National Institute of Environmental Health Sciences
Division of Translational Toxicology

Roots to Neurons: Evaluating Developmental Neurotoxicity (DNT) Potential of Botanicals Using an In Vitro Testing Battery

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RASS & MixSS Joint Webinar
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National Institutes of Health • U.S. Department of Health and Human Services

Disclaimer

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Tox Trivia!

In Season 2, Episode 5 of Will Trent, Will and Faith investigate the murder of a competitive Latin dancer who collapsed during a performance. The toxicology report reveals she was assassinated using a lethal, plant-derived neurotoxin hidden inside her body makeup. Which deadly poison was used to commit the murder?

a) Yohimbine

b) Strychnine

c) Aconitine

d) Mitragynine

Please place your answer in the chat! (e.g., A, B, C, or D)

Tox Trivia!

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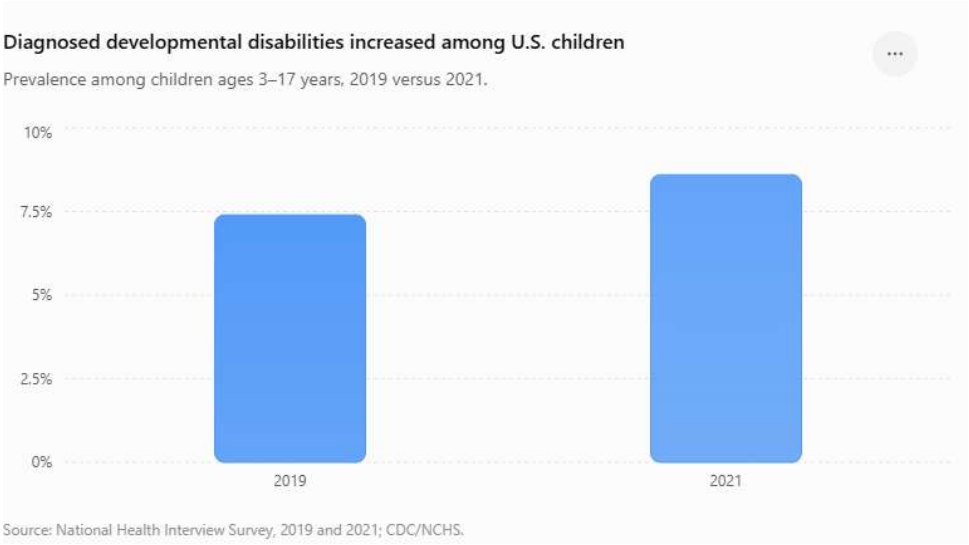
Neurodevelopmental Disorders (NDDs)

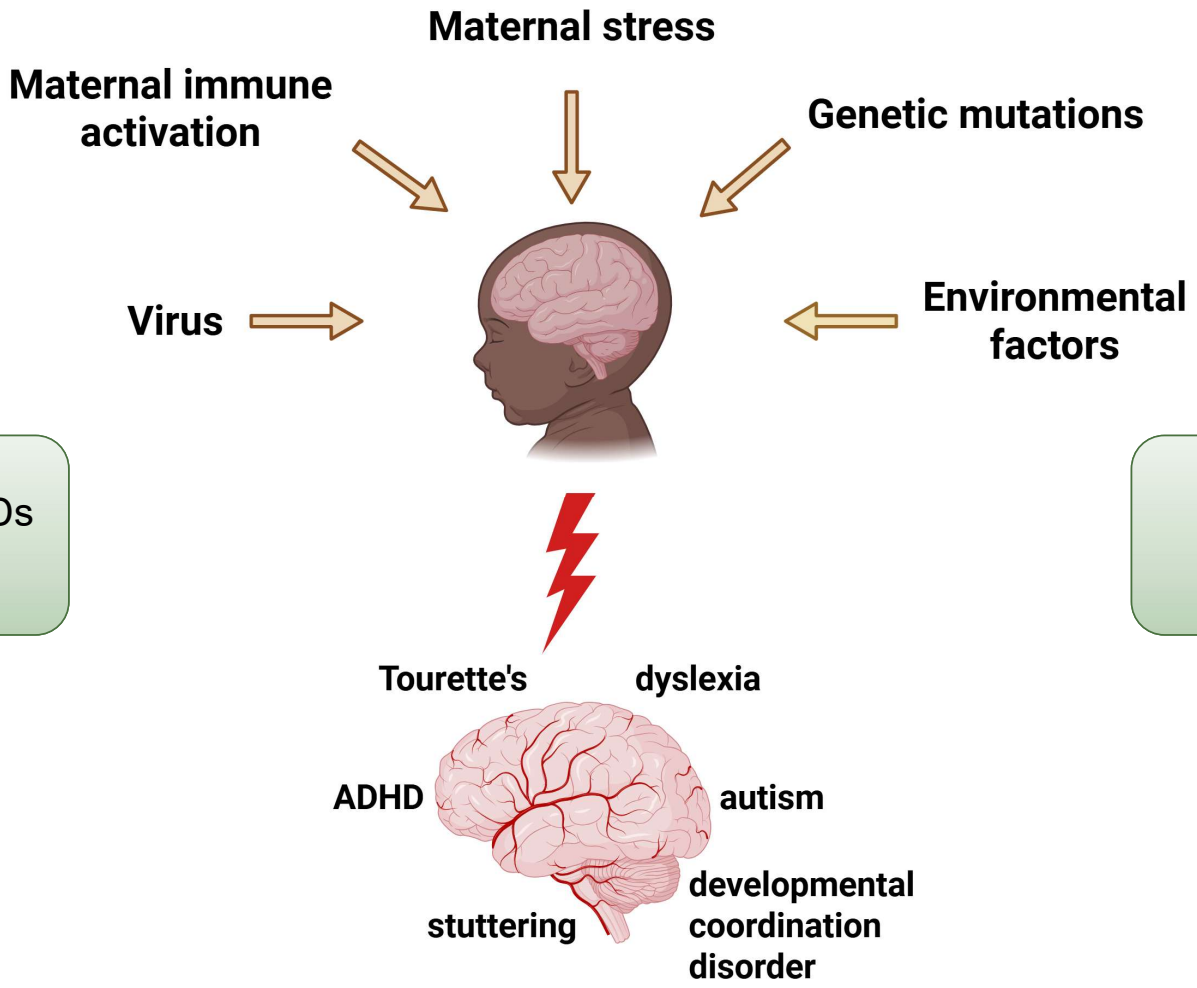
- NDDs encompass a group of conditions with onset in the developmental period
- These disorders manifest early in development often before the child enters school and are characterized by developmental deficits or differences in brain processes that can impact behavior, communication, and learning
- Types of NDDs
  - Autism spectrum disorder
  - Attention-deficit/hyperactivity disorder (ADHD)
  - Communication disorders (e.g., stuttering)
  - Motor disorders (e.g., Tourette's)

