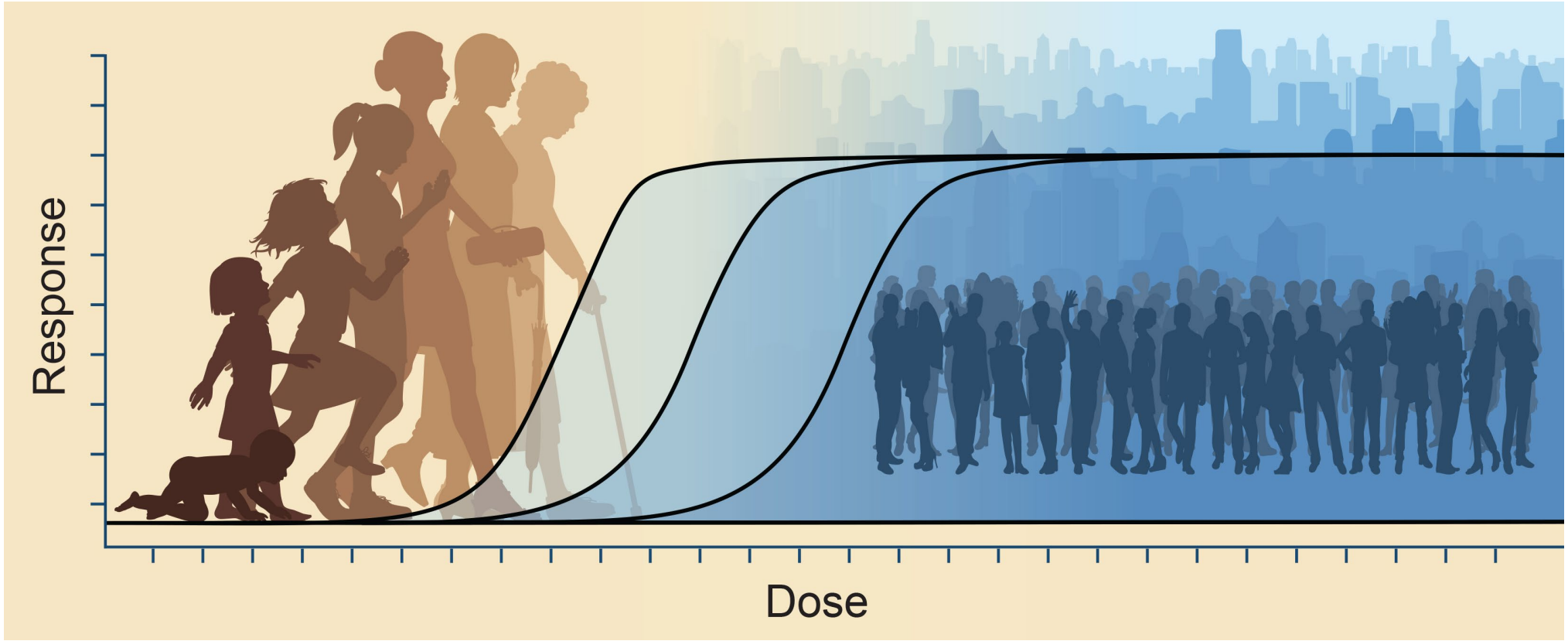




- Important for leveraging high-quality studies in the published literature
- Applications in systematic review of chemical effects
- Establishing reference datasets for validating new methods

Foster et al. 2023 under review

Using New Approach Methodologies to Address Variability and Susceptibility Across Populations



