DeTox: An *in silico* Alternative to Animal Testing for Predicting Developmental Toxicity Potential

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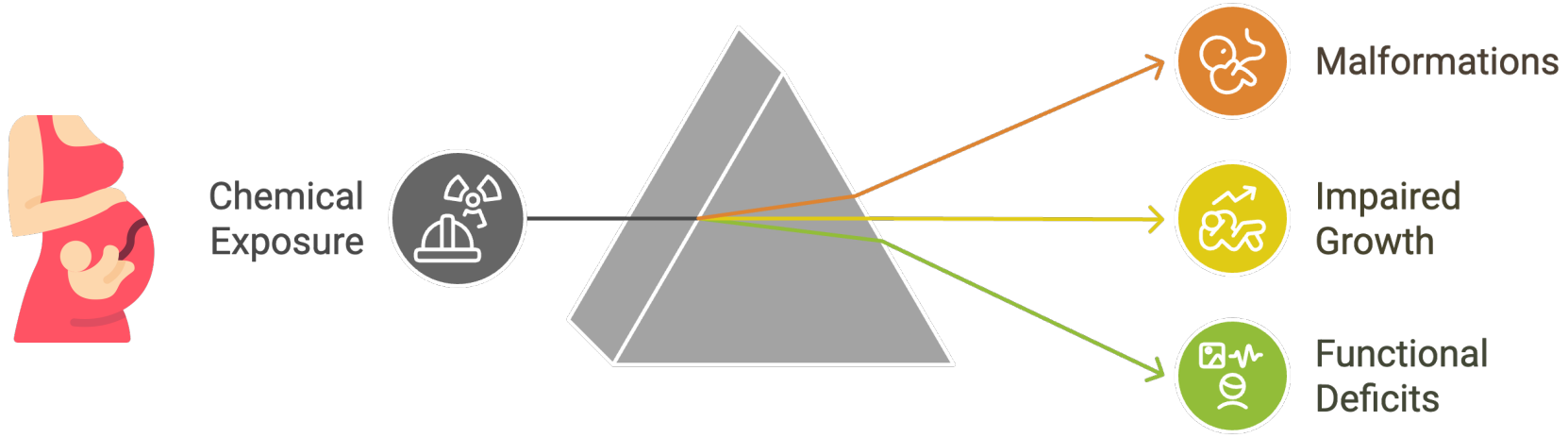
Conflict of Interest Statement

Alexander Tropsha and Eugene Muratov are co-founders of *Predictive LLC*, which develops novel computer methodologies for the prediction of toxicity endpoints.

All other authors declare no conflicts of interest.

Developmental Toxicity Definition

- Toxicological endpoint for hazard and risk assessment of chemicals affecting fetal growth, structural formation, organ function, or survival before maturity.
- Traditionally evaluated with ***in vivo*** studies such as the OECD Prenatal Development Toxicity Study (TG 414) and whole embryo culture assays in rats and rabbits.



Challenges of Animal Testing for Developmental Toxicity


- Expensive: $128,000+

- Time-consuming

- Raises ethical concerns

- Human-relevance uncertainty

US EPA Cost Estimates of Studies for Pesticide Registration. 2018.

Bailey, J., et al. (2005). Biogenic Amines.

Reported Predictive Power of *in vivo* Developmental Toxicity

Volume 149, Issue 20
October 2022

SPOTLIGHT | 22 SEPTEMBER 2022

***In vitro* models of human development and their potential application in developmental toxicity testing**

Mirjam Niethammer, Tanja Burgdorf, Elisa Wistorf, Gilbert Schönfelder, Mandy Kleinsorge

+ Author and article information

Development (2022) 149 (20): dev200933.
https://doi.org/10.1242/dev.200933

WellBeing International
WBI Studies Repository

2005

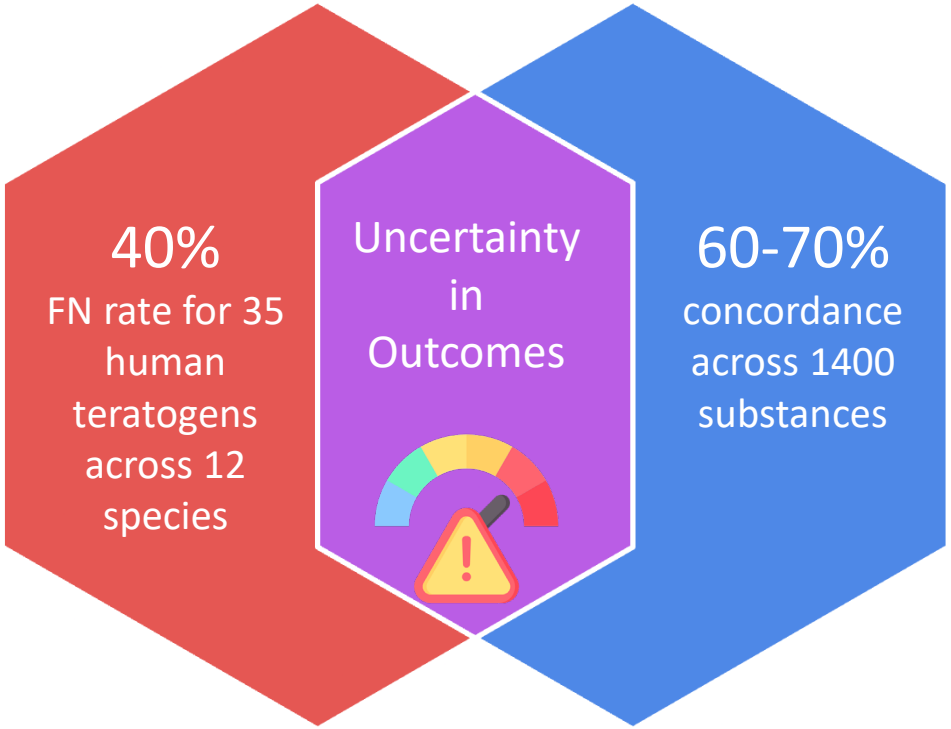
The Future of Teratology Research is In Vitro

Jarrod Bailey
University of Newcastle-upon-Tyne

Andrew Knight

Jonathan Balcombe
Physicians Committee for Responsible Medicine

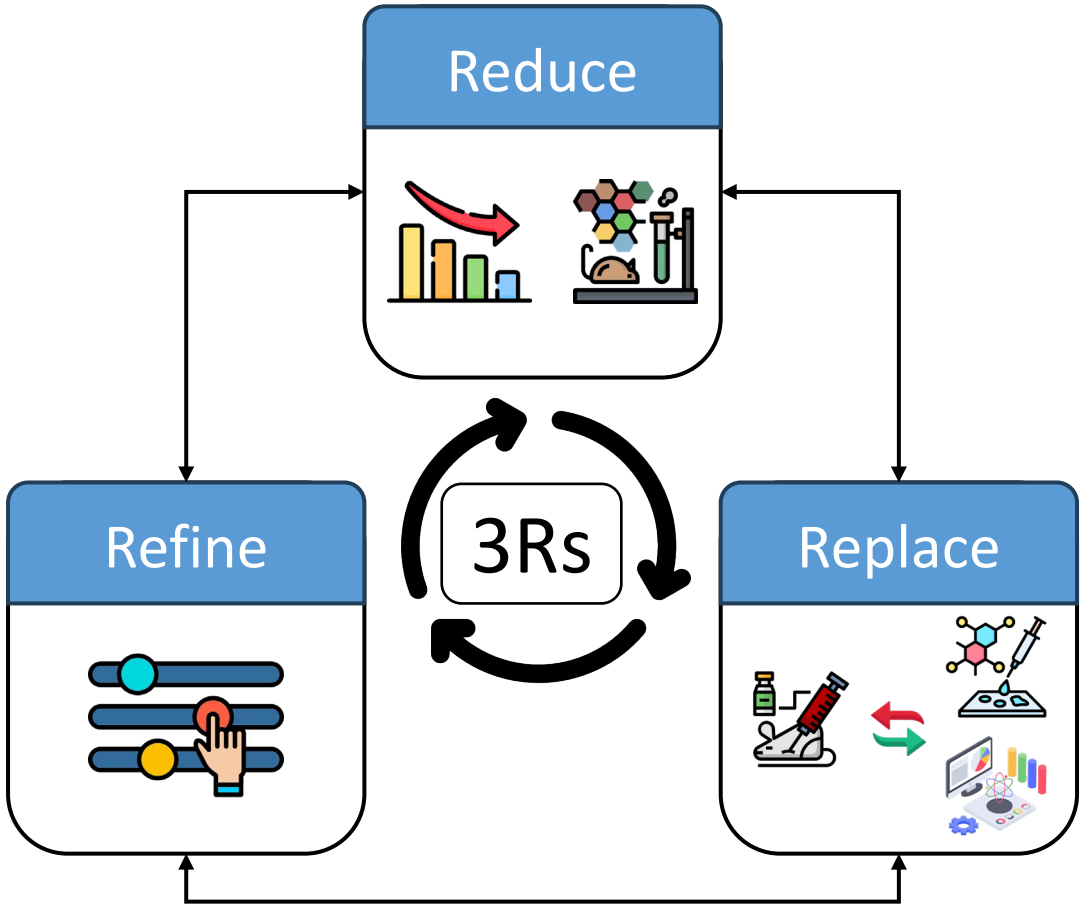
Positive Predictability Challenges



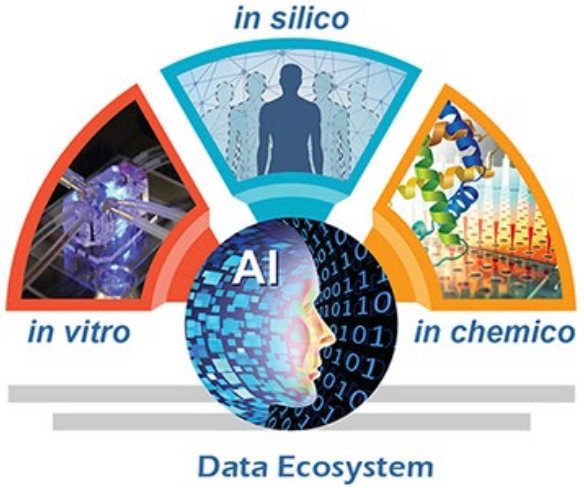
Inter-species Variability

Niethammer, Development (2022); Bailey, J., et al. (2005). Biogenic Amines.

