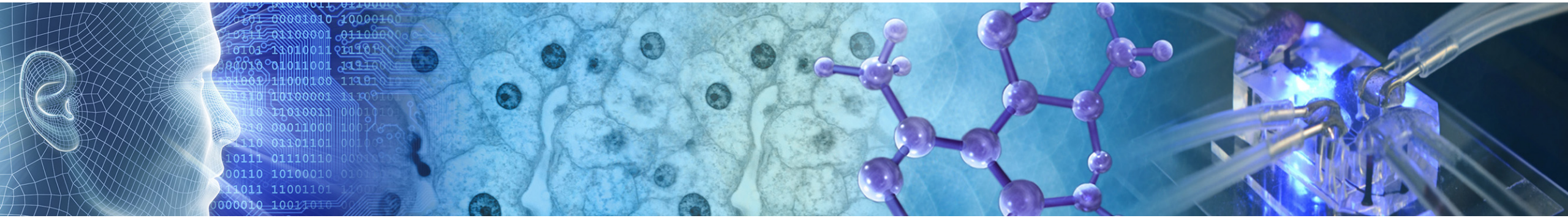
NTP
National Toxicology Program



DNTP/NICEATM Update

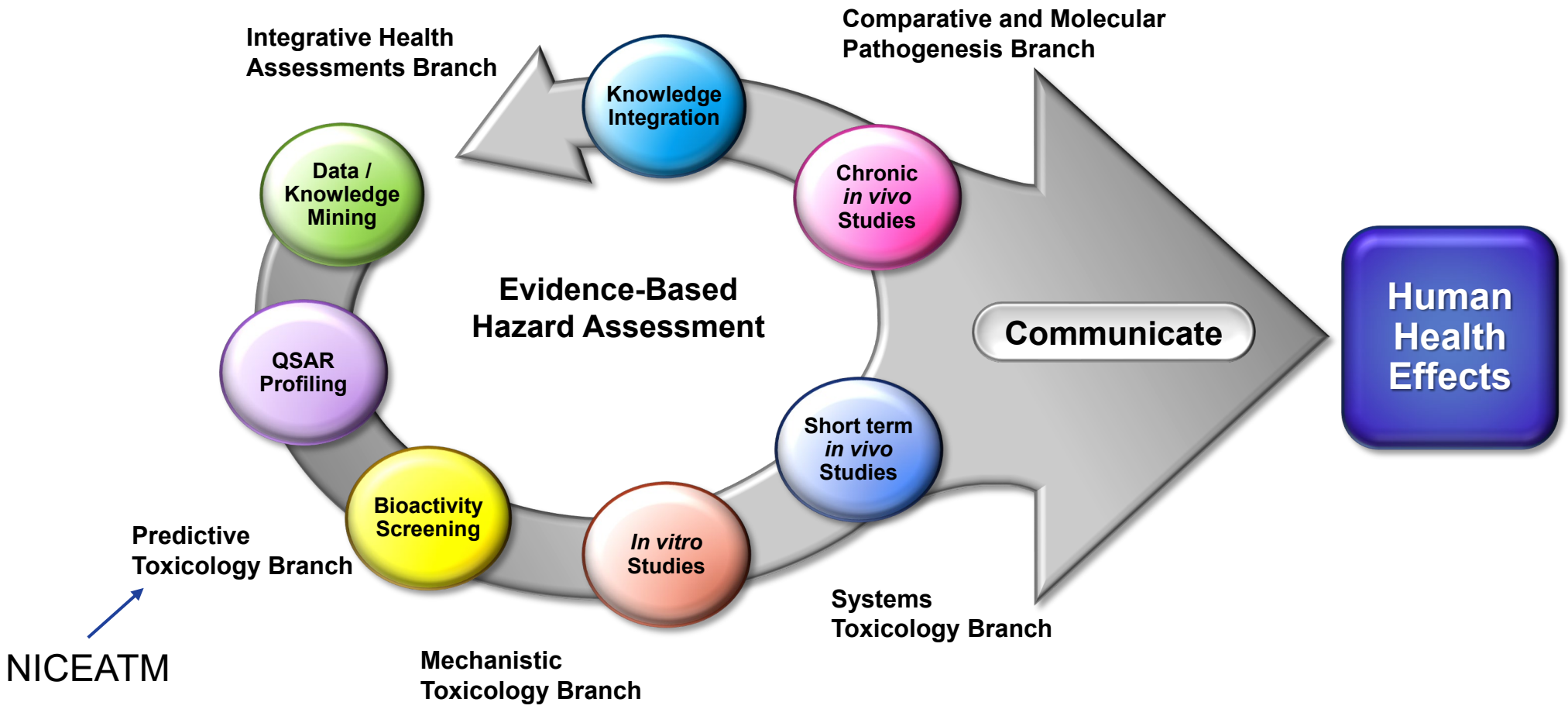
ICCVAM Public Forum
May 27, 2021

Nicole Kleinstreuer
Acting NICEATM Director

DNTP Translational Toxicology Pipeline

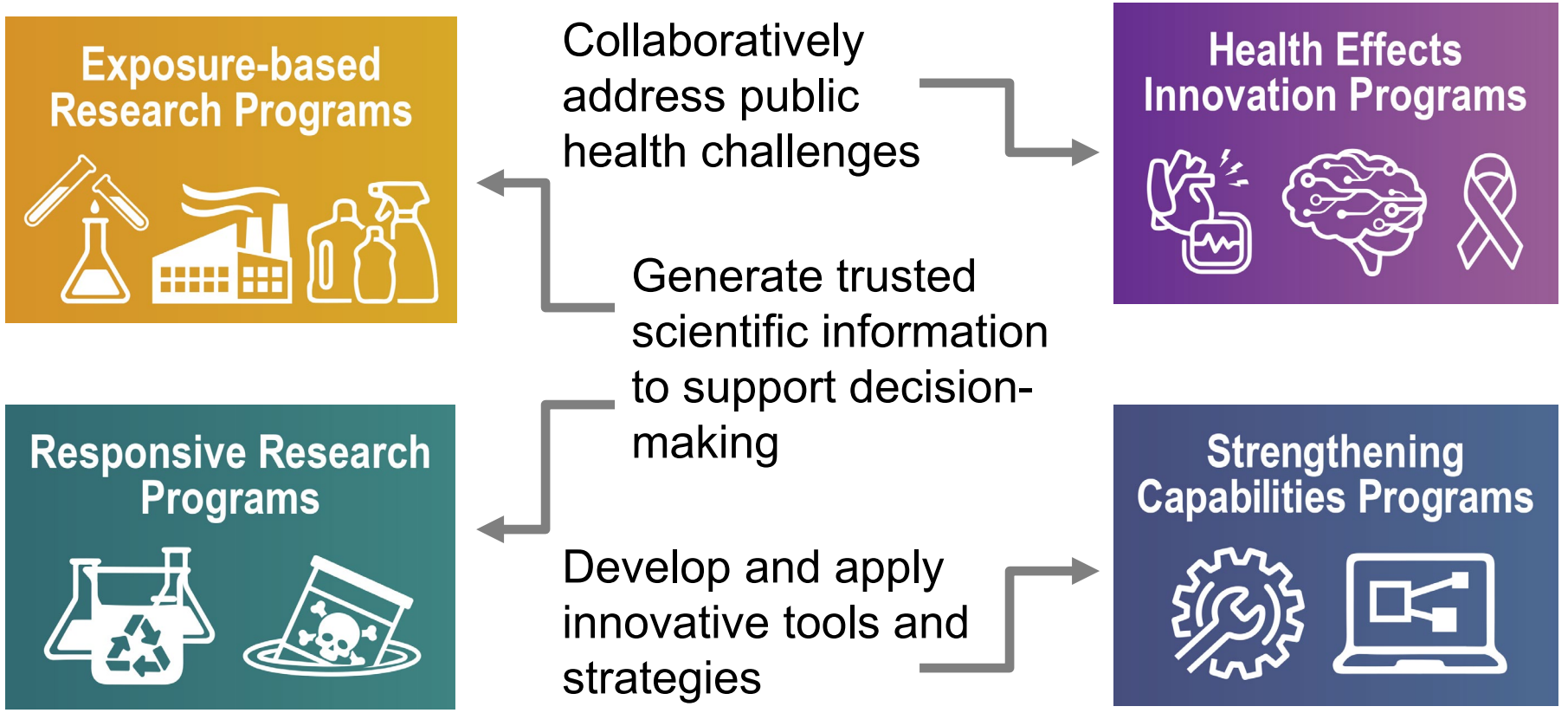
Each assessment in the pipeline qualifies the previous one.

→ Build confidence in the relationship between MOA and in vivo health effect



DNTP Strategic Areas of Focus

- Cardiovascular
- Carcinogenesis
- Developmental Neurotox

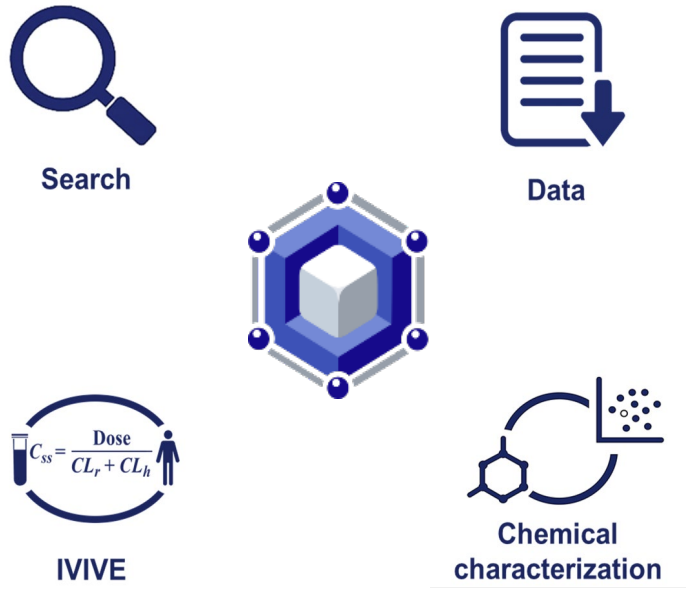


Resources to make existing information on carcinogens FAIR

- Curated data and search tools
  - Organized by toxicity endpoints
  - Standardized terminology, units, and formatting
- Curated chemical lists
  - Reference lists with classifications and bioactivity
  - In vitro assays linked with defined terminology
- Computational models
  - In vitro to in vivo extrapolation (IVIVE)
  - Quantitative structure-activity relationship (QSAR) models

Chemical Effects in Biological Systems (CEBS)
https://manticore.niehs.nih.gov/cebssearch

Integrated Chemical Environment (ICE)
https://ice.ntp.niehs.nih.gov/



Slide courtesy: Carci HEI PMT

Integrated Chemical Environment (ICE) database

Chemical Lists

- Tox21
- AR In Vitro Agonist (R)
- AR In Vitro Antagonist (R)
- AR In Vivo Agonist
- AR In Vivo Antagonist
- EPA IRIS Carcinogenicity Classifications
- EPA Pesticide Active Ingredients
- EPA Pesticide Inert Ingredients, Food and Nonfood Use
- ER In Vitro Agonist (R)
- ER In Vivo Agonist (R)
- Eye Irritation-Corrosion (R)
- Genotoxicity (R)
- IARC Classifications
- NTP Cancer Bioassay Chemicals
- RoC Classifications
- Skin Corrosion (R)
- Steroidogenesis - Androgen
- Steroidogenesis - Estrogen
- Thyroid

