

# NICEATM Update

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ICCVAM Public Forum

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- ICE 2.0 and tools
  - CATMoS
  - IVIVE
  - Determining pKa from Chemical Structure
- OECD Skin Sensitization Project
- Automating Reference Data Identification for Developmental Toxicity
- Alternatives for Rabies Vaccine Testing
- Implementation of the Monocyte Activation Test for Medical Devices
- Alternatives for Botulinum Neurotoxin Testing



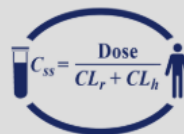
# Integrated Chemical Environment

- Updated Search
- Expanded IVIVE
- Data Updates

The screenshot shows the homepage of the National Toxicology Program's Integrated Chemical Environment. At the top, there is a navigation bar with links for 'Calendar & Events', 'News & Media', 'Get Involved', and 'Support'. Below this is the NTP logo and the text 'National Toxicology Program U.S. Department of Health and Human Services'. A search bar is located on the right side of the header. Below the header is a secondary navigation bar with links for 'HOME', 'SEARCH', 'TOOLS', 'DATA', 'ABOUT', and 'HELP'. The main content area features a 'News & Events' section with the headline 'ICE 2.0 is here!' and a sub-headline 'The updated ICE release includes expanded workflows, new predictions from OPERA and improved search.' Below this is a button labeled 'Learn about ICE updates' and a 'UPDATES' button. To the right of the text is a large image showing a human head profile with a grid overlay, a molecular structure, and a cell. Below the image is a 'PAUSE' button and three small thumbnail images. To the right of the image is a text box that reads 'ICE provides data to support development of new approaches for chemical safety testing. Click here to learn More about ICE!'.



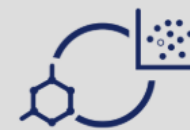
Search ›



IVIVE ›



Machine Learning ›



Chemical Characterization ›



# Assay Selection

Integrated Chemical Environment

HOME SEARCH TOOLS DATA ABOUT HELP

Chemicals Mixtures

Input

Results

Run Search Clear

Select Assays

Assay	Description	Assay Type	
	OPERA LogD, p...	Phys Chem	in silico
	OPERA fu	Phys Chem	in silico
	Hershberger, Ag...	Endocrine	in vivo
	Androgen Rece...	Tox21	in vitro

Union or Intersection

Union x v

Add Chemicals with same QSAR-structure

+ 1 chemical quick list selected.

Enter one CASRN per l

Select Assays

Endocrine x v

Select All Deselect All

∨ Androgen			<input type="checkbox"/>
Hershberger, Agonist rat	in vivo		<input checked="" type="checkbox"/>
Hershberger, Antagonist rat	in vivo		<input type="checkbox"/>
AR Binding	in vitro		<input type="checkbox"/>
AR Transactivation-Agonist	in vitro		<input type="checkbox"/>
AR Transactivation-Antagonist	in vitro		<input type="checkbox"/>
AR Potency Category	in vitro		<input type="checkbox"/>
∨ TOX21 Androgen	in vitro		<input checked="" type="checkbox"/>
Androgen Receptor Pathway	in vitro		<input checked="" type="checkbox"/>
AR Pathway Model, Agonist	in silico		<input type="checkbox"/>
AR Pathway Model, Antagonist	in silico		<input type="checkbox"/>
∨ Estrogen			<input type="checkbox"/>

Finished

Data updates include:

- New OPERA models for ADME and physchem properties
- Hershberger assay data



# Updated Search Table

Chemicals Mixtures

Input

Results



**Selected Assays: Hershberger, Agonist rat, Androgen Receptor Pathway, AR Pathway Model, Agonist, OPERA CLint**

**Selected Reference Lists: AR In Vitro Agonist 2016 (R), AR In Vivo Agonists 2018**

Download

Query Formulations

Clear Filter

Number of chemicals = 65.

QSAR Match	Substance Name	CASRN	DTXSID	Qsar Ready ID	Hershber... Agonist rat LOEL mg/kg/day	Hershber... Agonist rat NOEL mg/kg/day	Hershber... Agonist rat Call	Androgen Receptor Pathway Call (# Assays=11)	PhysChem Properties CLint uL/min/... hepatocy...
YES	Metalaxyl-M	70630-17-0	DTXSID80...	ZQEIXNIJ... UHFFFAO... N				Inactive:U...	0.218
	Metalaxyl	57837-19-1	DTXSID60...	ZQEIXNIJ... UHFFFAO... N		375	inactive	Active:cou...	0.218
	Dichlorodi...	50-29-3	DTXSID40...	YVGGHN... UHFFFAO... N				Active:cou...	0.56

New! Add chemicals with the same QSAR structure to query



# Expanded IVIVE Tools

Integrated Chemical Environment

HOME SEARCH TOOLS DATA ABOUT HELP

IVIVE Machine Learning Chemical Characterization

Input Results

### IVIVE Workflow Input

The IVIVE tool uses pharmacokinetic models to predict the daily equivalent administered dose from activity concentration of selected assays.

Run Workflow

+ Select Chemical Quick List

Enter one CASRN per line.

Select in vitro endpoint:

- AC50
- 1C
- Glu PBPK
- Solve\_3comp

Select model:

Solve\_3comp

Select Assays

- Androgen Receptor Pathway
- Cell Cycle
- Cytochrome P450
- Cytotoxicity (burst)
- Cytotoxicity (burst + stress)
- Estrogen Receptor Pathway
- G Protein-Coupled Receptors
- Mitochondrial
- Nuclear Receptor
- Steroidogenesis
- All Other Tox21 Assays