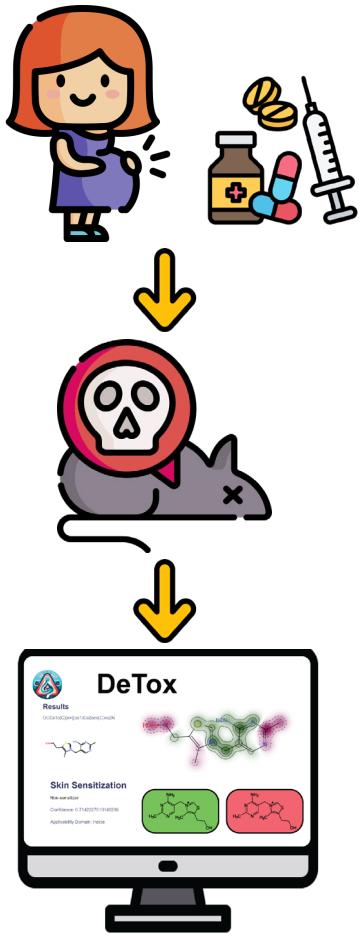
Alternatives to Animal Testing



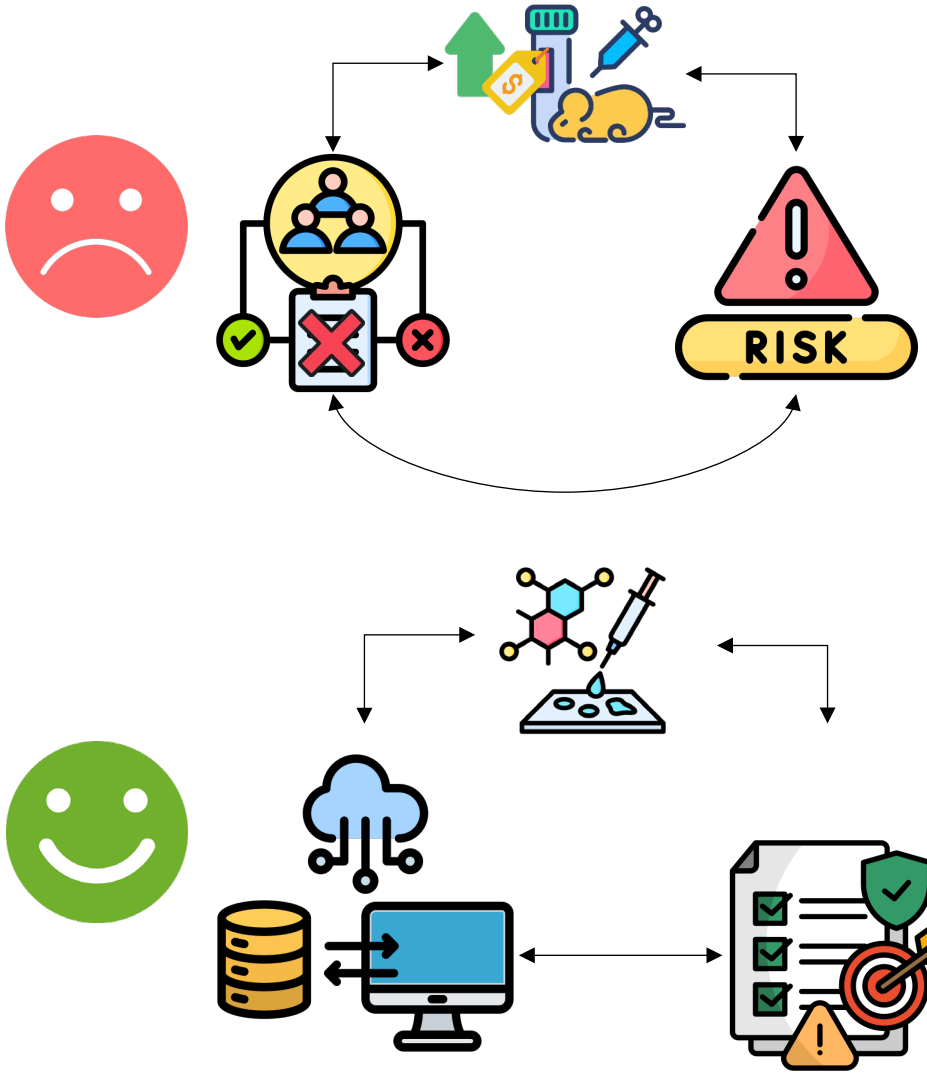
- NAMs: New Approach Methodologies
- NIH Complement-ARIE for NAMs
- QSAR: Quantitative-Structure Activity Relationship



Need for Improved Developmental Toxicity Testing



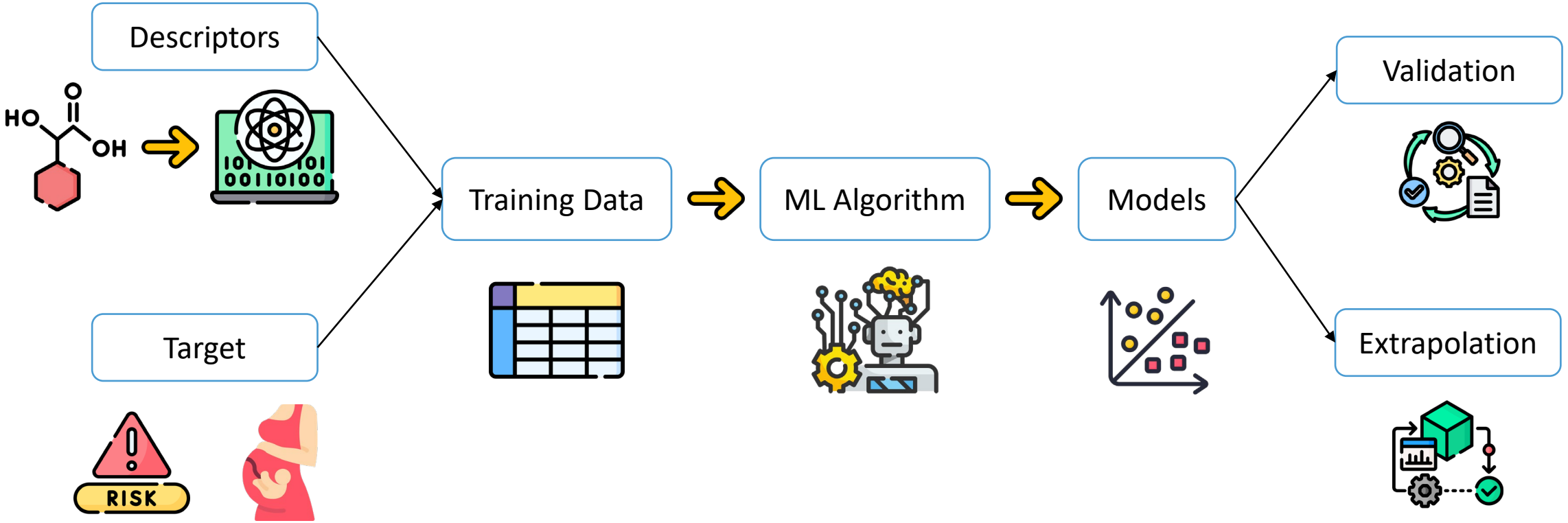
- 80% of pregnant women use prescription medication.[1]
- For known teratogens, animals testing only 60% positive predictivity.[2]
- DeTox offers an *in silico* alternative to animal testing for developmental toxicity



[1]*Lund, Addiction (2012).* [2]*Niethammer, Development (2022)*

Machine Learning (ML) Overview:

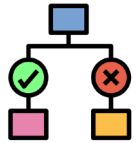
- Analyze impact of many factors on an outcome



Methodology

Data Collection

Definition of Developmental Toxicants:



- Binary Classifications (developmentally toxic vs. non-toxic)
  - Toxic: Presence of any developmental abnormality
  - Non-toxic: No significant adverse outcomes

- Adverse Outcomes:
  - Malformations, structural abnormalities
  - Spontaneous abortions
  - Cognitive deficits
  - Altered growth
  - Functional/behavioral changes

Schardein, J. L. *Teratogenesis, Carcinogenesis, and Mutagenesis* 1987, *7* (3), 255–271.

Data Collection and Outcome Labeling

47,000 records

Non-toxic + investigated

Removed

Toxic

Category	Definition
A	No risk in human studies
B	No risk in animal studies
C	Risk cannot be ruled out. There are no satisfactory studies in pregnant women, but animal studies demonstrated a risk to the fetus.
D	Evidence of risk (studies in pregnant women have demonstrated a risk to the fetus
X	Studies in pregnant women have demonstrated a risk to the fetus, and/or human or animal studies have shown fetal abnormalities.

Data Collection and Outcome Labeling

Reference compounds for alternative test methods to indicate developmental neurotoxicity (DNT) potential of chemicals: example lists and criteria for their selection and use

Michael Aschner[1], Sandra Ceccatelli[2], Mardas Daneshian[3], Ellen Fritsche[4], Nina Hasiwa[3], Thomas Hartung[3,5], Helena T. Hogberg[5], Marcel Leist[3,6,7], Abby Li[8], William R. Mundy[9], Stephanie Padilla[9], Aldert H. Piersma[10,11], Anna Bal-Price[12], Andrea Seiler[13], Remco H. Westerink[14], Bastian Zimmer[15], and Pamela J. Lein[16,17]

ELSEVIER

Neurotoxicology and Teratology

Volume 93, September–October 2022, 107117

Review article

An expert-driven literature review of "negative" chemicals for developmental neurotoxicity (DNT) *in vitro* assay evaluation

Melissa M. Martin [a], Nancy C. Baker [d], William K. Boyes [c 1], Kelly E. Carstens [a], Megan E. Culbreth [a], Mary E. Gilbert [c], Joshua A. Harrill [a], Johanna Nyffeler [a e], Stephanie Padilla [a], Katie Paul Friedman [b], Timothy J. Shafer [a]

REVIEW · Volume 368, Issue 9553, P2167-2178, December 16, 2006 Download Full Issue