EPA OPP to be added 2Q2021

Tox21 HTS assays mapped to Key Characteristics of Carcinogens (KCC)

cHTS | Acute Lethality | Sensitization | Irritation/Corrosion | Endocrine | **Cancer** | DART | Chemical Parameters

Search

Cancer

Mode of Action

KCC1: Electrophilic/Metabolically Activated	in vitro
KCC2: Genotoxic Effects	in vitro
KCC3: Alteration of DNA Repair/Genomic Stability	in vitro
KCC4: Epigenetic Alterations	in vitro
KCC5: Oxidative Stress	in vitro
KCC6: Chronic Inflammation	in vitro
KCC8: Receptor Mediated Effects	in vitro
KCC10: Cell Proliferation/Death/Energetics	in vitro

Data

CASRN	Chemical Name	DTXSID	Original SMILES	Original InChIKey	QSAR Ready SMILES	Technical Report No.	NTP Level Of Evidence Male Rats	NTP Level Of Evidence Female Rats	NTP Level Of Evidence Male Mice	NTP Level Of Evidence Female Mice	Tested in Tox21
67-66-3	Chloroform	DTXSID1020306	ClC(Cl)Cl	HEDRZPFGACZZDS-UHFFFAOYSA-N	ClC(Cl)Cl	TR-000	P	NE	P	P	Yes
143-50-0	Chlordecone (kepone)	DTXSID1020770	ClC12C(=O)C3(Cl)C4(Cl)C1(Cl)C1(Cl)C2(Cl)C3(Cl)C4(Cl)C1(Cl)Cl	LHHGDZSESBACKH-UHFFFAOYSA-N	ClC12C(=O)C3(Cl)C4(Cl)C1(Cl)C1(Cl)C2(Cl)C3(Cl)C4(Cl)C1(Cl)Cl	TR-001	P	P	P	P	Yes
79-01-6	Trichloroethylene	DTXSID0021383	ClC=C(Cl)Cl	XSTXAVWGXDQKEL-UHFFFAOYSA-N	ClC=C(Cl)Cl	TR-002	NE	NE	P	P	Yes

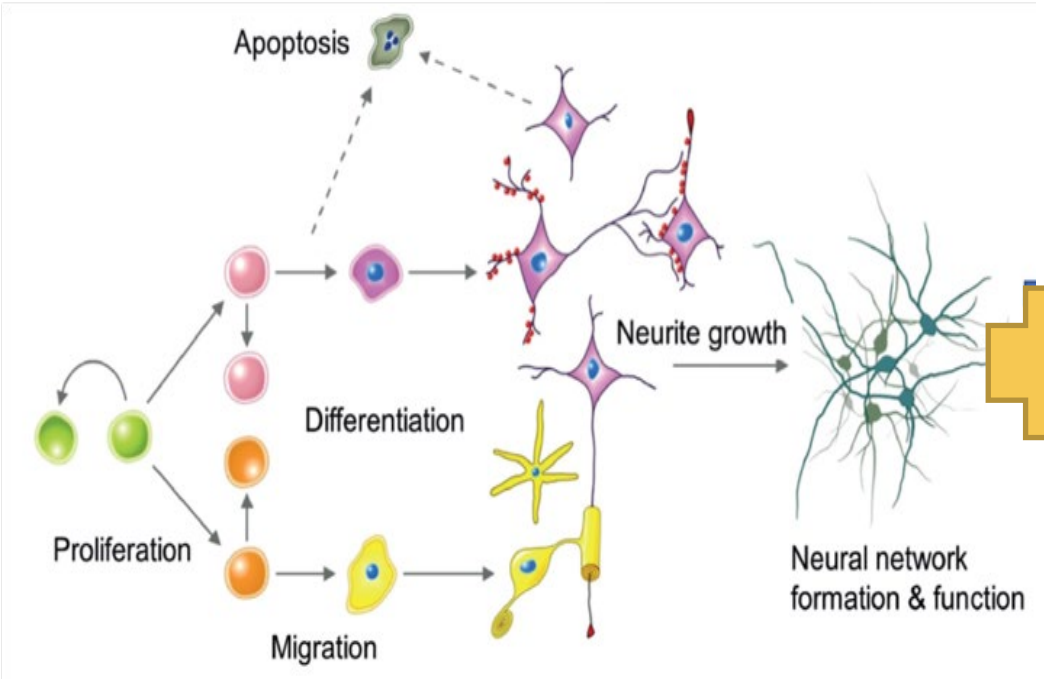
Additional information on each chemical:

- genotoxicity data
- highest dose tested
- dose and tissue used for level of evidence call
- type of lesion

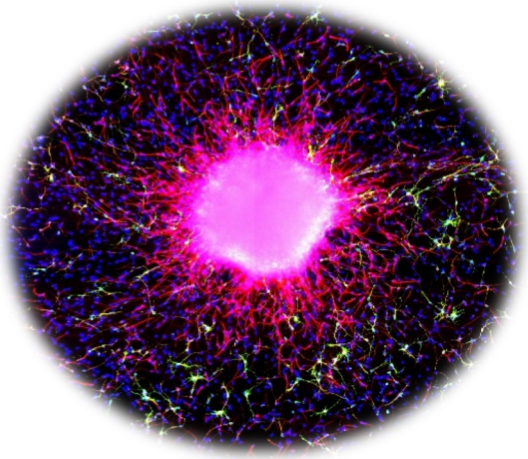
Slide courtesy: Carci HEI PMT

DNTP DNT HEI Objectives

Implement a DNT screening battery that covers key neurodevelopmental events



Human-derived + rodent + zebrafish



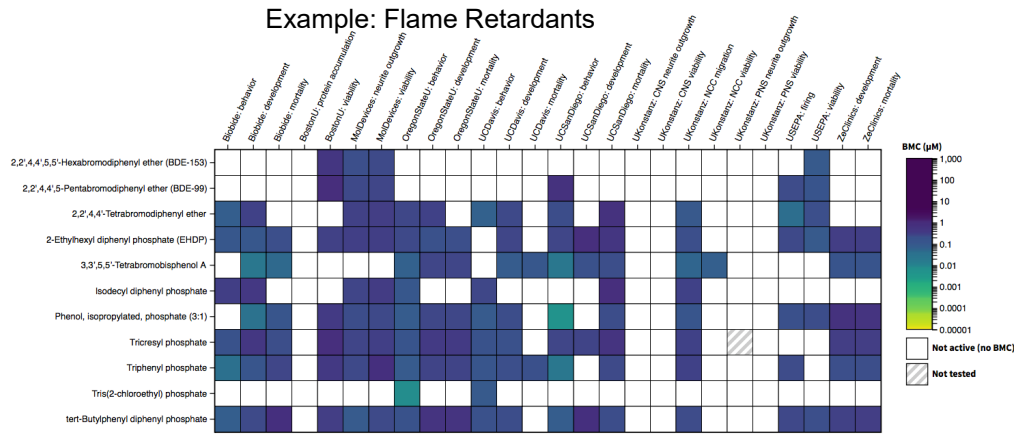
2-D assays **3D- Neurospheres** **Zebrafish**

DNTP's Proposed Battery: Initial Assay Selection

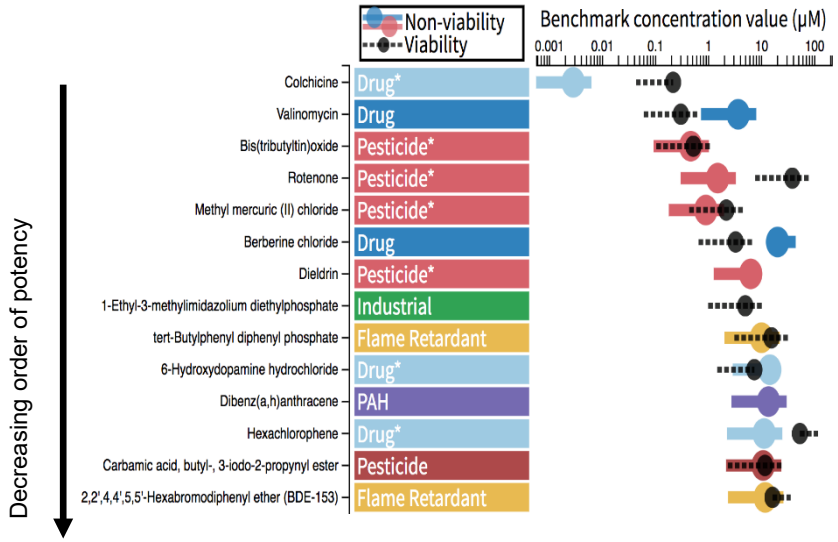
Slide courtesy: DNT HEI PMT

DNT- Data Integration and Visualization Enabling Resource

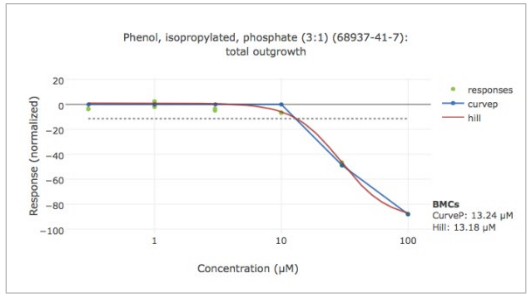
(DNT-DIVER)



