# Classifying and predicting developmentally neurotoxic chemicals using new approach methods and machine learning

Kelly Carstens, PhD
Email: Carstens.Kelly@epa.gov

U.S. Environmental Protection Agency
Research Triangle Park, NC

**Office of Research and Development**
**Center for Computational Toxicology and Exposure**
**Biomolecular and Computational Toxicology Division**
**Computational Toxicology and Bioinformatics Branch**

**The Safety Pharmacology Society**
**2022 Annual Meeting**

**Session: Current Status and Future Perspectives in AI-Based Drug Assessment**
**9/13/22**

# Conflict of Interest Statement

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

# Developmental neurotoxicity (DNT) new approach methods (NAMs)

❖Neurodevelopmental disability is the most prevalent chronic medical condition encountered in pediatrics (Zablotsky et al. 2019).

❖Both genetic and **environmental risk factors** have been identified as underlying causes driving this prevalence.

**DNT NAMs**: multi-dimensional DNT screening assays that cover complex neurobiological space: temporal, different 'key events' in neurodevelopment, cell-types, and species.
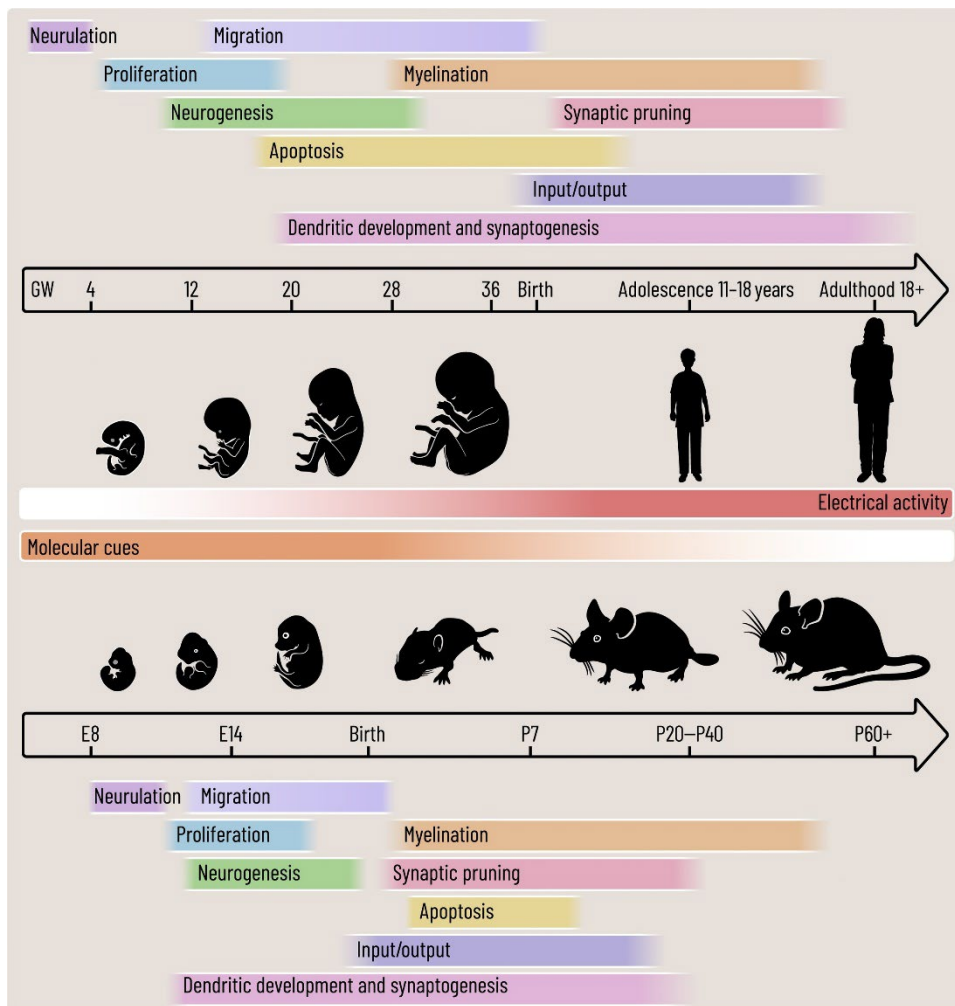


Chini and Hanganu-Opatz. 2021. Trends in Neuro.

# Overview

- Can we build a model to classify compounds that demonstrate *in vivo* DNT bioactivity?

- Can we improve the model performance by reducing the dimensions of the DNT NAM dataset?

- Current limitations to using DNT NAMs and machine learning models to predict DNT.

# Strengths of using a high-throughput, multi-dimensional screening battery for DNT

❖ No single *in vitro* screening assay can recapitulate all critical cellular events of neurodevelopment.

❖ Some compounds may disrupt specific cellular events at different stages of development.