THE LANCET

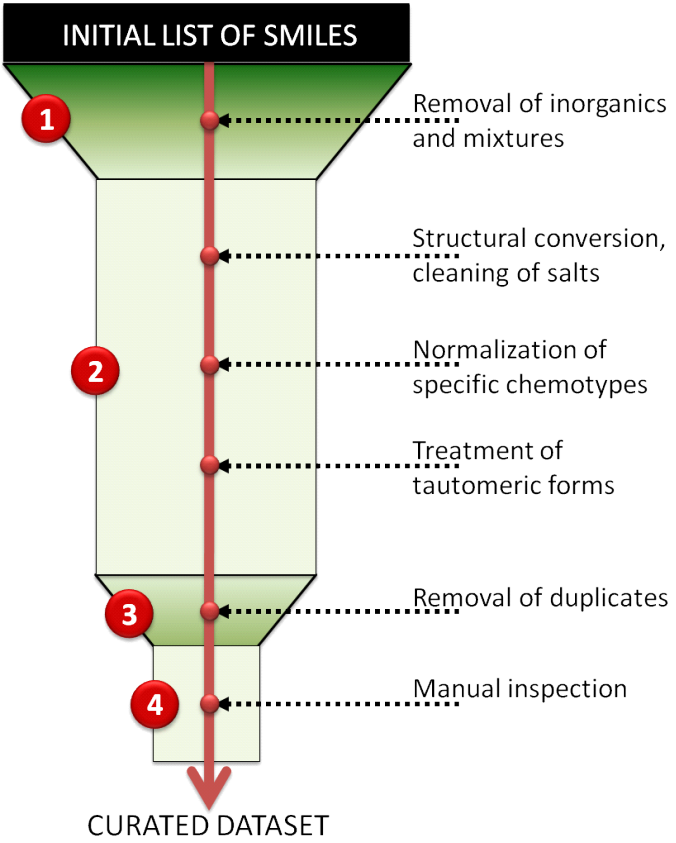
Developmental neurotoxicity of industrial chemicals

Dr, Prof P Grandjean, MD [a,b] · Prof PJ Landrigan, MD [c,d]

Affiliations & Notes Article Info

Additional 450 Records!

Results of Data Curation



	FDA - DailyMed	TERIS	Mundy 2009	Aschner
Initial number of records	**46943**	**293**	**108**	**75**
Removal of inconsistent data	42075	290	105	73
Removal of mixtures, inorganics, and cleaning/removal of salts	21316	275	89	62
Normalization of specific chemotypes	21023	275	87	62
Removal of Unclassified Compounds	4023	-	86	-
Final number of unique compounds after removing duplicates	**221**	**275**	**86**	**61**

Fourches D, et al. J Chem Inf Model. 2010 50(7):1189-204.

Verification of Non-Toxic Compounds and Trimester-Specific Literature Search

- Literature search
  - “Compound” AND “Trimester” AND “teratogen” or ”developmental toxicity”

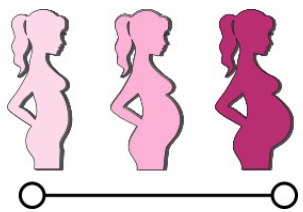
- Only included studies done under OECD or EPA guidelines
  - Testing done in rabbits, rats, or mice OR
  - Human studies with 50+ individuals

- Updated compound outcome if newer studies showed **any** of the adverse effects linked to developmental toxicity

Datasets Summary

Evaluated collected literature for **overall** and **trimester-specific** developmental toxicity