Neurodevelopmental Disorders (NDDs)

- Increase in prevalence of NDDs in the US and globally – CDC: 1 in 6 kids diagnosed with NDD
- Between 2019 and 2021, the prevalence of diagnosed developmental disabilities increased from 7.4% to 8.6% among U.S. children between the ages of 3 and 17 years old



Etiology of NDDs

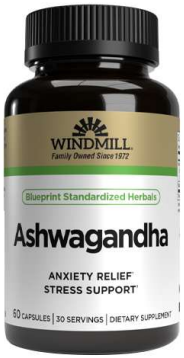


The etiology of NDDs is not fully known.

Multifactoral:
-environmental
-genetics
-neurological

Created using Biorender

Botanicals



Botanical: *a plant or plant part (e.g., leaf, root, seed) valued for its medicinal or therapeutic properties, flavor, and/or scent*

Three Common Forms of Botanicals

- **Botanical Preparations**
  (e.g., herbal/dietary supplements, teas)
- **Botanical Drugs**
  (e.g., green tea extract [Sinecatechins])
- **Essential Oils**
  (e.g., lavender, tea tree, rosemary)

Widespread of Use of Botanical Dietary Supplements

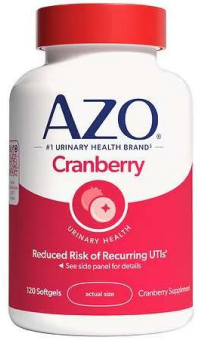
US Sales of Herbal Supplements Increased by 5.4% in 2024

American Botanical Council 2024 Herb Market Report

- The usage of botanical supplements is widespread and growing
- Retail sales of herbal dietary supplements in US reached a record of $13.231 billion in 2024 and projected to climb past $16 billion by 2026

Top 10 Botanical Dietary Supplements Sold – U.S. Mainstream Retail

Rank	Botanical/Ingredient	2024 Sales
1	**Psyllium** (*Plantago ovata*)	**$289.5 M**
2	**Elderberry** (*Sambucus nigra*, *S. canadensis*)	**$145.2 M**
3	**Ashwagandha** (*Withania somnifera*)	**$144.5 M**
4	**Turmeric** (*Curcuma longa*)	**$141.8 M**
5	**Beet root** (*Beta vulgaris*)	**$117.0 M**
6	**Apple cider vinegar** (*Malus* spp.)	**$91.1 M**
7	**Cranberry** (*Vaccinium macrocarpon*)	**$74.4 M**
8	**Wheatgrass/Barley grass** (*Triticum aestivum/Hordeum vulgare*)	**$46.9 M**
9	**Ginger** (*Zingiber officinale*)	**$44.6 M**
10	**Green tea** (*Camellia sinensis*)	**$42.7 M**



American Botanical Council 2024 Herb Market Report

With increased usage of botanicals, there is presumed safety…..