Compare activity of compounds/classes across multiple assays



Compare activity of compounds within an assay



Individual dose-response curves

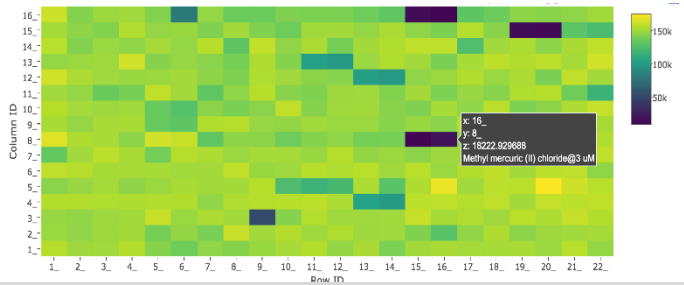


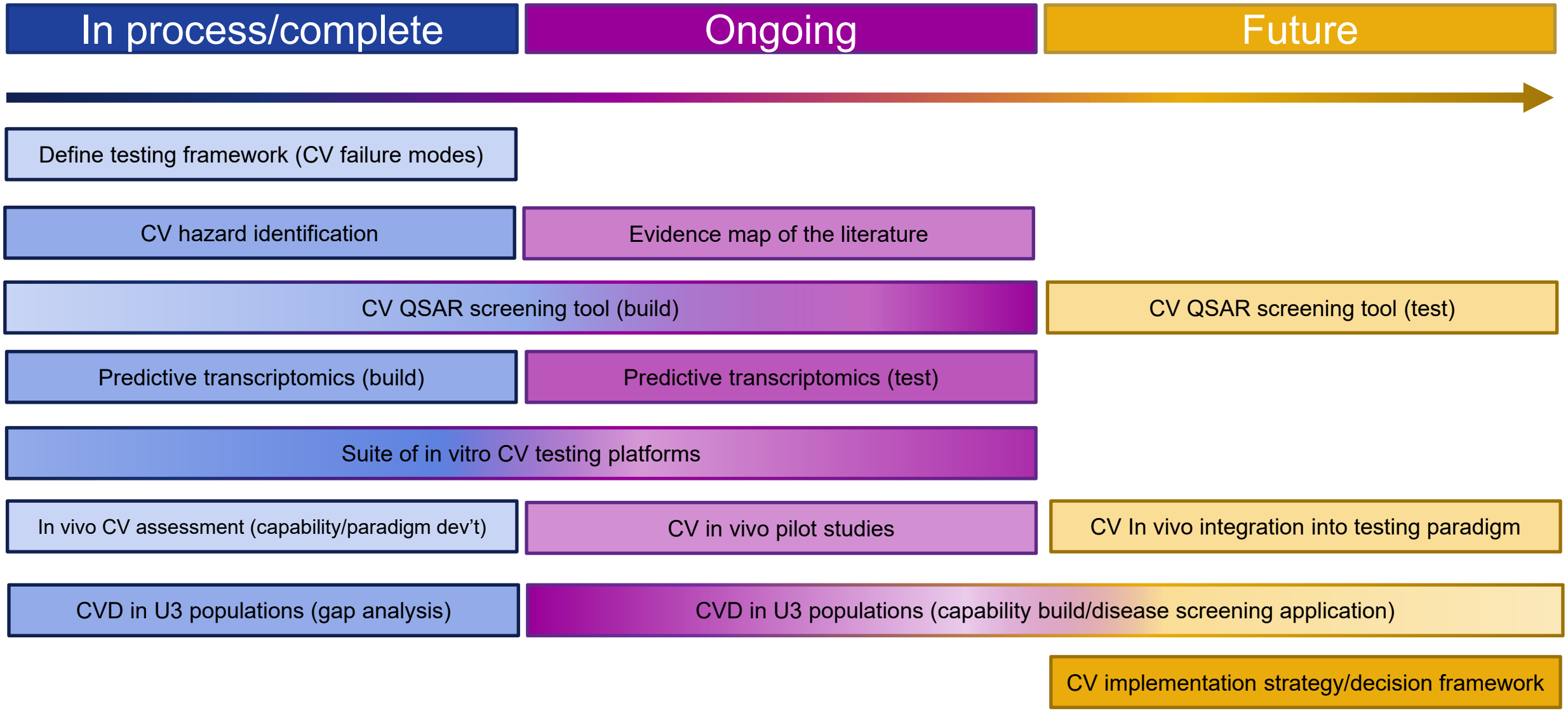
Plate and well level information

Control variability in assay

https://sandbox.ntp.niehs.nih.gov/neurotox/

Slide courtesy: DNT HEI PMT

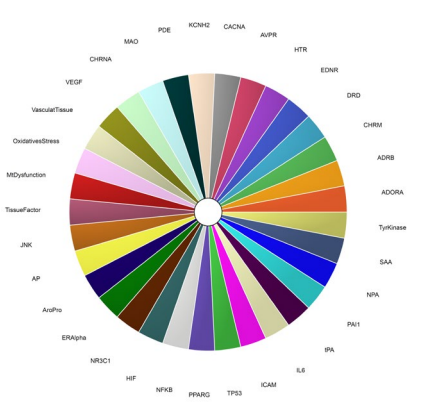
CV HEI Portfolio



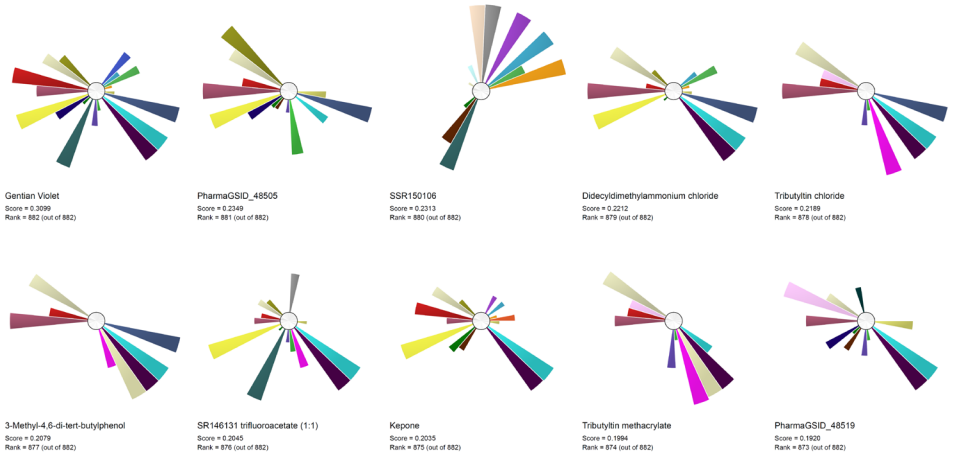
Slide courtesy: CV HEI PMT

CardioToxPi: Using Tox21 qHTS Data and AI

Krishna et al. 2020, Chem Res Tox

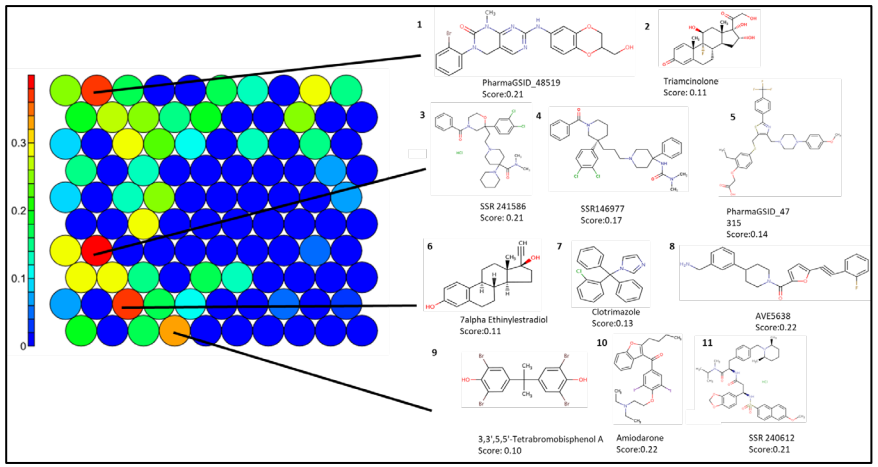


Slice	Traget name	Effect	Slice Color
ADORA	Adenosine Receptor	Vasodialation, alterations in BP	
ADRB	Adrenergic Receptor	Arrhythmia, Alterations in BP	
CHRM	Muscarinic Acetylcholine Receptor	Alterations in BP and HR, tachycardia	
DRD	Dopamine Receptor	Alterations in BP and HR, Vascular relaxation	
EDNR	Endothelin Receptor	Alterations in BP, Can exert adverse effects during	
HTR	Serotonine Receptor	Alterations in BP, Potential cardiac valvulopathy	
AVPR	Vasopressin Receptor	Alterations in BP and HR, Cardiac hypertrophy	
CHRNA	Cholinergic receptor	Alterations in BP and HR	
CACNA	Voltage-Gated Calcium Channel	Alterations in BP, QT prolongation, Arrhythmia	
KCNH2	Potassium Voltage Gated Channel	Prolongation of QT interval of ECG	
VEGF	Vascular Endothelial Growth Factor	Alterations in BP , Cardiac Ischemia	
VascularTissue	Vascular Tissue	Myocardial ischemia, cardiac Arrhythmias	
OxidativeStress	Oxidative Stress	Cellular Hypertrophy; Cardiac Cell Death	
MtDysFunction	Mitochondrial Dysfunction	Cardiac dysfunction; Cardiomyopathy	
TissueFactor	Tissue Factor	Alterations in BP and ventricular hypertrophy	
PDE	Phosphodiesterase	Alterations in cardiac contractility, HR and BP	
MAO	Monoamine Oxidase	Alterations in BP	
JNK	c-Jun N-terminal kinase	Vascular injury, cardiac hypertrophy	
TyrKinase	Tyrosine Kinase	Alterations in BP, LV dysfunction, conduction	
AroPro	Aromatase Protein	Ischemic heart disease	
ERAlpha	Estrogen receptor Alpha	Abnormal cardiac contractility, cardiac hypertrophy	
NR3C1	Glucocorticoid receptor	Alterations in BP; Arrhythmia	
PPARG	Peroxisome Proliferator Activated Receptor γ	Cardiac hypertrophy , Atherosclerosis	
AP	Activating Protein	Atherosclerosis	
HIF	Hypoxia Inducible Factor 1	Ischaemia disease	
NFKB	NF Kappa B	Atherosclerosis	
TP53	Tumor Protein p53	Alteration in cardiac function	
ICAM1	Intercellular adhesion molecule 1	Markers of endothelial dysfunction	
IL6	Interleukin 6	Markers of inflammation and oxidative stress	
t-PA	Tissue Type plasminogen activator	Markers of endothelial dysfunction	
PAI -1	Plasminogen activator inhibitor type	Markers of endothelial dysfunction	
NPA	Natriuretic peptide A	Release in response to elevation in LV filling pressure	
SAA1	Serum amyloid A1	Direct promotion of vascular dysfunction through SAA within vascular tissues	

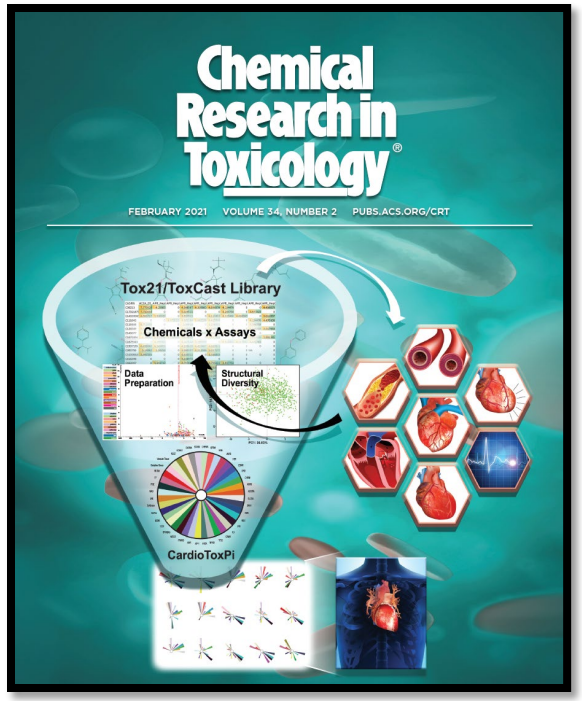


CardioToxPi for 10 most active chemicals

Self Organizing Map: structural clusters enriched for CV activity



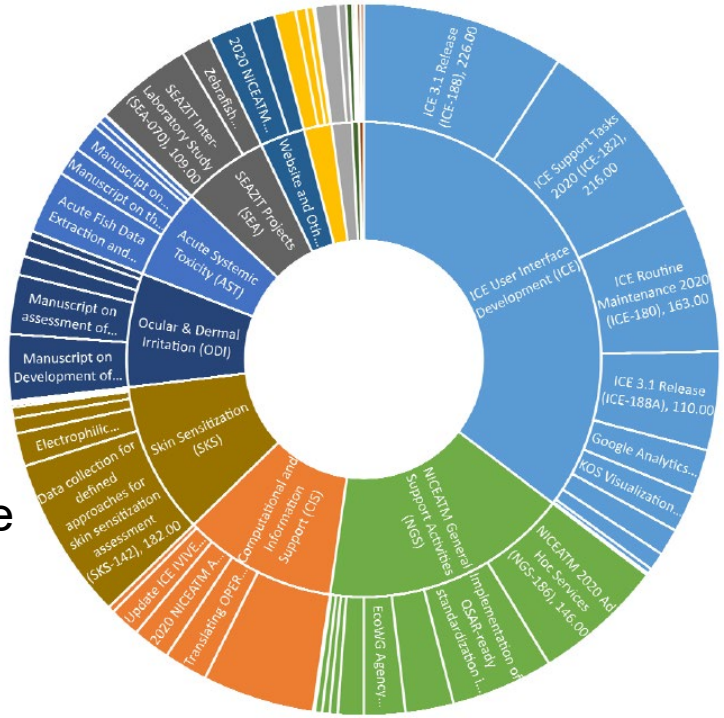
- Special Issue Cover: **Computational Toxicology**
- Published February 2021
  - QSAR and other in silico studies
  - IVIVE methods
  - Application of NextGen sequencing and HTS data
  - Use of artificial intelligence and machine learning to model critical in vivo toxicity endpoints.