Overall Developmental Toxicity

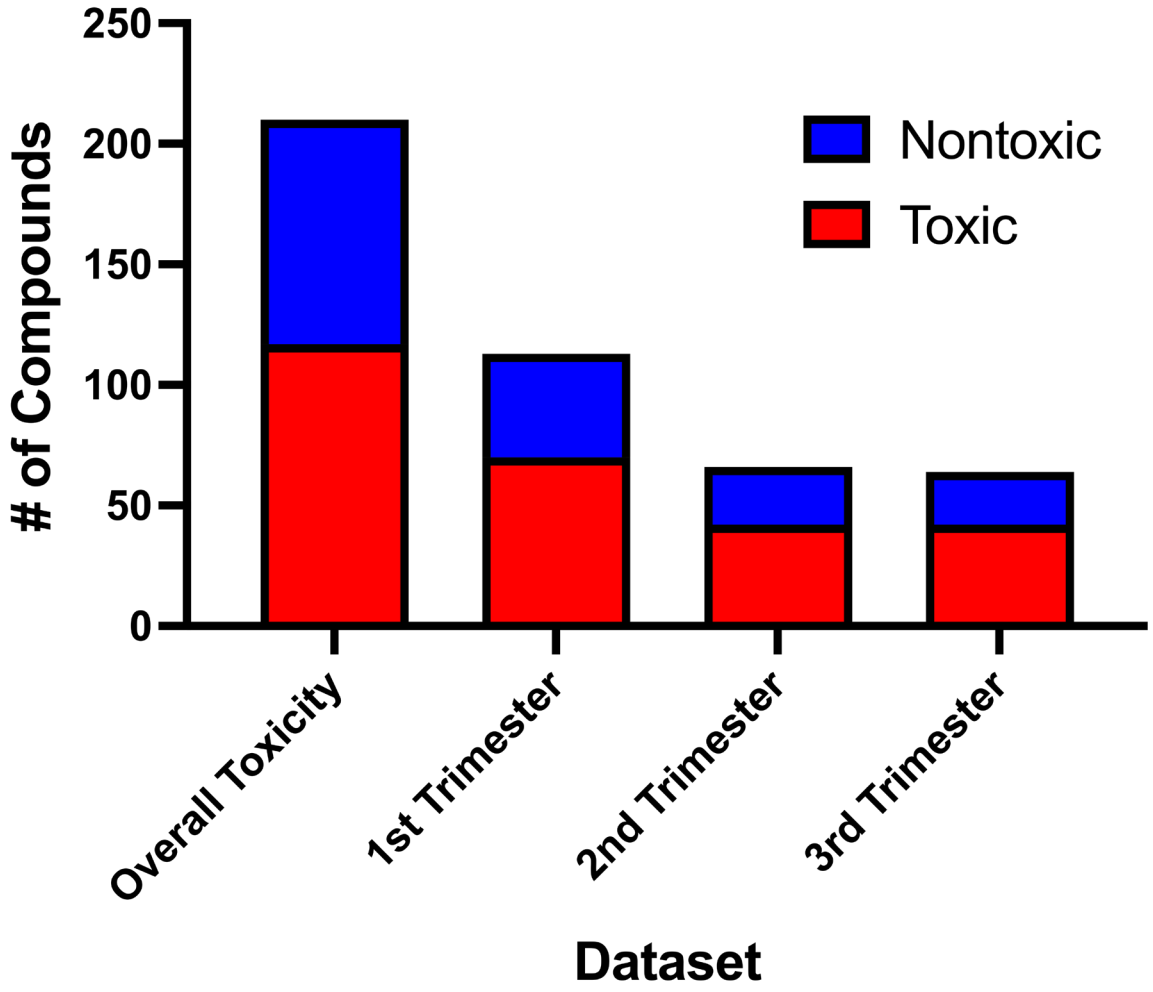


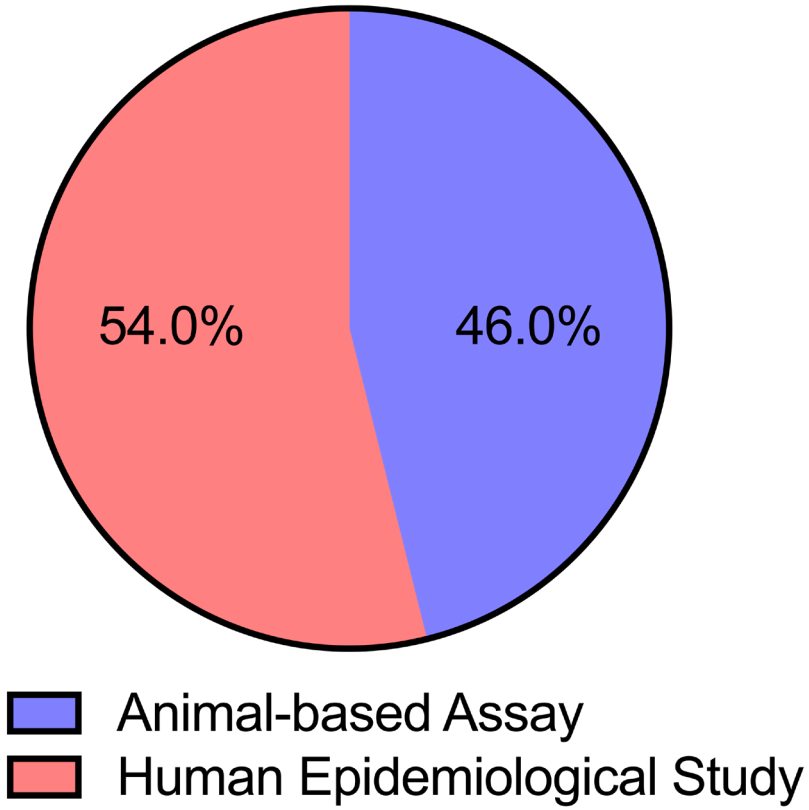
First Trimester Dev. Toxicity

Second Trimester Dev. Toxicity

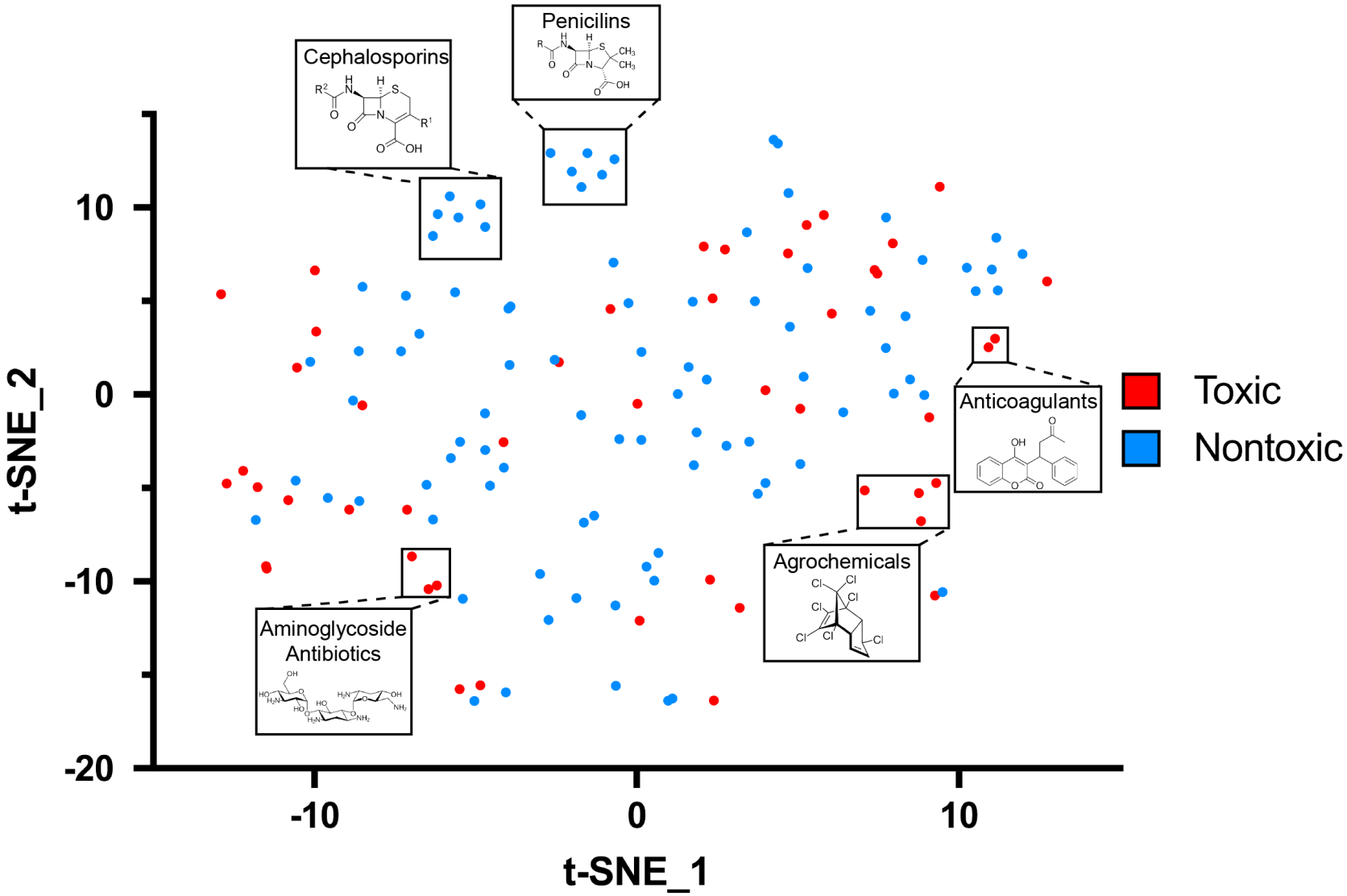
Third Trimester Dev. Toxicity

384 Compounds Collected After Curation and Activity Verification

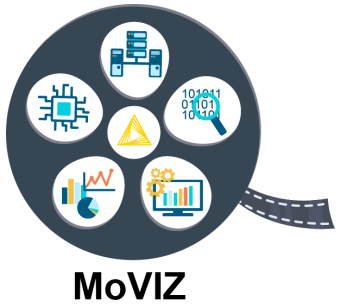




Chemical Space Analysis: Class-Specific Grouping

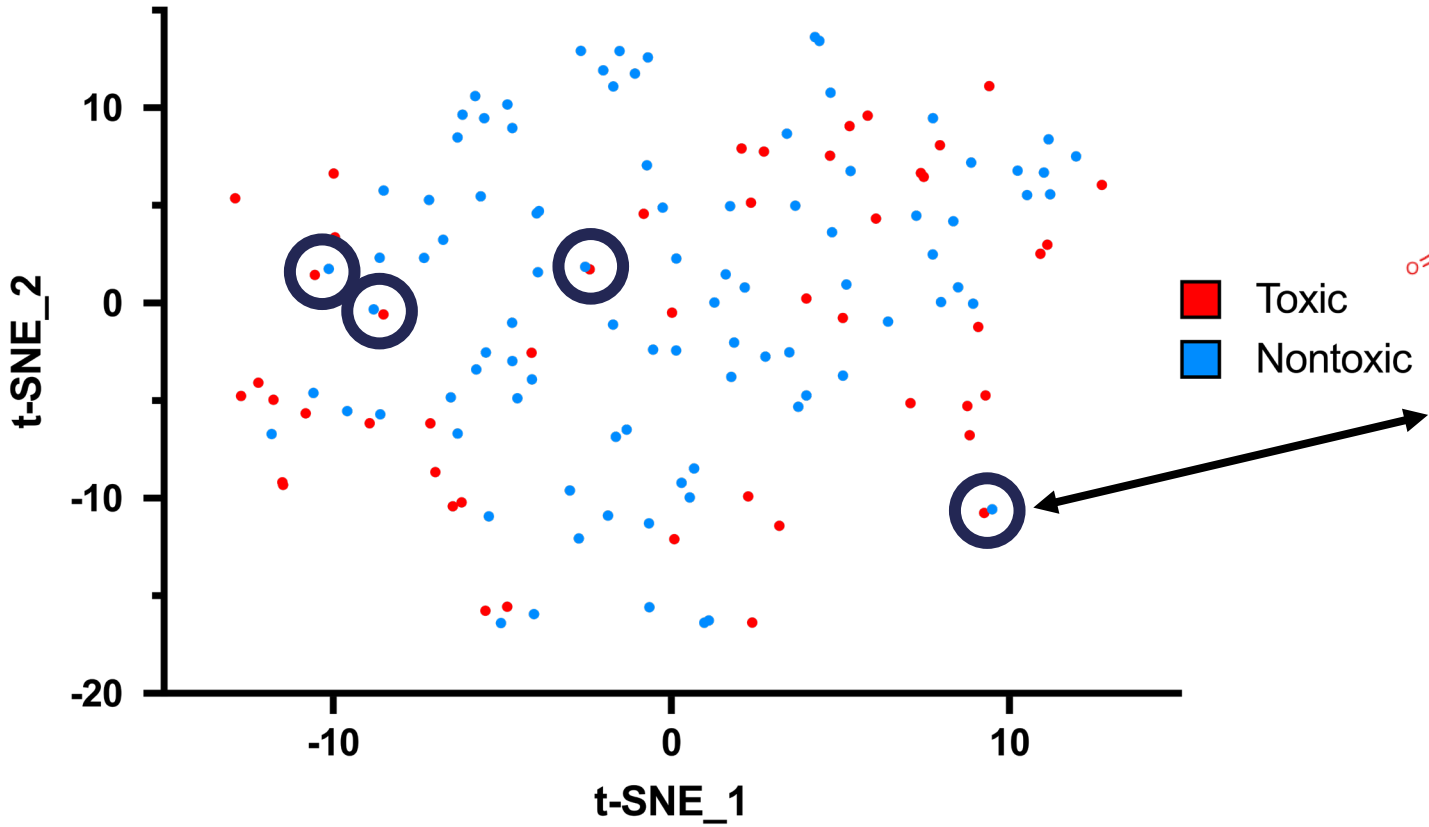


- Supervised Classification
- Morgan descriptors 2048 bits and radius 2
- Filtered low var. descriptors.
- Dimensionality reduction using SVM.

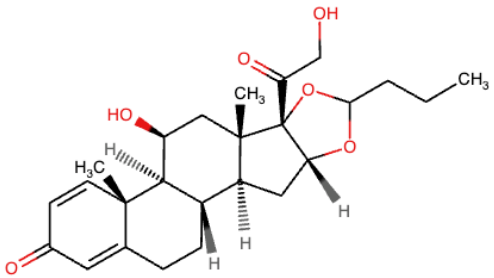


Moreira-Filho, J. et al *J Cheminform* **2024**, *16* (1), 101.

Chemical Space Analysis Reveals Few Activity Cliffs



Nontoxic

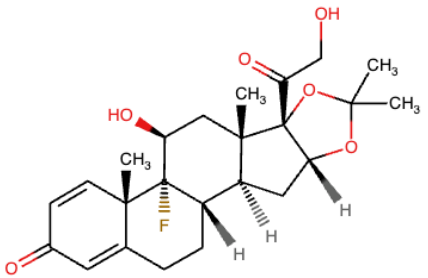


Budesonide

source:

Human epidemiological

(6600+ births)



Triamcinolone acetonide

Source:

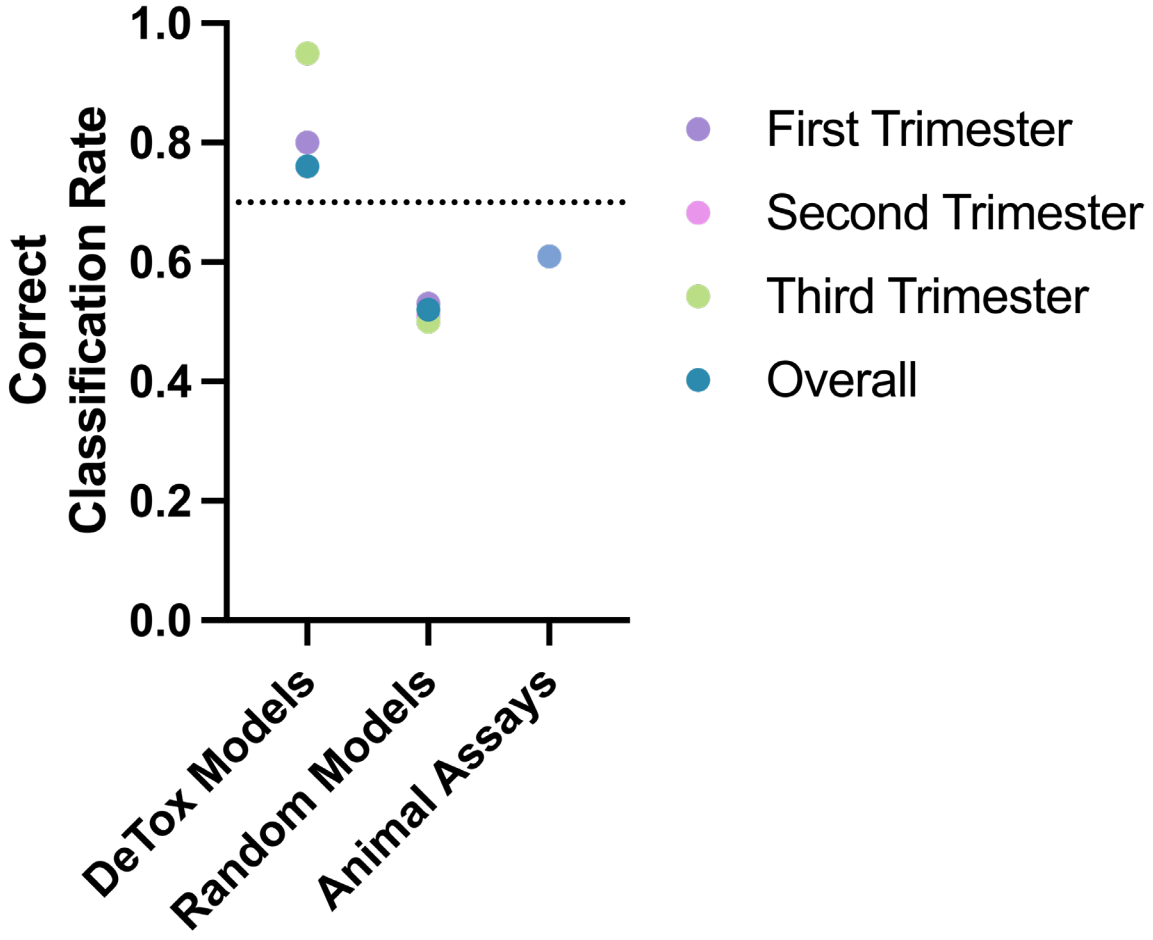
Rats

Toxic

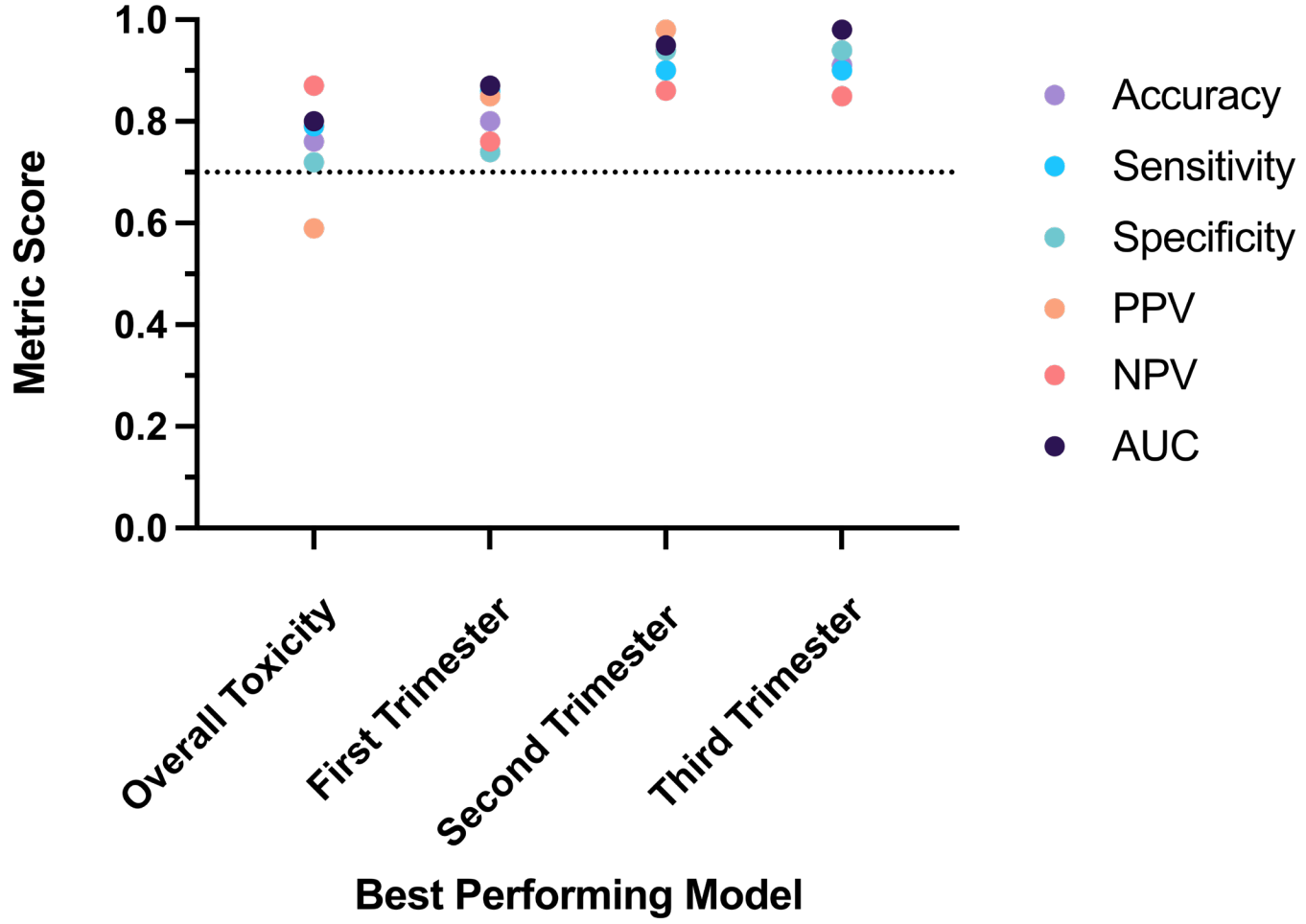
Moreira-Filho, J. et al *J Cheminform* **2024**, *16* (1), 101.

24 Binary Classification Models Developed

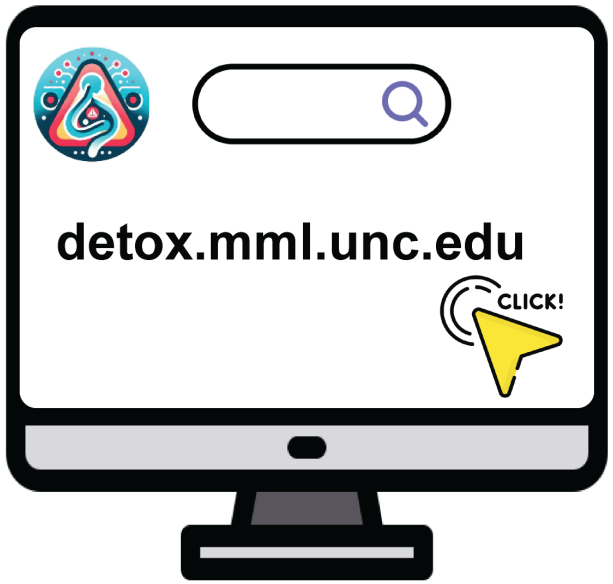
- 24 Binary Classification Models
- **Descriptors:**
  - Topological Fingerprints (ECFP4)
  - Structural Fingerprints (MACCS)
- **ML Algorithms:**
  - Random Forest (RF)
  - Light Gradient-Boosting Machine (LightGBM)
  - Support Vector Machine (SVM)



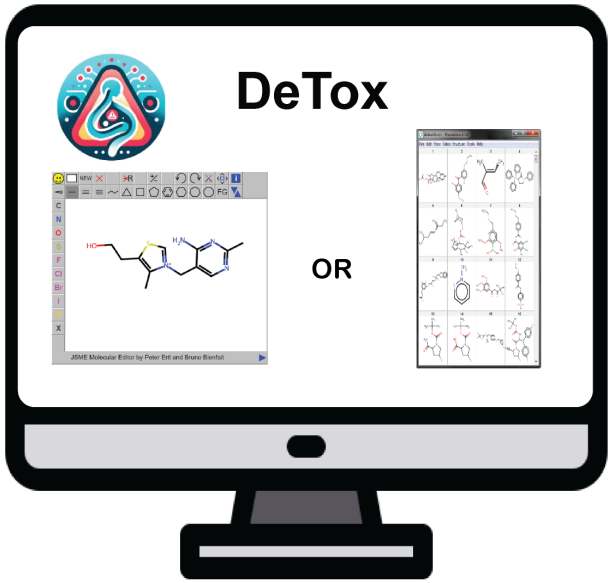
Trimester-Specific Model Performance



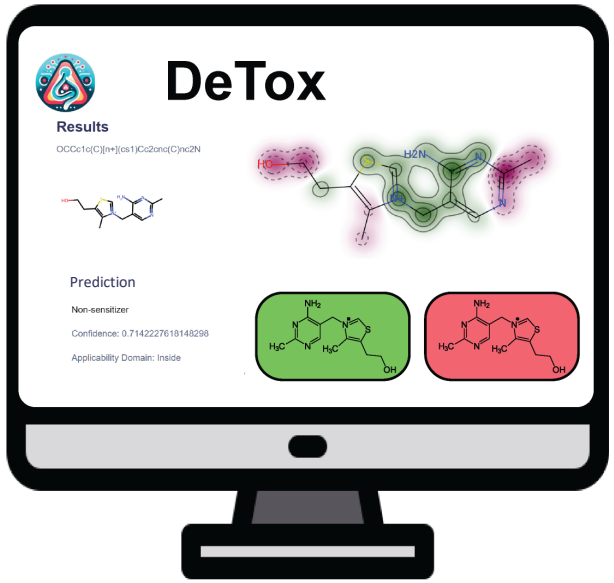
Model Deployment



DeTox
Web tool



Sketch Compound
OR
Input SMILES List



Get Report with
Fragment Contribution Maps

DeTox: Predicting Developmental Toxicity Potential

This is an online web portal to predict developmental toxicity, described in "PregPred: an In-Silico Alternative to Animal Testing for Predicting Developmental Toxicity Potential". To use, enter SMILES in the box below, or draw a compound and hit load SMILES, then click "Get Properties". Results will appear below. By default all models for all endpoints will be run. You can choose to turn off certain endpoints in the options sidebar. Fragment contribution maps are generated with RDKit. To turn on the maps, check the "Display contribution maps" in the options sidebar. It defaults to off because the maps will increase the runtime significantly, so if using please be patient. More information about these maps can be found here.

For the applicability domain calculation (AD), an ensemble confidence approach is used such that if the average prediction confidence of the ensemble of models is above 0.6, the prediction is considered "inside" the AD

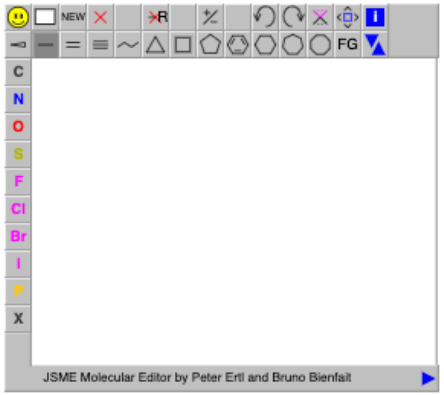
Please cite "PregPred: an In-Silico Alternative to Animal Testing for Predicting Developmental Toxicity Potential". Models and code for this webserver can be found here.