❖ Some neural cell-types may be differentially sensitive to perturbation.
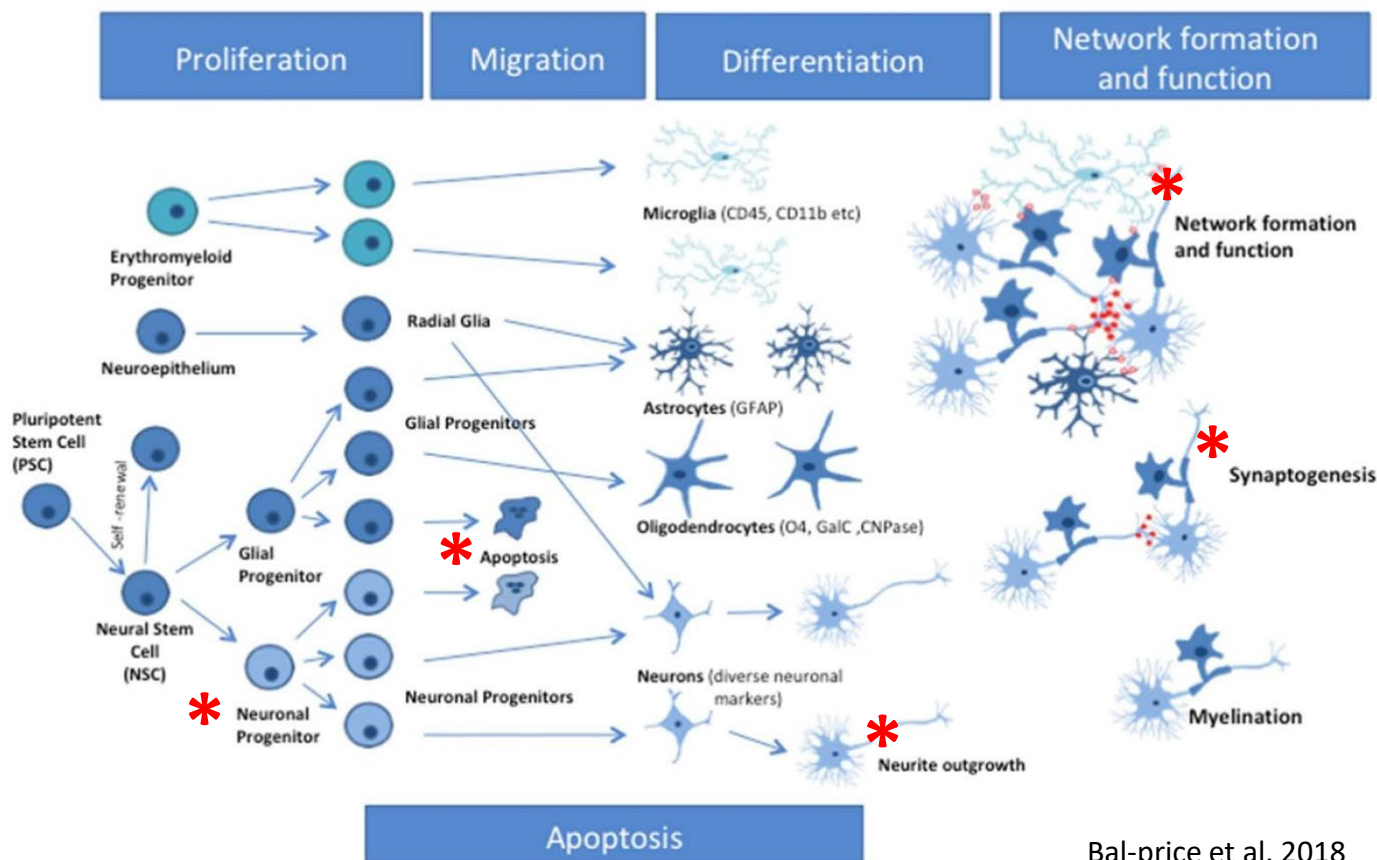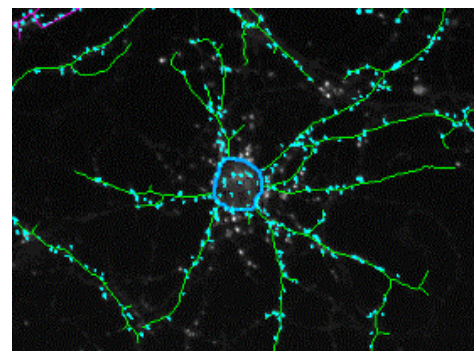