Select Route

iv

Dosing Intervals, hours

24

Days of exposure, days

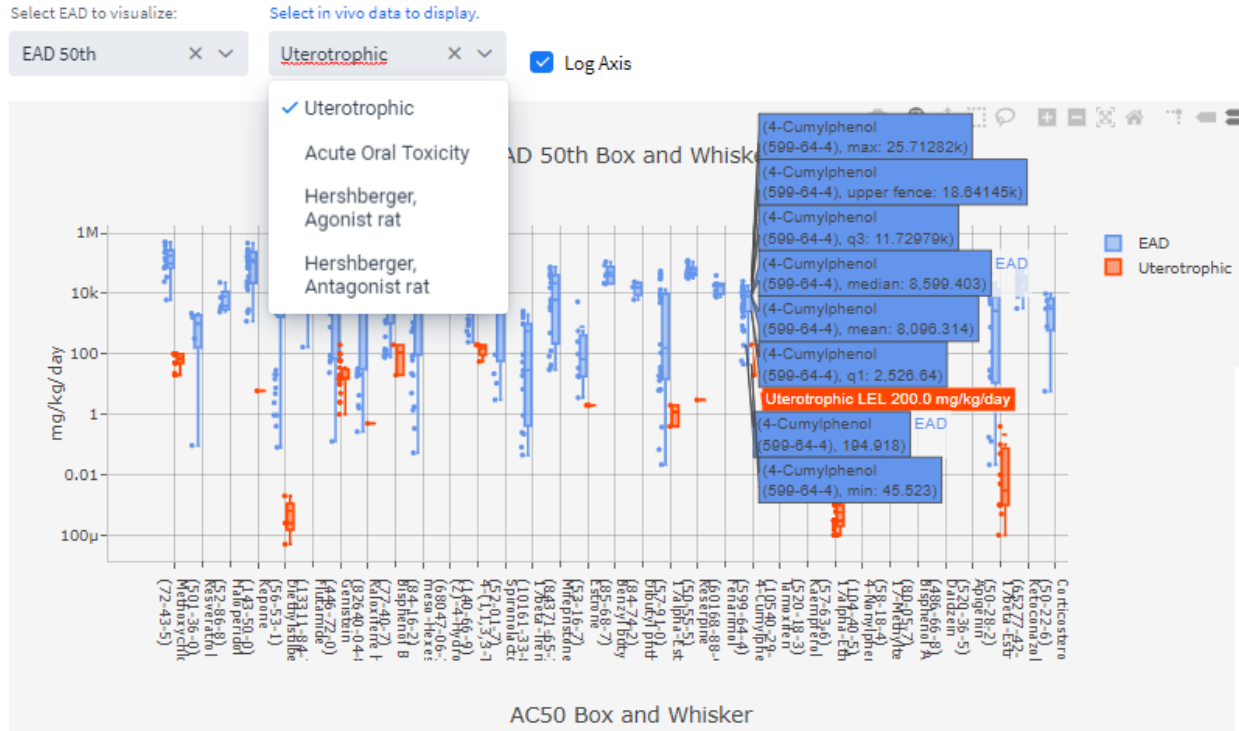
3

These models come from the US EPA [httk 1.9.2](#) package. For details see [Userguide](#)

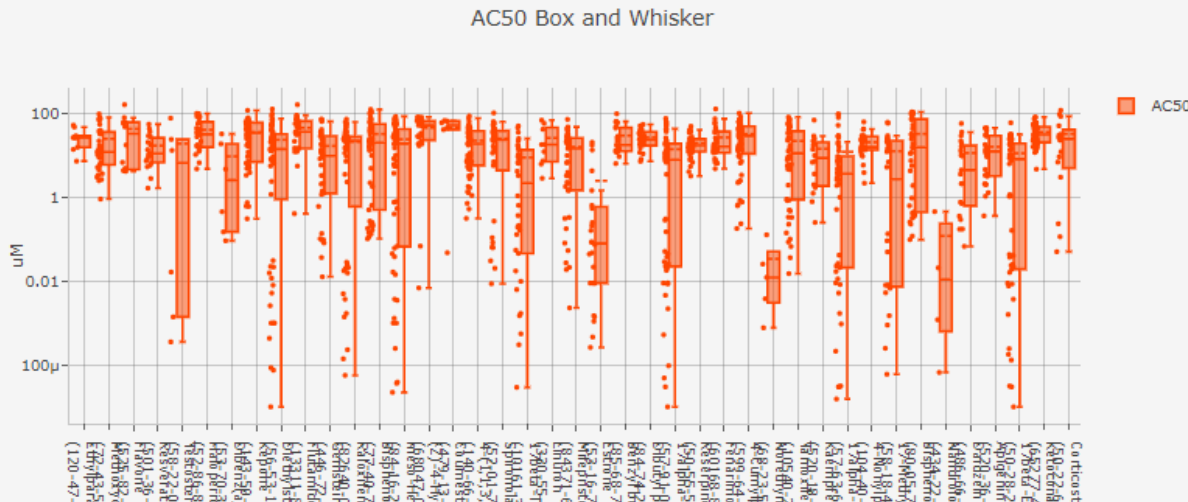
- IVIVE tool has been updated to include PBPK models
- Solve\_3comp from EPA's htkk package
  - Glucuronidation PBPK model in **BETA**
  - Currently limited to 50 chemicals per query



# Overlay In Vivo Data on IVIVE Results



- Overlay in vivo data
- Update the plots with assay filtering
- Download data to explore locally



assays: 22/85

assay

- ER|
- TOX21\_Era\_LUC\_BG1\_Antagonist
- TOX21\_Era\_BLA\_Agonist\_ratio
- OT\_ER\_EraERb\_0480
- OT\_Era\_EREGFP\_0480
- NVS\_NR\_mERa
- OT\_ER\_ERbERb\_1440
- TOX21\_Era\_BLA\_Antagonist\_ratio
- BSK\_CASM3C\_Proliferation\_down
- OT\_ER\_EraERa\_0480
- ATG\_FRa\_TRANS\_in

Close



# Global Collaborative Projects

## CERAPP

Collaborative Estrogen Receptor  
Activity Prediction Project (2015/16)

## CoMPARA

Collaborative Modeling Project for Androgen  
Receptor Activity (2017/18)

## CATMoS

Collaborative Acute Toxicity Modeling Suite  
(2018/19)



Endocrine Disruptor Screening Program (EDSP)