OCCc1c(C)[n+](cs1)Cc2cnc(C)nc2N

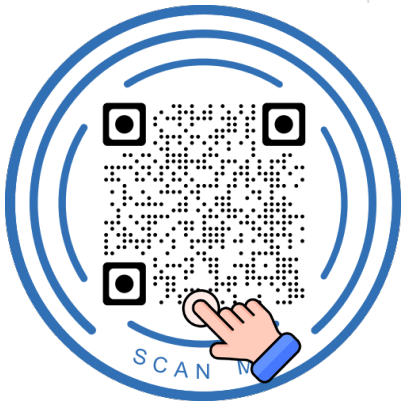
GET PROPERTIES

You can also generate a CSV of the results by entering the compounds below, separated by commas or new lines. fragment contribution maps will not be generated for CSV. Large numbers of SMILES and models wil take a long time to process, be patient.

Enter multiple SMILES strings as a comma or new line separated list and generate a .csv file.



https://detox.mml.unc.edu/



Model Options

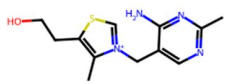
- Calculate AD
- Display Fragment Contribution Maps

Enabled Models

- Overall Toxicity
- First Trimester Toxicity
- Second Trimester Toxicity
- Third Trimester Toxicity

Results

OCCc1c(C)[n+](cs1)Cc2cnc(C)nc2N



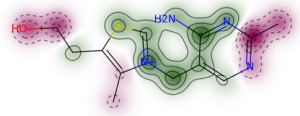
Overall Toxicity

Non-toxic

Confidence: 0.7142227618148298

Applicability Domain: Inside

Green contributes to toxicity, purple contributes to non-toxicity



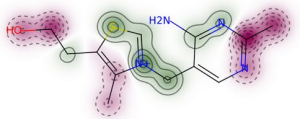
First Trimester Toxicity

Non-toxic

Confidence: 0.7278686807369527

Applicability Domain: Inside

Green contributes to toxicity, purple contributes to non-toxicity



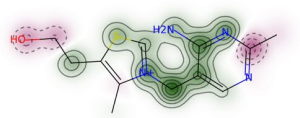
Second Trimester Toxicity

Non-toxic

Confidence: 0.6848660113886743

Applicability Domain: Inside

Green contributes to toxicity, purple contributes to non-toxicity



Third Trimester Toxicity

Non-toxic

Confidence: 0.7149612270008872

Applicability Domain: Inside

Green contributes to toxicity, purple contributes to non-toxicity

Model Performance Comparison to CAESAR

Cassano et al. Chemistry Central Journal 2010, 4(Suppl 1):S4
http://www.journal.chemistrycentral.com/content/4/S1/S4

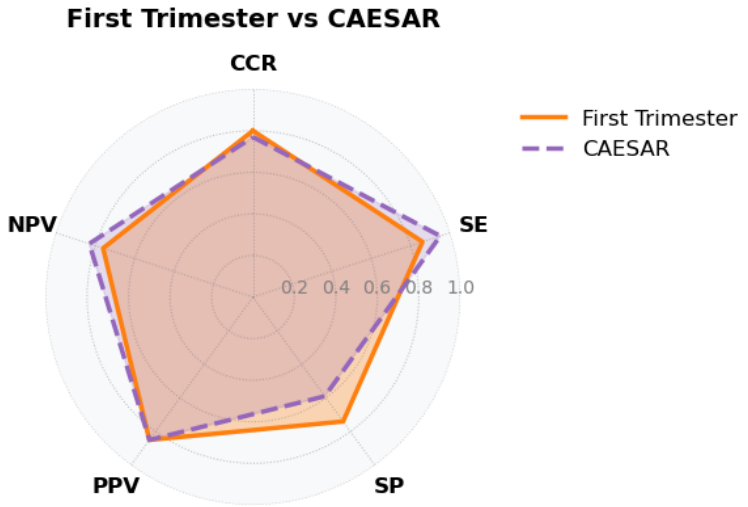
Chemistry Central Journal

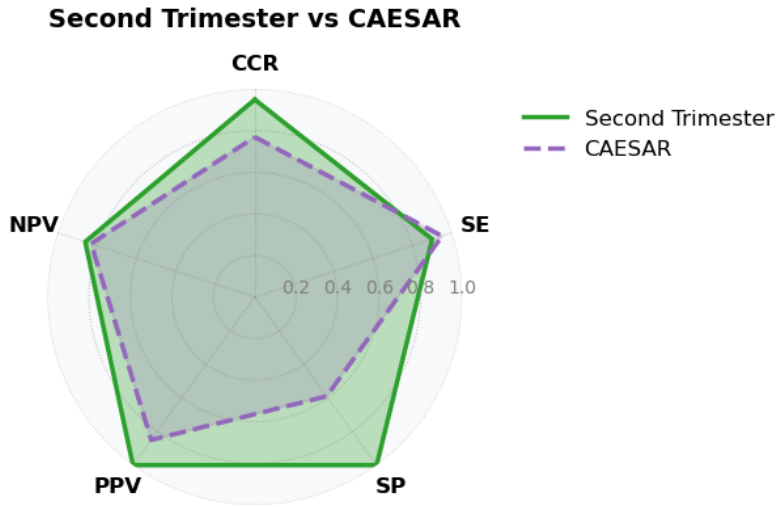
PROCEEDINGS Open Access

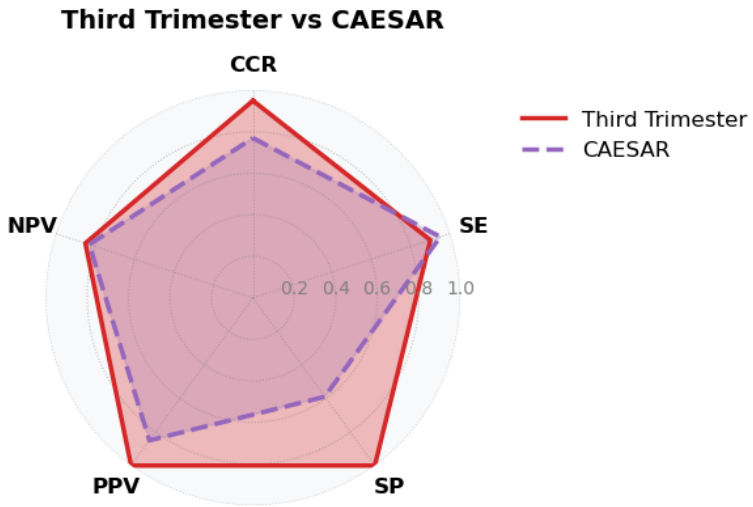
CAESAR models for developmental toxicity

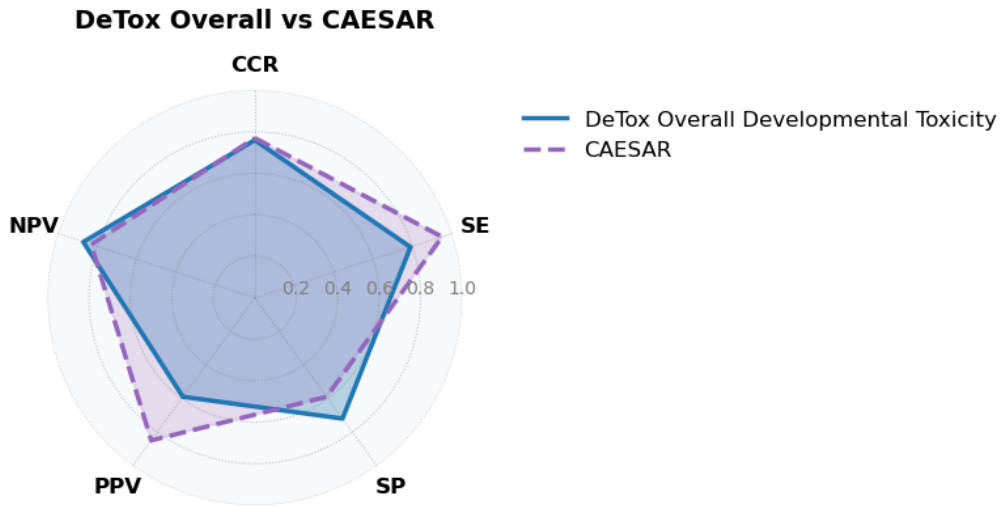
Antonio Cassano[1], Alberto Manganaro[1], Todd Martin[2], Douglas Young[2], Nadège Piclin[3], Marco Pintore[3], Davide Bigoni[4], Emilio Benfenati[1*]

From CAESAR Workshop on QSAR Models for REACH









[3]*Cassano, Chemistry Central (2009).*

Model External Validation Performance

Cassano *et al. Chemistry Central Journal* 2010, **4**(Suppl 1):S4
http://www.journal.chemistrycentral.com/content/4/S1/S4