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

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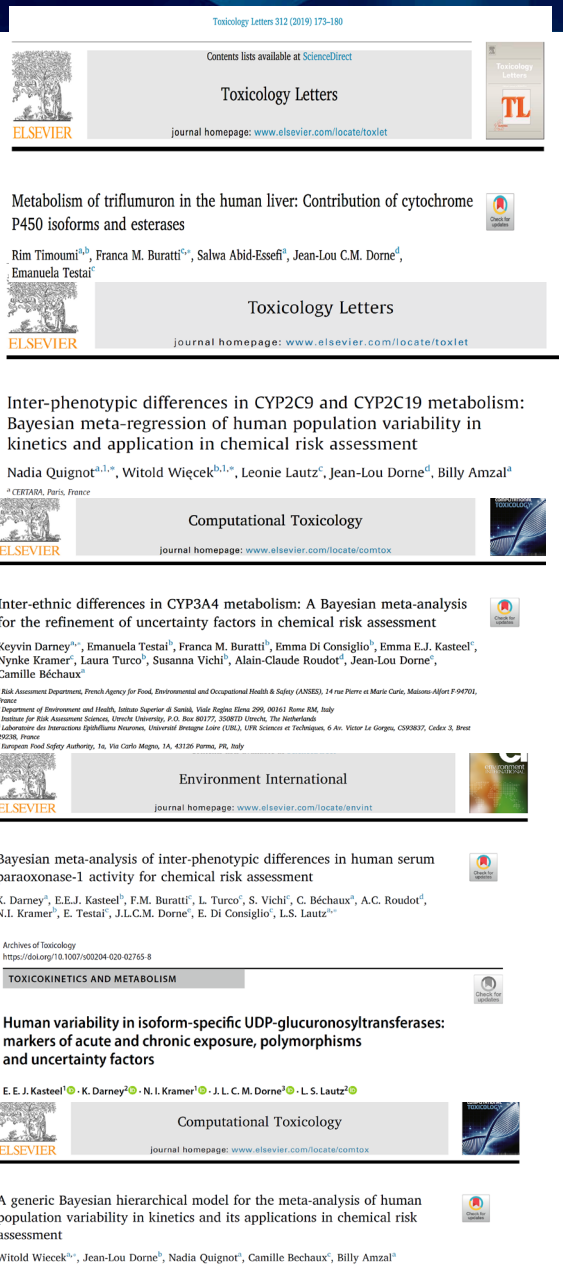
TK to Connect Metabolism and Variability


Toxicology Letters 312 (2019) 173–180

Contents lists available at ScienceDirect

Toxicology Letters

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

Metabolism of triflumuron in the human liver: Contribution of cytochrome P450 isoforms and esterases

Rim Timoumi[a,b], Franca M. Buratti[c,*], Salwa Abid-Essefi[a], Jean-Lou C.M. Dorne[d], Emanuela Testai[c]

Toxicology Letters

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

Inter-phenotypic differences in CYP2C9 and CYP2C19 metabolism: Bayesian meta-regression of human population variability in kinetics and application in chemical risk assessment

Nadia Quignot[a,1,*], Witold Więcek[b,1,*], Leonie Lautz[c], Jean-Lou Dorne[d], Billy Amzal[a]

[a] CERTARA, Paris, France

Computational Toxicology

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

Inter-ethnic differences in CYP3A4 metabolism: A Bayesian meta-analysis for the refinement of uncertainty factors in chemical risk assessment

Keyvin Darney[a,*], Emanuela Testai[b], Franca M. Buratti[b], Emma Di Consiglio[b], Emma E.J. Kasteel[c], Nynke Kramer[c], Laura Turco[b], Susanna Vichi[b], Alain-Claude Roudot[d], Jean-Lou Dorne[e], Camille Béchaux[e]

[a] Risk Assessment Department, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 14 rue Pierre et Marie Curie, Maisons-Alfort F-94701, France
[b] Department of Environment and Health, Istituto Superior di Sanità, Viale Regina Elena 299, 00161 Rome RM, Italy
[c] Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, 3508TD Utrecht, The Netherlands
[d] Laboratoire des Interactions Epithélium Neurones, Université Bretagne Loire (UBL), UFR Sciences et Techniques, 6 Av. Victor Le Gorgeu, CS93837, Cedex 3, Brest 29238, France
[e] European Food Safety Authority, 1a, Via Carlo Magno, 1A, 43126 Parma, PR, Italy

Environment International

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

Bayesian meta-analysis of inter-phenotypic differences in human serum paraoxonase-1 activity for chemical risk assessment

K. Darney[a], E.E.J. Kasteel[b], F.M. Buratti[c], L. Turco[c], S. Vichi[c], C. Béchaux[a], A.C. Roudot[d], N.I. Kramer[b], E. Testai[c], J.L.C.M. Dorne[e], E. Di Consiglio[c], L.S. Lautz[a,*]

Archives of Toxicology
https://doi.org/10.1007/s00204-020-02765-8

TOXICOKINETICS AND METABOLISM

Human variability in isoform-specific UDP-glucuronosyltransferases: markers of acute and chronic exposure, polymorphisms and uncertainty factors