ICCVAM Acute Toxicity Workgroup

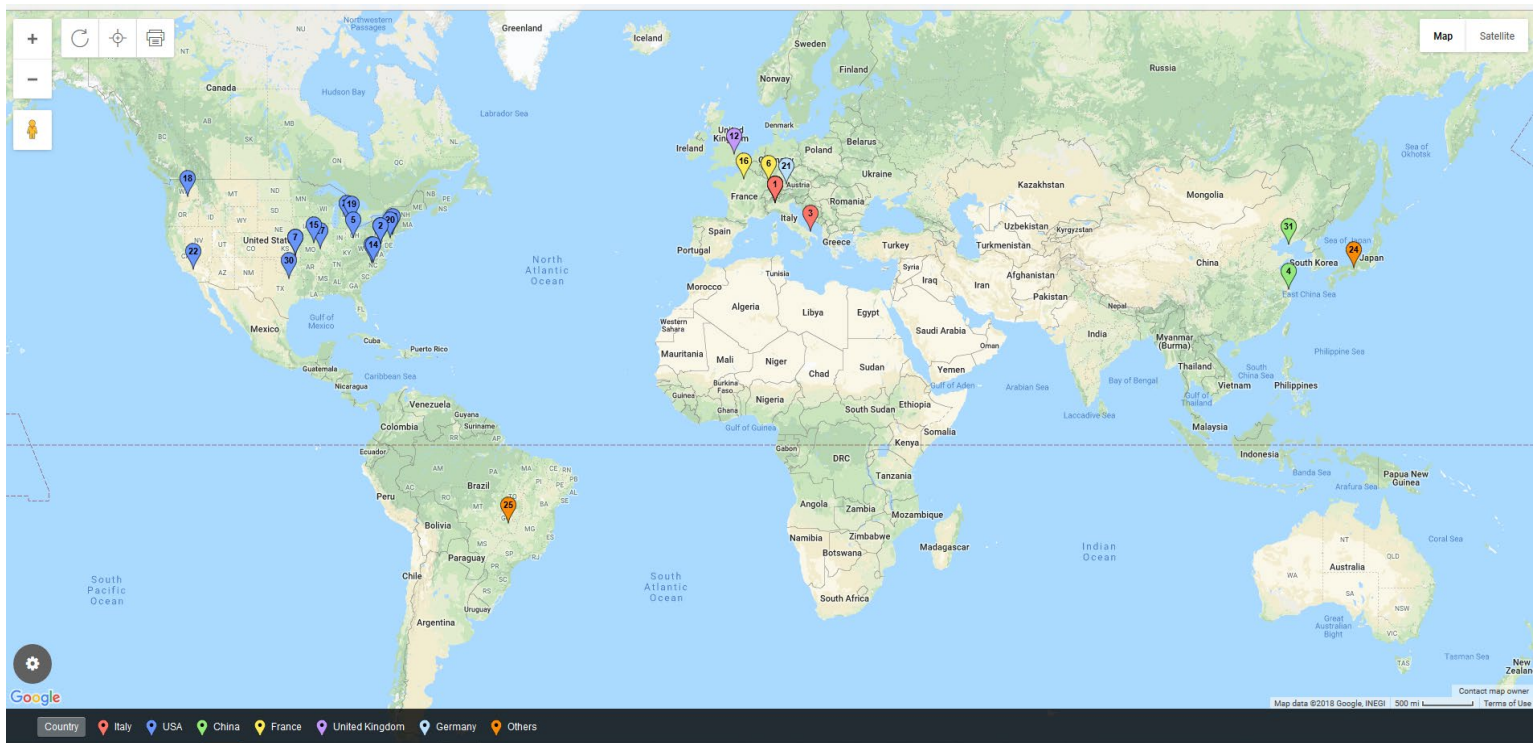
Over 100 international participants representing academia, industry, and government contributed.





## Consortium:

- **35 Participants/Groups** from around the globe representing academia, industry, and government contributed



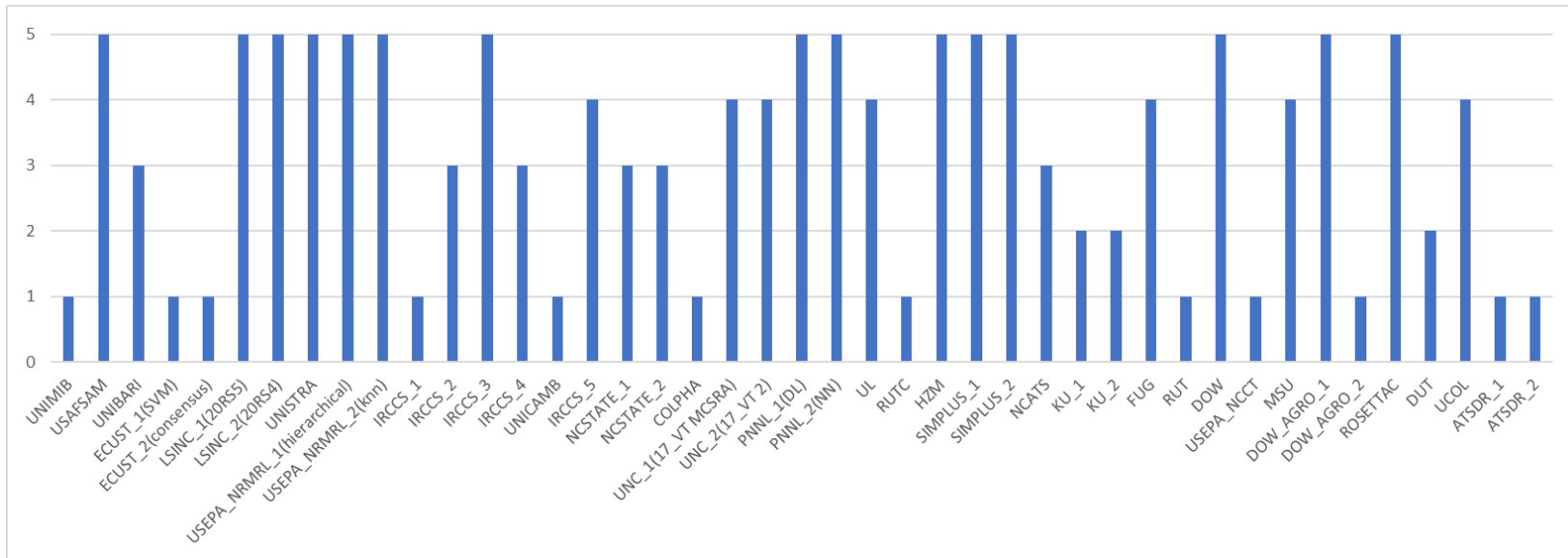
(<https://batchgeo.com/map/d06c5d497ed8f76ecfee500c2b0e1dfa>)



# CATMoS Submitted Models

- GHS categories: 23 models
- Very Toxic: 32 models
- Non-toxic: 33 models
- EPA categories: 26 models
- LD50: 25 models

**Total: 139 models**





## Qualitative evaluation:

- Documentation
- Defined endpoint
- Unambiguous algorithm
- Availability of code
- Applicability domain definition
- Availability of data used for modeling
- Mechanistic interpretation

## Quantitative evaluation:

- Goodness of fit: training statistics
- Evaluation set predictivity: statistics on the evaluation set
- Robustness: balance between (Goodness of fit) & (Test set predictivity)

$$S = 0.3 * (\textit{Goodness of fit}) + 0.45 * (\textit{Test set predictivity}) + 0.25 * (\textit{Robustness})$$

### Categorical models (binary and multi-class):

$$\textit{Goodness of fit} = 0.7 * (BA_{Tr}) + 0.3 * (1 - |Sn_{Tr} - Sp_{Tr}|)$$

$$\textit{Test set predictivity} = 0.7 * (BA_{Tst}) + 0.3 * (1 - |Sn_{Tst} - Sp_{Tst}|)$$