Toxic Properties of Botanicals

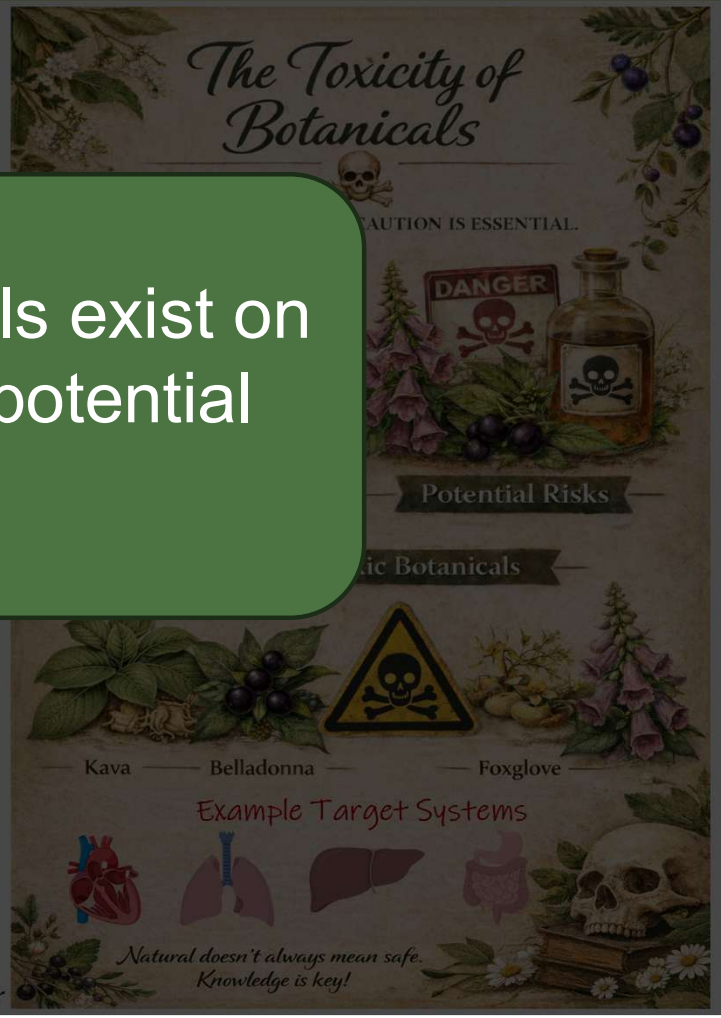
- Some botanicals have been shown to adversely affect the central nervous system
- Most chemicals are not routinely evaluated for developmental neurotoxicity (DNT)
- The potential for natural products to result in damage to the nervous system raise the concern for safety and increases the need for evaluating the toxic properties of botanicals

Made by Zakiyah, ChatGPT, and Biorender

Toxic Properties of Botanicals

- Some botanicals have been shown to adversely effect the central nervous system
- Most chemicals evaluated for d neurotoxicity (D
- The potential fo result in damage to the nervous system raise the concern for safety and increases the need for evaluating the toxic properties of botanicals

Why can certain botanicals exist on the market with known potential toxic effects?



Made by Zakiyah, ChatGPT, and Biorender

Dietary Supplement Health and Education Act of 1994

Under the Dietary Supplement Health and Education Act (DSHEA) of 1994, the FDA is not authorized to approve products containing dietary ingredients that were on the market prior to 1994 for safety and effectiveness before they are marketed.

Limitations of Current In Vivo DNT Testing

Primary regulatory approach

Used by EPA and OECD

***A priori* trigger required**

Studies are typically initiated only when triggered by existing evidence

Time- and resource-intensive

Require substantial animal use, time, and cost

Human relevance questioned

Species differences and limited mechanistic understanding can complicate translation of animal findings to humans

Global Effort to Develop a New Framework for DNT Testing

Toxicology and Applied Pharmacology 354 (2018) 3–6

Contents lists available at ScienceDirect

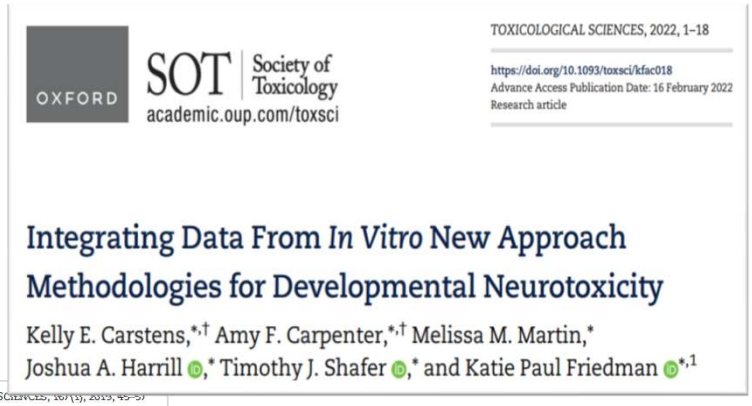
Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