Chemistry Central Journal

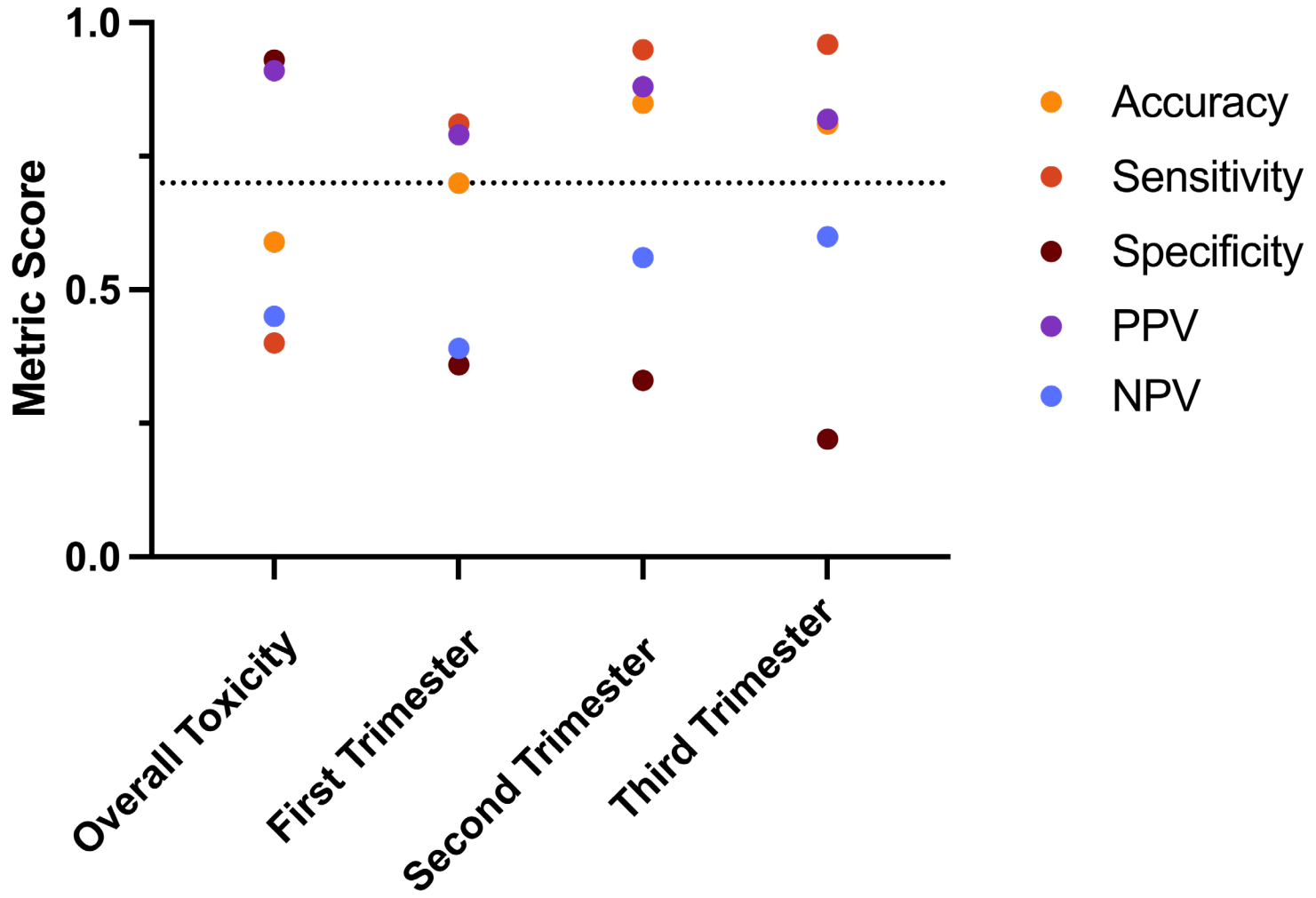
PROCEEDINGS Open Access

CAESAR models for developmental toxicity

Antonio Cassano[1], Alberto Manganaro[1], Todd Martin[2], Douglas Young[2], Nadège Piclin[3], Marco Pintore[3], Davide Bigoni[4], Emilio Benfenati[1*]

From CAESAR Workshop on QSAR Models for REACH

- **292 chemicals with FDA labels for developmental toxicity**
- Compounds outside model's training set were predicted using web platform
  - ~200 compounds per dataset



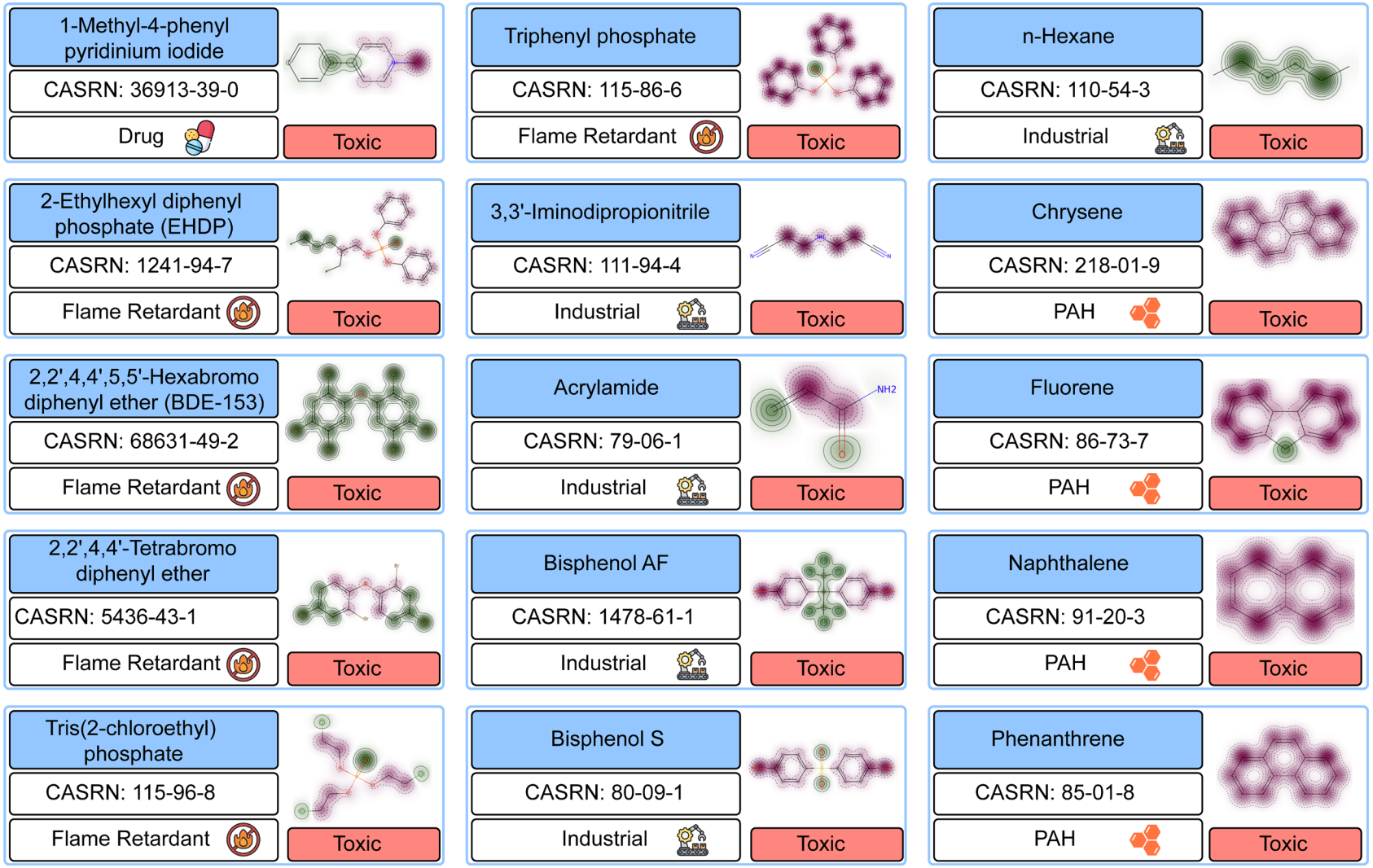
[3]*Cassano, Chemistry Central (2009).*

Virtual Screening of DNT-DIVER

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

DNT-DIVER

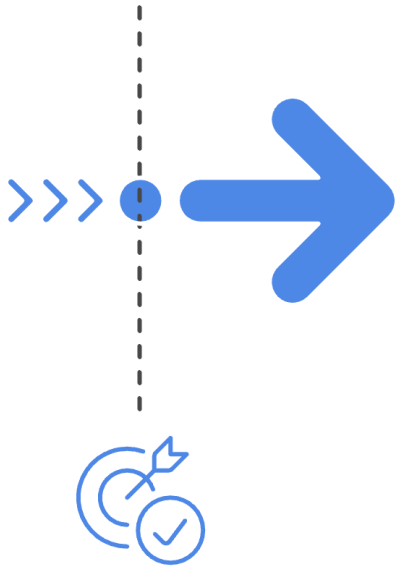
- **87 neurodevelopmental toxicants**
- Compounds **not included in training set** were predicted
- 15 shown are inside Applicability Domain for all 4 models
- Consensus predictions by the web platform



Conclusions:

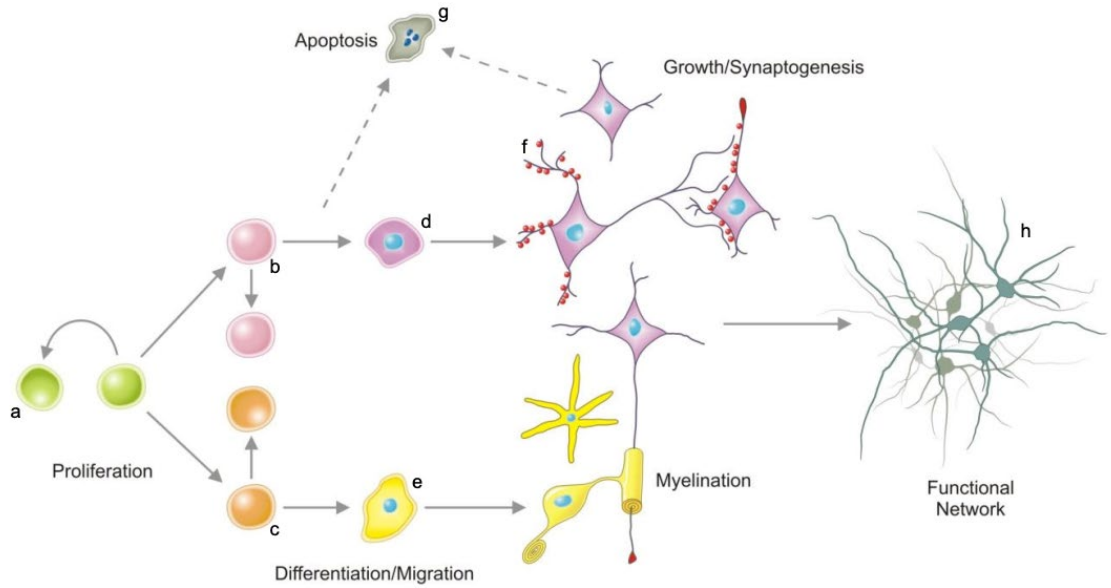
In silico approaches

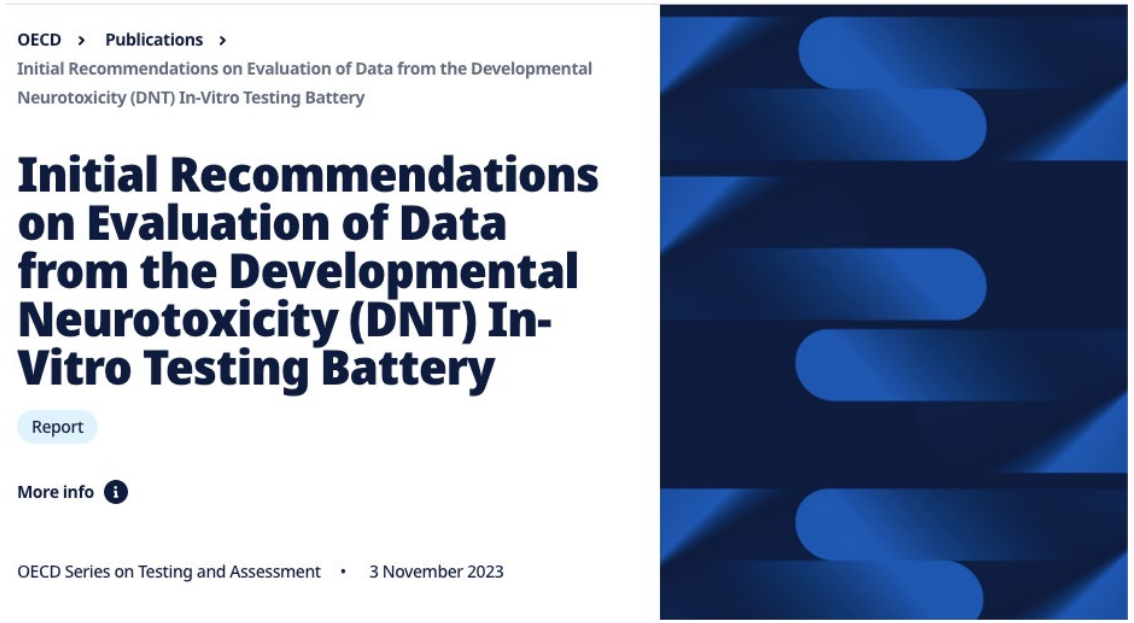
Fast and cost-effective alternative to animal testing.



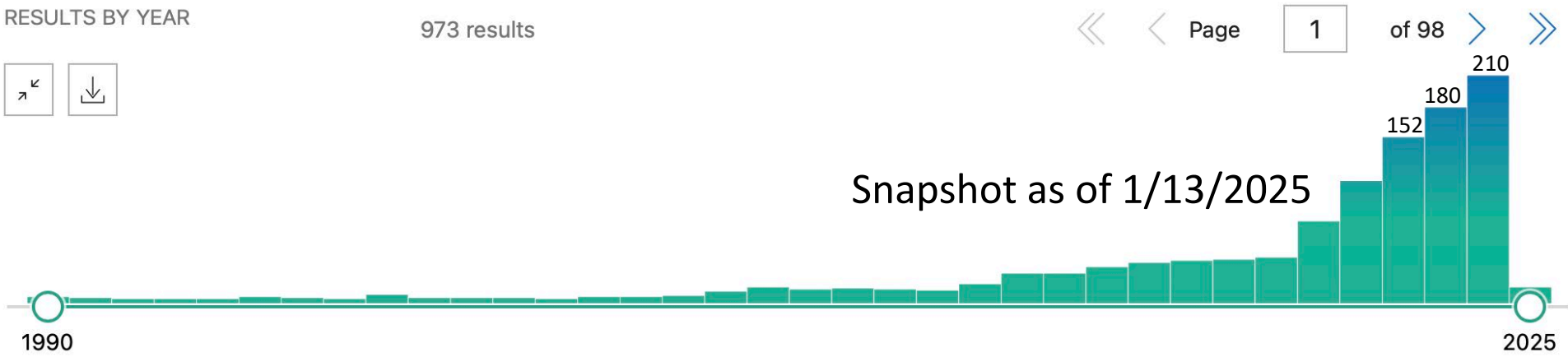
Future Directions: NAMs for Developmental Neurotoxicity Mechanistic Analysis

- Initial Guidance published in **Nov. 2023**.
- Integrates a battery of 17 *in vitro* assays – covering key DNT processes

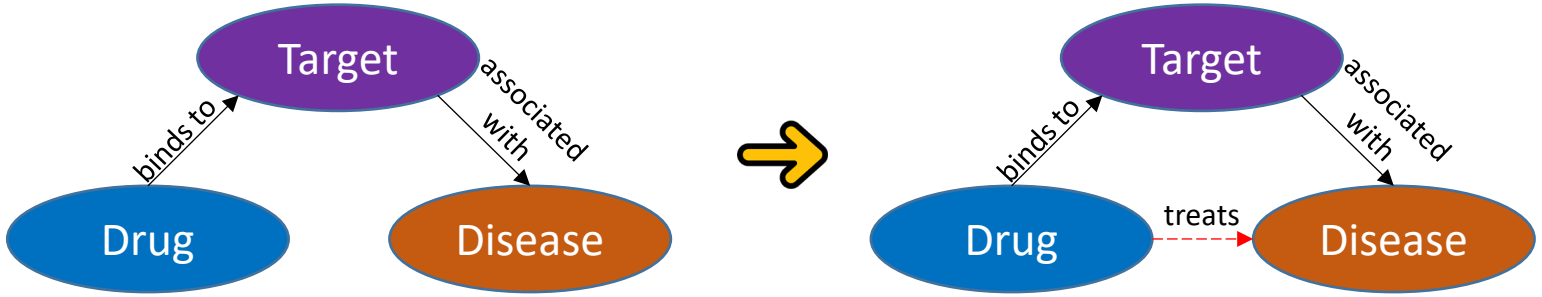




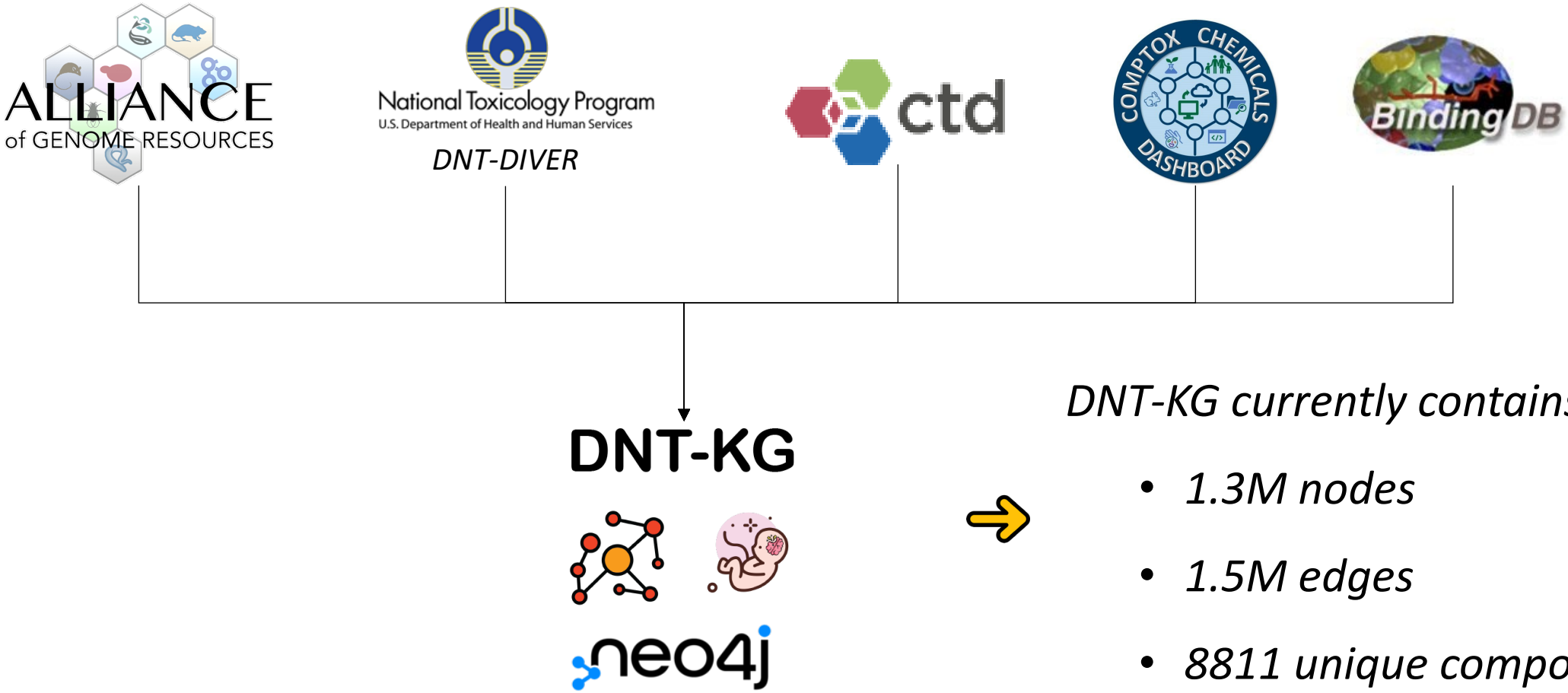
Constructing a Developmental Neurotoxicity Knowledge Graph (DNT-KG)



NSF invests $26.7 million in building the first-ever prototype open knowledge network



Knowledge Graphs Can Integrate Toxicology Datasets for Mechanistic Insights



DNT-KG currently contains:

- *1.3M nodes*
- *1.5M edges*
- *8811 unique compounds*

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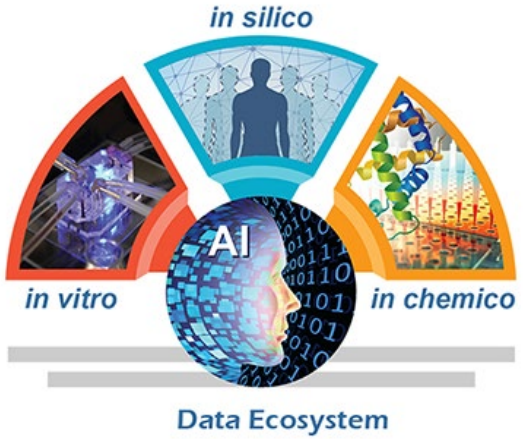
https://detox.mml.unc.edu/



Complement-ARIE