$$\textit{Robustness} = 1 - |BA_{Tr} - BA_{Tst}|$$

### Continuous models:

$$\textit{Goodness of fit} = R_{Tr}^2$$

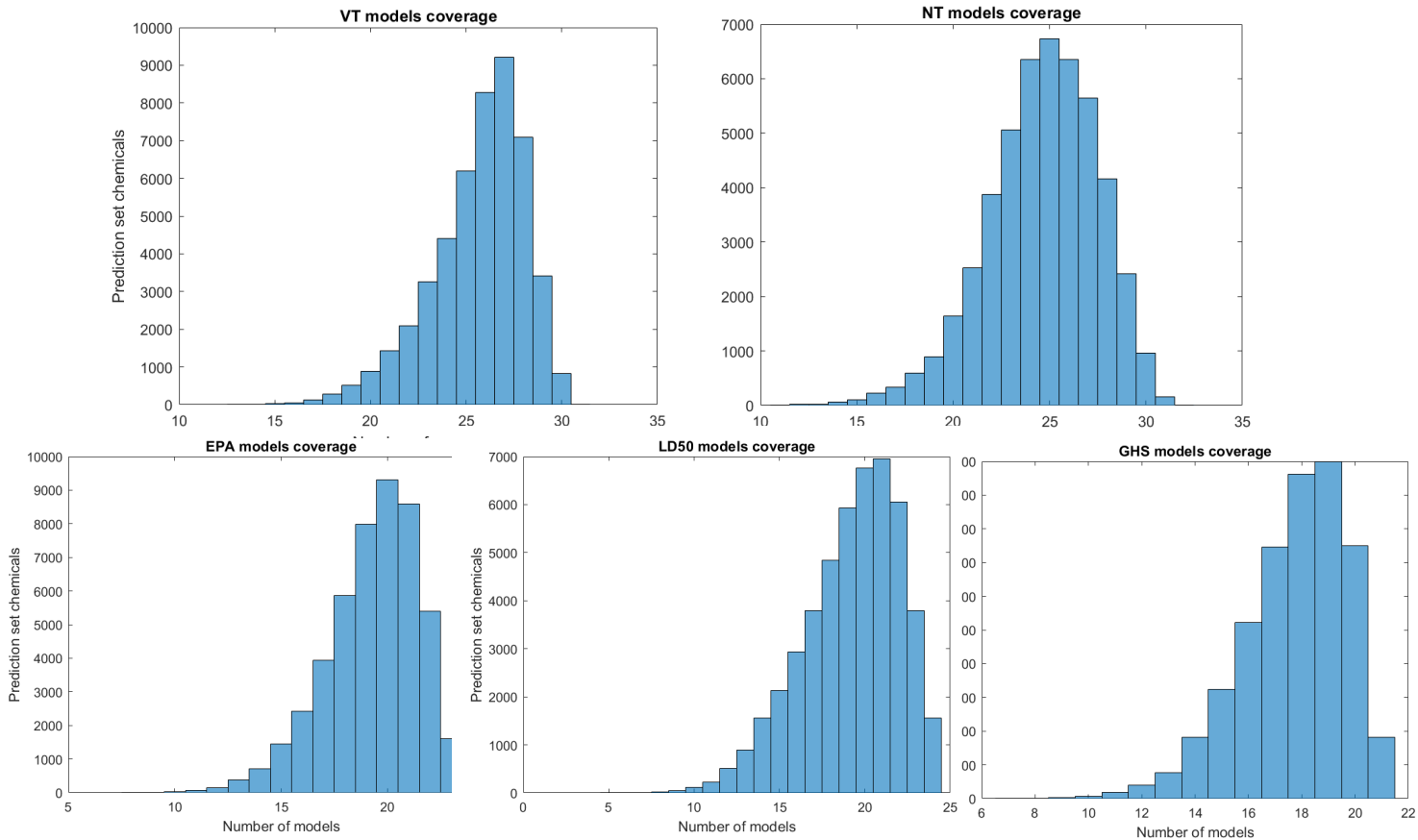
$$\textit{Test set predictivity} = R_{Tst}^2$$

$$\textit{Robustness} = 1 - |R_{Tr}^2 - R_{Tst}^2|$$



# Coverage of the models

## Distribution of the number of models/chemical





# Generation of Consensus Predictions

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- Models passing qualitative evaluation (requirement for transparency; description of approach was sufficient)
- Integrating only *in-domain* predictions across chemicals in the prediction set (48,137 chemicals) for each model, respectively
  - Categorical models: weighted majority rule
  - Continuous model: weighted average



# Performance Assessment

## CATMoS Consensus Model Statistics

	Very Toxic		Non-Toxic		EPA		GHS	
	Train	Eval	Train	Eval	Train	Eval	Train	Eval
Sensitivity	0.87	0.67	0.93	0.70	0.73	0.50	0.63	0.45
Specificity	0.94	0.96	0.96	0.88	0.96	0.91	0.91	0.92
Balanced Accuracy	0.93	0.81	0.94	0.79	0.83	0.71	0.77	0.68
<b><i>In vivo</i> Balanced Accuracy</b>	0.81		0.89		0.82		0.79	

	LD50 values		LD50 values
	Train	Eval	<i>In Vivo</i>
R2	0.84	0.64	0.80
RMSE	0.32	0.51	0.42

The consensus predictions perform as well as replicate *in vivo* data at predicting oral acute toxicity outcome



## Generalized CATMoS models: datasets

- LD50: 28954
- VT: 23767
- NT: 30971
- EPA: 25487
- GHS: 25720

- High concordance among models
- Proportional distribution of:
  - LD50 values
  - VT/NT classes
  - EPA/GHS categories

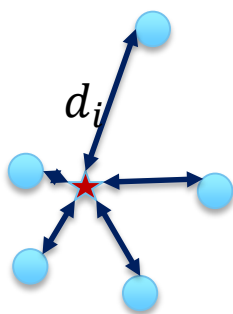


- Split into 75% training and 25% test set
- Calculate PaDEL & CDK2 descriptors
- Dimensionality reduction (missing values & low variance)
- Feature selection (most relevant descriptors for each endpoint)

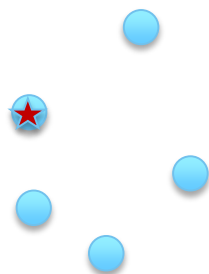


# Consensus implementation

## Generalized CATMoS models: new chemical predictions



$$d_1 \neq 0$$
$$w_i = f(d_i)$$
$$Pred_i = f(w_i, N_i)$$



$$d_1 = 0$$
$$Pred_i = N_i$$

★ New chemical to be predicted

● Nearest neighbors ( $N_i$ )

$d_i$ : Euclidean distance based on the selected descriptors for each endpoint

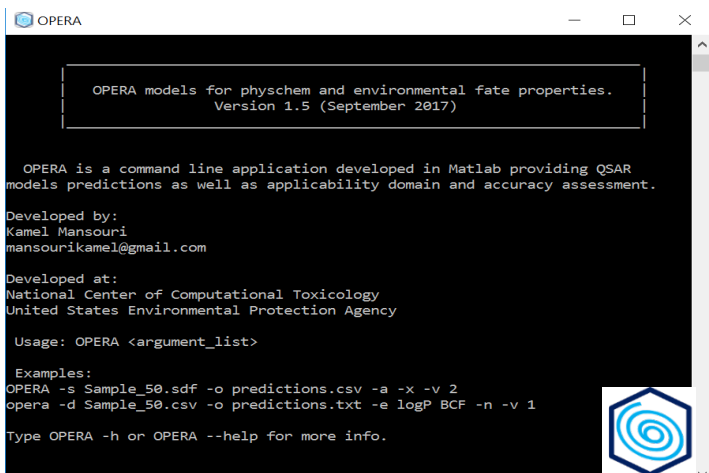
➔ Automated, weighted-endpoint dependent read-across: weighted kNN