ELSEVIER

Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes

Ellen Fritsche[a], Philippe Grandjean[b], Kevin M. Crofton[c], Michael Aschner[d], Alan Goldberg[e,w], Tuula Heinonen[f], Ellen V.S. Hessel[g], Helena T. Hogberg[h], Susanne Hougaard Bennekou[i], Pamela J. Lein[j], Marcel Leist[k], William R. Mundy[l], Martin Paparella[m], Aldert H. Piersma[n], Magdalini Sachana[o], Gabriele Schmuck[p], Roland Solecki[q], Andrea Terron[r], Florianne Monnet-Tschudi[s], Martin F. Wilks[t], Hilda Witters[u], Marie-Gabrielle Zurich[s], Anna Bal-Price[v,*]



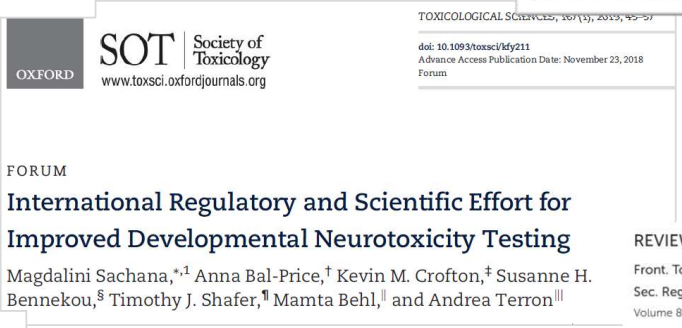
OXFORD SOT Society of Toxicology academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2022, 1–18

https://doi.org/10.1093/toxsci/kfac018
Advance Access Publication Date: 16 February 2022
Research article

Integrating Data From *In Vitro* New Approach Methodologies for Developmental Neurotoxicity

Kelly E. Carstens,*,† Amy F. Carpenter,*,† Melissa M. Martin,* Joshua A. Harrill,* Timothy J. Shafer,* and Katie Paul Friedman*,1



OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(1), 2019, 45–57

doi: 10.1093/toxsci/kfy211
Advance Access Publication Date: November 23, 2018
Forum

FORUM

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing

Magdalini Sachana,*,1 Anna Bal-Price,† Kevin M. Crofton,‡ Susanne H. Bennekou,§ Timothy J. Shafer,¶ Mamta Behl,‖ and Andrea Terron‖

Toxicology and Applied Pharmacology 354 (2018) 7–18

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Toxicology and Applied Pharmacology

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ELSEVIER

Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using *in vitro* methods

Anna Bal-Price[a,*], Francesca Pistollato[a], Magdalini Sachana[b], Stephanie K. Bopp[a], Sharon Munn[a], Andrew Worth[a]

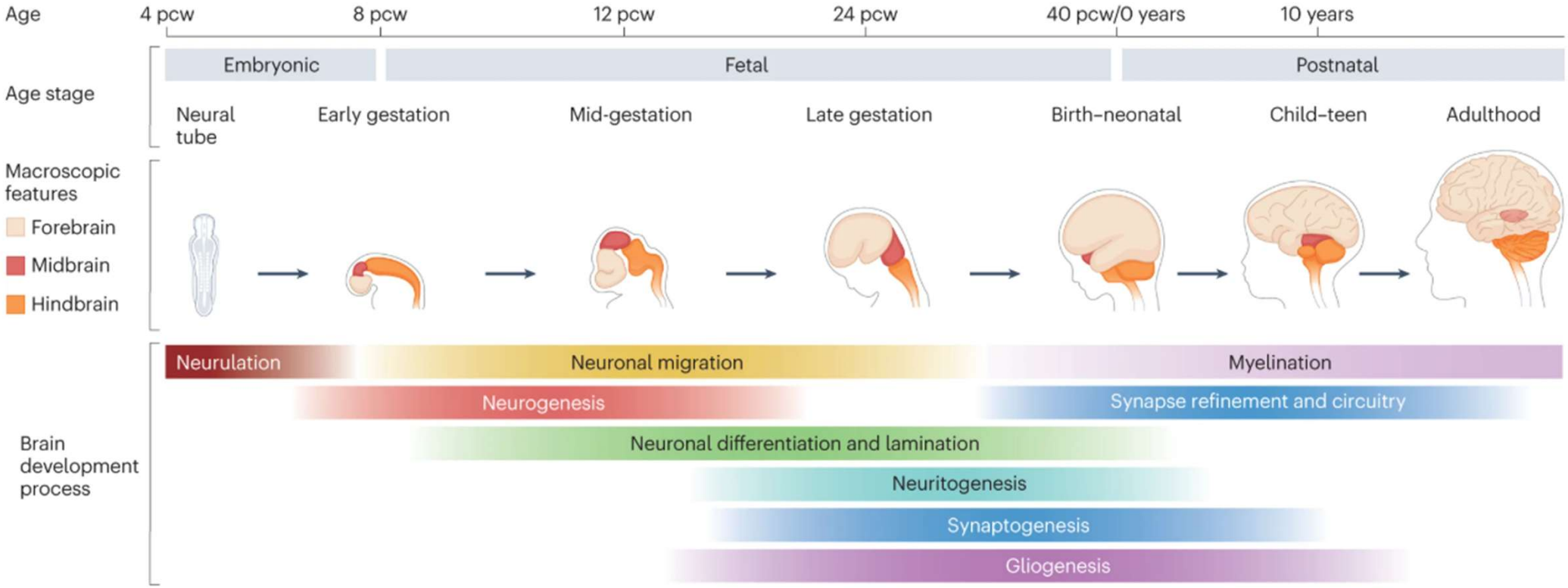
REVIEW article

Front. Toxicol., 19 March 2026
Sec. Regulatory Toxicology
Volume 8 - 2026 | https://doi.org/10.3389/ftox.2026.1774469

From concept to regulation: OECD's role in the development of the DNT *in vitro* battery

Magdalini Sachana [1*] Helena T. Hogberg [2] Iris Mangas [3]

Key Processes of Human Brain Development



Zhou, Y., Song, H. & Ming, Gl. Genetics of human brain development. Nat Rev Genet 25, 26–45 (2024). https://doi.org/10.1038/s41576-023-00626-5

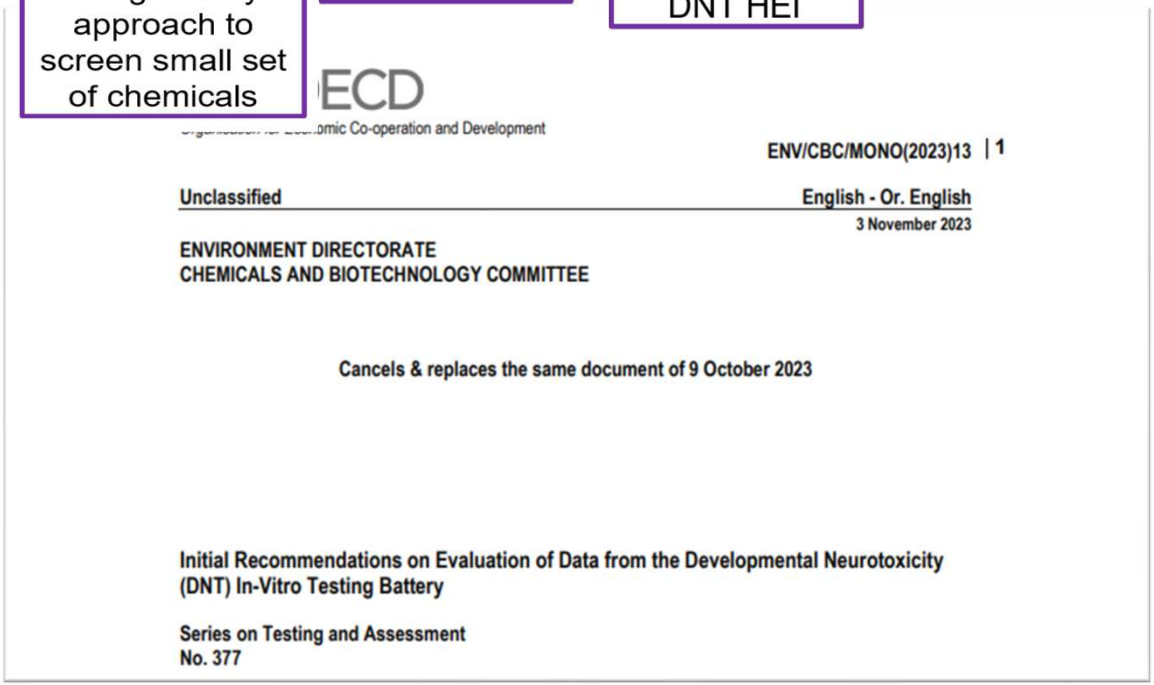
Global Effort to Develop a New Framework for DNT Testing

Created "NTP80" list to screen for compounds with potential for DNT/NT

Published paper using battery approach to screen small set of chemicals

NTP Integrated DNT Testing Workshop

Formation of DNT HEI



ECD

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ENV/CBC/MONO(2023)13 | 1

Unclassified

English - Or. English

3 November 2023

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 9 October 2023

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

Series on Testing and Assessment
No. 377

Phase 1 testing **2021**

Phase 2 testing **2023**

Phase 3 testing **2024**

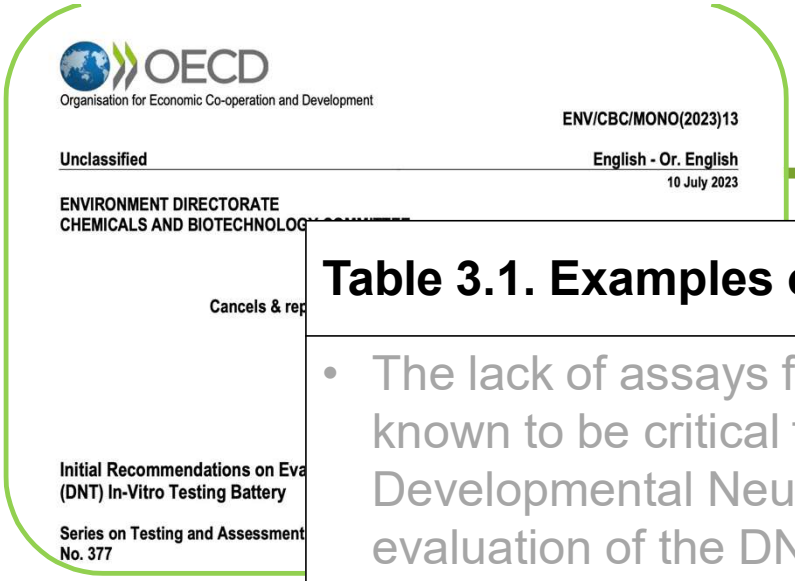


Table 3.1. Examples of weight of evidence (WoE) limitations of the DNT IVB
• The lack of assays for several cellular processes and systemic processes known to be critical for normal neurological development (see Sections Developmental Neurotoxicity In Vitro Battery (description of assays) and evaluation of the DNT IVB for chemical testing).
• Need for development of additional AOPs to increase mapping of KEs covered in the DNT IVB.
• **Relatively limited number of tested chemicals as compared to current accepted batteries (e.g. ER activation).**
• Uncertainty in the overall specificity and sensitivity of the DNT IVB due to limited testing of DNT reference chemicals and comparison of results to curated in vivo developmental neurotoxicity database.
• A need for consensus-based and regulatory driven tiered testing strategy to be used in IATAs

Chemical Nominations Received From Multiple Stakeholders

Federal Agencies

NIH National Institute of Environmental Health Sciences
Division of Translational Toxicology

FDA CFSAN

Consumer Product Safety Commission United States

United States Environmental Protection Agency

European Agencies

ECHA European Chemicals Agency

efsa European Food Safety Authority

OECD

Universities

The University of North Carolina at Chapel Hill

UC Davis University of California

Duke University

VU Vrije Universiteit Amsterdam

scaht Swiss Centre for Applied Human Toxicology

UFZ Helmholtz Centre for Environmental Research

Non-profits

HESI

Botanical Safety Consortium

NRDC Natural Resources Defense Council

Escher Fund for Autism
Humans start as molecules
GermlineExposures.org

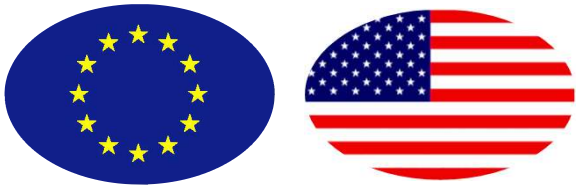
Objective:

Screen botanicals in a battery of assays to determine DNT potential.

Made with Biorender

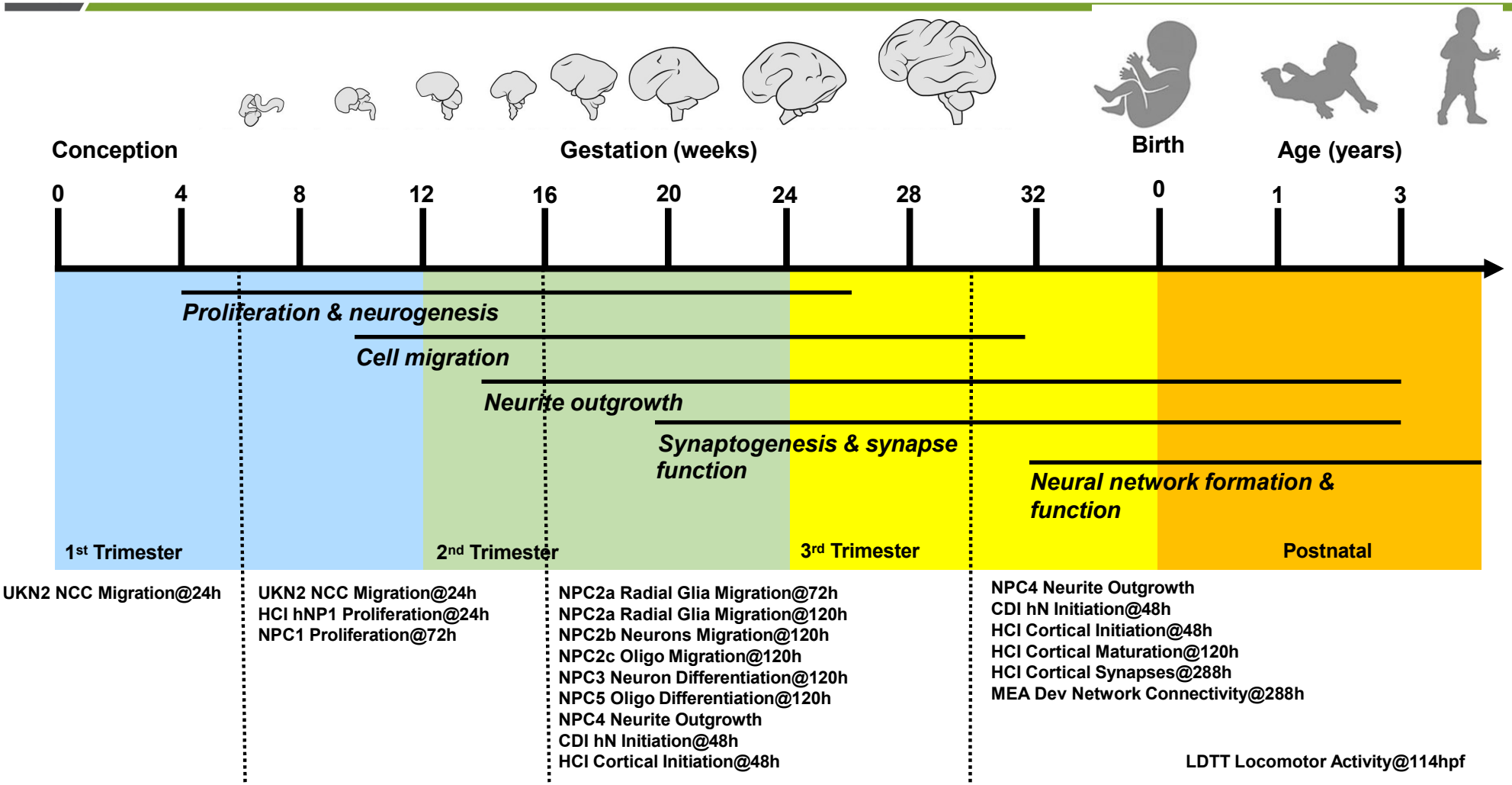
Refined Battery: OECD DNT IVB vs DTT DNT Battery

- US EPA (7assays)
- IUF Dusseldorf University (7 assays)
- Konstanz University (OECD DNT IVB =3 vs. 1 in DNT Battery)
- Zebrafish behavior assay (Added to DNT Battery)



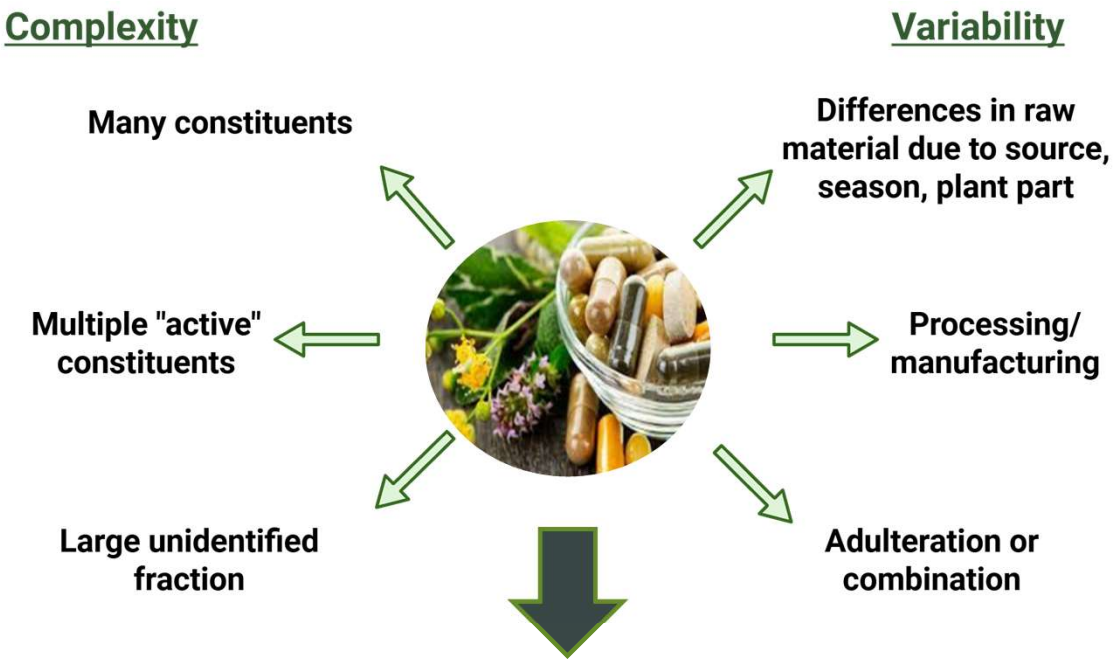
Neurodevelopmental Process	Assay: Human				Rat	Data Gaps	
NPC Proliferation	*NPC1* Neural Progenitor (IUF)	*hNP1 Prolif* Neural Progenitor (EPA)				hiPSC-derived Neural Progenitor	Radial Glia Neural Progenitor
NPC Apoptosis	*hNP1 Apop* Neural Progenitor (EPA)						
Cell Migration	*UKN2* Neural Stem line (UKON)	*NPC2a* Radial Glia (IUF)	*NPC2b* Neuronal (IUF)	*NPC2c* Oligodendrocyte (IUF)			
NPC-Neuronal Differentiation	*NPC3* Neuron (IUF)					Neuronal Subtype	hiPSC-derived Neural Progenitor
Neurite Outgrowth	*NPC4* Neuron (IUF)	*UKN4* NSC line Neuron (UKON)	*UKN5* Peripheral Neuron (UKON)	*hN Initiation* Neuron (EPA)	*Cortical Initiation* Primary Neuron (EPA)		
Neurite Maturation					*Cortical Maturation* Primary Neuron (EPA)	Human Neuron	
Synaptogenesis					*Cortical Synapto* Primary Neuron (EPA)	Human Neuron	
NPC-Glial Differentiation	*NPC5* Oligo (IUF)					Astrocyte	Radial glia
Myelination						Oligodendrocyte	
Neural Network Formation					*Cortical MEA* Primary Neuron (EPA)	Human Neuron Based	

Battery Captures Embryonic and Early Fetal Timepoints



Whole Mixture Approach For Assessing Botanicals

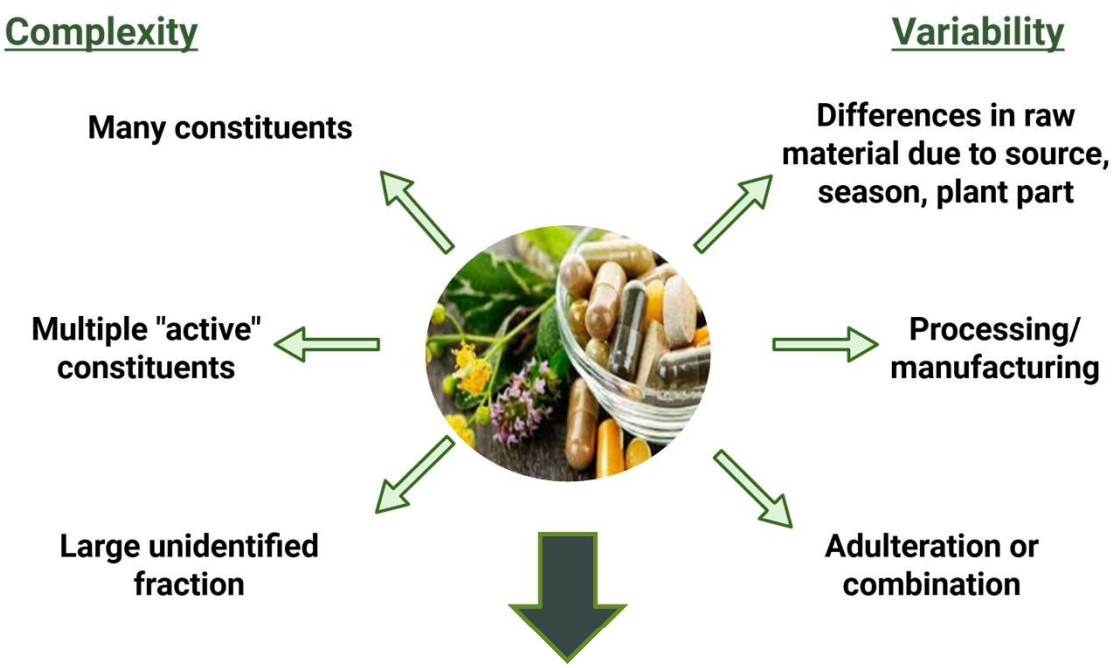
Botanicals are complex mixtures….



Test article selection, procurement, preparation, and evaluation

Considerations and Approach for Assessing Botanicals

Botanicals are complex mixtures….



Test article selection, procurement, preparation, and evaluation

Whole mixture approach

Diverse products in the marketplace

Sufficient similarity

Mixture of Interest "Representative"

Food and Chemical Toxicology 118 (2018) 328–339

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

ELSEVIER

How similar is similar enough? A sufficient similarity case study with *Ginkgo biloba* extract

Natasha R. Catlin[a,1], Bradley J. