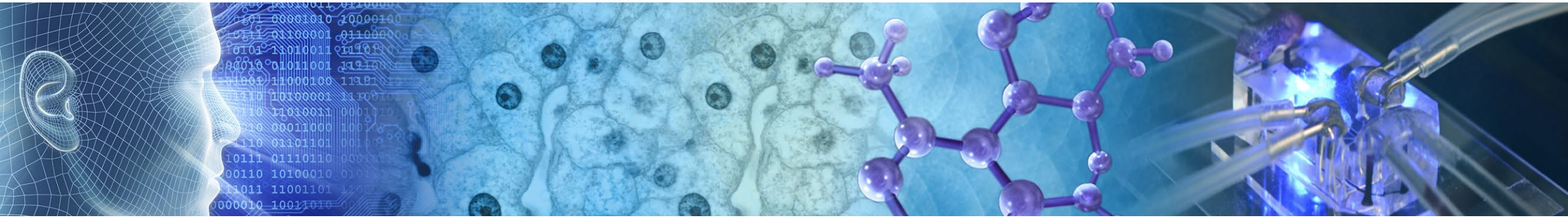
NTP
National Toxicology Program



NICEATM Update

ICCVAM Public Forum
27 May 2022

Nicole Kleinstreuer
Acting NICEATM Director

NICEATM and ICCVAM

- National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (**NICEATM**), supporting the Interagency Coordinating Committee for the Validation of Alternative Methods (**ICCVAM**)
- ICCVAM Authorization Act of 2000: To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new and revised toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing (**3Rs**) animal tests and ensuring human safety and product effectiveness.

7 Regulatory Agencies

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

10 Research Agencies

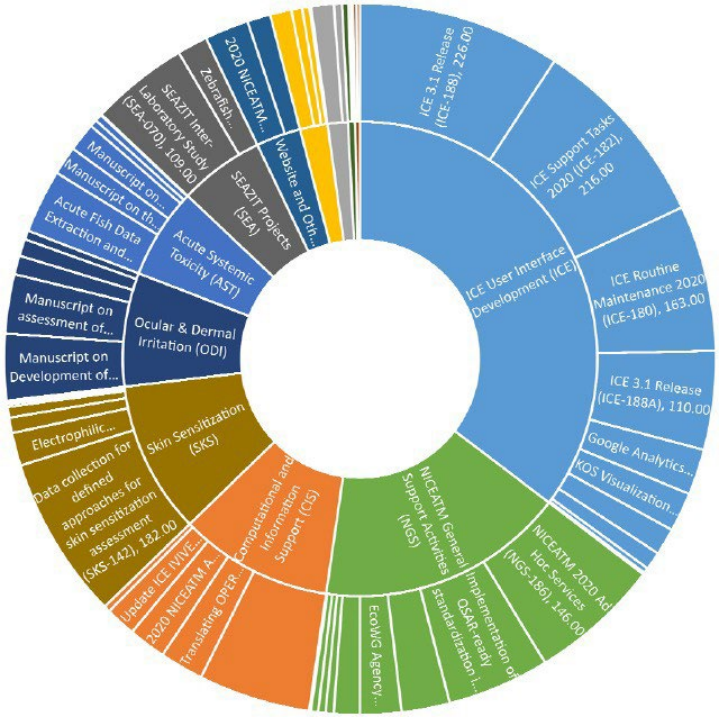
Agency for Toxic Substances and Disease Registry
National Institute for Occupational Safety and Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
National Institutes of Health
Department of Defense
Department of Energy
National Institute of Standards and Technology
Veterans Affairs Office of Research and Development

*Other participants include: NCATS, Tox21 Representatives

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

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



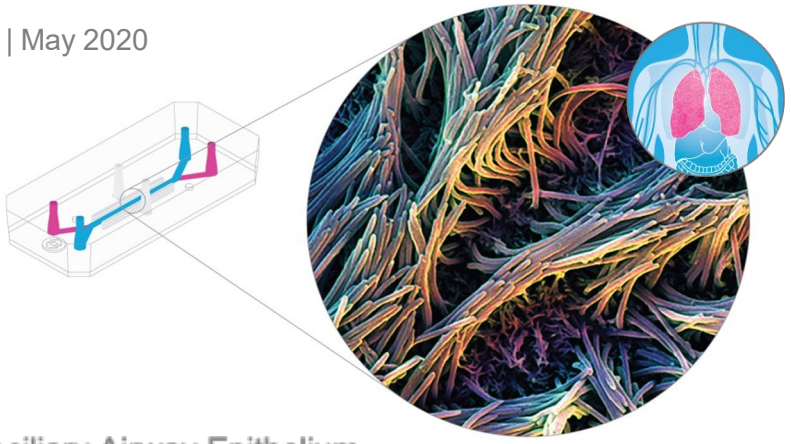
- Summarizes US agency activities to promote alternatives or reduce animal use
- 2018-2019 report published in July 2020, available online at: https://ntp.niehs.nih.gov/go/2019iccvamreport

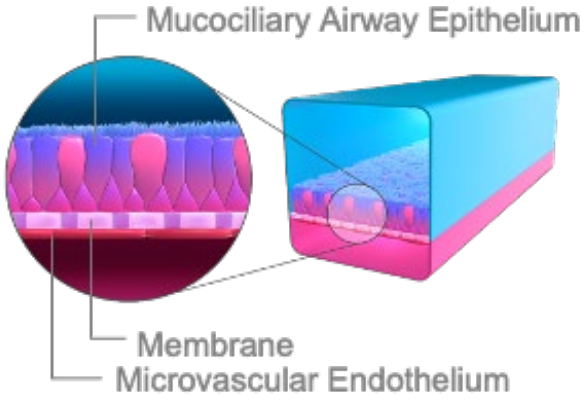
Report for 2020-2021 will be out soon!

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

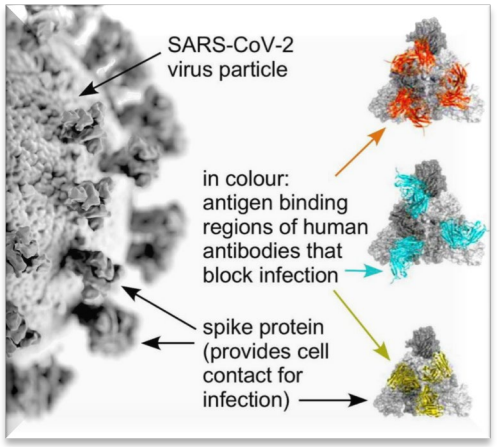
MPSCoRe: Microphysiological Systems for COVID-19 Research

Emulate, Inc. | May 2020





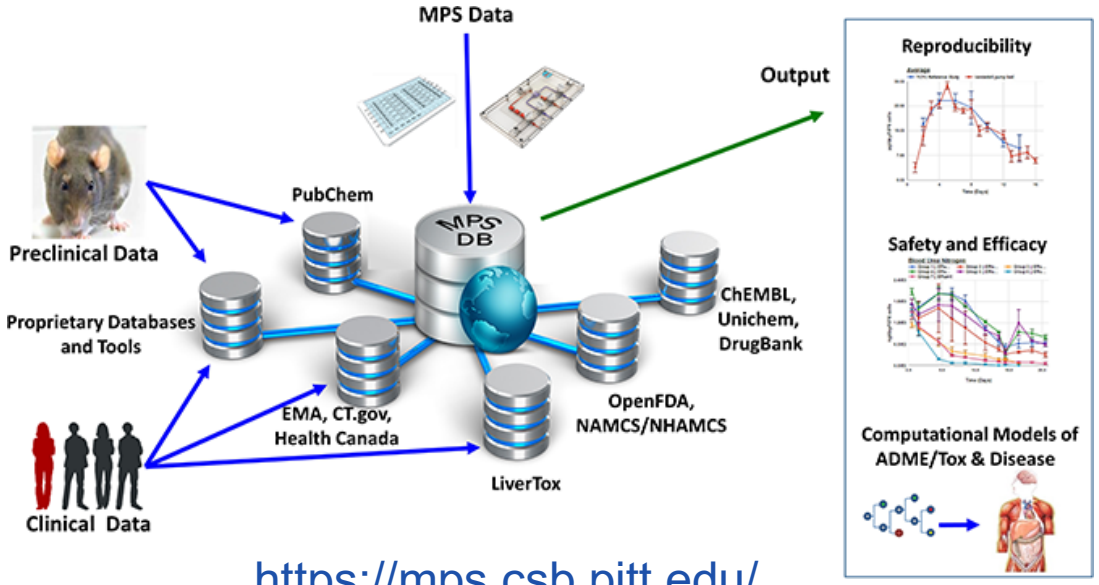
Test novel therapeutics in tissue chip & MPS models and compare with pre/clinical data



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



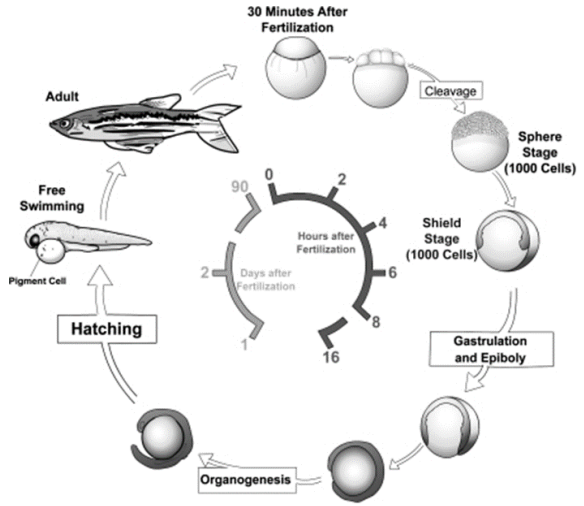
https://mps.csb.pitt.edu/

Donnelly Centre for Cellular • Biomolecular Research UNIVERSITY OF TORONTO | UNIVERSITY OF TORONTO | YUMAB | CORAT | NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

SEAZIT: Systematic Evaluation of the Application of Zebrafish in Toxicology

SEAZIT Activity:

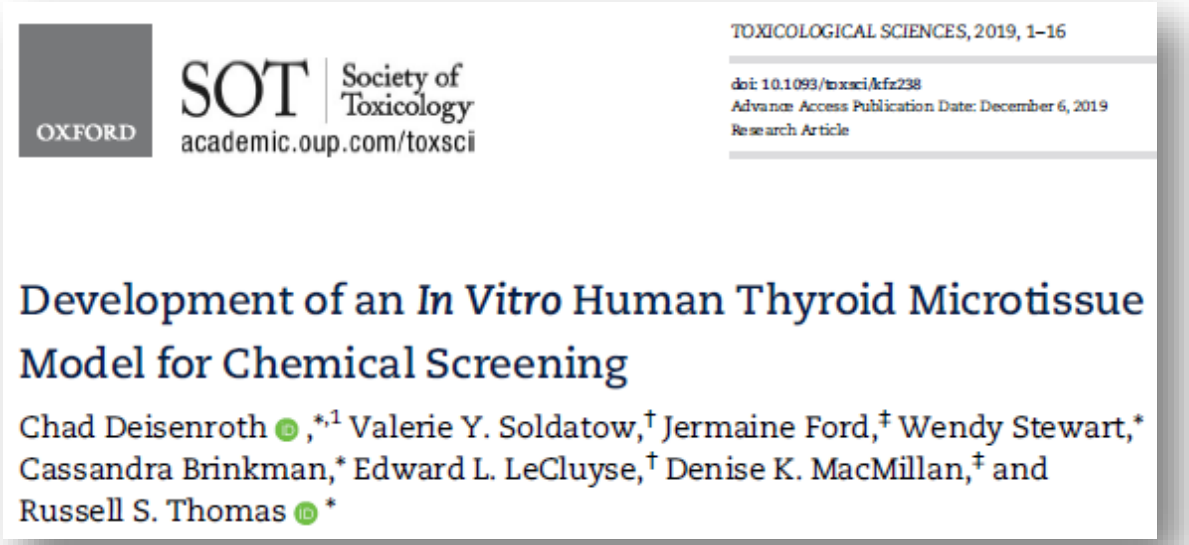
- Interlaboratory study to compare the influence of exposure conditions on study outcome
  - Chorion on/off and static versus repeat exposure
  - Dose-range finder is complete
  - Definitive study in-progress

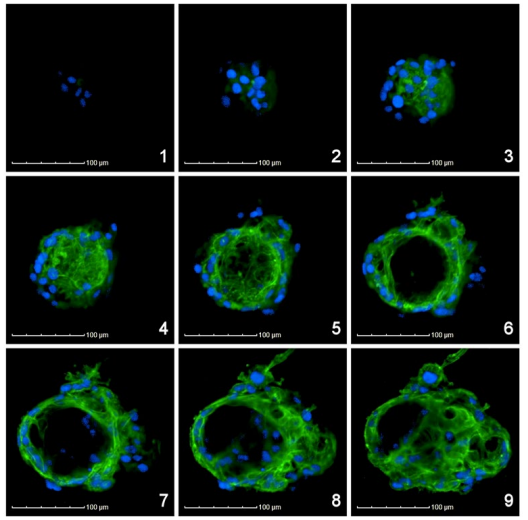


- Completed Manuscript:
  - Implementation of Zebrafish Ontologies for Toxicology Screening published: *Frontiers in Toxicology, March 2022,* https://doi.org/10.3389/ftox.2022.817999

- Manuscripts in preparation:
  - Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT): Inter-Laboratory Study Design
  - Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) Database in National Toxicology Program (NTP)

Establishing an In Vitro Human Thyroid Microtissue Model for Chemical Screening





Objective: Form Validation Management Team to help support interlaboratory study for the transferability and reproducibility of a thyroid microtissue model developed by EPA CCTE (Deisentroth *et al.* 2019) including the overall study design, analytical approaches, and data interpretation to the optimize the human thyroid microtissue model system.

1. Develop a minimum characterization strategy and acceptance criteria for qualifying suitable lots of primary human cells.
2. Determine optimal culture conditions, medium formulation, time points, endpoints, and analytical methods for developing a final chemical testing protocol.
3. Establish a suitable reference compound set representing diverse mechanisms-of-action and their optimal dosing ranges, exposure frequency, duration and endpoint analysis for the assay.

Private-Public Partnerships: Prospective Testing

Agchems and Eye Irritation

syngenta

CORTEVA agriscience

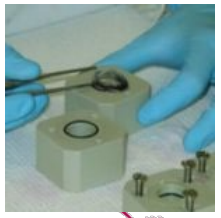
BASF We create chemistry

FMC

BAYER

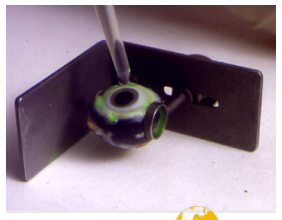
N=16

BCOP



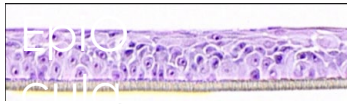
IIVS Institute for In Vitro Sciences

ICE



CiToxLAB

EpiOcular

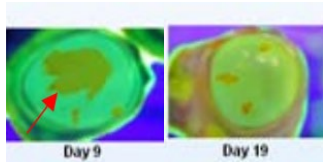


MatTek CORPORATION

NRR

IIVS Institute for In Vitro Sciences

PorCORA



MB Research Labs

Agchems, AMCPs and Skin Irritation

N=65

CLOROX

ECOLAB

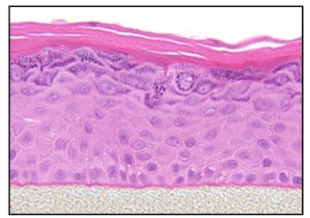
scJohnson

P&G

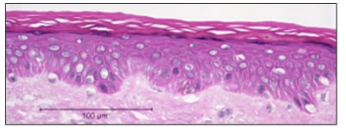
COLGATE-PALMOLIVE

CHURCH & DWIGHT CO., INC.

IIVS Institute for In Vitro Sciences



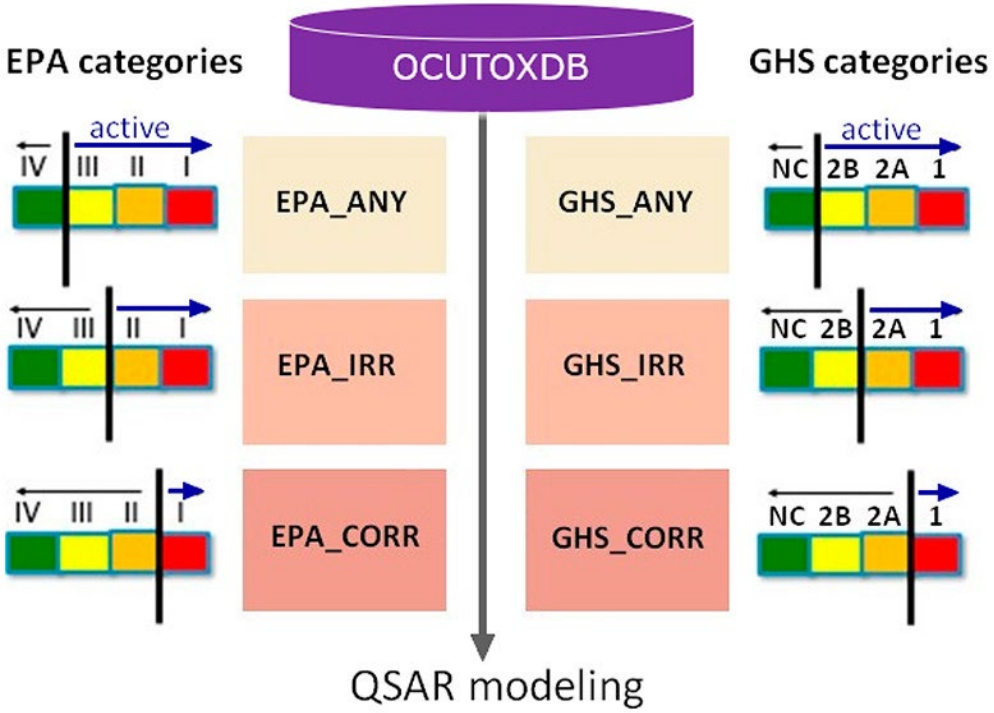
MatTek Corp. EpiDerm™ (EPI-200)



Native human skin

Addressing Mixtures Computationally

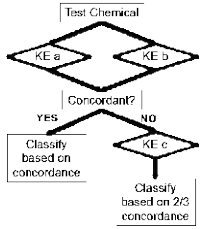
Mixtures-Inclusive Ocular QSAR Models

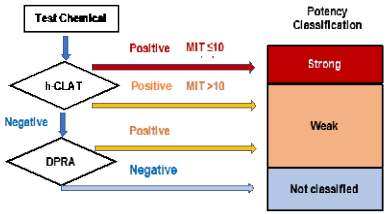


- Set of *in silico* models to predict EPA and GHS hazard classifications for chemicals and mixtures
- Accuracies in the range of 72 – 94% depending on approach and purity threshold.

Sedykh et al. Chem Res Tox 2022

Application of Defined Approaches for Skin Sensitization to Agrochemical Products



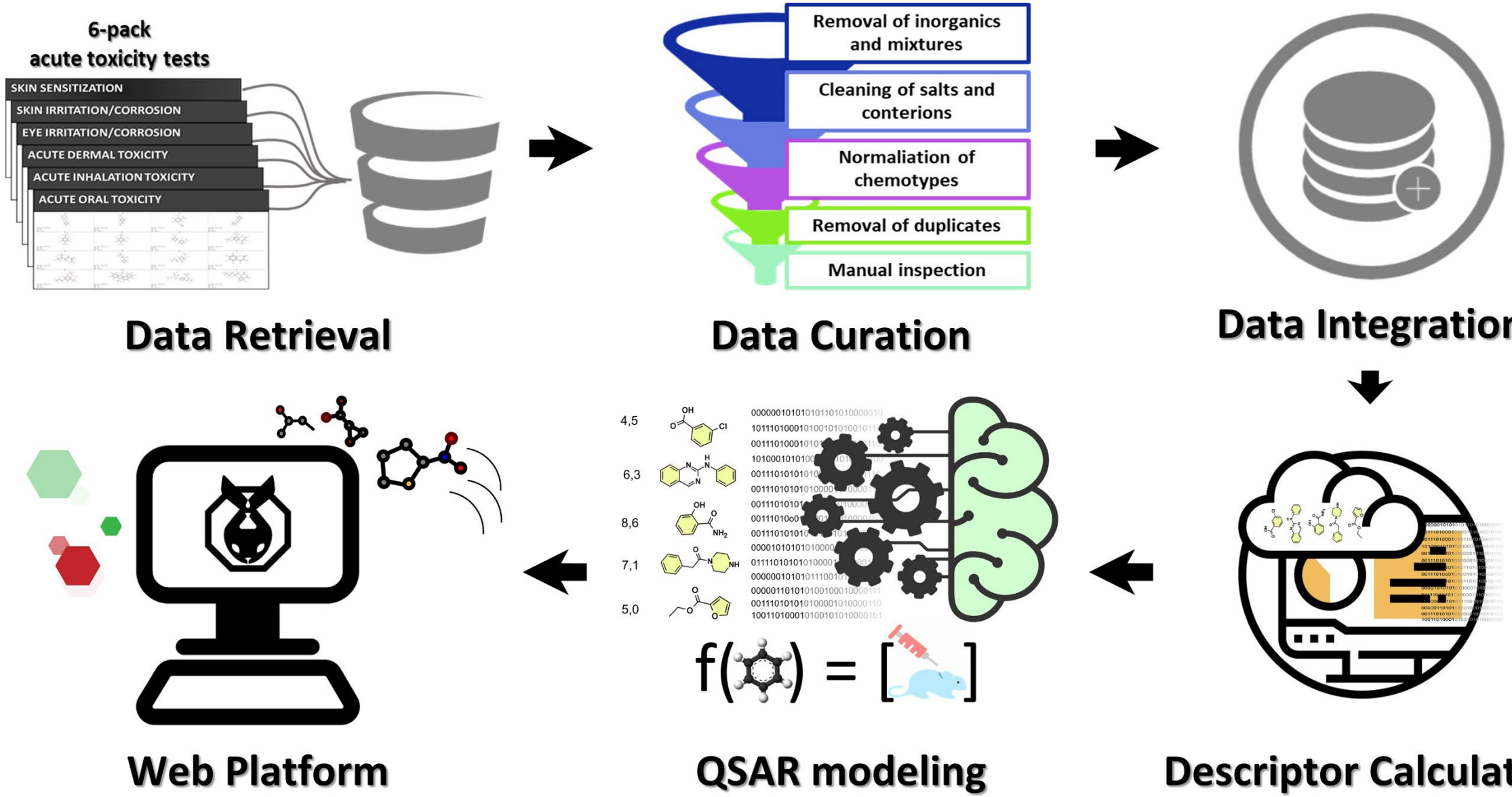


Score	h-CLAT MIT (µg/ml)	DPRA Mean Cysteine and Lysine Depletion (%)	DPRA Cysteine Depletion (%)	QSAR Toolbox Hazard Prediction
3	≤10	≥42.47	≥98.24	-
2	>10, ≤150	≥22.62, <42.47	≥23.09, <98.24	-
1	>150, ≤5000	≥6.38, <22.62	≥13.89, <23.09	Positive
0	Negative	<6.38	<13.89	Negative

- Tested 27 agrochemical formulations in the DPRA, the KeratinoSens™ assay, and the h-CLAT
- Data used as inputs to evaluate three DAs for hazard classification of skin sensitization potential and two DAs for potency categorization

Strickland et al. Front Toxicol 2022

STopTox: In Silico 6-Pack Alternative



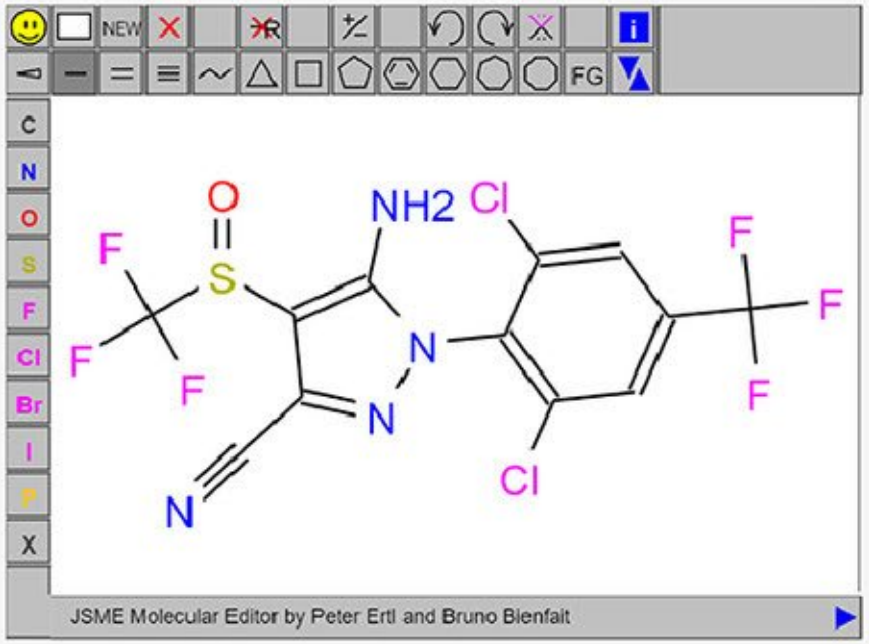
Borba et al. EHP 2022

BeeTox AI Web App

BeeTox

an artificial intelligence web app to assess acute toxicity of chemicals in honey bees

Draw molecule or load file



SUBMIT ANALYSIS

Results

Prediction

Toxic (+)