E. E. J. Kasteel[1] · K. Darney[2] · N. I. Kramer[1] · J. L. C. M. Dorne[3] · L. S. Lautz[2]

Computational Toxicology

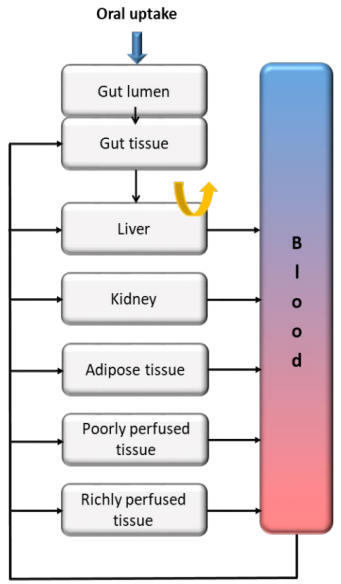
journal homepage: www.elsevier.com/locate/comtox

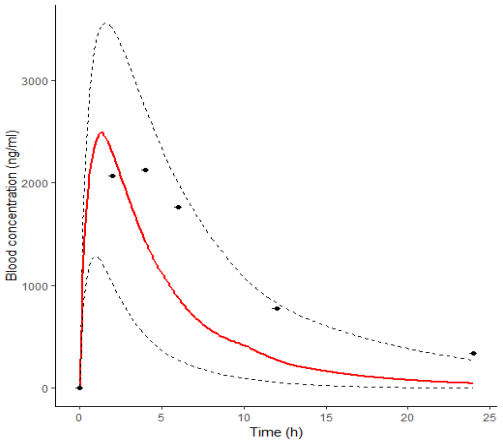
A generic Bayesian hierarchical model for the meta-analysis of human population variability in kinetics and its applications in chemical risk assessment

Witold Wiecek[b,*], Jean-Lou Dorne[b], Nadia Quignot[a], Camille Bechaux[c], Billy Amzal[a]

Covering Phase I CYP450 and Phase II UGTs enzymes

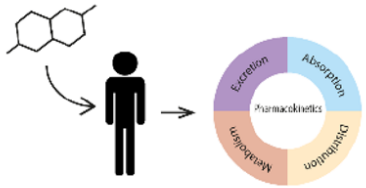
PBPK models + virtual population





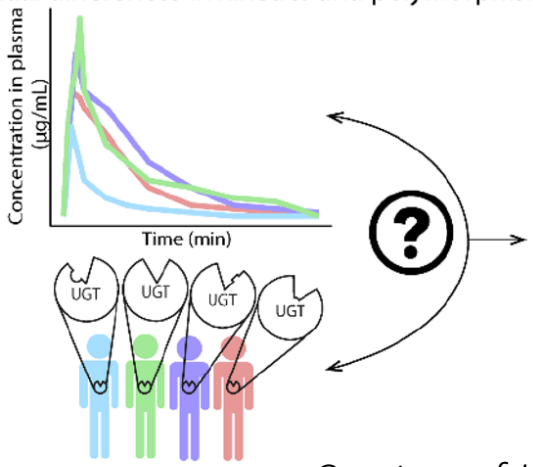
https://ice.ntp.niehs.nih.gov/

Pharmacokinetic data on compounds metabolised by UGT isoforms is collected and summarised in a database



Data on polymorphism frequencies in different populations is collected and summarised