Bal-price et al. 2018

# Experimental models in the DNT NAMs battery

## Microelectrode array (MEA) network formation assay (NFA)



Axion Biosystems
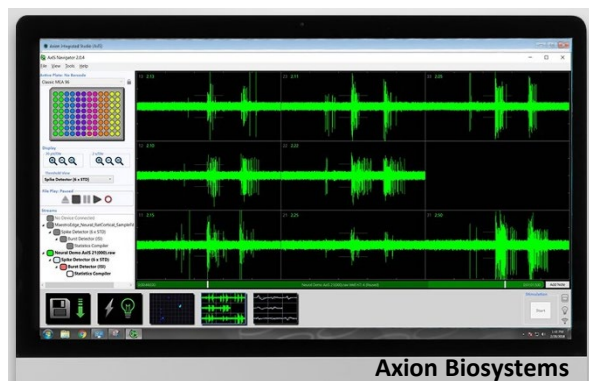
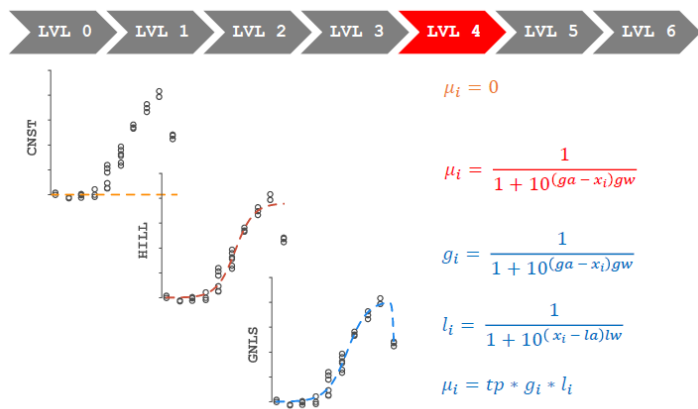| Activity type | Cell Culture |
|---|---|
| General activity | Primary rat cortical neurons (DIV 5, 7, 9, 12) |
| Network connectivity | |
| Bursting | |
| Cytotoxicity | |

## High-content imaging



| Assays | Cell culture |
|---|---|
| Neurite Outgrowth (NOG) | Primary rat neurons, human hN2 neural cells |
| Synaptogenesis and Neurite maturation | Primary rat neurons |
| Proliferation | Human hNP1 neuroprogenitors |
| Apoptosis | Human hNP1 neuroprogenitors |

# Defining bioactivity using the ToxCast pipeline

Model fitting (constant, hill, gain-loss)



$\mu_i = 0$

$\mu_i = \dfrac{1}{1 + 10^{(ga - x_i)gw}}$

$g_i = \dfrac{1}{1 + 10^{(ga - x_i)gw}}$

$l_i = \dfrac{1}{1 + 10^{(x_i - la)lw}}$

$\mu_i = tp * g_i * l_i$

Select winning model and

**Selectivity:** bioactivity occurring below cytotoxicity



**Cytotoxicity AC$_{50}$**
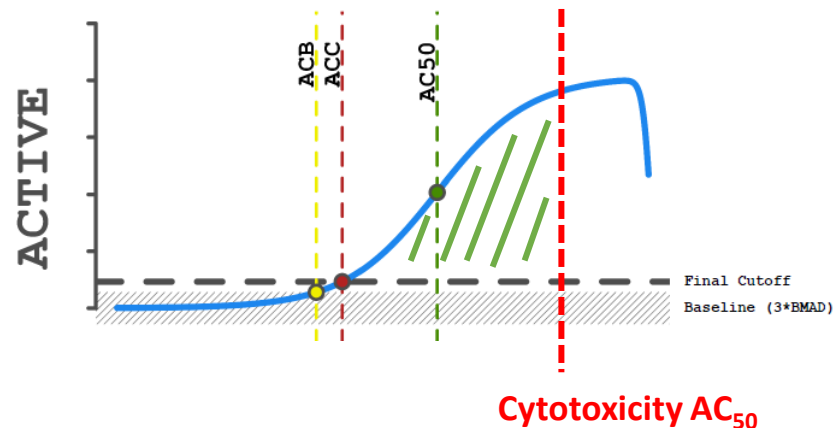
https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing.html#level-4

