Analyzing multi-dimensional developmental neurotoxicity new approach methodologies: computational approaches to identify phenoytpes.

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Introduction

Background:

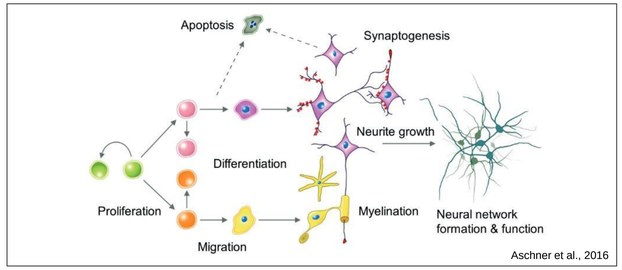
- A 15-year international-coordinated research effort has been under way to develop a battery of *in vitro* developmental neurotoxicity new approach methodologies (DNT-NAMs) to inform the understanding of DNT-related bioactivity.
- Here, we describe the collective performance of a subset of DNT-NAMs developed at the US-EPA for describing putative DNT-relevant bioactivity for 92 chemicals, including 53 reference DNT positives and 13 putative DNT negatives.

DNT-NAMs development → **DNT-NAMs evaluation** → DNT-NAMs implementation

Aims:

- Evaluate patterns of potency and selectivity for the 92 substances using hierarchical clustering to inform the understanding of DNT-related bioactivity.
- Determine the accuracy of the DNT-NAM battery in classifying 53 DNT reference positives[1] and 13 putative negatives.
- Utilize *in vitro* to *in vivo* extrapolation (IVIVE) approaches with high-throughput toxicokinetic modeling[2] (HTTK) to assess the maximal concentration tested of false negatives compounds.

DNT-NAM battery for key neurodevelopmental processes

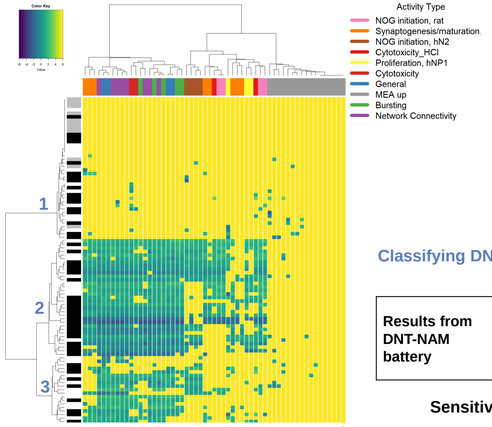


Aschner et al., 2016

Assay technology	Chemicals screened	Cell culture model	Assay/ key neurodevelopmental events	Endpoints measured
Microelectrode array (MEA)	92 (+28 repeats)	Primary rat cortical neurons (DIV 5, 7, 9, 12)	Network Formation assay (NFA); Decreasing neuronal activity	17
			Increasing neuronal activity	17
			Cytotoxicity	2
High-content imaging assays (HCI)	92	Primary rat cortical neurons	Neurite outgrowth (NOG)	4
			Synaptogenesis and Neurite maturation	8
		Human hN2 neural cells	NOG	4
		Human hNP1 neuroprogenitors	Proliferation	3
			Apoptosis	2

Evaluating potency collectively informs DNT-relevant bioactivity.

Heatmap of the DNT-NAM battery activity (NFA and HCI assays)



Color key: Potency (AC_{50} values): Yellow = screened negative; Teal = 1-100 µM; Dark blue = 0.0001-0.01 µM

Row label bar (left): DNT reference positives (black) and negatives (gray)

Activity Type (right): Activity associated with DNT-NAM endpoints

Chemical clustering (rows):

Cluster 1: Little to no activity chemicals. **Cluster 2:** High activity and potency chemicals. **Cluster 3:** Moderate activity and lower potency chemicals

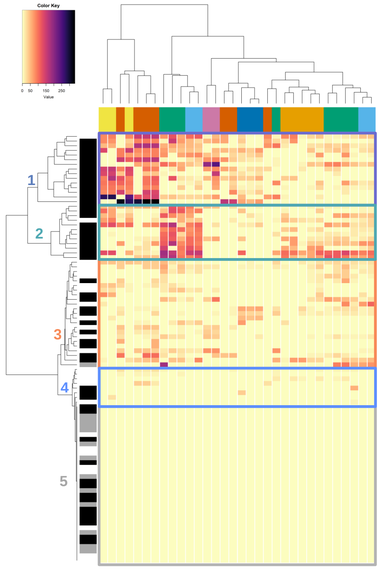
Classifying DNT Reference Chemicals

		Negatives (13)	Positives (53)
Results from DNT-NAM battery	**Potent and high activity** (Clusters 2,3)	False positive: 4	True positive: 49
	Inactive/ equivocal (Cluster 1)	True Negative: 9	False negative:4

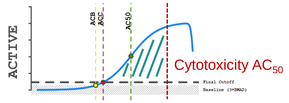
Sensitivity= 93%, Specificity= 69%, Accuracy= 88%

Selective activity reveals clear differential patterns of DNT-relevant bioactivity.