Slide courtesy: CV HEI PMT

Ongoing NICEATM and ICCVAM Projects

- Integrated Chemical Environment
- OPERA (QSAR/QSPR)
- Reference data curation
- Variability of in vivo data
- Acute Systemic Toxicity
- Dermal absorption
- Eye and skin irritation
- Skin sensitization
- ZF models (SEAZIT)
- Acute Fish Retrospective
- Carcinogenesis
- Cardiovascular toxicity
- Developmental Toxicity
- Animal-free affinity reagents
- Microphysiological Systems
- Evolving Process of Validation



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

- **Subscribe to NICEATM News email list**

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

Acute 6-Pack Alternatives

Dermal lethality	• US EPA Waiver guidance available
Oral lethality	• In silico (CATMoS) for single chemicals; additivity for formulations under consideration
Inhalation lethality	• 3D models being evaluated; LC50 database for in silico model development being built
Eye irritation	• NAMs for Cat I and/or Cat IV (TG 437, 438, 460, 491, 492, 494); Prospective testing ongoing
Skin irritation	• NAMs for Cat I or Cat IV (TG 430, 431, 435, 439); Prospective testing ongoing
Skin sensitization	• EPA science policy, draft risk assessment, and OECD international DASS guideline

Mansouri et al. 2021 EHP; Clippinger et al. 2021 Cut Ocu Tox; Rooney et al. 2021 Reg Tox Pharm; Allen et al. 2021 ALTEX; Hamm et al. 2021 Reg Tox Pharm under review

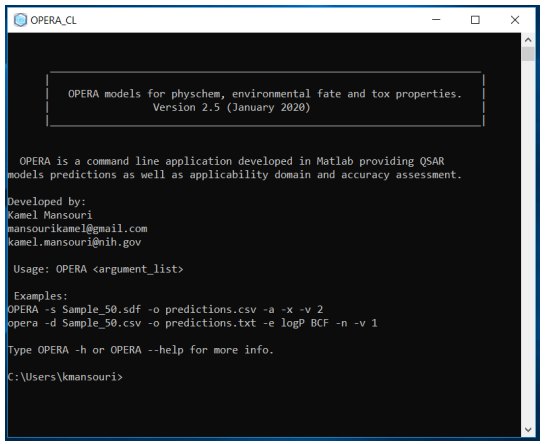
Collaborative Modeling Project for Predicting Acute Oral Toxicity (CATMoS)

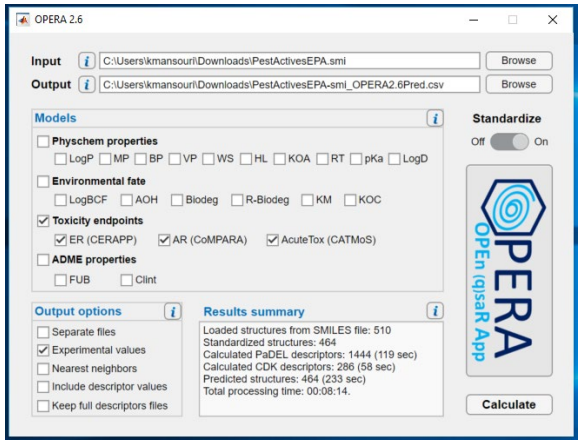
Mansouri et al. 2021 EHP

CATMoS implementation in OPERA

OPERA suite of models:

- Free, open-source, and open-data
- Command line and GUI
- Single chemical and batch mode
- Windows OS and Linux
- Embeddable wrapper libraries in Java, C, C++, and Python





Collaboration with ATWG partners and ICCVAM agencies

Agency	No. Substances	Agency	No. Substances
Air Force	421	EPA OPP	36
Army Public Health Command	18	EPA OPPT	8
Army Edgewood Chemical Biological Center	42	EPA NCCT	4815
CPSC	110	EPA EFED	160
DOT	3671	FDA CFSAN	22

Progress made with EPA EFED

- Compare CATMoS predictions to acute oral toxicity data on 160 pesticides registered in the last 25 years.
- Determine impact on risk assessments, leading to additional curation and characterizing confidence in predictions.

Human-relevant approaches for eye corrosion/irritation potential

Clippinger et al. 2021 Cut Ocu Tox

Prior GHS category	1	2A	2B	NC
1 (serious eye damage)	73%	16%	0%	10%
2A (irritant)	4%	33%	4%	59%
2B (mild irritant)	0%	4%	16%	80%
NC (non-irritant)	1%	4%	2%	94%

Adapted from Luechtefeld et al., ALTEX 33(2), 2016.

- The rabbit test should not be used as a reference method to demonstrate the validity of *in vitro/ex vivo* assays
- *In vitro/ex vivo* methods are as or more reliable and relevant than the rabbit test

Consider strengths and limitations of all available methods with respect to:

- their relevance to human ocular anatomy
- the mechanisms of eye irritation/corrosion in humans

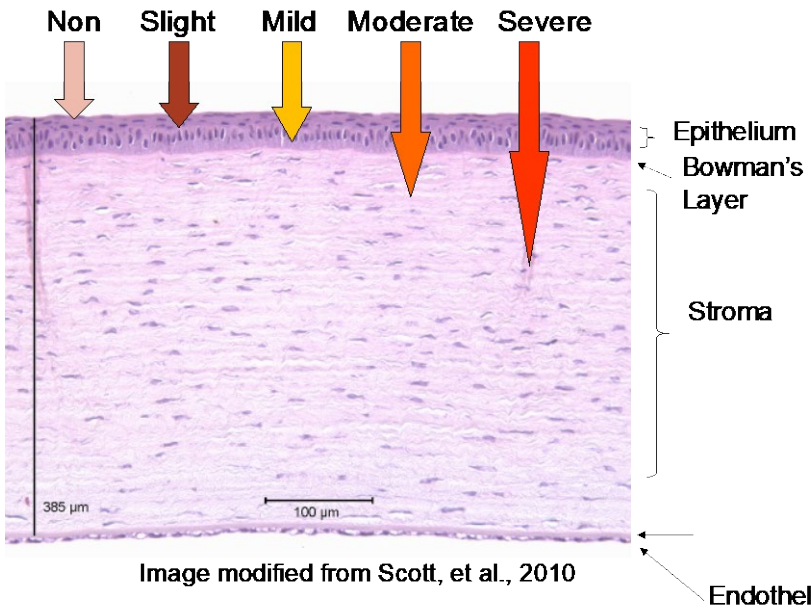
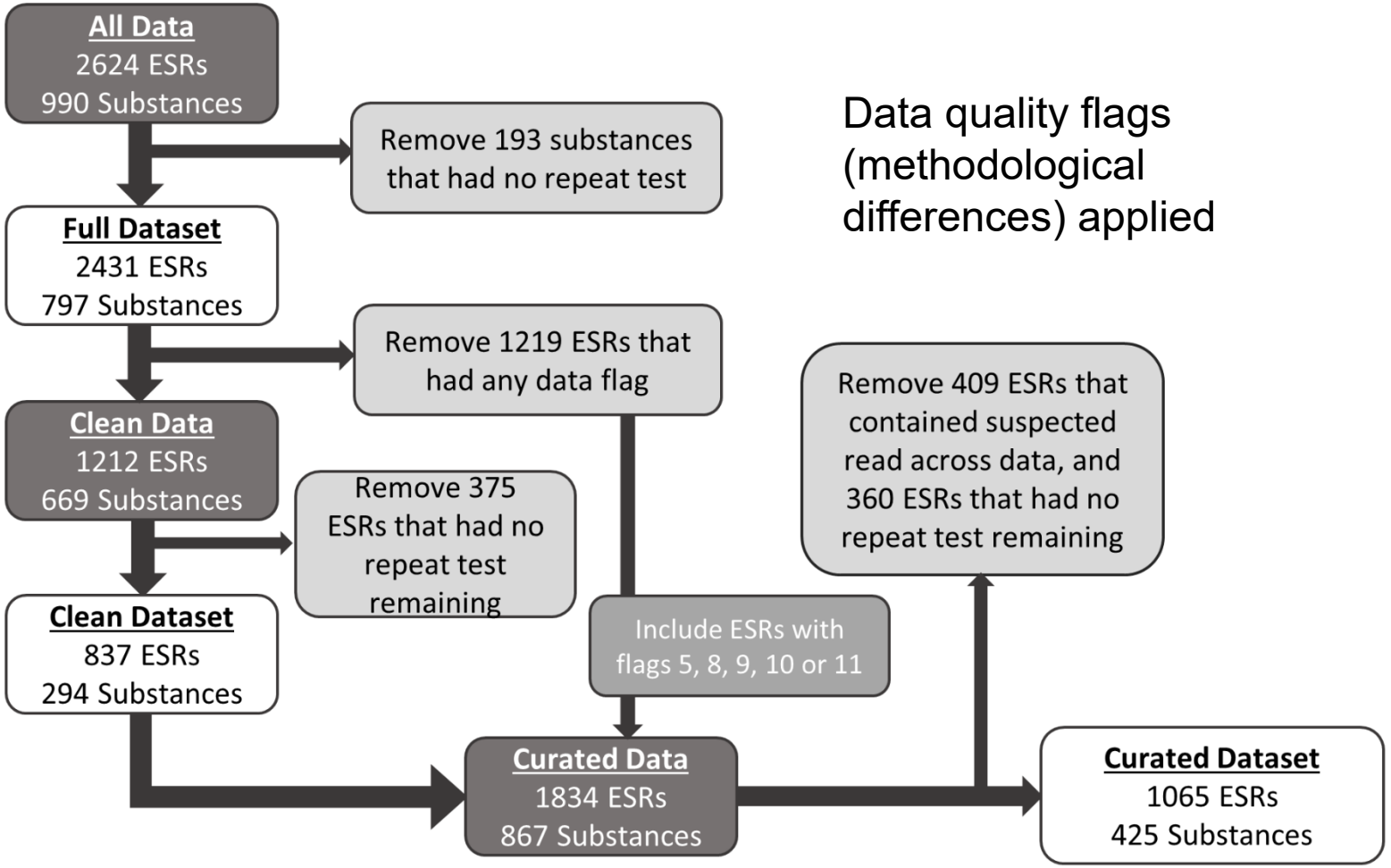