# Running CATMoS Consensus models

## OPERA Standalone app



```
OPERA
-----
OPERA models for physchem and environmental fate properties.
Version 1.5 (September 2017)

OPERA is a command line application developed in Matlab providing QSAR
models predictions as well as applicability domain and accuracy assessment.

Developed by:
Kamel Mansouri
mansourikamel@gmail.com

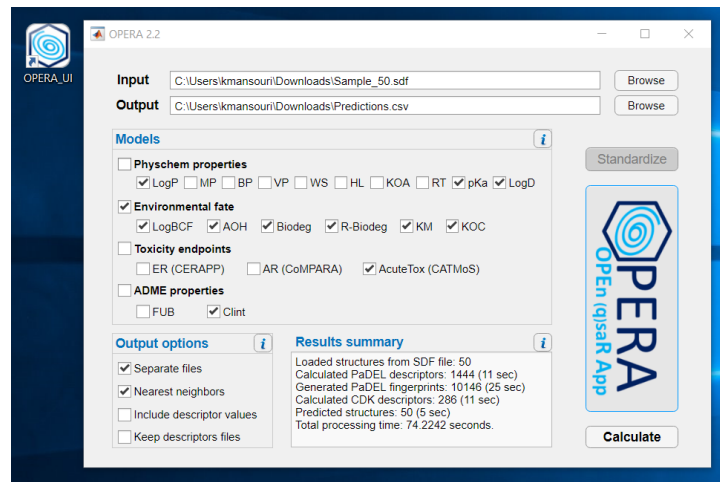
Developed at:
National Center of Computational Toxicology
United States Environmental Protection Agency

Usage: OPERA <argument_list>

Examples:
OPERA -s Sample_50.sdf -o predictions.csv -a -x -v 2
opera -d Sample_50.csv -o predictions.txt -e logP BCF -n -v 1

Type OPERA -h or OPERA --help for more info.
```

### Command line



### Graphical user interface

- Free, open-source & open-data
- Single chemical and batch mode
- Multiple platforms (Windows and Linux)
- Embeddable libraries (java, C, C++, Python)

Mansouri et al. J Cheminform (2018). <https://doi.org/10.1186/s13321-018-0263-1>

<https://github.com/NIEHS/OPERA>



# Available on ICE and EPA CompTox dashboard

<https://ntp.niehs.nih.gov/>

**National Toxicology Program**  
U.S. Department of Health and Human Services

Integrated Chemical Environment

Chemicals

Input  
Results

Assay	Description	Assay Type
NHK NRU	Acute Oral Toxicity	in vitro
3T3 NRU	Acute Oral Toxicity	in vitro

Select EAD to visualize: EAD 95th  
Select in vivo data to display: Acute Oral Toxicity  
 Log Axis

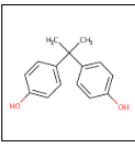
EAD 95th Box and Whisker

Search • NIVE • Machine Learning • Chemical Characterization •

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

**EPA** United States Environmental Protection Agency

Home Advanced Search Batch Search Lists Predictions



## Bisphenol A

80-05-7 | DTXSID702

Searched by DSSTox Substance Id.

Property  
Summary

Download Columns

Property	Experimental average	Predicted
LogP: Octanol-Water	3.32 (1)	3.29
Melting Point	155 (7)	139
Boiling Point	200 (1)	363
Water Solubility	5.26e-4 (1)	9.62e-4
Vapor Pressure	-	8.37e-7
Flash Point	-	190
Surface Tension	-	46.0
Index of Refraction	-	1.60
Molar Refractivity	-	68.2

DETAILS  
EXECUTIVE SUMMARY  
**PROPERTIES**  
ENV. FATE/TRANSPORT  
HAZARD  
▶ ADME  
▶ EXPOSURE  
▶ BIOACTIVITY  
SIMILAR COMPOUNDS  
GENRA (BETA)  
RELATED SUBSTANCES  
SYNONYMS



# OPERA predictions on EPA's CompTox dashboard

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

Chemistry Dashboard

OPERA Models: LogP: Octanol-Water

Bisphenol A  
80-05-7 | DTXSID7020182

Cc1ccc(O)cc1C(C)c2ccc(O)cc2

**Model Results**  
Predicted value: 3.35  
Global applicability domain: PASS  
Local applicability domain index: 0.88  
Confidence level: 0.75

Calculation Result for a chemical

**Model Performance**  
Model Performance with full QMRF

Weighted KNN model

6-fold CV (76%)		Training (76%)		Test (26%)	
Q2	RMSE	R2	RMSE	R2	RMSE
0.85	0.69	0.86	0.67	0.86	0.78

Nearest Neighbors from the Training Set

Chemical	Measured	Predicted
Bisphenol A	3.32	3.35
BUTANOIC ACID 2-(4-BIPHENYLYL)-3-HYDROXY-2-METHYL-	3.25	3.45
Flutoprotin	4.16	3.83
2,2-Diphenylpropionic acid	2.69	2.93
3-(4-(2-(4-BIPHENYLYL)HEXANOIC ACID	3.75	3.68

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Mansouri et al. OPERA models  
(<https://doi.org/10.1186/s13321-018-0263-1>)  
Williams et al. CompTox Chemistry Dashboard  
(<https://doi.org/10.1186/s13321-017-0247-6>)



## OPERA 1.5

### Physchem & Environmental fate:

Model	Property
<b>AOH</b>	Atmospheric Hydroxylation Rate
<b>BCF</b>	Bioconcentration Factor
<b>BioHL</b>	Biodegradation Half-life
<b>RB</b>	Ready Biodegradability
<b>BP</b>	Boiling Point
<b>HL</b>	Henry's Law Constant
<b>KM</b>	Fish Biotransformation Half-life
<b>KOA</b>	Octanol/Air Partition Coefficient
<b>LogP</b>	Octanol-water Partition Coefficient
<b>MP</b>	Melting Point
<b>KOC</b>	Soil Adsorption Coefficient
<b>VP</b>	Vapor Pressure
<b>WS</b>	Water solubility
<b>RT</b>	HPLC retention time

## New in OPERA2:

- Physchem properties:
  - General structural properties
  - pKa
  - Log D
- Toxicity endpoints
  - ER activity (CERAPP)  
<https://ehp.niehs.nih.gov/15-10267/>
  - AR activity (CoMPARA)  
<https://doi.org/10.13140/RG.2.2.19612.80009>
  - Acute toxicity (CATMoS)  
<https://doi.org/10.1016/j.comtox.2018.08.002>
- ADME properties
  - Plasma fraction unbound (FuB)
  - Intrinsic clearance (Clint)