Interindividual differences in kinetics and polymorphisms



UGT-related uncertainty factors

Courtesy of Jean-Lou Dorne

European Food Safety Authority

National Institute of Environmental Health Sciences
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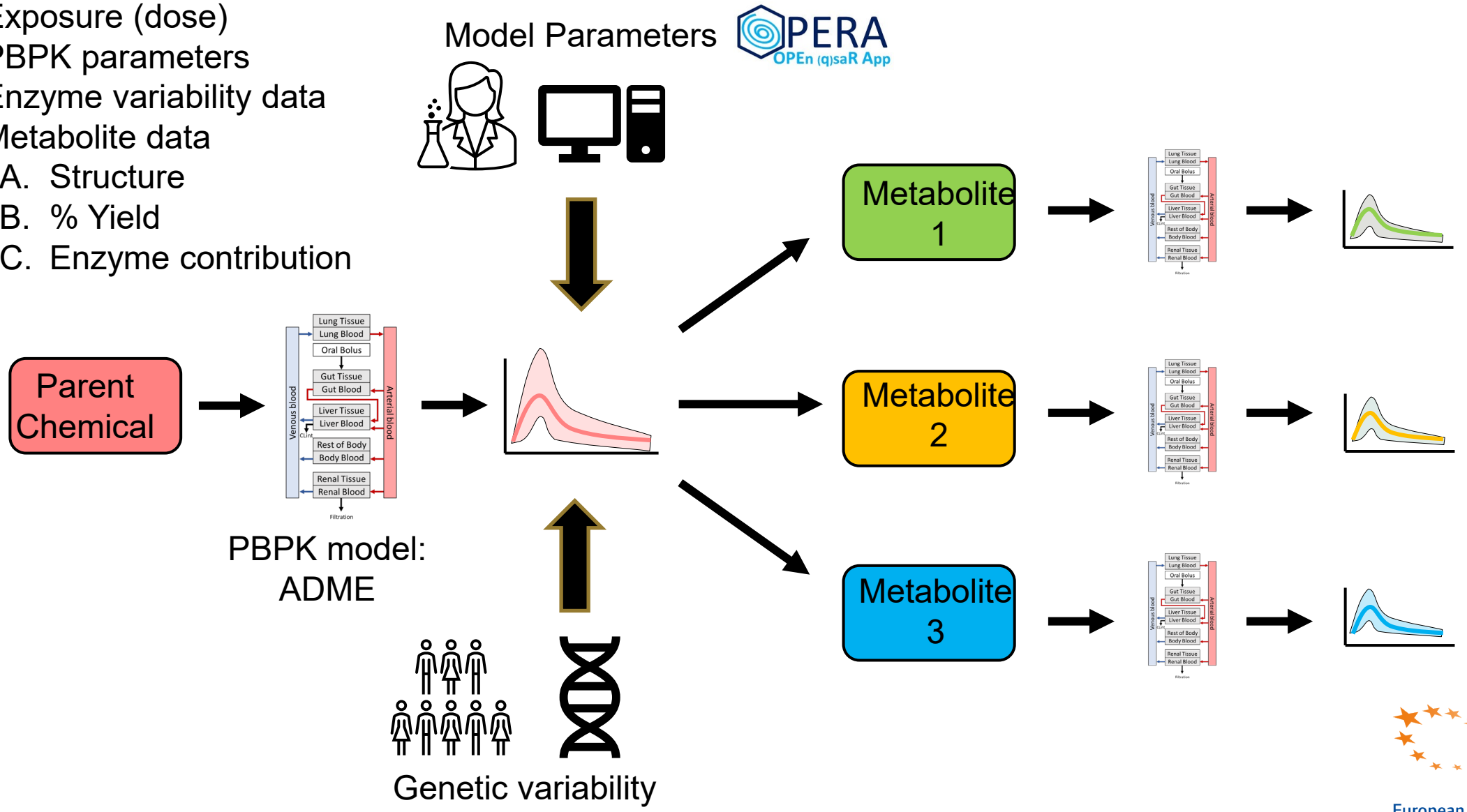
Incorporating Population Variability in Metabolism

Inputs needed:

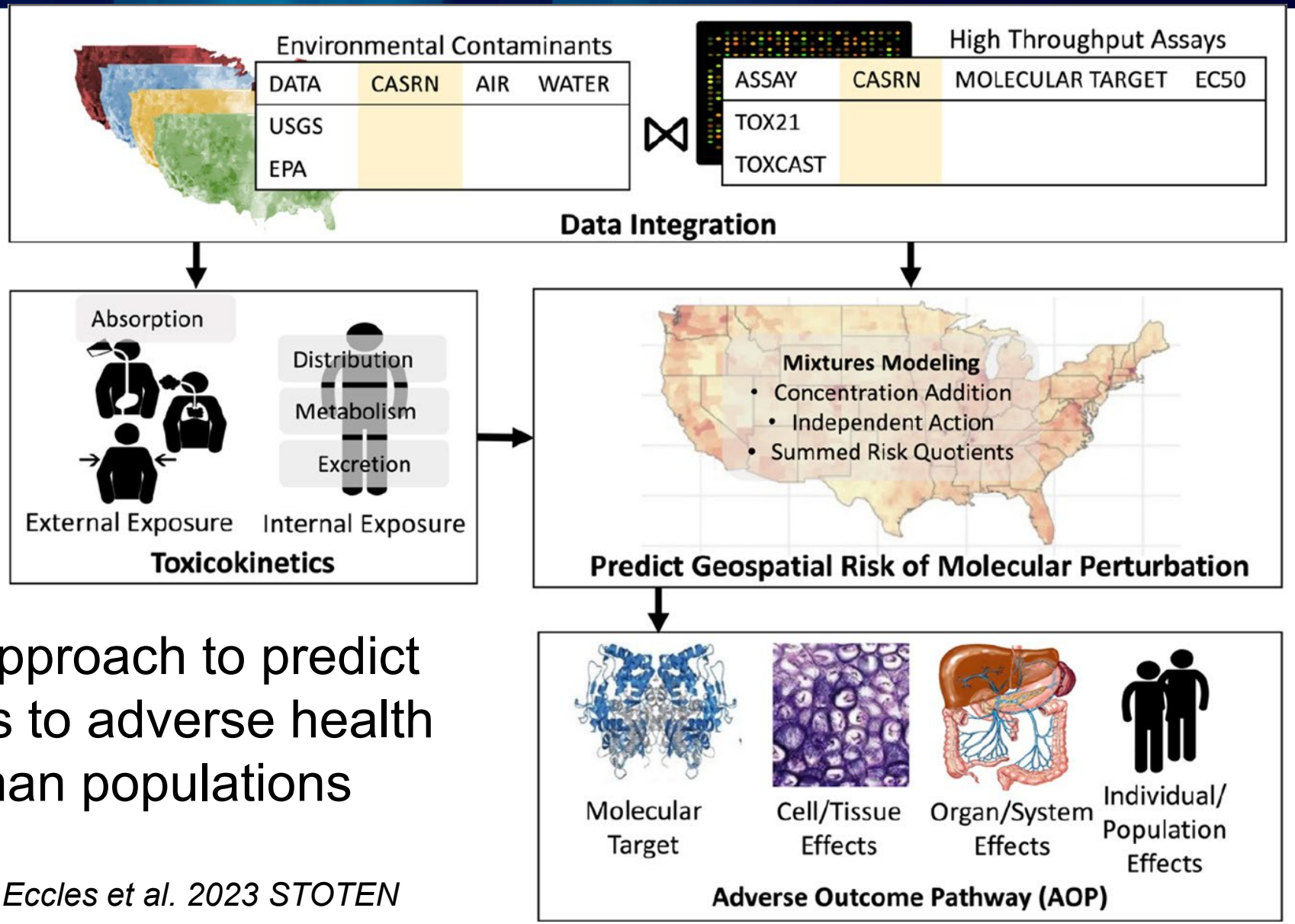
1. Exposure (dose)
2. PBPK parameters
3. Enzyme variability data
4. Metabolite data
   A. Structure
   B. % Yield
   C. Enzyme contribution



efsa
European Food Safety Authority

Geospatial Modeling Approach

Linking in vitro HTS data and exposure information to predict geospatial risk of molecular perturbation.



Eventual goal: use approach to predict chemical contributions to adverse health outcomes across human populations

Eccles et al. 2023 STOTEN

National Institute of Environmental Health Sciences
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Inter-laboratory Pre-validation Study of Human Thyroid Microtissue Assay

OXFORD SOT Society of Toxicology
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Research Article

Development of an *In Vitro* Human Thyroid Microtissue Model for Chemical Screening

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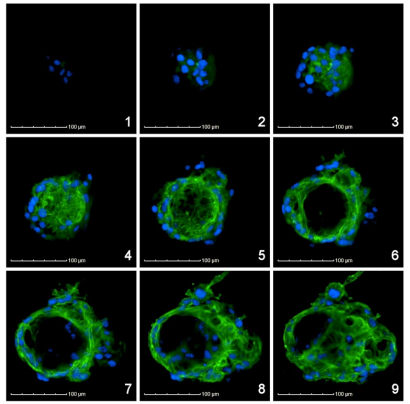