Image modified from Scott, et al., 2010

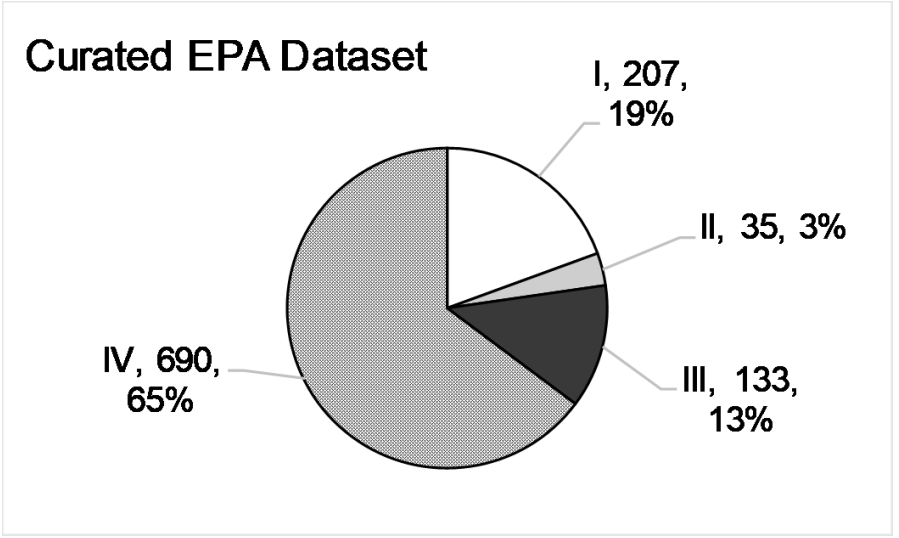
Skin Irritation Variability: EPA Regulatory Categories

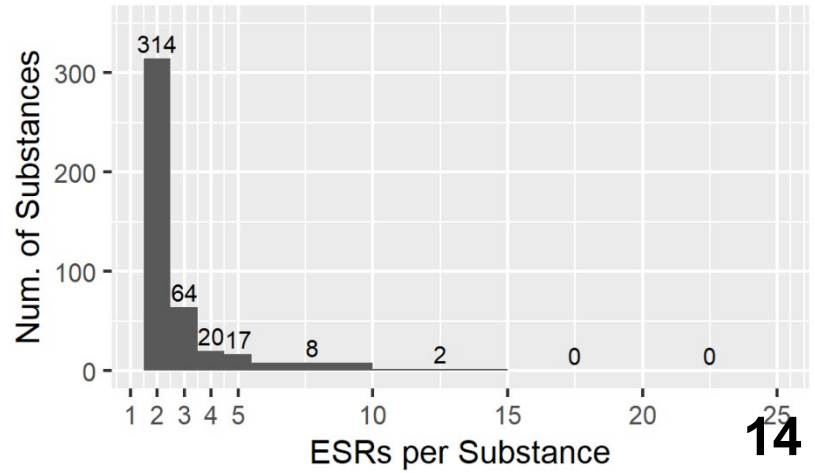
Rooney et al. 2021 Reg Tox Pharm

- Curated data for chemicals with multiple study reports
- Calculated PDII (avg. erythema + avg. edema)



Data quality flags (methodological differences) applied





Skin Irritation Conditional Probability Tables

EPA	Category I	Category II	Category III	Category IV
PDII	Corrosive	>5.0	2.1-5.0	0-2.0
Signal Word	DANGER	WARNING	CAUTION	CAUTION
PPE Required	Coveralls worn over long-sleeved shirt and long pants	Coveralls worn over short-sleeved shirt and short pants	Long-sleeved shirt and long pants	Long-sleeved shirt and long pants
	socks	socks	socks	socks
	Chemical-resistant footwear	Chemical-resistant footwear	Shoes	Shoes
	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	Waterproof or chemical resistant gloves	No minimum

Irritant

Non-irritant

Curated Dataset with Binary Approach

Prior Result	Irritant (Cat I or II)	Non-irritant (Cat III or IV)
Irritant (Cat I or II)	75.6%	24.4%
Non-irritant (Cat III or IV)	3.9%	96.1%

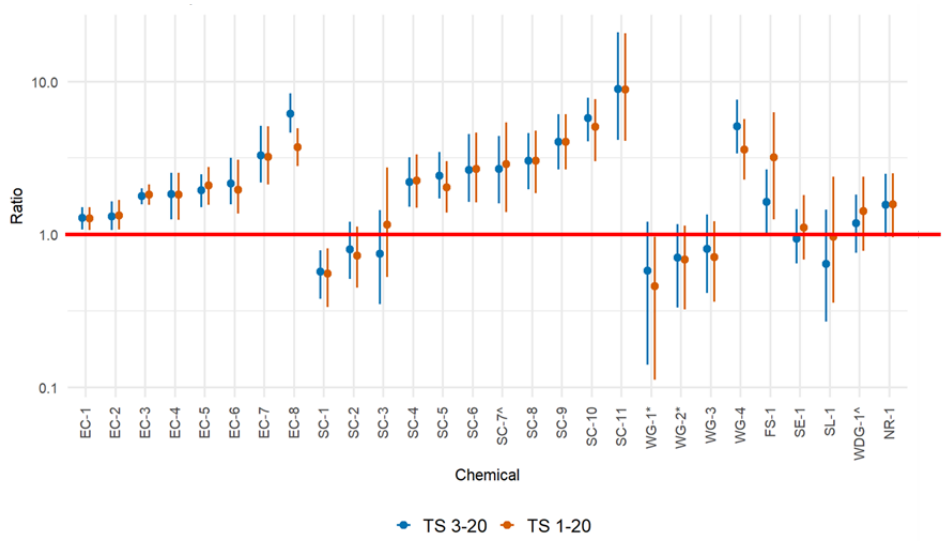
Curated Dataset

Prior result	COR	II	III	IV
COR	86.3%	4.2%	7.1%	2.5%
II	14.1%	44.9%	20.5%	20.5%
III	6.9%	5.2%	53.6%	34.3%
IV	0.9%	2.0%	9.1%	88.0%

Dermal Absorption Analyses

Allen et al. 2021 ALTEX

- Absorption through in vitro human skin was found to be similar to, or less than, that observed in rat skin (in vitro and in vivo) for all formulations.
- The human in vitro assay provided a similar or higher estimate of dermal absorption than the triple pack
- For human health risk assessment, in vitro assays using human skin would be preferable. Such tests would be directly relevant to the species of interest (humans) and avoid any overestimation of dermal absorption using rat models.

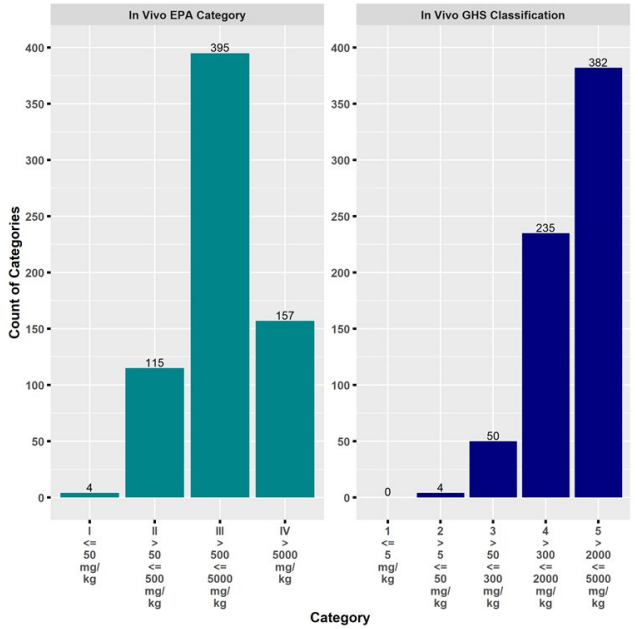


- However, rat in vitro studies would still have utility if human in vitro data were not available.
- In vitro rat data provide estimates of dermal absorption that are at least as protective as in vivo rat data, and thus could also be considered adequate for use in establishing dermal absorption factors.

$$triple\ pack\ DAF = rat\ in\ vivo \times (human\ in\ vitro \div rat\ in\ vitro)$$

Acute Toxicity Mixtures Equation Analyses

- GHS Mixtures Equation - mathematical approach to calculating toxicity of mixtures based on components
- Compare LD50s predicted for formulations based on the GHS Mixtures Equation to those determined from in vivo results with the complete formulation.
- Data set consisted of 671 formulations produced by eight companies:
  - 51 antimicrobial cleaning products (AMCPs), 620 agrochemical formulations



In vivo Classification	EPA Additivity Classification				Within-class Concordance
	I	II	III	IV	
I	3	1	0	0	75%
II	4	30	61	20	26%
III	1	34	197	163	50%
IV	0	1	19	137	87%
Total	8	66	277	320	**55%**

In vivo Classification	GHS Additivity Classification					Within-class Concordance
	1	2	3	4	5/NC	
1	0	0	0	0	0	NA
2	0	3	1	0	0	75%
3	0	4	10	26	10	20%
4	0	0	17	134	85	57%
5/NC	0	1	4	39	337	88%
Total	0	8	32	199	432	72%

- 79% (128/163) of “discordant” substances (EPA Cat III predicted as Cat IV, yellow highlight) had in vivo LD50 values measured between 2000 and 5000 mg/kg or a limit test LD50 > 2000 mg/kg.

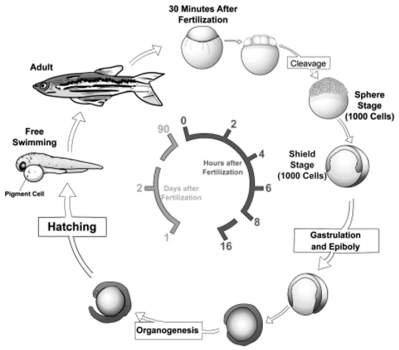
Supplementary Analysis & Conclusions

In vivo LD_{50}	Additivity LD_{50} Prediction (mg/kg)			Within-class Concordance
	≤50	>50 to ≤500	>500	
≤50	3	1	0	75%
>50 to ≤500	4	30	81	26%
>500	1	35	514	93%
Total	8	66	595	82%

- Precautionary statements and associated PPE are much more stringent with LD50 < 500 mg/kg; supplementary analysis combined all substances with LD50 > 500 mg/kg together.

- Most “discordant” substances had in vivo LD50s values measured between 2000 and 5000 mg/kg or a limit test LD50 > 2000 mg/kg.
- When considering formulations with LD50 >500 mg/kg together, overall concordance increased from 55% to 82%.
- Within-class concordance for less toxic substances was consistently over 85% regardless of classification system.
- Animal tests are inherently variable. Similar underclassification could also be observed following a repetition of the animal test.
- Our results suggest the mixtures equation is promising for identifying substances that would not be expected to induce toxicity.
- However, the lack of more toxic formulations in the dataset preclude us from reaching definitive conclusions across the spectrum of hazard categories.