Confidence

83%

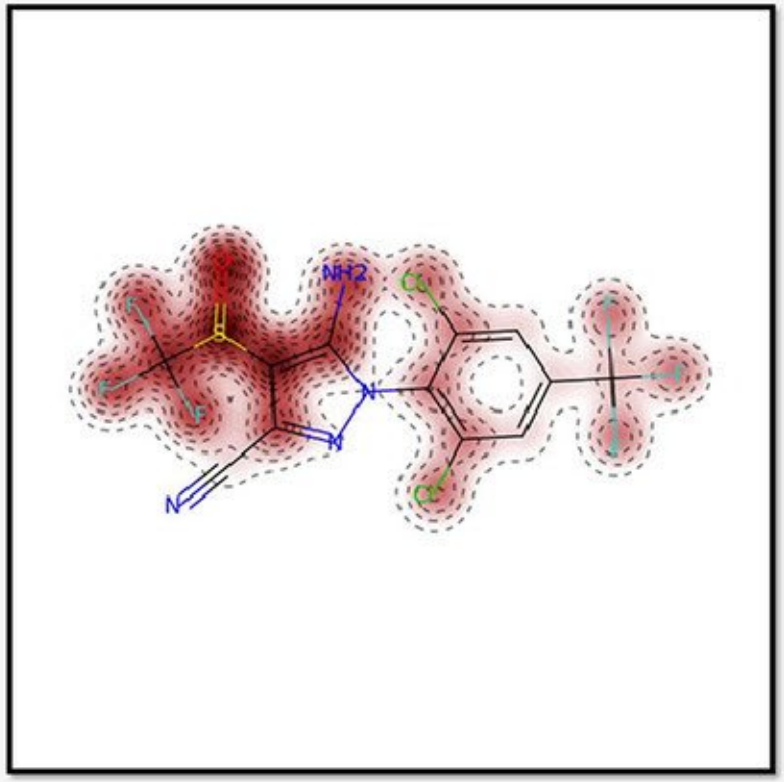
Predicted pLD_{50}

5.12

Aplicability domain

Inside

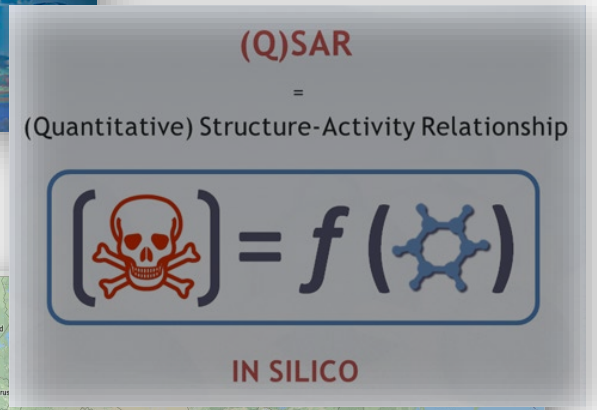
Contribution map

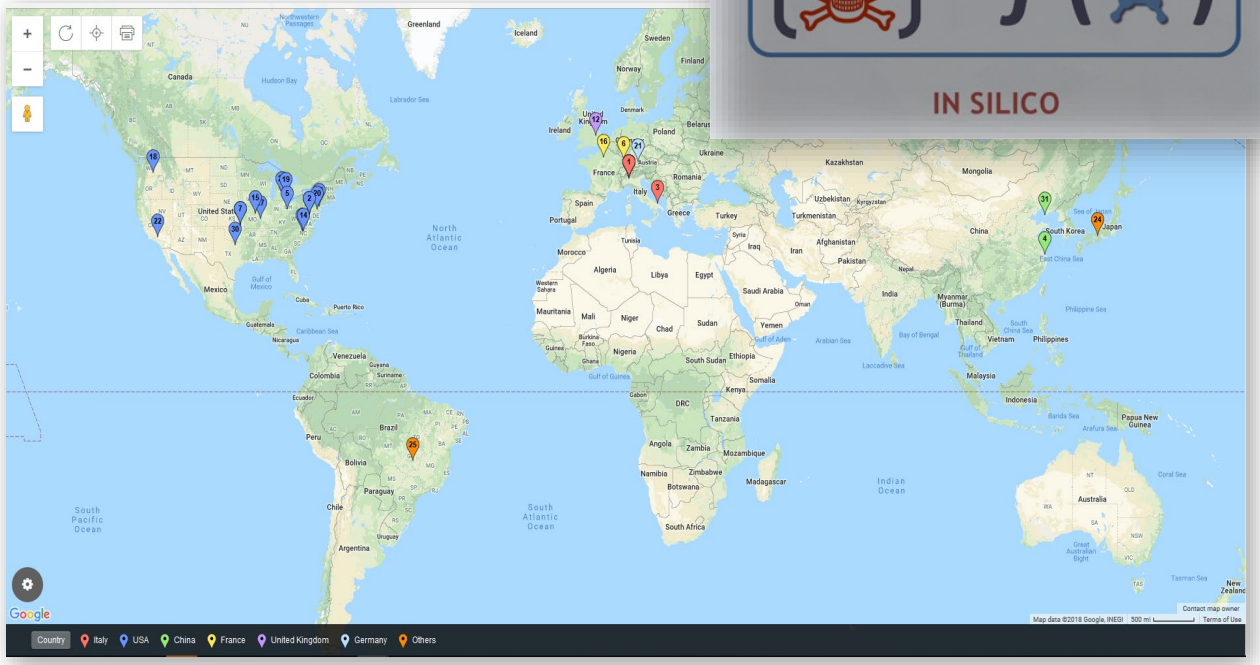


Moreira-Filho et al. AI in Life Sciences, 2021

http://beetoxai.labmol.com.br/

Global Crowdsourcing Predictive Models





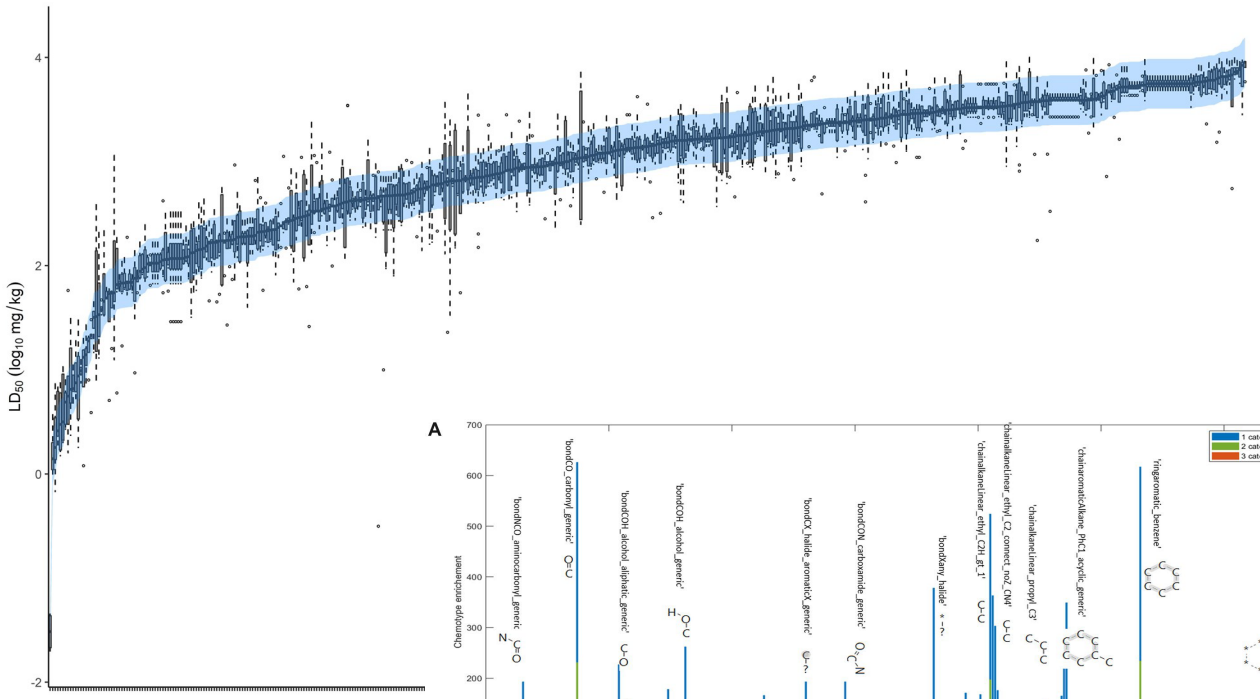
- 35 Groups: academia, industry, govt
- Curate reference data to train & test models: >10k chemicals
- Use molecular structure and chemical properties to predict toxicity
- Combine best models together into “ensemble” approaches
- Create open access AI/ML modeling suite

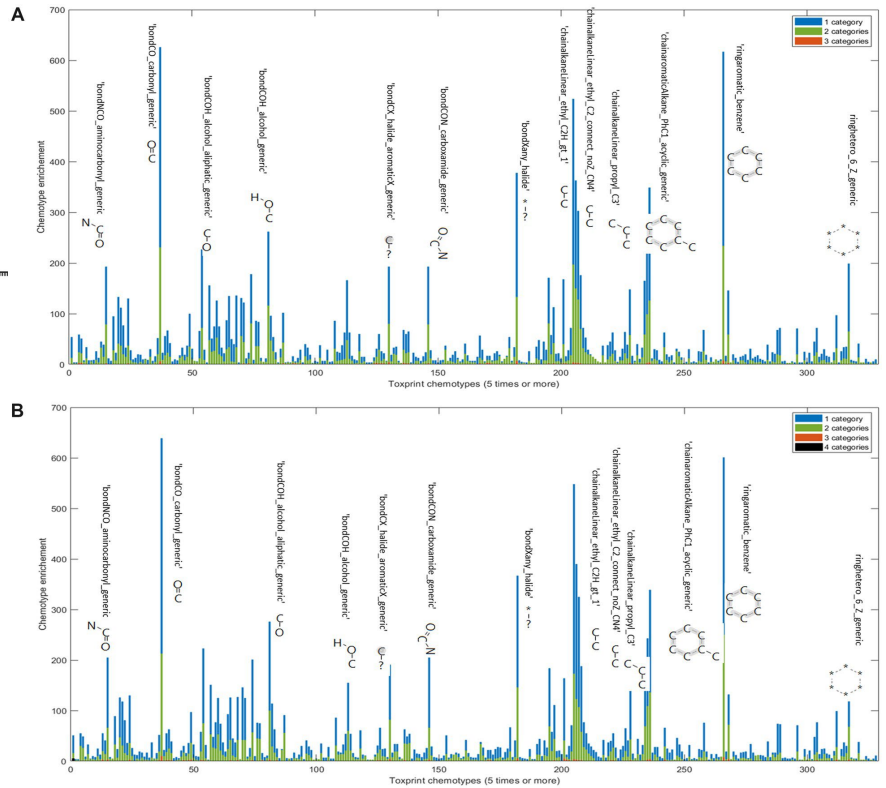
OPERA
OPEn (q)saR App

https://github.com/NIEHS/OPERA

Mansouri et al. J Cheminform (2018); Kleinstreuer et al. Comp Tox (2018); Mansouri et al. Env Health Persp (2020, 2022); Chushak et al. 2021

Characterizing Variability and Applying to NAM 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	

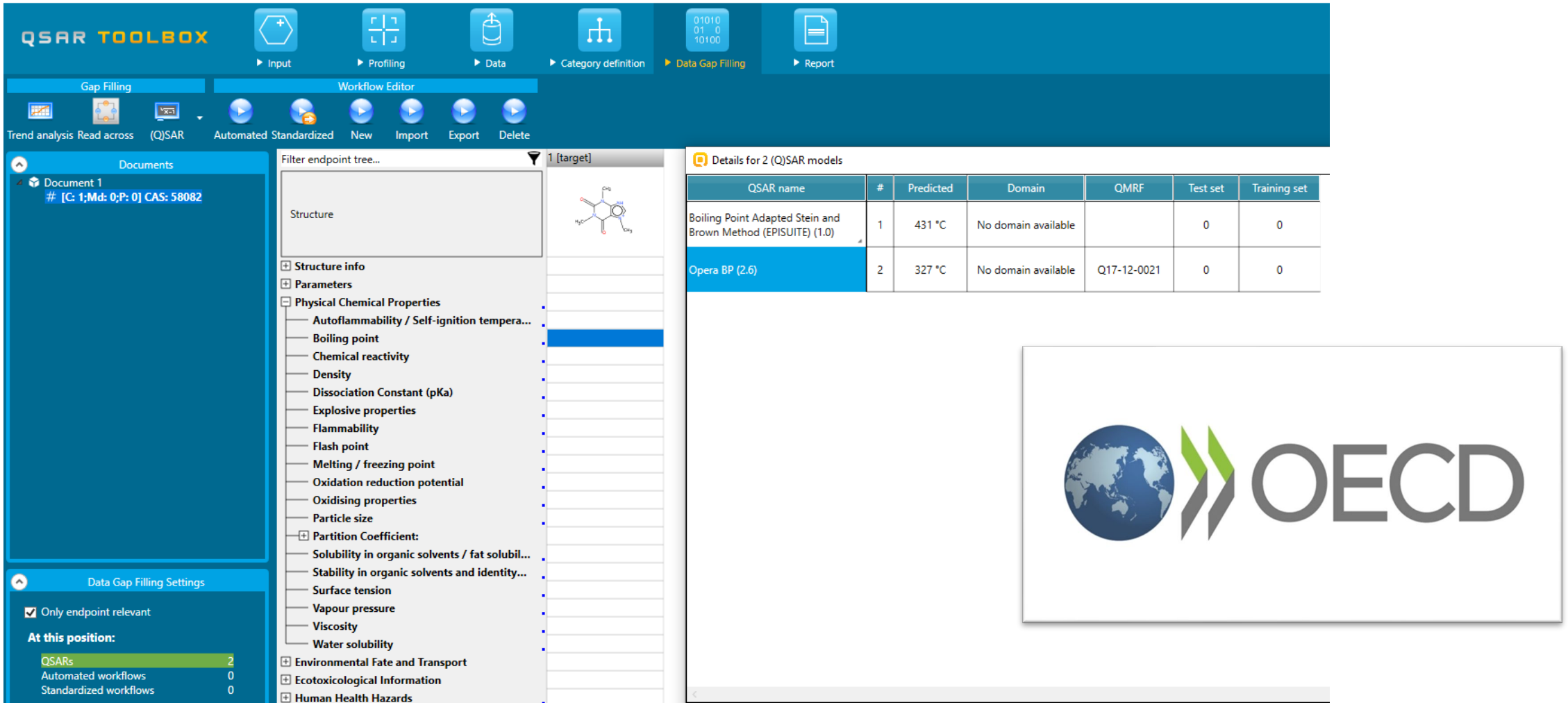
	LD50 values		LD50 values
	Train	Eval	***In Vivo***
R2	0.85	0.65	0.80
RMSE	0.30	0.49	0.42

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

OPERA-TB: An OECD QSAR Toolbox Plug-in

Available soon



ICEv3.6 https://ice.ntp.niehs.nih.gov/

- Democratizing access to data
  - Organized by toxicity endpoints and mechanisms
  - Standardized terminology, units, and formatting
- Curated information
  - Reference chemical lists with classifications and bioactivity
  - In vitro assays annotated with defined terminology
- Computational models
  - Internal and external exposure predictions
  - Chemical characterization, and toxicity predictions

Bell et al. 2017 EHP; Bell et al. 2020 Tox In Vitro; Abedini et al. 2021 Comp Tox

Data in ICE

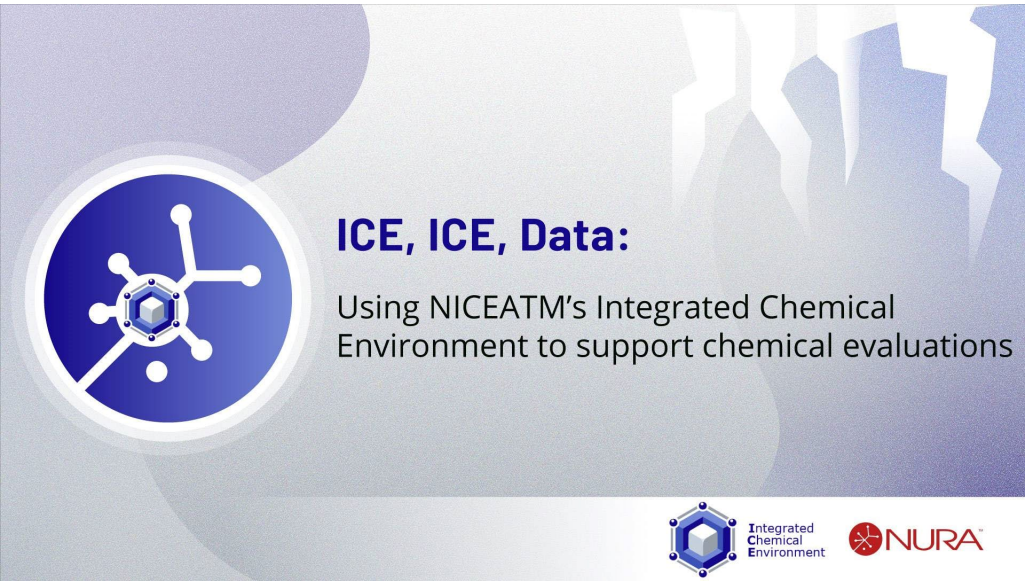
Toxicity endpoint	Assays	# of chemicals
Chemical Parameters	Physchem, ADME, and toxicity endpoints	~10000
Acute Toxicity	In vivo acute oral, dermal, and inhalation toxicity	~10000
Cancer	In vivo and in vitro Cancer, and Weight of Evidence	3042
DART	In vivo and in vitro DART	607
Skin Sensitization	In vivo and in vitro skin sensitization	2181
Skin Irritation	In vivo and in vitro skin irritation/corrosion	1664
Eye Irritation	In vivo and in vitro eye irritation/corrosion	796
Endocrine	In vivo and low throughput in vitro data on AR and ER agonist and antagonist activity	281
cHTS	Curated ToxCast and Tox21 assays	9213

In Silico Models/Integrated Approaches

Endpoint	Model	# of chemicals*
Physicochemical Properties	OPEn (q)saR App (OPERA) *Mansouri et al. J Cheminform 2018*	800,000+
Acute Oral Toxicity	Collaborative Acute Toxicity Modeling Suite (CATMoS) - Rat acute oral toxicity. *Mansouri et al. EHP 2021*	800,000+
Endocrine	Estrogen Receptor pathway Model. *Judson et al. Tox Sci 2015*	1812
	Androgen Receptor Pathway Model. *Kleinstreuer et al. Chem Res Tox 2017*	1855
	Collaborative Estrogen Receptor Activity Prediction Project (CERAPP). *Mansouri et al. EHP 2016*	800,000+
	Collaborative Modeling Project for Androgen Receptor Activity (COMAPRA). *Mansouri et al. EHP 2020*	800,000+

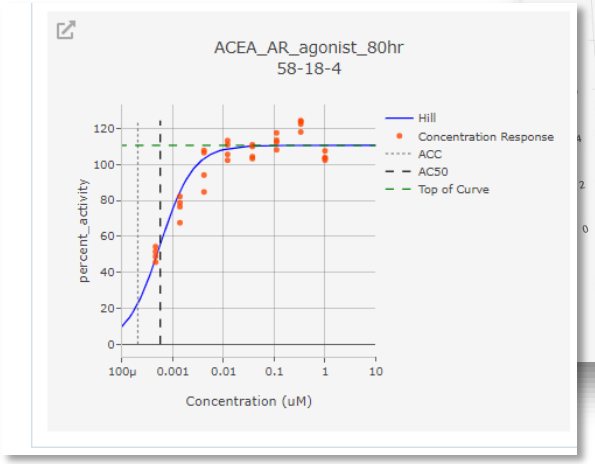
ICE Tools

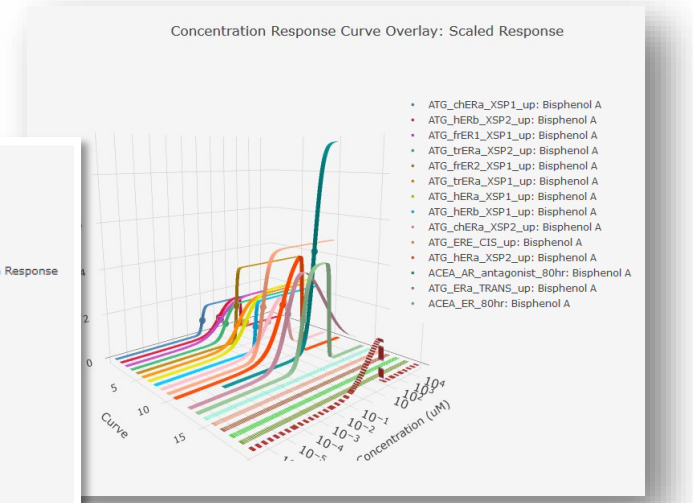
1. Perform a search
   - Search
2. Expand inventory: Identify similar chemicals
   - Chemical Quest
3. Characterize chemical inventory
   - Chemical characterization
4. Review in vitro data details
   - Curve Surfer
5. Generate chemical concentration predictions
   - PBPK
6. Predict equivalent administered dose from in vitro data
   - IVIVE



Training available online and via https://ice.ntp.niehs.nih.gov/

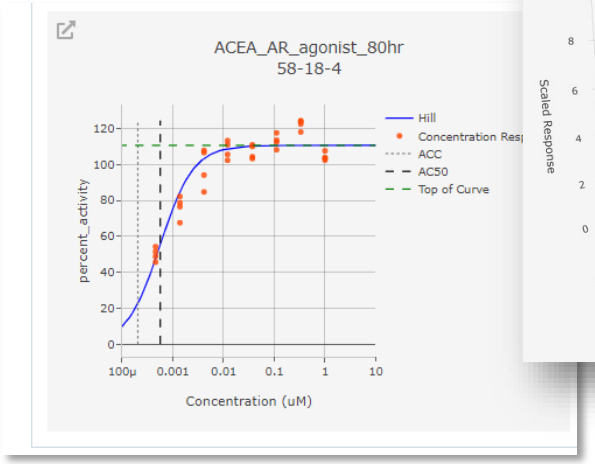
HTS Data Exploration

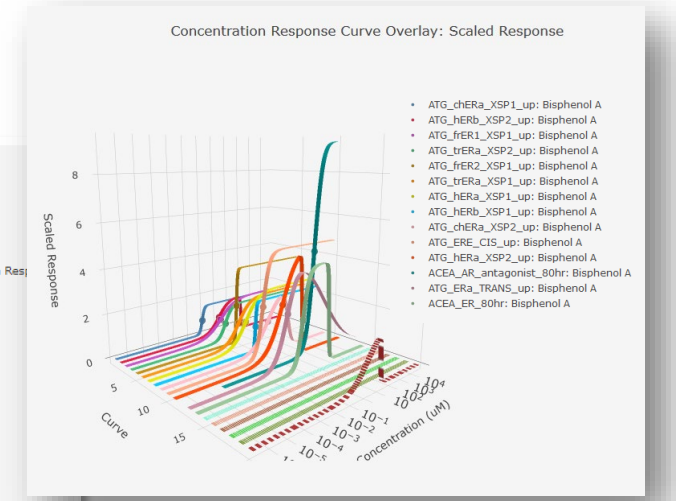




ICE Functionalities

HTS Data Exploration

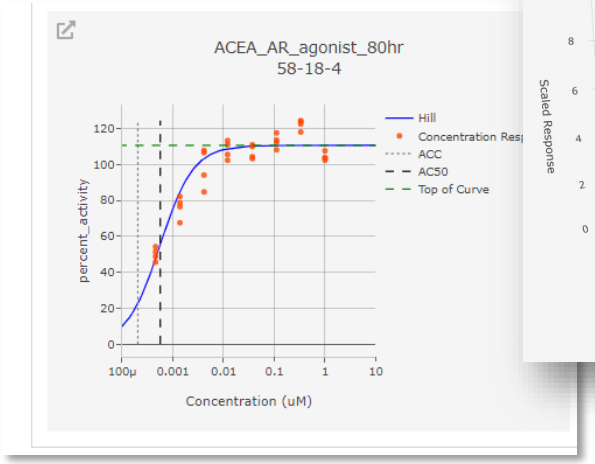


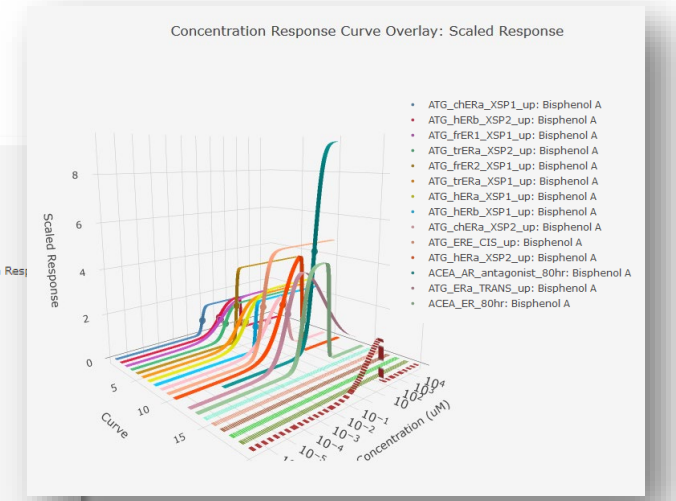


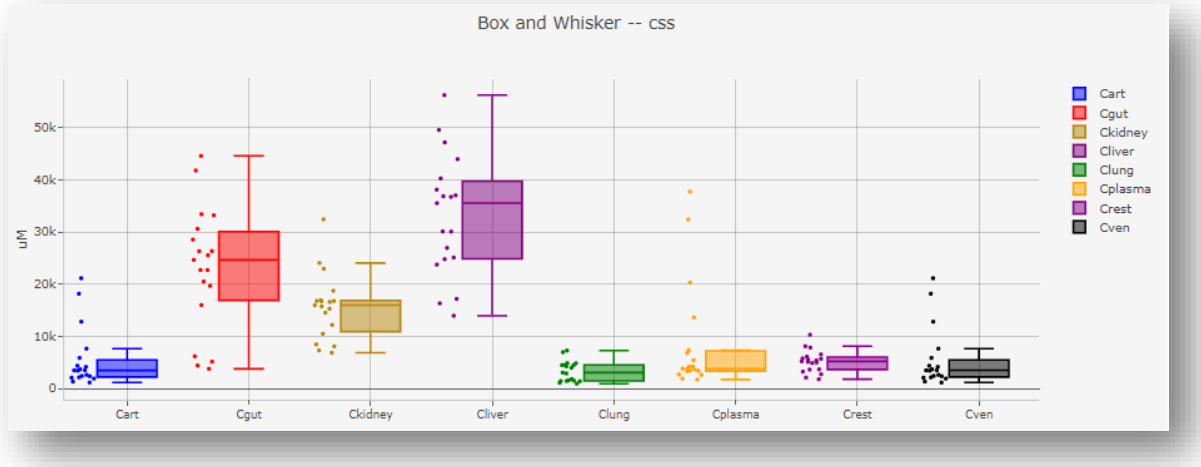
Chemical Similarity

ICE Functionalities

HTS Data Exploration





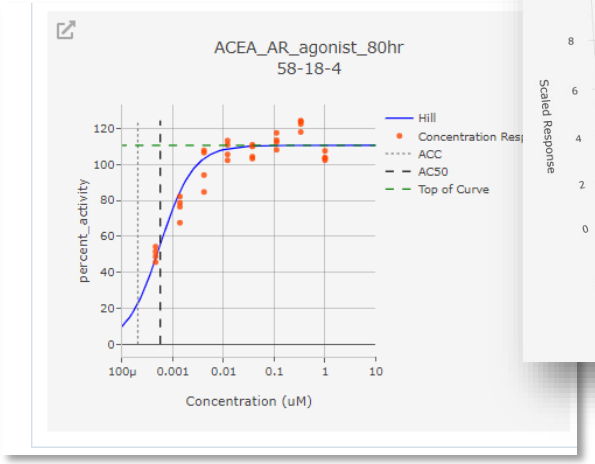


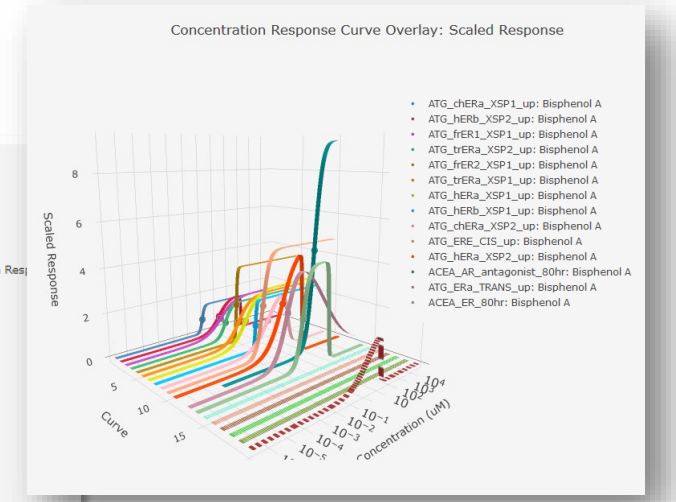
Predicting Chemical Exposure:
Body Tissues, Consumer Products

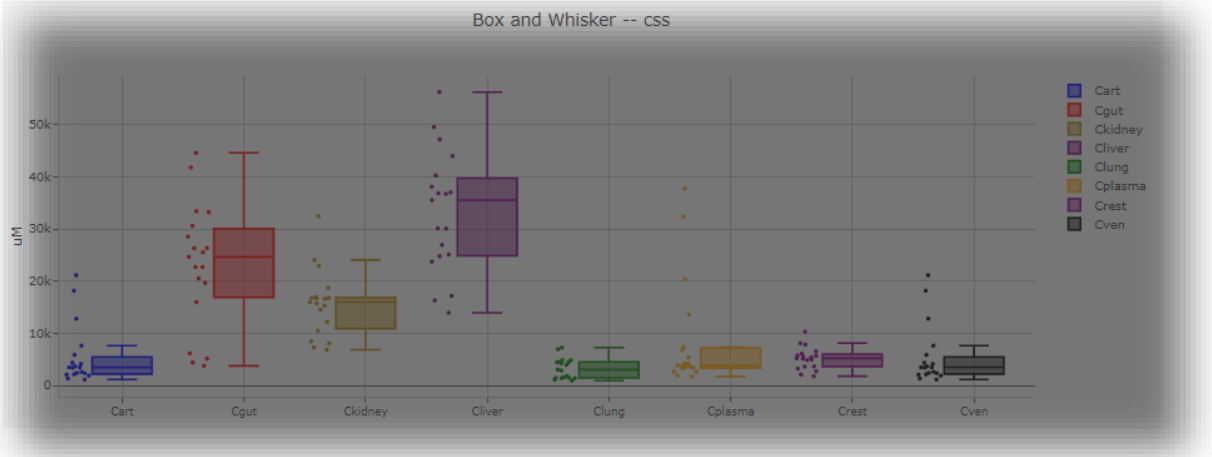
Chemical Similarity

ICE Functionalities

HTS Data Exploration

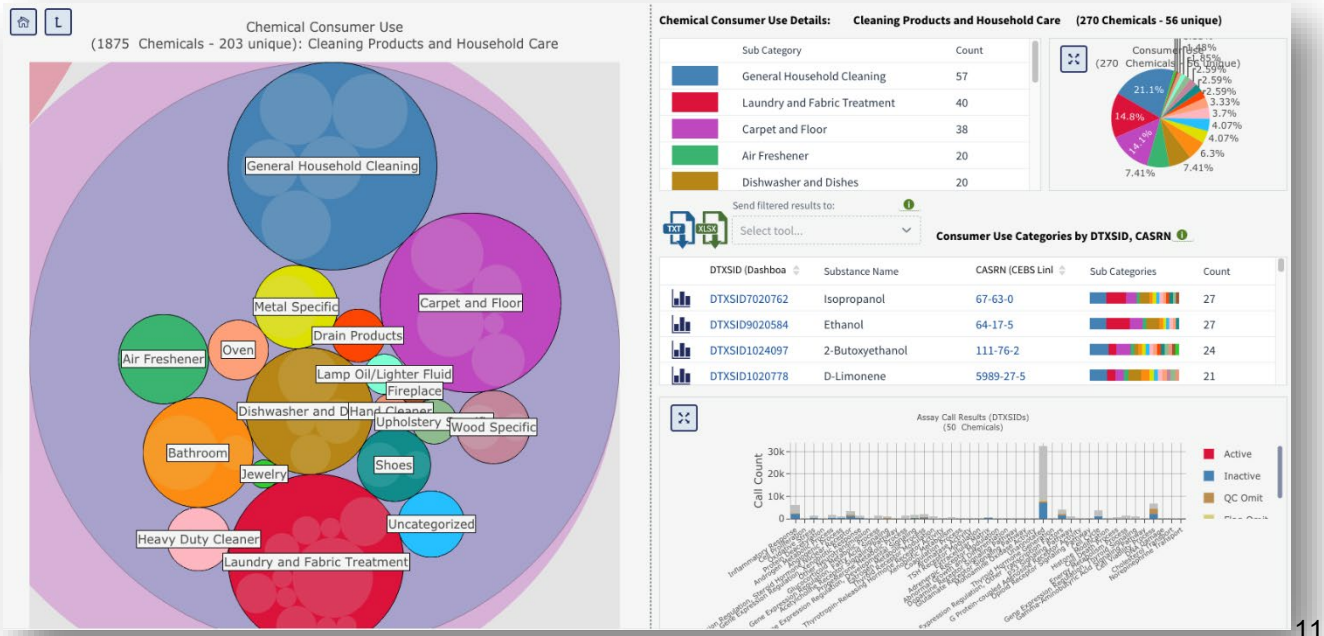






Predicting Chemical Exposure: Body Tissues, Consumer Products

Chemical Similarity



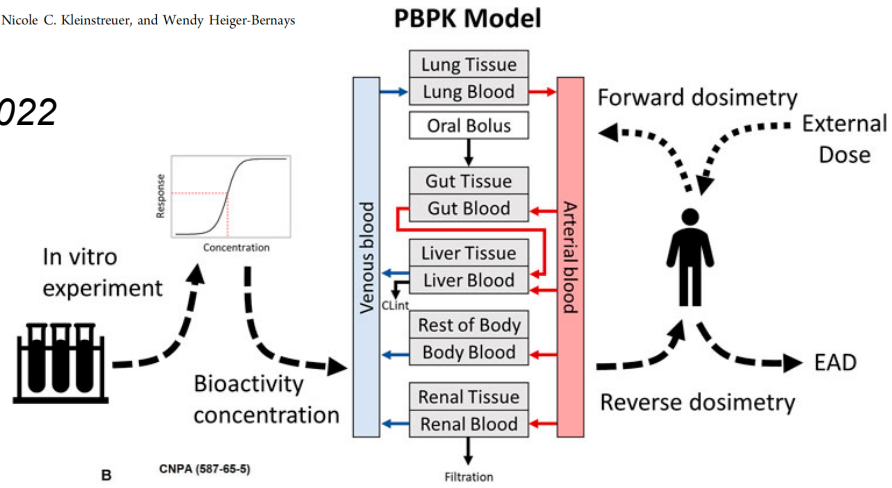
Applications of IVIVE

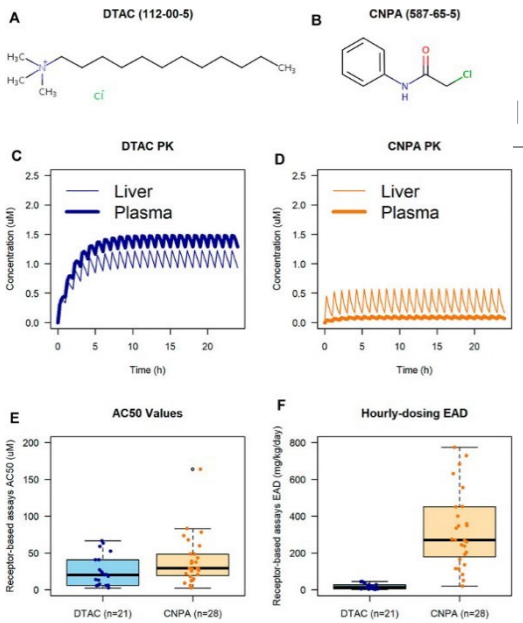
Environmental Science & Technology
pubs.acs.org/est Article

Impact of High-Throughput Model Parameterization and Data Uncertainty on Thyroid-Based Toxicological Estimates for Pesticide Chemicals

Jeffrey M. Carlson,* Patricia A. Janulewicz, Nicole C. Kleinstreuer, and Wendy Heiger-Bernays

Carlson et al. 2022





Frontiers in Pharmacology
PERSPECTIVE
published: 13 April 2022
doi: 10.3389/fphar.2022.864742

Application of an Accessible Interface for Pharmacokinetic Modeling and *In Vitro* to *In Vivo* Extrapolation

David E. Hines[1], Shannon Bell[1], Xiaoqing Chang[1], Kamel Mansouri[2], David Allen[1] and Nicole Kleinstreuer[2]*

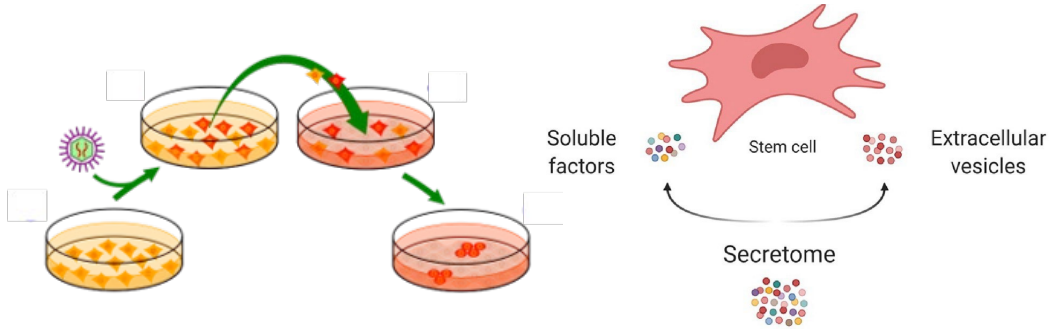
[1]Inotiv-RTP, Research Triangle Park, Durham, NC, United States, [2]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, Durham, NC, United States

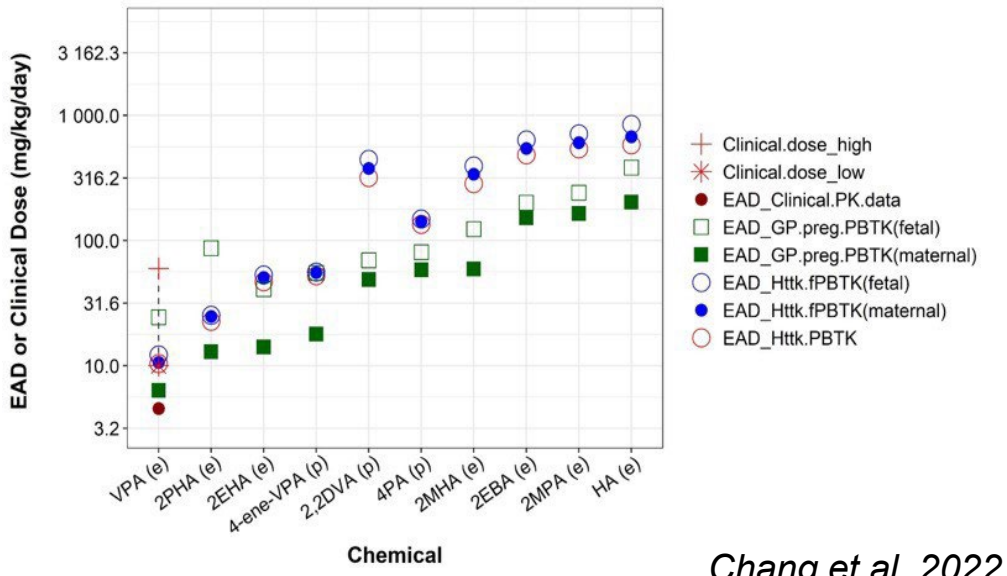
Hines et al. 2022

RESEARCH ARTICLE
Birth Defects Research | Society for Birth Defects Research & Prevention | WILEY

Quantitative in vitro to in vivo extrapolation for developmental toxicity potency of valproic acid analogues

Xiaoqing Chang[1] | Jessica Palmer[2] | Annie Lumen[3] | Un Jung Lee[3] |
Patricia Ceger[1] | Kamel Mansouri[4] | Catherine Sprankle[1] |
Elizabeth Donley[2] | Shannon Bell[1] | Thomas B. Knudsen[5] |
John Wambaugh[5] | Bethany Cook[1] | David Allen[1] | Nicole Kleinstreuer[4]





Chang et al. 2022

TK to Connect Metabolism and Variability in Humans

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]

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[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[b,*]

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]

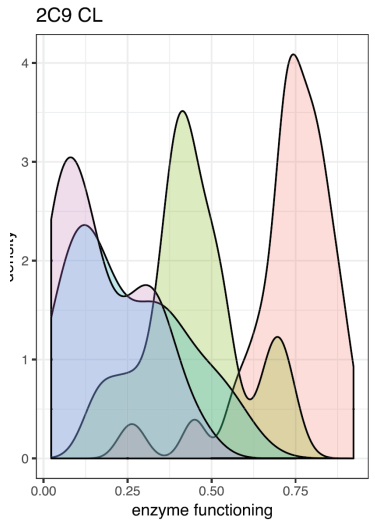
Computational Toxicology

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

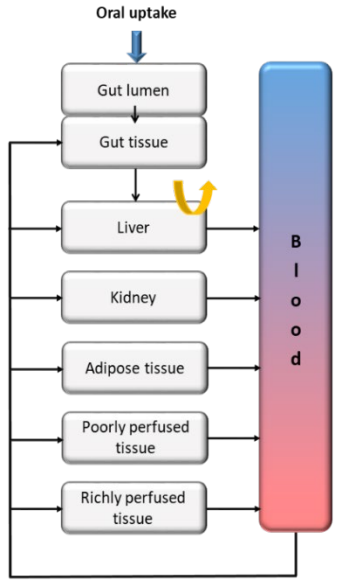
A generic Bayesian hierarchical model for the meta-analysis of human population variability in kinetics and its applications in chemical risk assessment

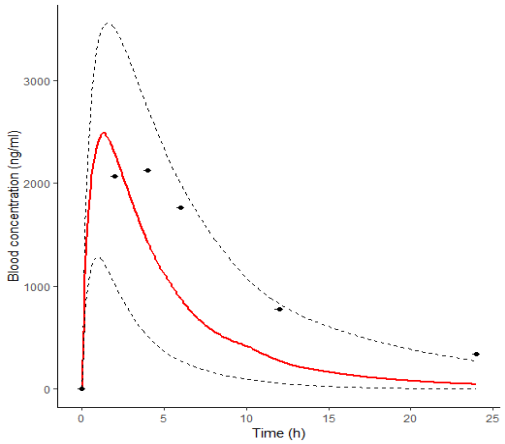
Witold Wiecek[b,*], Jean-Lou Dorne[b], Nadia Quignot[a], Camille Bechaux[c], Billy Amzal[a]

Covering Phase I CYP450 and Phase II UGTs enzymes



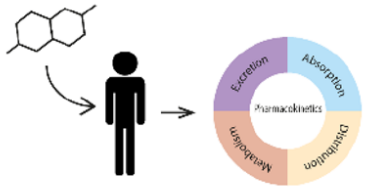
PBPK models + virtual population





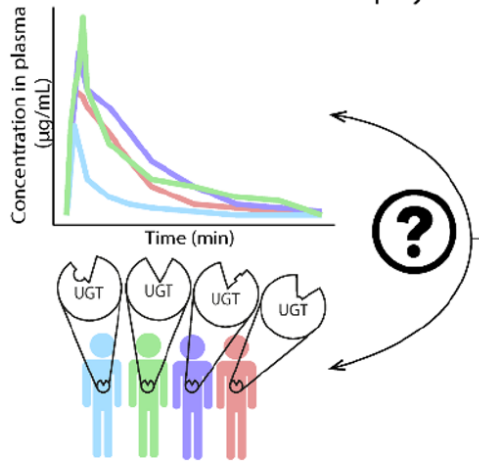
https://ice.ntp.niehs.nih.gov/

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



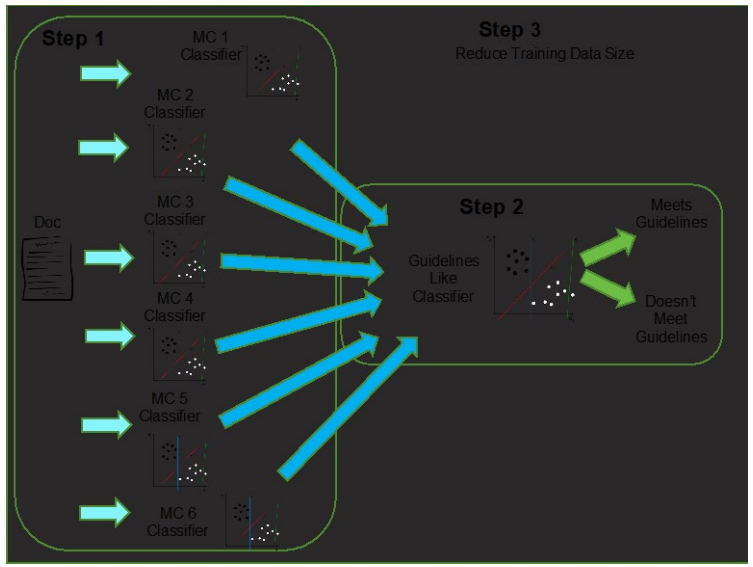
UGT-related uncertainty factors

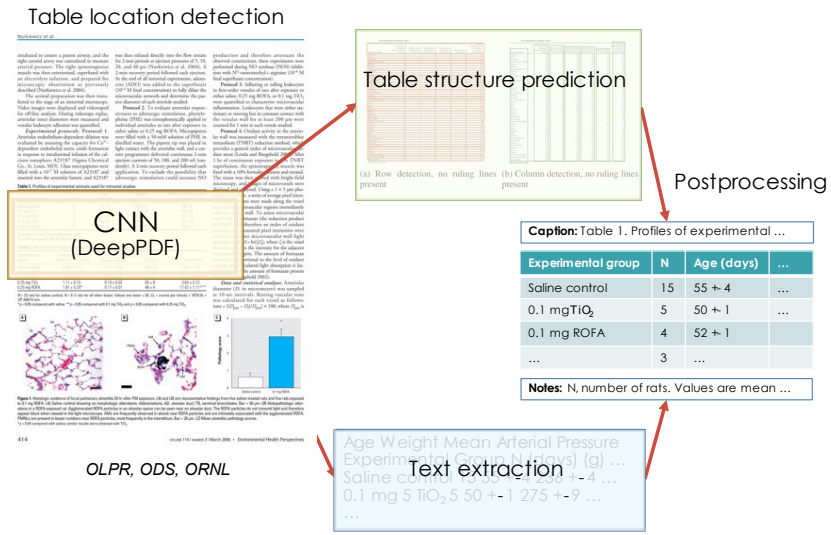
Courtesy of Jean-Lou Dorne

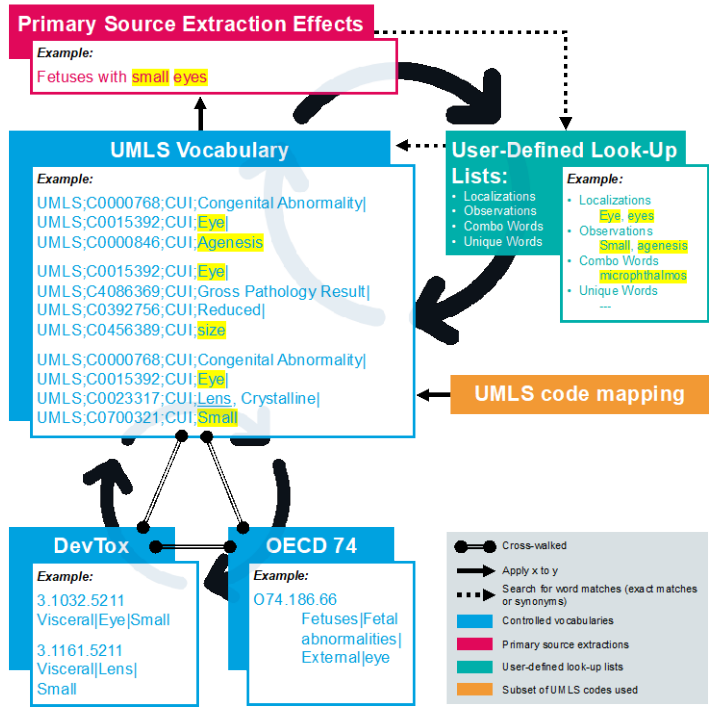
European Food Safety Authority

Machine Automating Study Data Curation

Identification → Extraction → Annotation



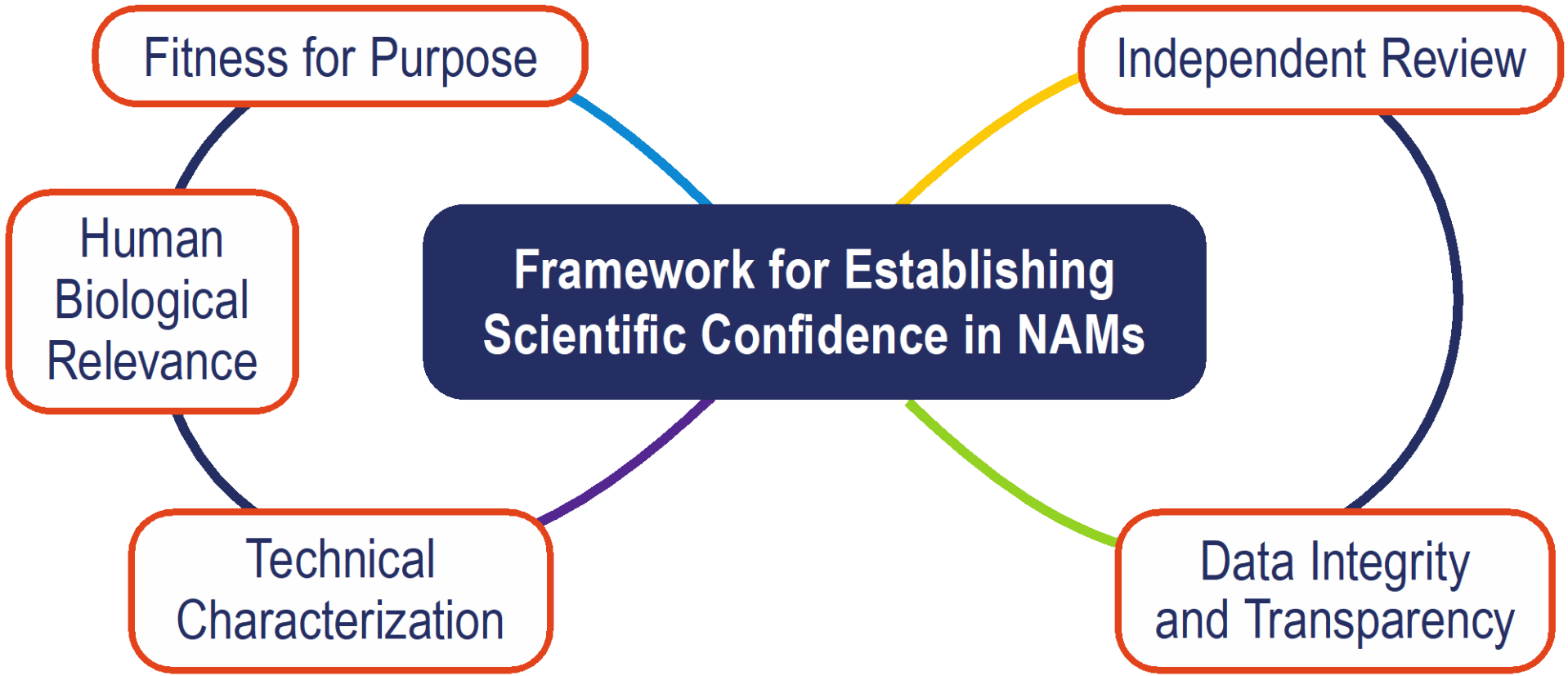




Foster et al. 2022 under review

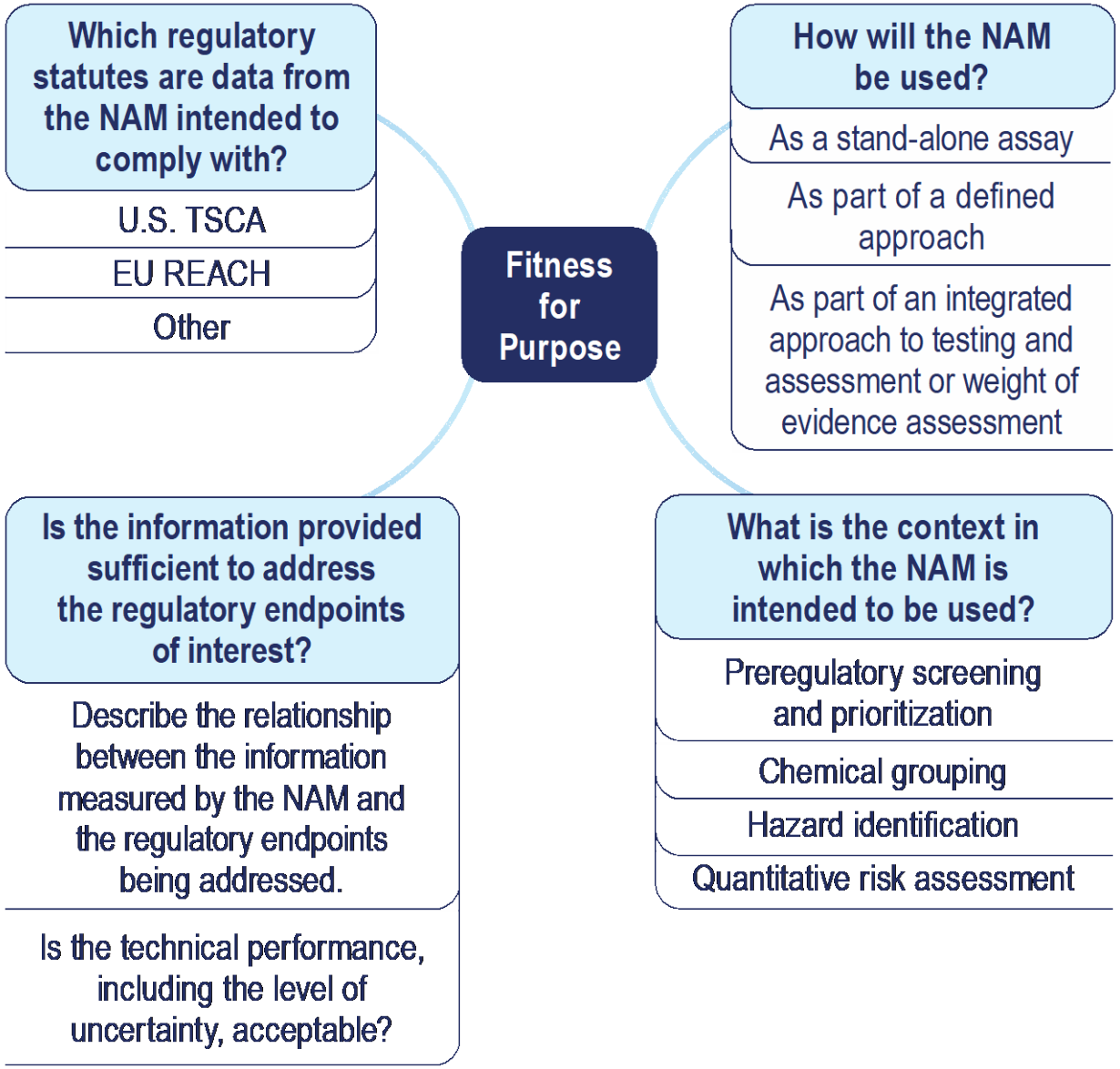
- Important for identifying high-quality studies in the published literature
- Applications in systematic review of chemical effects
- Establishing reference datasets for validating new methods

Essential Elements of a NAMs Scientific Confidence Framework



van der Zalm et al. 2022 under review

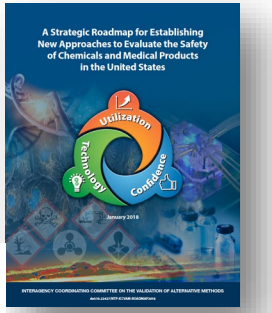
Understanding Fitness for Purpose



van der Zalm et al. 2022 under review

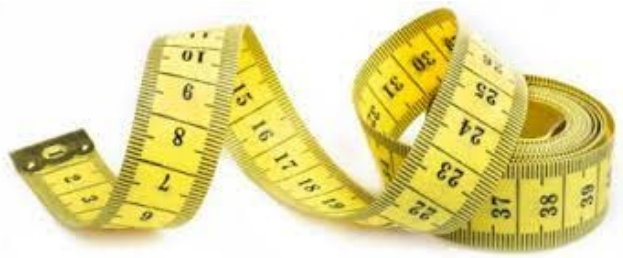
Lessons (Continuously) Learned & Being Applied

- Roadmap 101: Engagement with regulatory stakeholders



- Fit for purpose, performance-based evaluations

- Opportunity for tailored assessments, where data requirements are driven by use cases



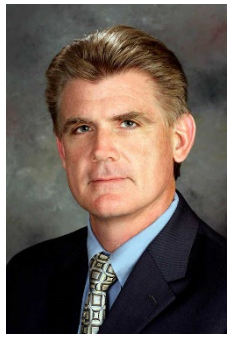
- Communication is key

- There are multiple NAMs that are ready for use now!

Acknowledgments

The NICEATM Group
(current and former)

ICCVAM Leadership
(current and former)



- ICCVAM Agency Reps/WGs
- Contract Support Staff (Inotiv, Sciome, ICF, Kelly)
- OECD Partners
- Academic Collaborators
- Global QSAR Consortium Participants
- Stakeholders (Public, NGO, etc.)

QUESTIONS?