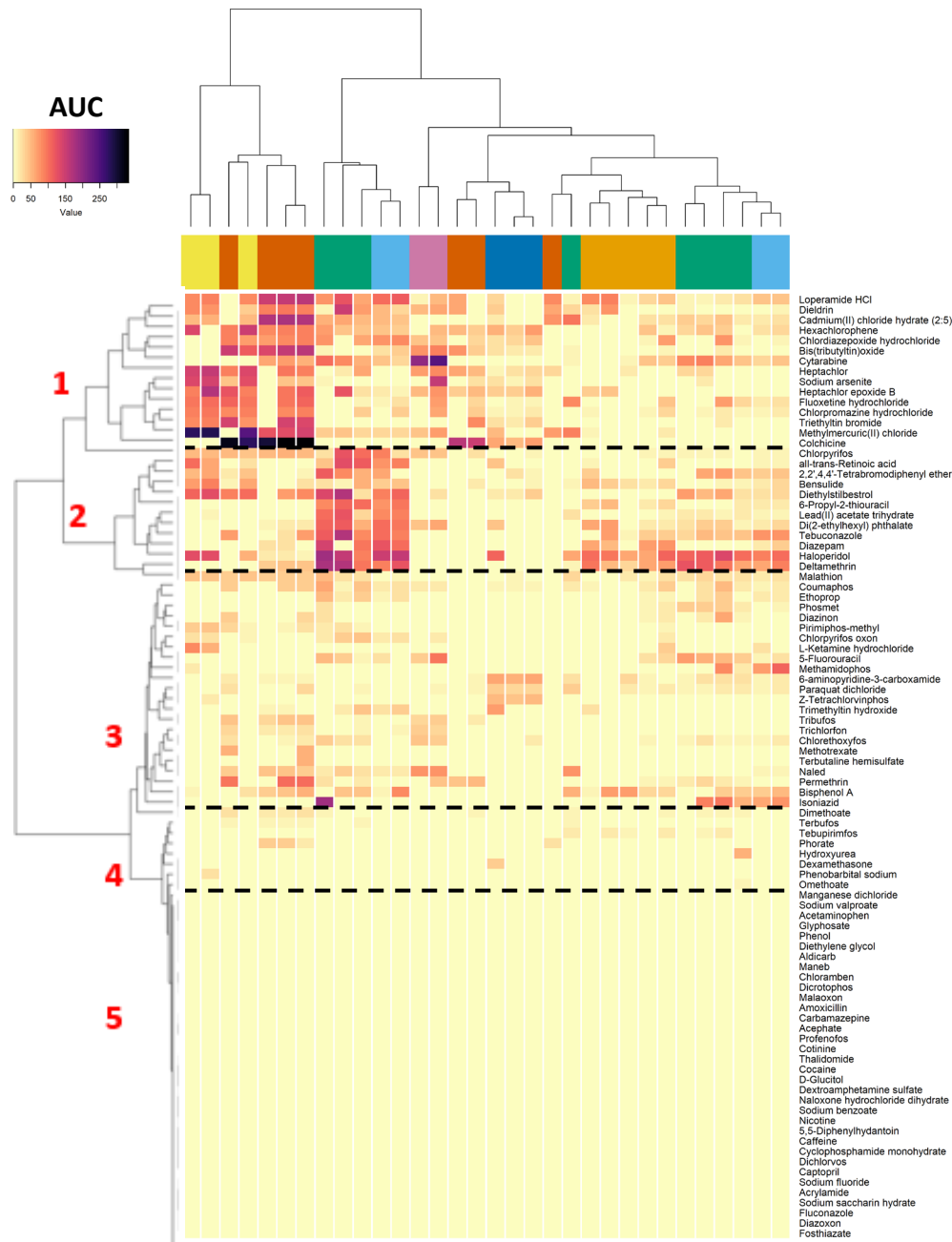ToxCast pipeline (tcpl) R package (version 2.0.3) (Filer et al. 2017)

*Selective* bioactivity is informative for identifying patterns of biological activity

Activity Type
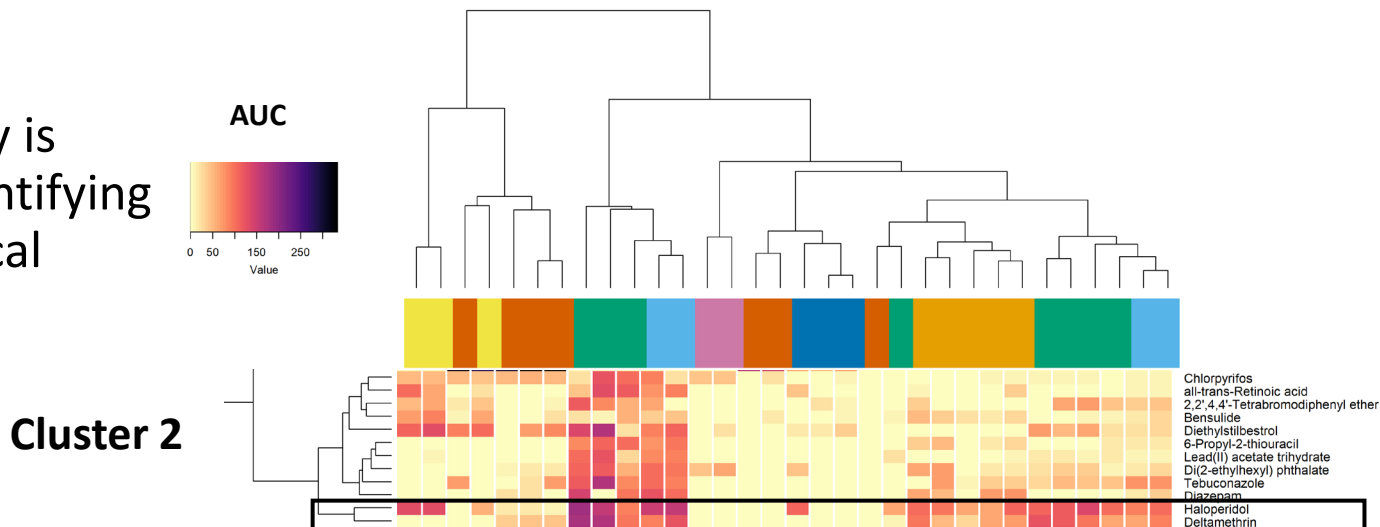- ▬ NOG initiation, rat
- ▬ Synaptogenesis/maturation, rat
- ▬ NOG initiation, hN2
- ▬ Proliferation, hNP1
- ▬ General
- ▬ Bursting
- ▬ Network Connectivity

**NOG**: Neurite outgrowth
**AUC**: Area under the curve

Carstens et al. 2022 ToxSci.

*Selective* bioactivity is informative for identifying patterns of biological activity

**AUC**

Cluster 2

Chlorpyrifos
all-trans-Retinoic acid
2,2',4,4'-Tetrabromodiphenyl ether
Bensulide
Diethylstilbestrol
6-Propyl-2-thiouracil
Lead(II) acetate trihydrate
Di(2-ethylhexyl) phthalate
Tebuconazole
Diazepam
Haloperidol
Deltamethrin

### Activity Type

- ▬ NOG initiation, rat
- ▬ Synaptogenesis/maturation, rat
- ▬ NOG initiation, hN2
- ▬ Proliferation, hNP1
- ▬ General
- ▬ Bursting
- ▬ Network Connectivity

**NOG**: Neurite outgrowth
**AUC**: Area under the curve

| High selectivity | Moderate/ Low selectivity |
|---|---|
| Network connectivity | NOG (hN2) |
| Neuronal activity | Bursting |

**Haloperidol**: antipsychotic- Dopamine $D_2$ receptor antagonist

**Deltamethrin**: pyrethroid insecticide- voltage-gated sodium channels modulators

Carstens et al. 2022 ToxSci.

# Can we build a model to classify compounds that demonstrate *in vivo* DNT bioactivity?



**DNT Reference**
— Positive
— Negative

AUC
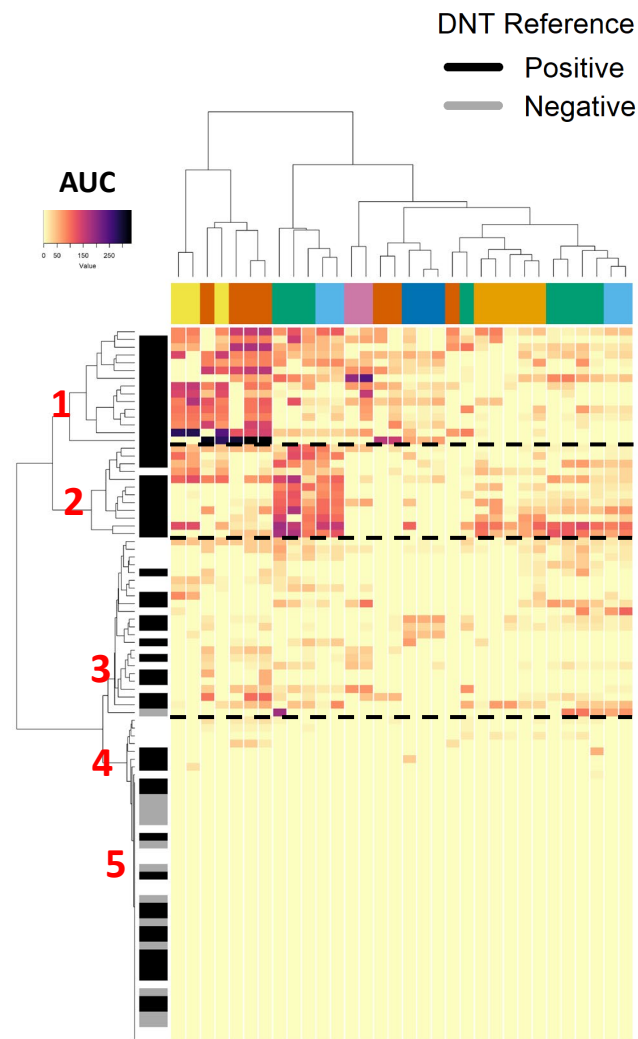
| | *In vivo* evaluation chemicals | |
|---|---|---|
| **Classification** | **Positive (53)** Mundy et al. 2015 Aschner et al. 2016 Harrill et al. 2018 | **Negative (13)** Martin et al. 2022. |
| **Cluster 1** Synap/ prolif/ NOG/ Neurite maturation | 14 | 0 |
| **Cluster 2** General/ network/ bursting activity/ synap | 11 | 0 |
| **Cluster 3** General/ network activity/ bursting/ synap/NOG | 11 | 1 |
| **Cluster 4** General/ network activity/ bursting/ synap/ NOG | 3 | 0 |
| **Cluster 5** 'Inactive/ equivocal' | 14 | 12 |

| | **Positive** | **Negatives** |
|---|---|---|
| **Selective activity** (Clusters 1,2,3,4) | True positive: 39 | False positive:1 |
| **Inactive/ equivocal** (Cluster 5) | False negative: 14 | True Negative: 12 |

**Selective bioactivity:**

**Sensitivity= 74%**

**Specificity= 92%**

**Bioactivity including cytotoxicity:**

**Sensitivity= 93%**

**Specificity= 69%**

Carstens et al. 2022 ToxSci.

# How accurate is our DNT NAMs model in predicting chemicals with evidence of *in vivo* DNT using machine learning?
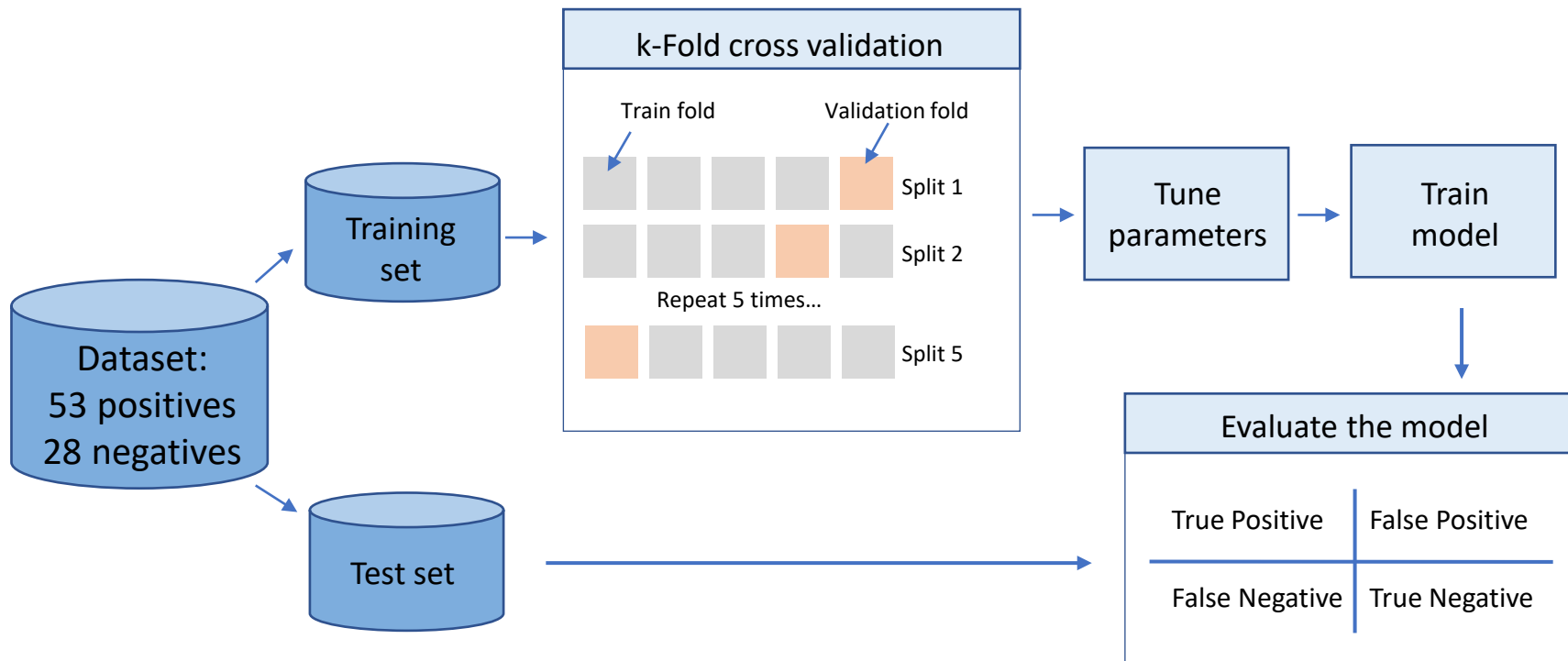
**Dataset**:

Variables: 19 MEA NFA endpoints and 16 HCI endpoints

Data: selective bioactivity; area under the concentration response curve below cytotoxicity

Outcome: categorical; evidence of *in vivo* DNT as 'positive' or 'negative'.