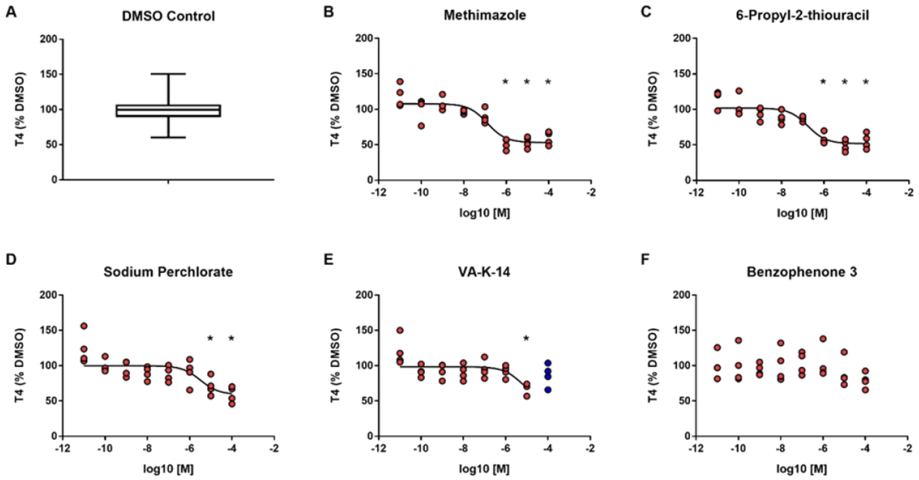

Team Members

Coordinator: NICEATM

Method Developer Lab 1

Lab 2 — LifeNet Health® Saving Lives. Restoring Health. Giving Hope.

Lab 3 — CORTEVA™ agriscience

Lab 4 — BAYER

Status:

- Materials Cooperative Research and Development Agreement (MCRADA) in place
- Validation Management Team (VMT) recruited
- SOPs reviewed by VMT
- Transferability to lab 2 to start shortly

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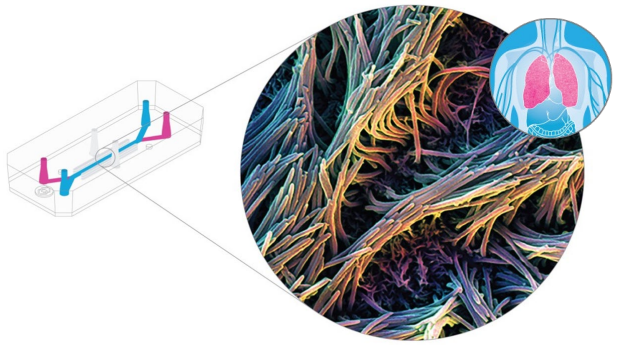
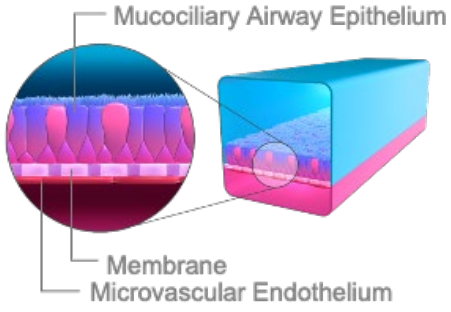
MPSCoRe: Microphysiological Systems for COVID-19 Research

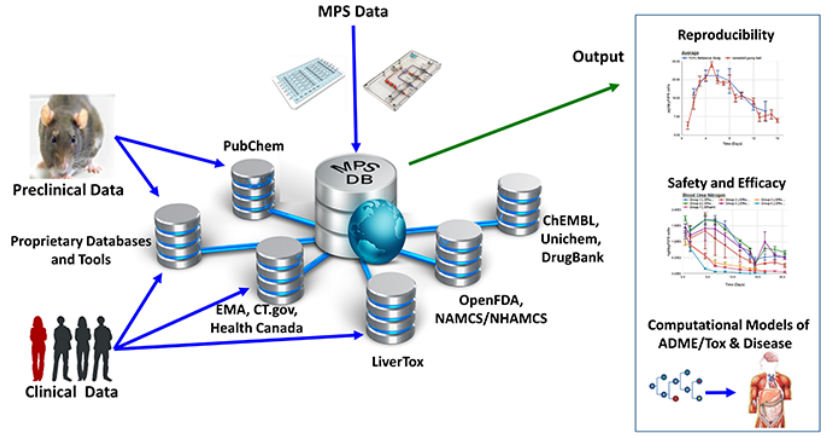


Joint working group to support global COVID-19 tissue chip research activities
Partnership with NC3Rs, DoD, NIAID, NCATS, others.