SEAZIT: Systematic Evaluation of the Application of Zebrafish in Toxicology



SEAZIT Goals:

- Provide the scientific basis on which to make a programmatic decision on the further routine use of zebrafish in toxicological evaluation of chemicals
- Provide fundamental knowledge on the use of zebrafish in toxicology, which will support further research endeavors by the academic community

SEAZIT Activities:

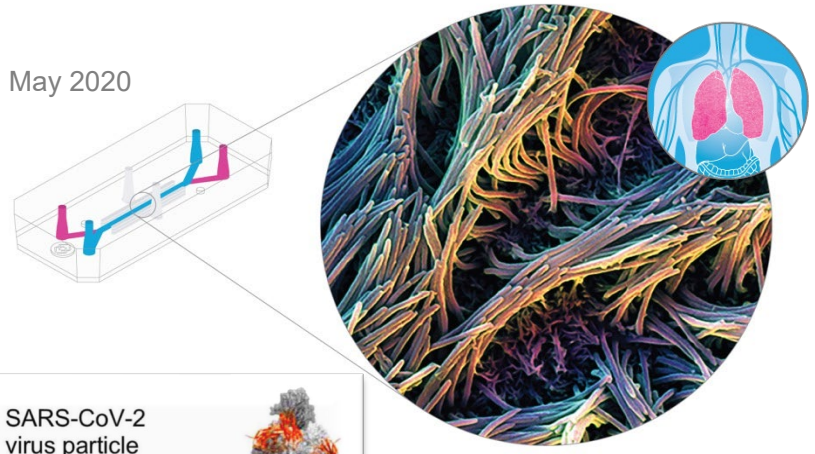
- Inter-laboratory study to compare the impact of **chorion-on v. chorion-off and single v. repeat exposure**
- Reference phenotype atlas for zebrafish screening assays along with a means of differentiating abnormal from normal

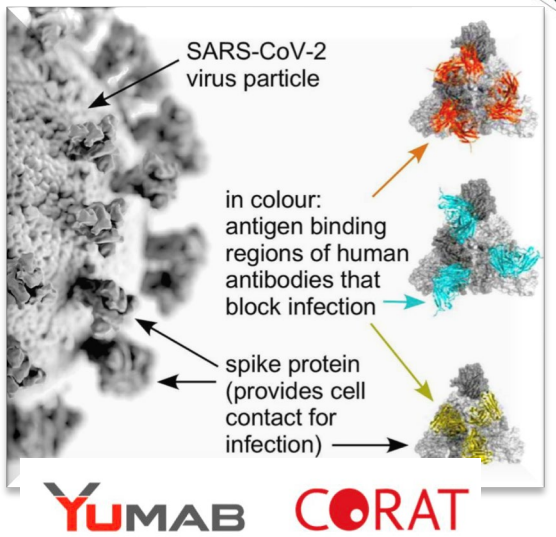
	**Normal** Body axis of larvae is linear from snout to caudal fin
	**Normal** Body axis of larvae is linear from snout to caudal fin
	**Borderline** Slight curvature in body axis
	**Mild** Slight curvature in body axis
	**Moderate** Noticeable curvature in body axis
	**Severe** Noticeable bend in body axis. Trunk is at a 90 degree angle to the rest of the axis.

https://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/dev-tox/seazit/index.html

MPSCoRe: Microphysiological Systems for COVID-19 Research

Emulate, Inc. | May 2020

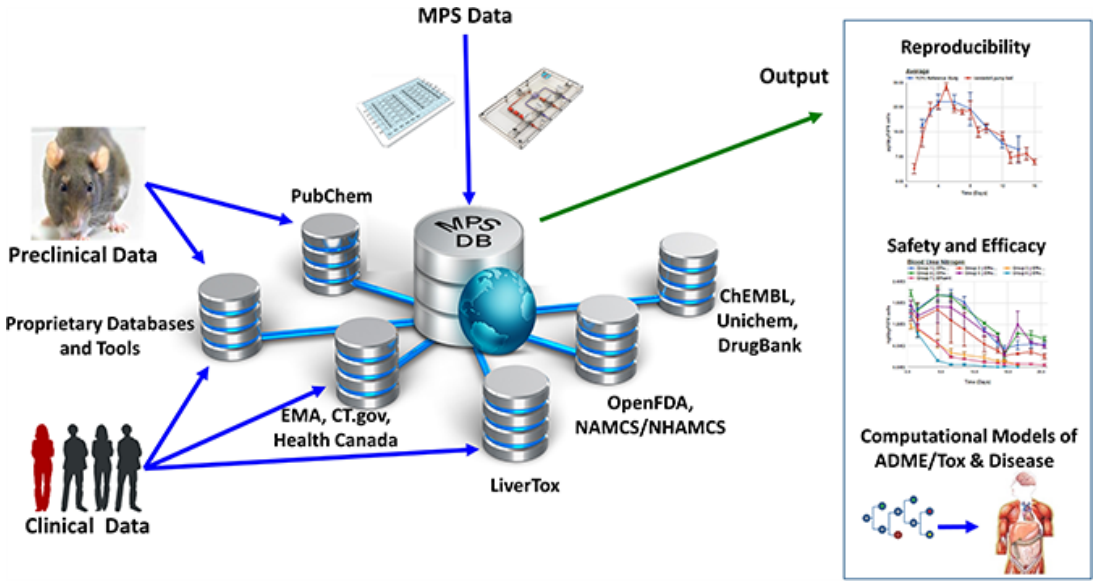




Establish organ-on-chip technology at the NIAID Integrated Research Facility

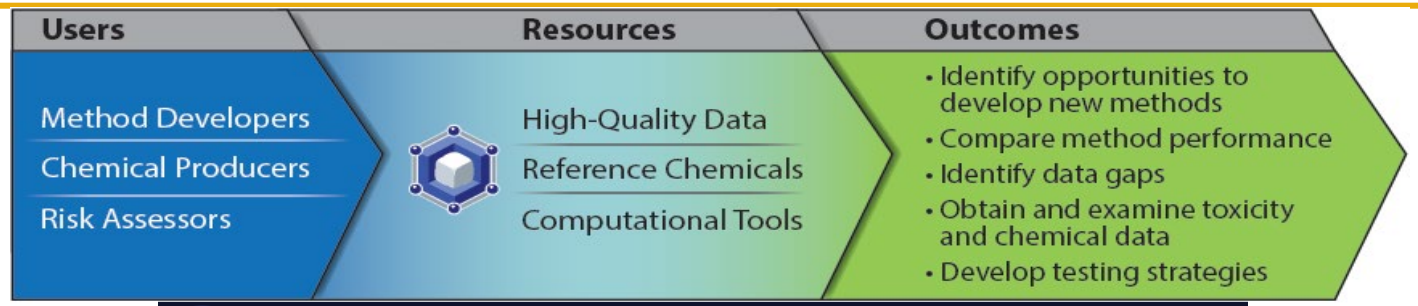
Joint working group to support global COVID-19 tissue chip research activities
Partnership with UK NC3Rs, DoD, NIAID, NCATS.

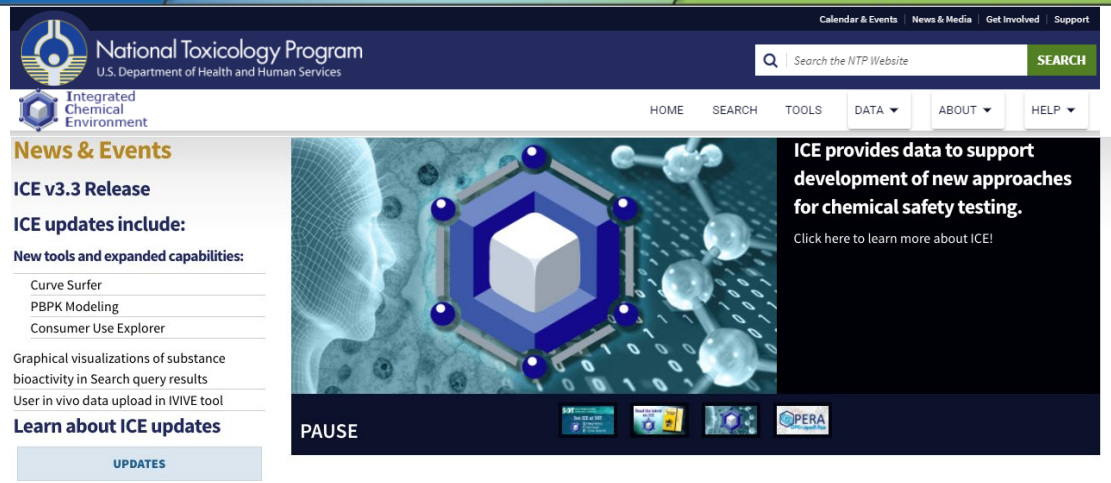
https://ntp.niehs.nih.gov/go/mps

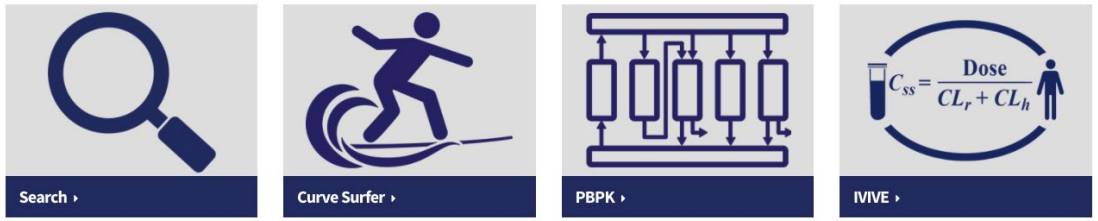


Development of a COVID-19 Disease Portal in the Microphysiology Systems (MPS) Database (University of Pittsburgh)