**Model Development:**



Training, Validation, and Testing

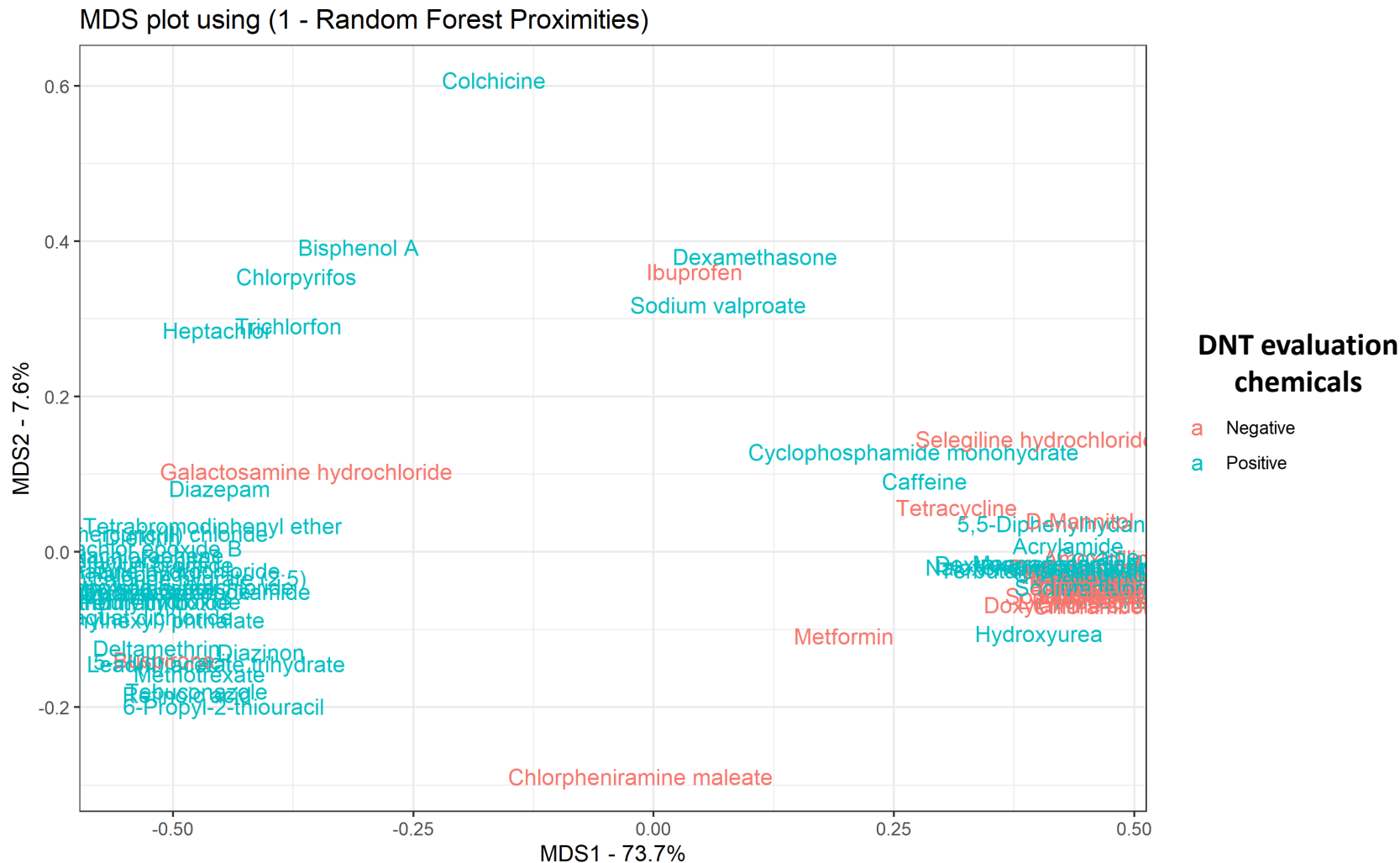# How accurate is our DNT NAMs model in predicting chemicals with evidence of *in vivo* DNT using machine learning?

| | Type | Split | Accuracy % | Balanced accuracy % | Sensitivity % | Specificity % |
|---|---|---|---|---|---|---|
| **RF** | Classification | 70/30 | **78.3** | 83.3 | 100 | 66.7 |
| **SVM** | Classification | 70/30 | **69.6** | 73.8 | 87.5 | 60.0 |

**Abbreviations**: RF, random forest; SVM, support vector machine.

**Model Performance:**
The random forest (RF) model performed the best and achieved an accuracy of 78.3%, a sensitivity of 100% and a specificity of 66.7%.

# How accurate is our DNT NAMs model in predicting chemicals with evidence of *in vivo* DNT using machine learning?



MDS plot using (1 - Random Forest Proximities)

**DNT evaluation chemicals**

a  Negative

a  Positive

# Can we improve the accuracy of our RF model by reducing the dimensions of the dataset?

## Correlation Matrix



## Feature Reduction

❖ Remove endpoints with zero variance
❖ Remove highly correlated endpoints

## Feature Importance with RF

❖ Identify most important features (endpoints) for identifying a chemical with evidence of *in vivo* DNT

# RF model performance improves when using the 10 most important features in classifying a chemical with evidence of *in vivo* DNT.