Heatmap of *selective* DNT-NAM battery activity (NFA and HCI assays)



Selectivity: area under the curve (AUC) at concentrations lower than the cytotoxicity AC50.



Color key: selectivity AUC (purple = high, pink = low to medium, yellow = negative or no selective activity

Row label bar (left): DNT reference positives (black) and negatives (gray)

Activity Type (right): Activity associated with DNT-NAM endpoints

Chemical clustering (rows):

Cluster 1: High selectivity for decreased neurite outgrowth (NOG) in human hN2 neurons and synaptogenesis.

Cluster 2: High selectivity for decreased network connectivity, general neuronal activity, and bursting activity.

Cluster 3: Moderate selectivity in various activity types across the battery.

Cluster 4: Low selectivity.

Cluster 5: Low selectivity to inactive.

Classifying DNT Reference Chemicals

		Negatives (13)	Positives (53)
Results from *selective* DNT-NAM battery	**Selective activity (Clusters 1-4)**	False positive: 1	True positive: 39
	Inactive (Cluster 5)	True Negative: 12	False negative:14

Sensitivity= 74%, Specificity= 92%, Accuracy= 77%

In vitro to *in vivo* extrapolation (IVIVE) identifies false negatives that may not have been tested at high enough concentrations.

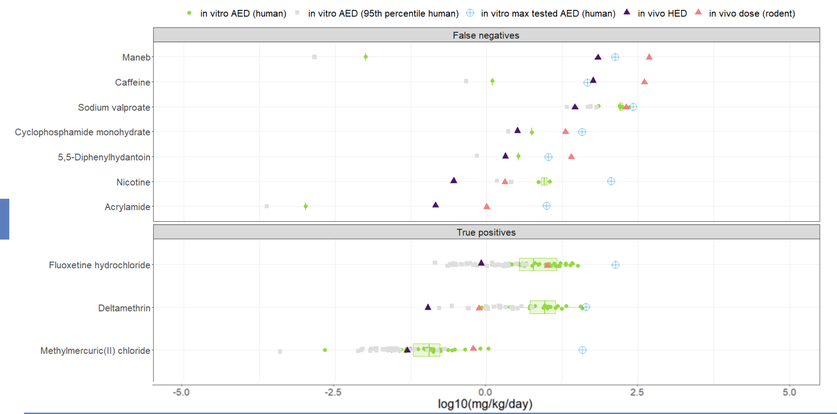
- Based on reported DNT-relevant *in vivo* rodent doses[1] and converted to HEDs[3], caffeine, maneb, and 5,5'-diphenylhydantoin may have not been screened at a high enough concentration.
- The AED based on the maximum screened concentration for sodium valproate approached doses associated with *in vivo* adversity, and cyclophosphamide monohydrate, acrylamide, and nicotine appeared to have been screened at sufficient concentrations.
- Three true positives were included for comparison, demonstrating *in vivo* points-of-departure that were several orders of magnitude below the maximum screened concentration.

AED: administered equivalent dose

In vitro AC_{50} → Human AED

HED: human equivalent dose

In vivo animal lowest effect dose → *In vivo* HED



Summary and Future Directions

- Potency in the DNT-NAM battery alone does well to capture any effect on DNT-relevant processes, but does little to distinguish patterns of effect in terms of network formation and function.
- Hierarchical clustering of DNT-NAM battery selective activity classifies DNT reference chemicals with 74% sensitivity, with 14 false negatives that may be due to biological or screening limitations, such as the maximal concentration tested. The limited number of true negative reference chemicals may bias specificity.

Conclusion: This preliminary evaluation of the DNT-NAM battery reveals differential patterns of DNT-relevant bioactivity that are informative for elucidating substrate-specific biological effects, contributing to a larger effort to use NAMs for identification and prioritization of putative DNT chemicals.

Future Direction: A larger screened chemical dataset, the addition of assays that cover more neurobiological space, and a more balanced DNT reference chemical set will continue to improve data interpretation and model building of the DNT-NAM battery.

References:

1: Mundy, W. et al., (2015). Expanding the test set: Chemicals with potential to disrupt mammalian brain development. *Neurotoxicol Teratol, 52*(Pt A), 25-35. doi:10.1016/j.ntt.2015.10.001
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3: Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm, 7*(2), 27-31. doi:10.4103/0976-0105.177703

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