# Open Source QSAR Models For pKa

- The logarithmic dissociation constant, pKa, strongly influences a chemical's pharmacokinetic and biochemical properties:
  - Reflects the ionization state of a chemical,
  - Affects lipophilicity, solubility, protein binding, tissue:plasma partition coefficients and blood-brain barrier.



pKa is important for ADMET properties, PBPK modeling and IVIVE

**Problem statement:**

No currently available free and transparent predictors for heterogeneous chemical classes



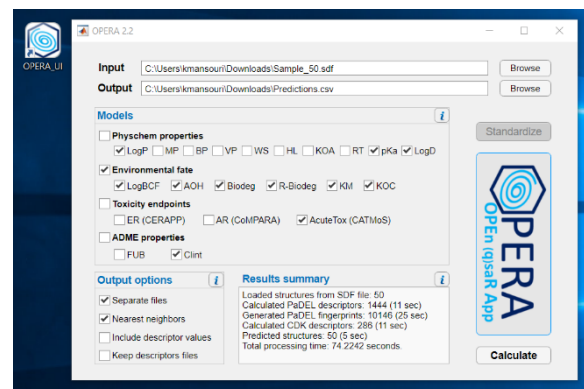
## Building free & open source models using free & open source tools:

- pKa values for 7912 chemicals collected from DataWarrior.
- Data curated and chemical structures were standardized for QSARs
- PaDEL software to calculate molecular descriptors and fingerprints.
- Several machine learning approaches were applied:
  - DNN: deep neural networks
  - SVM: support vector machine
  - XGB: extreme gradient boosting.
- Models were 5-fold cross-validated and evaluated against an external test set.
- The best models for each algorithm were compared to each other and to predictions from ACD/Labs and ChemAxon



# pKa project outcome

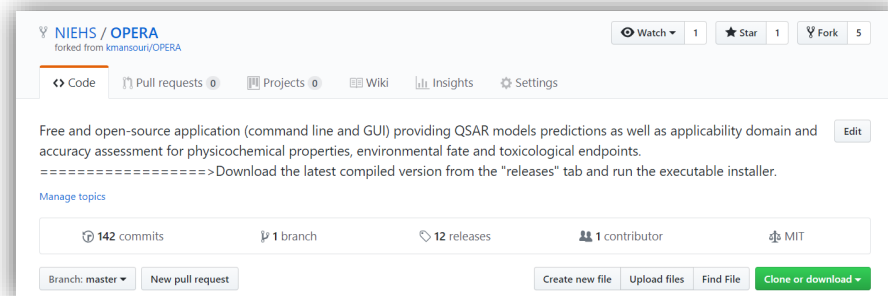
- Free and open source QSAR models for acidic and basic pKa
- Accuracy reaching an RMSE of 1.5 and an R<sup>2</sup> of 0.8
- Best models applied on DSSTox chemicals (~850k)
  - <https://ice.ntp.niehs.nih.gov/>
  - <https://comptox.epa.gov/dashboard>
- Manuscript submitted to Journal of Cheminformatics
- New chemicals can be predicted using OPERA (with applicability domain and accuracy estimates)



Code and executables freely available on

Github: <https://github.com/NIEHS/OPERA>

(Windows and Linux versions)





# Skin Sensitization Reference Databases

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- In support of OECD Defined Approach Guideline project
- LLNA database
  - DASS Expert Group expressed concerns about reference classifications
  - Project leads are re-evaluating; eliminating tests without supporting concentration/SI data and those using modified protocols
- Human database
  - Being expanded and QC'd with help from German National Institute for Risk Assessment (BfR)





# Human Skin Sensitization Data Project

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- Purpose: to provide reference human data to support the evaluation of alternative skin sensitization test methods
- Objectives
  1. Curate human predictive patch test database for DA performance assessment
  2. Analyze data to understand uncertainty and sources of variability
  3. Develop/apply a transparent, reproducible system for human skin sensitization potency categorization
    - Review/build on Basketter et al. 2014 & Api et al. 2017, which used data from predictive and diagnostic human patch tests



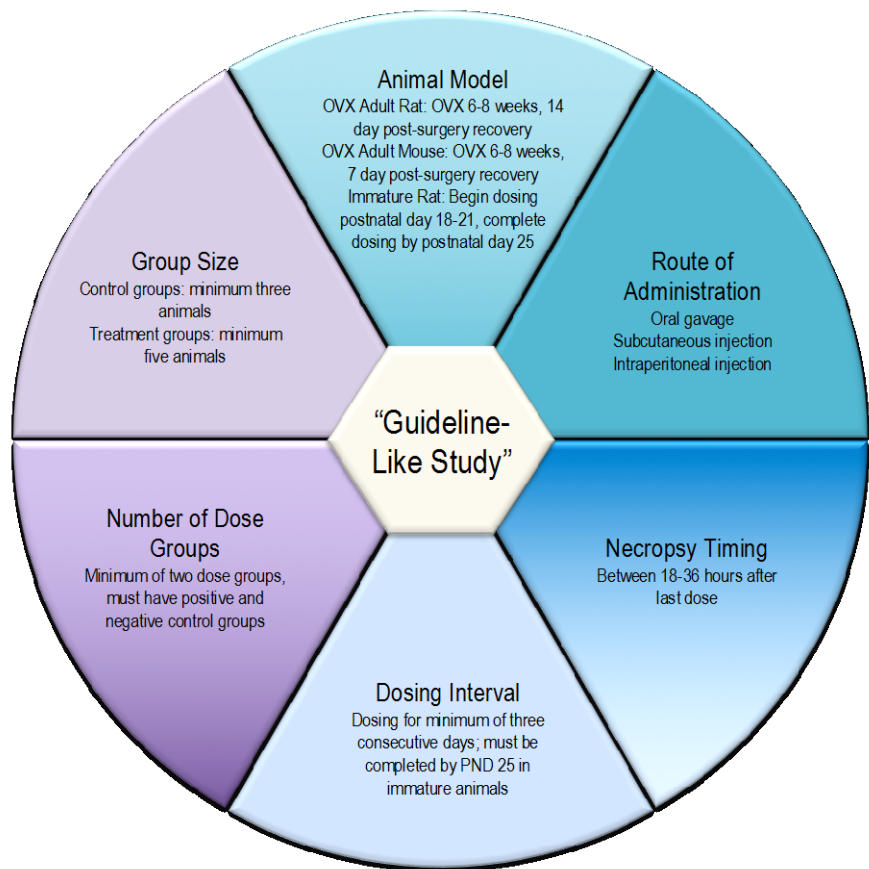
# Human Data Subgroup: Reviewers

---

- Anne Marie Api, Research Institute for Fragrance Materials
- Nicole Kleinstreuer, U.S. National Toxicology Program  
Interagency Center for the Evaluation of Alternative  
Toxicological Methods
- Hon Sum Ko, U.S. Food and Drug Administration, Division of  
Dermatology and Dental Products
- Joanna Matheson, John Gordon, U.S. Consumer Product  
Safety Commission, Health Sciences Directorate
- Judy Strickland, Integrated Laboratory Systems, contractor  
supporting NICEATM
- Matthias Herzler, Herrmann-Josef Thierse, German Federal  
Institute for Risk Assessment (BfR), Chemical and Product Safety