## Ranked RF feature importance

| Rank | endpoints | Importance |
|:---:|:---|:---:|
| 1 | CCTE_Mundy_HCl_NOG_NeuronCount_loss | 1.10 |
| 2 | CCTE_Shafer_MEA_dev_network_spike_duration_std_dn | 0.91 |
| 3 | CCTE_Shafer_MEA_dev_LDH_dn | 0.90 |
| 4 | CCTE_Shafer_MEA_dev_per_burst_spike_percent_dn | 0.89 |
| 5 | CCTE_Mundy_HCl_Cortical_Synap.Neur_Matur_NeuronCount_loss | 0.86 |
| 6 | CCTE_Mundy_HCl_Cortical_Synap.Neur_Matur_NeuriteSpotCountPerNeuron_loss | 0.86 |
| 7 | CCTE_Shafer_MEA_dev_bursting_electrodes_number_dn | 0.82 |
| 8 | CCTE_Shafer_MEA_dev_network_spike_peak_dn | 0.78 |
| 9 | CCTE_Shafer_MEA_dev_burst_rate_dn | 0.78 |
| 10 | CCTE_Shafer_MEA_dev_per_network_spike_spike_number_mean_dn | 0.74 |

## Model Performance

| | Type | Split | Accuracy % | Balanced accuracy % | Sensitivity % | Specificity % |
|:---:|:---:|:---:|:---:|:---:|:---:|:---:|
| **RF** | Classification | 70/30 | **87.0** | 90.0 | 100 | 80.3 |

R package 'caret'
https://cran.r-project.org/web/packages/caret/

# Current limitations to using DNT NAMs and machine learning models to predict DNT

❖ Limited number of chemicals screened across all DNT NAM assays

❖ Imbalanced set of reference chemicals, particularly few chemicals identified as DNT negatives

❖ Assay suite is currently limited to a subset of neurodevelopmental processes

❖ Lack of physicochemical information and toxicokinetic data currently incorporated into the model, e.g. blood brain barrier.
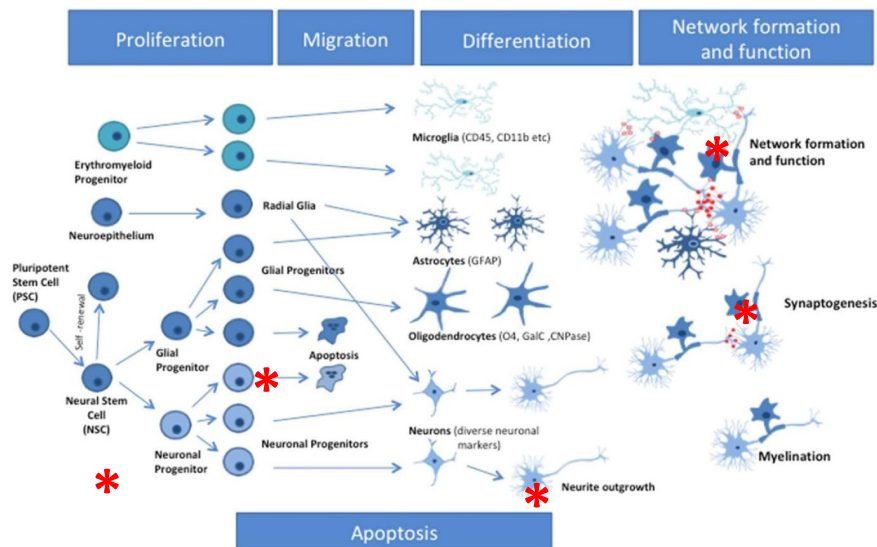


Bal-price et al. 2018

# Future Directions

❖ Screen additional chemicals, representing overlapping and diverse chemical structures and biological targets

❖ Integrate additional assays from our collaborators representing neurodevelopmental processes not currently covered, e.g. neuron differentiation and oligodendrocyte differentiation and maturation.
- Lab of Marcel Leist @ University of Konstanz
- Lab of Ellen Fritsche @ IUF- Leibniz Research Institute for Environmental Medicine

❖ Generate a DNT model that functions to reduce the complexity of a large set of endpoints and identify sensitive endpoints that may be informative for health protective points of departure.

# Acknowledgments

Tim Shafer

Katie Paul Friedman

Melissa Martin

Amy Carpenter

Theresa Freudenrich

Kathleen Wallace

Seline Choo

Jackson Keever

Josh Harrill

Megan Culbreth

Cina Mack

**Contact Info:**
Kelly Carstens, PhD
U.S. Environmental Protection Agency
Research Triangle Park, NC

Email: carstens.kelly@epa.gov
Office: 919-541-3834

**Assay data:**
Available in ToxCast invitrodb v 3.5
https://doi.org/10.23645/epacomptox.6062623.v8