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

Kleinstreuer & Holmes (2021) Drug Discovery Today





- Understand pathophysiology and disease mechanisms
- Test novel therapeutics in tissue chip & MPS models and compare with pre/clinical data

https://mps.csb.pitt.edu/

Advancing Research and Regulatory Acceptance of Microphysiological Systems for Infectious Disease Applications
May 31 – June 1, 2023

https://ntp.niehs.nih.gov/go/mps-2023wksp

NC 3Rs National Centre for the Replacement Refinement & Reductio of Animals in Research

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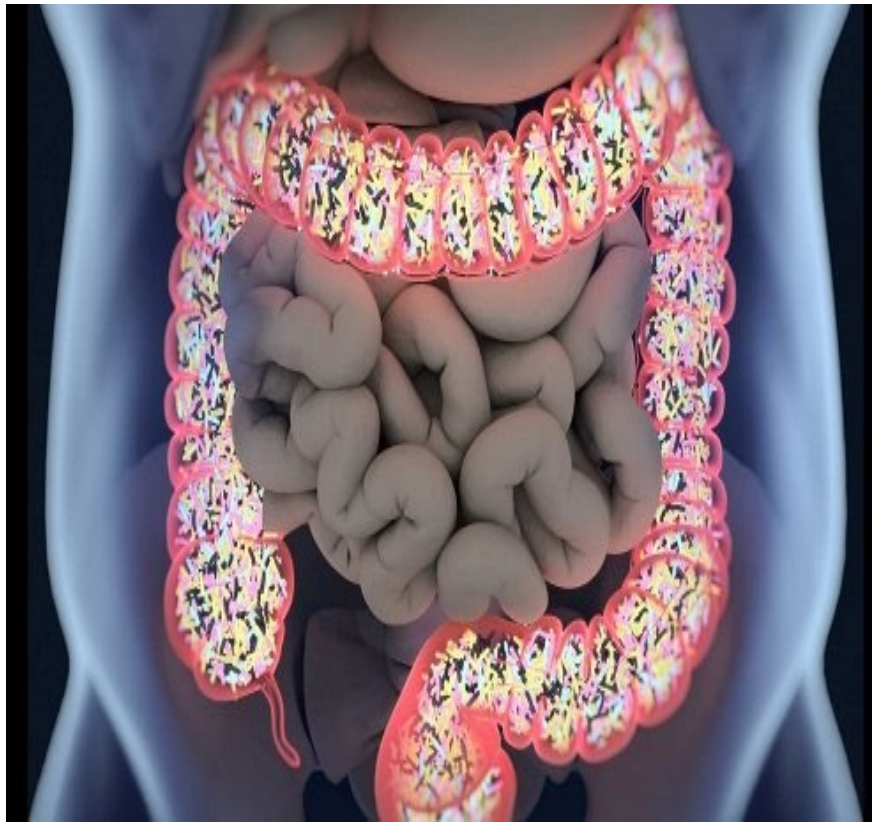
Workshop on NAMs for the Gastrointestinal Tract
October 11-12, 2023

An in-person workshop to examine the state of the science for NAMs modeling the gastrointestinal tract and their context for regulatory consideration.

- **Focal Areas:**
  - General “state of the science” for NAM gut models
  - Models for de-risking chemicals for systemic toxicity (regulatory relevance and application)
  - Gastrointestinal toxicity
  - Systemic absorption and distribution
  - Gut allergenicity
- **A webinar series to provide background information is planned for September (dates TBD)**
- **In-person Day 1:** Scientific talks/state of the science
- **In-person Day 2:** Breakout groups covering the following themes:
  - Establishing confidence in existing models
  - Strengths and limitations of different model systems



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Acknowledgments

The NICEATM Group

NIEHS/DTT Contributors

UNITED STATES ICCVAM
Advancing Alternatives to Animal Testing

https://ntp.niehs.nih.gov/go/2021iccvamreport

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