# Manually Identifying Reference Data

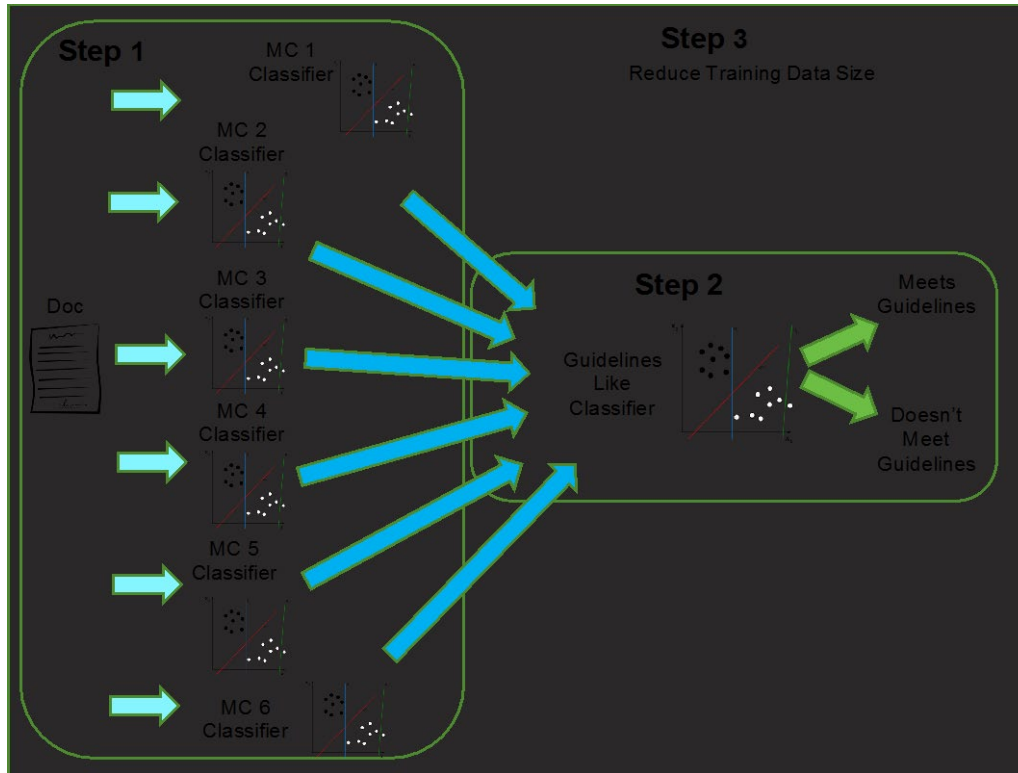


- Systematic literature search of publically available data (e.g. PubMed)
- Identify chemical activities measured in “guideline-like” uterotrophic studies
- Identify a subset of *in vivo* reference chemicals
  - Active chemicals verified in  $\geq 2$  independent studies
  - Inactive chemicals verified in  $\geq 2$  independent studies (with no positive results in any study)

*Kleinstreuer et al. EHP (2015)*



# Automating Reference Data Identification



- Project with Oak Ridge National Labs (ORNL) and FDA CFSAN to apply text-mining (NLP) approaches & ML to identify high-quality data
- Semi-automated retrieval and evaluation of published literature (trained on uterotrophic database)
- Apply to developmental toxicity studies



- Define literature search keywords
  - Use to search for developmental and reproductive toxicity studies
- Identify regulatory guidelines for conducting developmental and reproductive toxicity studies
- Extract study protocol details from guidelines
- Characterize study protocols to identify guideline components and minimum criteria
  - Group into tiered sets
    - 1) Required (appear in all TGs)
    - 2) Preferred
    - 3) Nice-to-have



# Workshop on MAT for Medical Devices

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- NICEATM and the PETA International Science Consortium (PISC) co-organized a workshop to discuss the use of the human cell-based monocyte activation test (MAT) as a standalone release test for medical devices.
  - September 18-19, 2018; NIH, Bethesda, MD, USA
- ~50 regulators, test developers, medical device manufacturers
  - discussed approaches to support use of the MAT for batch release testing of medical devices.
- Participants recommended studies needed to fill information gaps.
- Stakeholder subgroup will be convened to use the workshop conclusions as the basis for a proposal to the U.S. Food and Drug Administration's Medical Device Development Tools Program to consider the MAT as a non-animal alternative for pyrogen testing
- Finalizing workshop report for submission to ALTEX in the coming weeks



# Workshop on Rabies Vaccine Testing

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- NICEATM and the International Alliance for Biological Standardization – North America (IABS-NA) co-organized workshop.
  - October 16-17, 2018; NIH, Bethesda, MD, USA
- ~60 scientists from government, academia and industry developed recommendations to advance alternative methods for human and veterinary rabies virus vaccine testing.
- Detailed the current state of the science of nonanimal alternatives to traditional animal-based rabies virus vaccine potency and safety tests.
- Breakout group discussions focused on the steps necessary for implementing alternatives for veterinary and human rabies virus vaccine potency testing.
- Identified actions and data needed for further progress
- Finalizing workshop report to summarize meeting outcomes and associated conclusions – will be submitted to the Biologicals for publication in the next few weeks.



# Non-animal approaches to detect botulinum neurotoxin

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- Current assay to detect BoNT is a mouse bioassay
- A BoNT/E-specific ELISA-based kit has been developed by BioSentinel to detect BoNT/E in avian blood samples.
  - Current effort focused on establishing transferability to the National Wildlife Health Center (NWHC), part of the U.S. Geological Survey; also demonstrating long-term performance and suitability
- Additionally, a BoNT/C-specific ELISA-based kit has also been developed by BioSentinel.
  - Initial studies indicated that BoNT/C could not be detected in avian blood samples
  - Follow-up spike-recovery testing indicated that components in the blood were interfering with detection.
  - Current effort focused on additional development to reduce assay interference effects
- Collaborative effort with BioSentinal and NWHC





# Non-animal Affinity Reagents

- Challenge: Identifying alternatives to current animal-based methods for producing monoclonal and polyclonal antibodies (i.e., development of non-animal affinity reagents)
- EURL-ECVAM Scientific Advisory Committee (ESAC) reviewed the scientific validity of antibodies and non-antibody affinity reagents generated using animal-free technologies for use in research and diagnostics
- The ESAC WG has circulated their complete report for internal commenting and finalization.
  - Publication of the ESAC Opinion and Final Report scheduled for Spring 2019
- In general, there is a lack of any scientific evidence that these methods cannot be used in place of animal-based antibodies
- Based on the ESAC report, next steps for ICCVAM?
  - Facilitate implementation
  - Identify barriers and actionable solutions
  - Planned workshop for December 2019 (NIH – Porter Neuroscience Center)