Integrated Chemical Environment: ICEv3.3



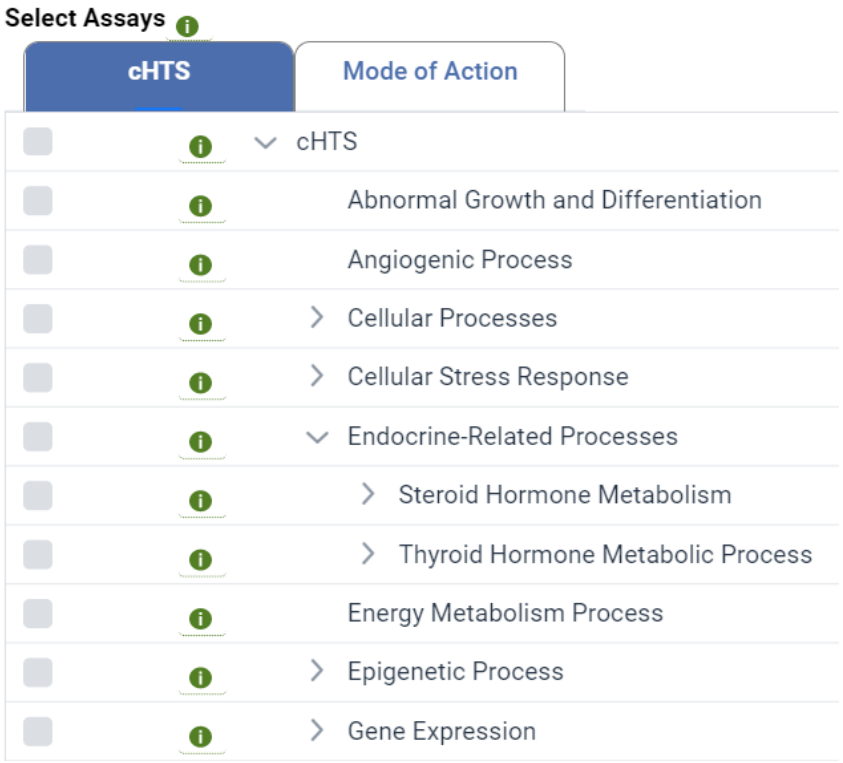


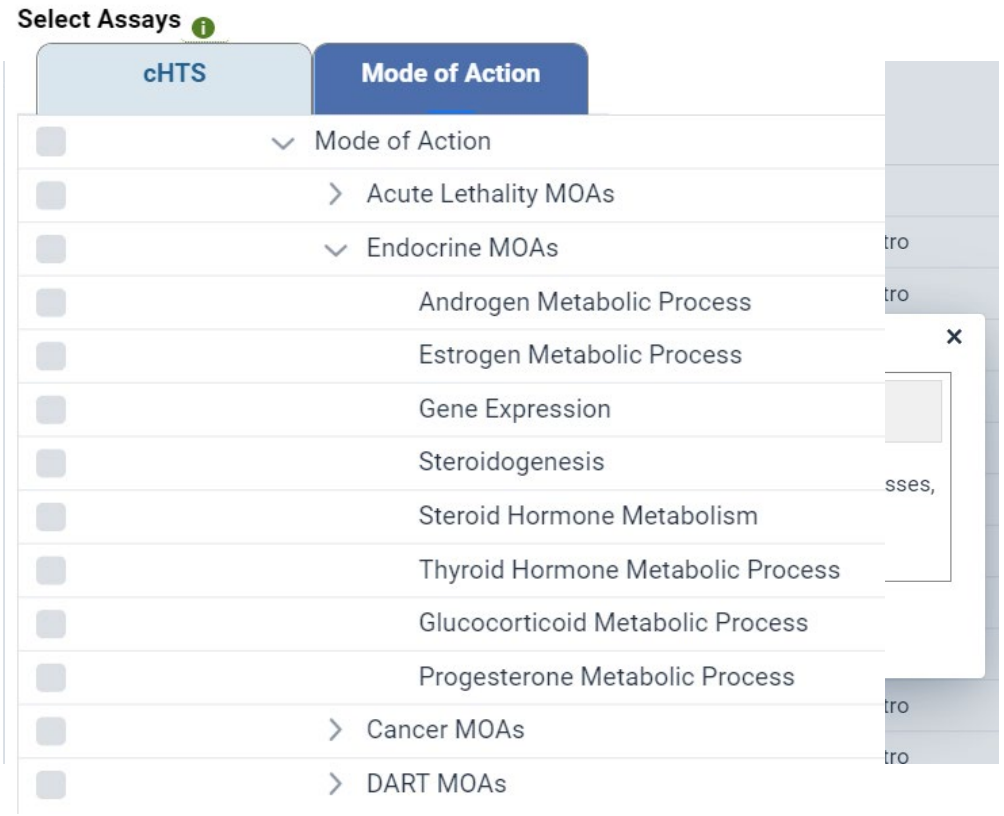


https://ice.ntp.niehs.nih.gov/

Bell et al. 2017 EHP
Bell et al. 2020 Tox In Vitro

Curation to assist meaningful assay selection and model building





- Curated high-throughput screening data (cHTS) starts with EPA invitrodb and incorporates chemical QC information and technology-specific flags
- Assays are grouped by biological process, mechanistic target, and MoA, and linked to ontologies

Curve Surfer is an interactive concentration response visualization tool for cHTS data

- Select/filter assays based on Mechanistic Target
- View specific assays/chemicals
- Filter on activity call, AC50

Integrated Chemical Environment

HOME SEARCH TOOLS DATA ABOUT HELP

Curve Surfer | PBPK | IVIVE | Chemical Characterization

Input
Results
Report an Issue

The Curve Surfer tool allows you to view and interact with concentration response curves from cHTS.

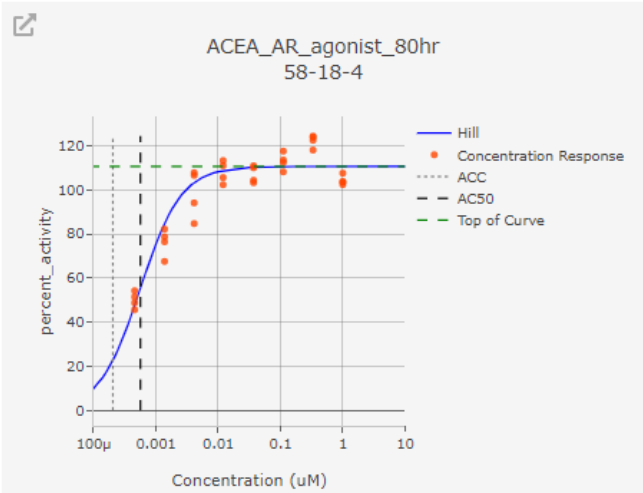
Select Page 1 of 21

Order By: Chemical Name, Asc

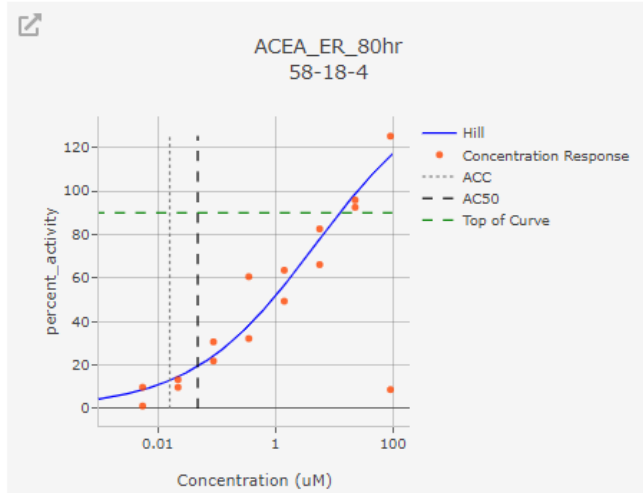
Only showing curves for 200 chemicals. Please reduce your query to view all chemicals.

Select Mechanistic Target To View Curves: All | Assay Text Fil... | Select Assay(s): 0 values | Select CASRN(s): 0 values | Select Call(s): Active, Choose...

Assay: ACEA_AR_agonist_80hr
Mechanistic Target: Androgen Metabolic Process
CASRN: 58-18-4
DTXSID: DTXSID1033664
Chemical Name: 17-Methyltestosterone
Winning Curve-Fit Model: Hill
AC50: 5.7E-4
ACC: 2.0E-4
Top of Curve: 110.66
Call: Active
View EPA curve (testing purposes only)

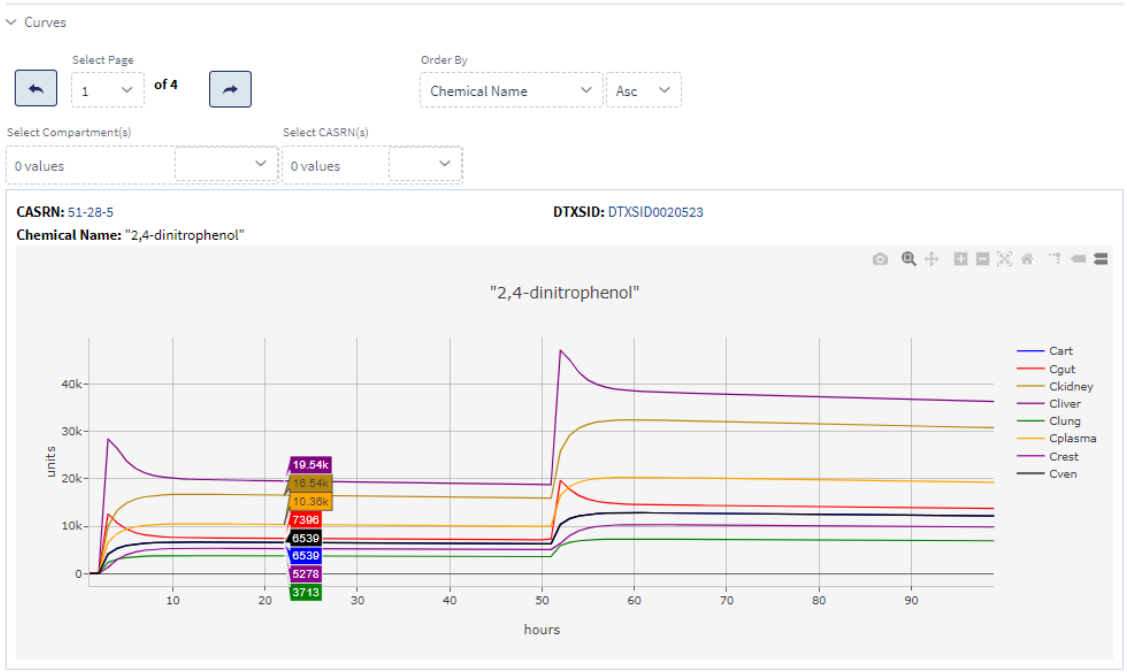


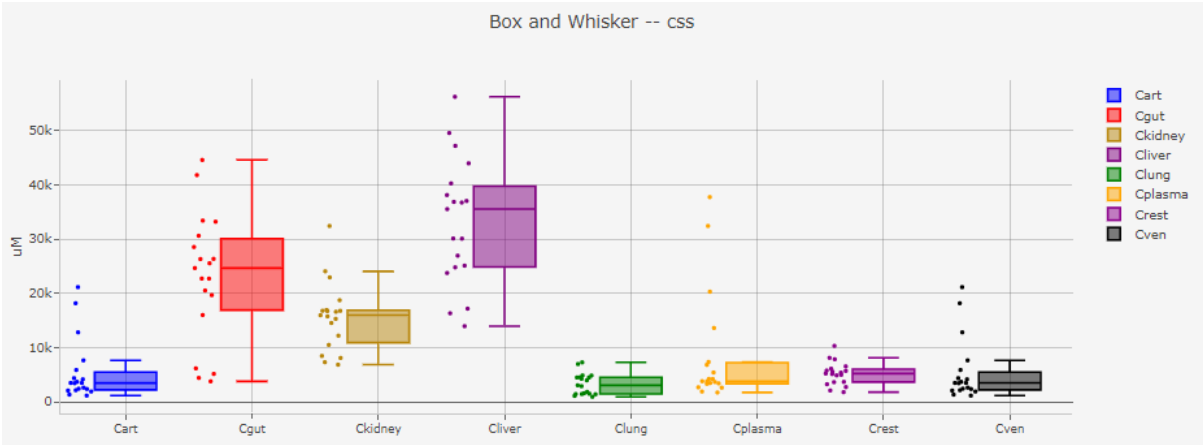
Assay: ACEA_ER_80hr
Mechanistic Target: Estrogen Metabolic Process
CASRN: 58-18-4
DTXSID: DTXSID1033664
Chemical Name: 17-Methyltestosterone
Winning Curve-Fit Model: Hill
AC50: 0.048
ACC: 0.016
Top of Curve: 89.94
Call: Active
View EPA curve (testing purposes only)

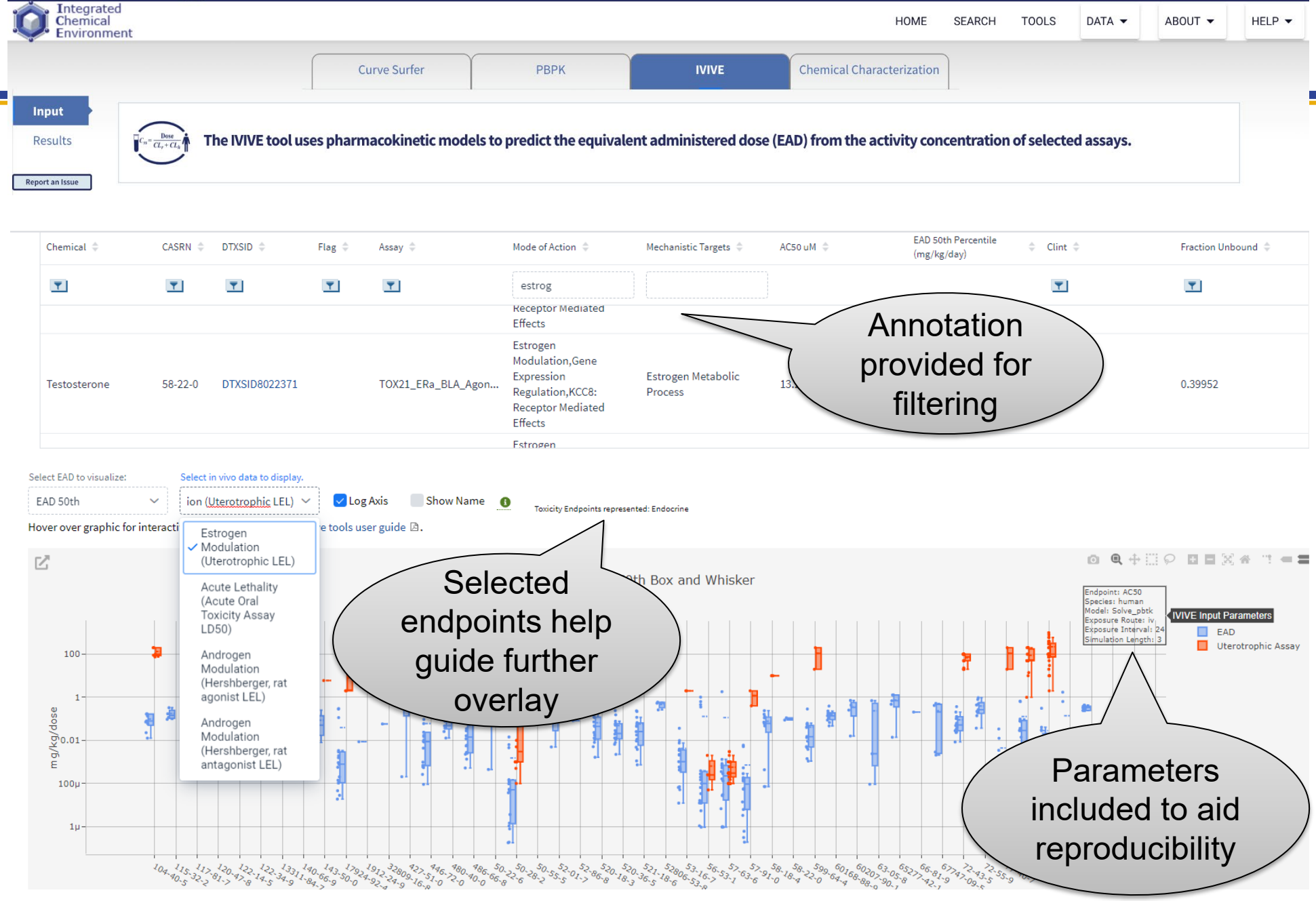


PBPK tool allows users to calculate internal chemical concentrations using PBPK models from the EPA httk R package and in-house code

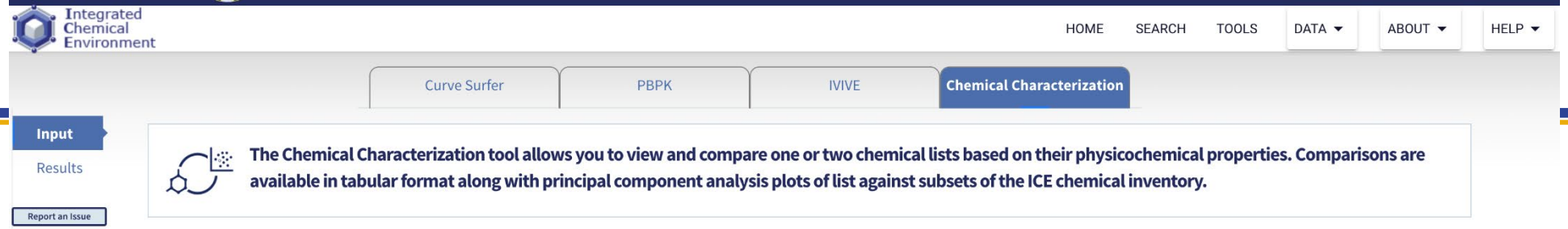
- Tissue level concentrations
- View individual chemical curves
- View overall distribution in different tissue compartments for all query chemicals





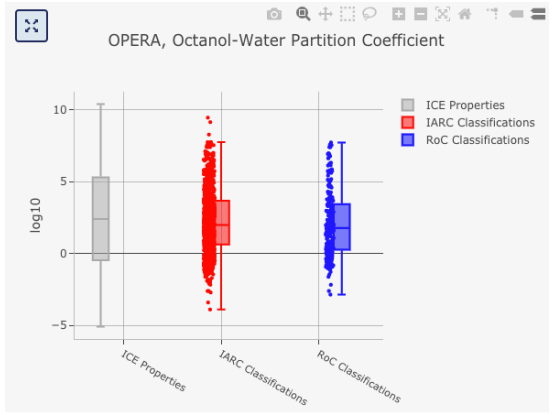


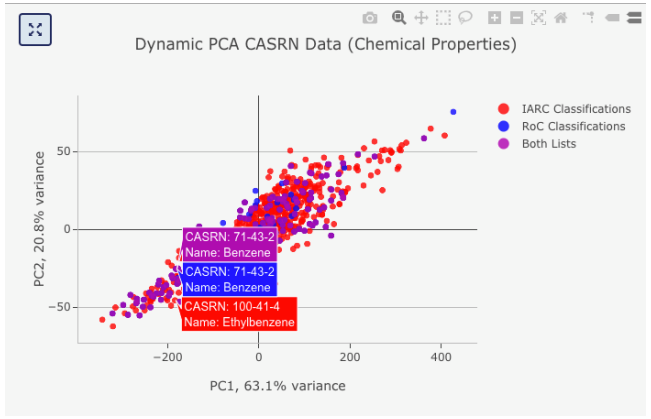
Transparency and annotation to help guide use and interpretation

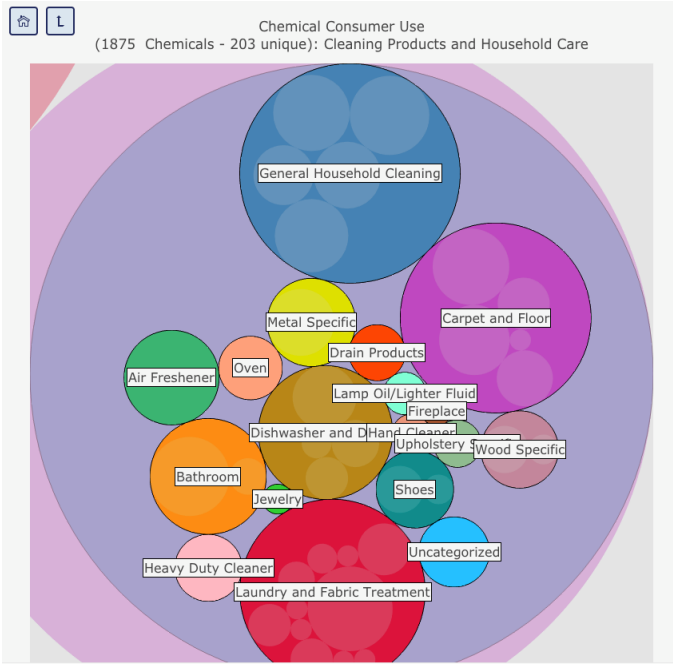


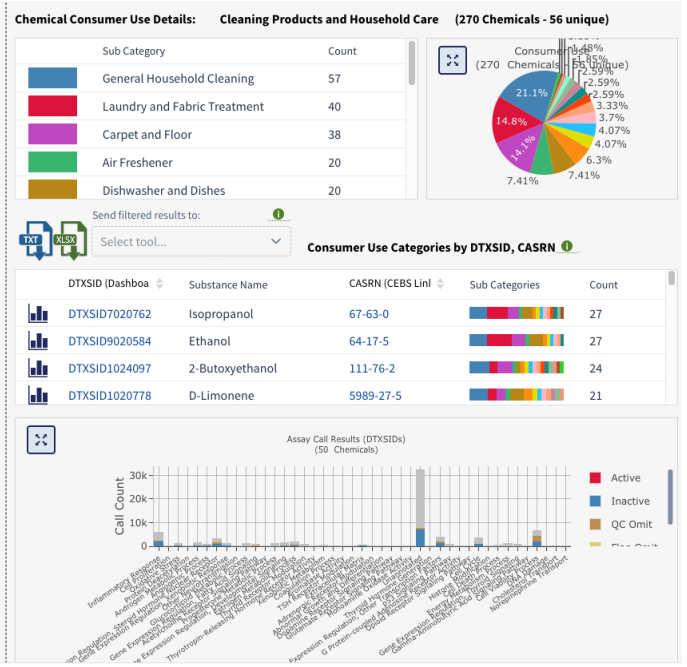
Chemical Characterization tool allows users to explore one or two chemical lists.

- Physicochemical property distributions
- Interactive PCA plots of chemical space coverage
- Presence in consumer products (EPA CPDat)









Acknowledgments: The NICEATM Group

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شكراً جزيلاً
ngiyabonga
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謝謝
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teşekkür ederim
Баярлалаа
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kia ora
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tack
спасибо
faafetai lava
vinaka
спасибі
blagodaram
dank je
misaotra
matondo
paldies
grazzi
mahalo
tapadh leat
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nanni
nandri
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tenki
bedankt
bayarlalaa
gràcie
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তোমাকে ধন্যবাদ
감사합니다
xièxie
merci

Questions?