# Acknowledgments

- ILS/NICEATM
- ICCVAM partners
- ICATM partners
- Modeling consortium participants

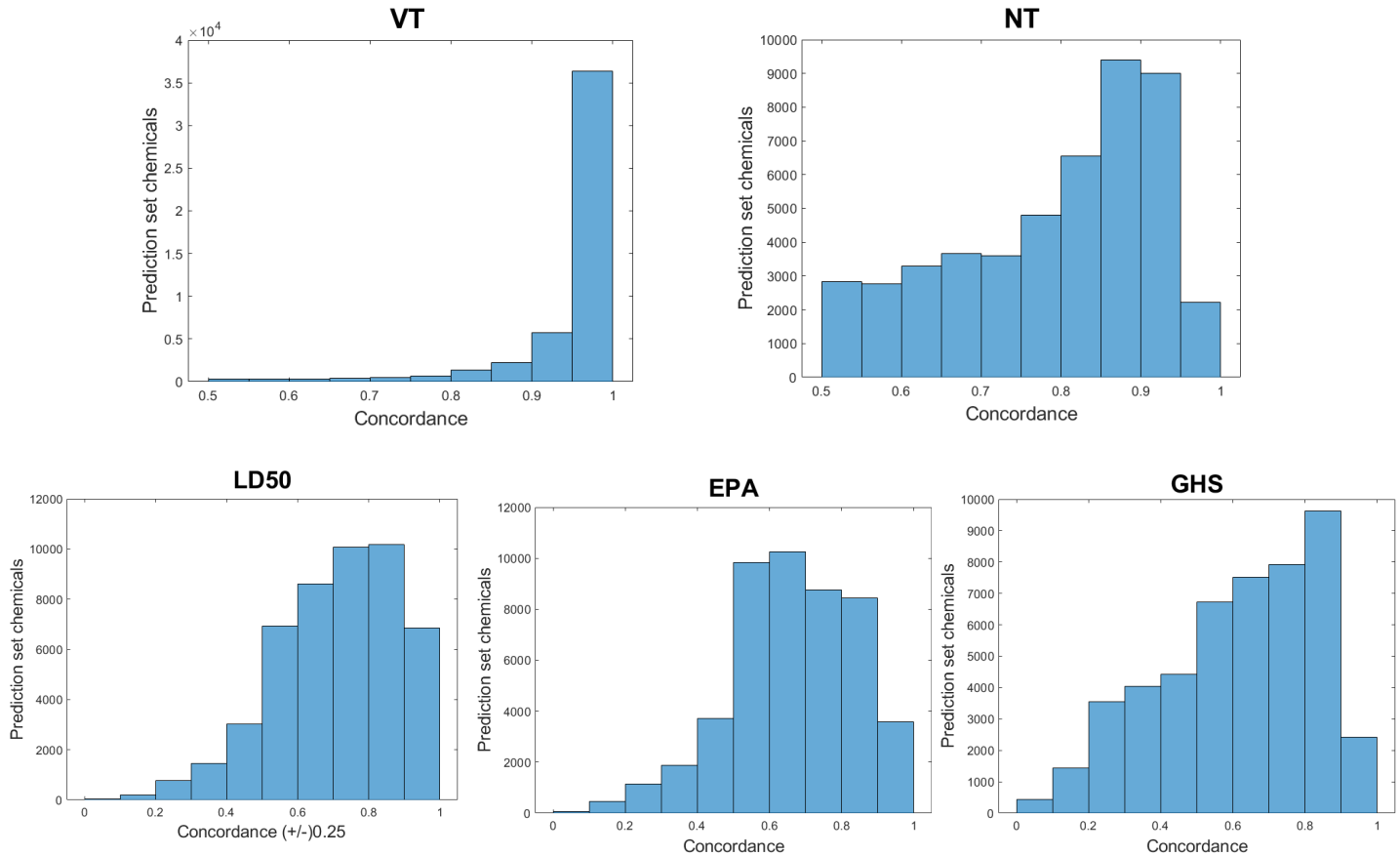






# Consensus concordance

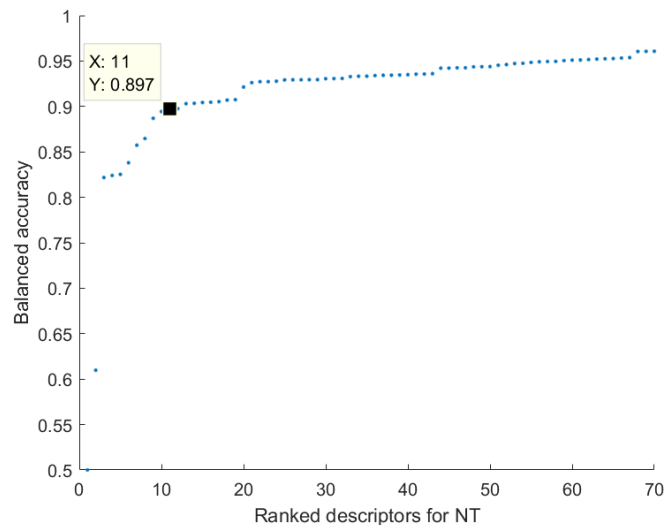
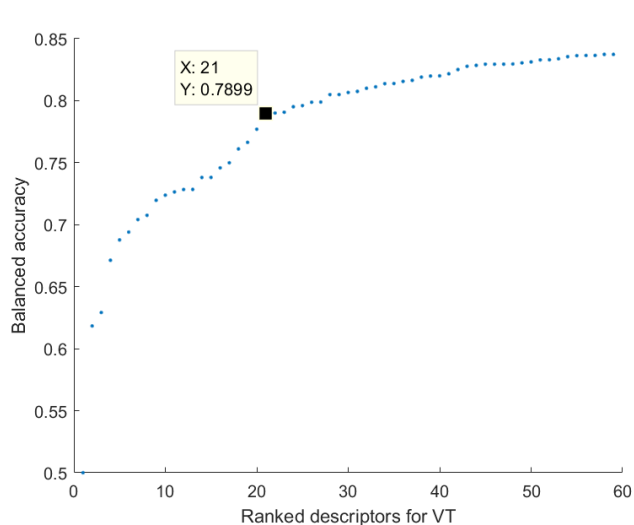
## Distributions of the concordance between models





# Consensus implementation

## Generalized CATMoS models: statistics



Endpoint	Descriptors	Training (5-f CV)	Test set
VT (BA)	21	0.79	0.77
NT (BA)	11	0.90	0.89
EPA (BA)	15	0.79	0.81
GHS (BA)	15	0.78	0.79
LD50 (Q <sup>2</sup> ,R <sup>2</sup> )	23	0.79	0.81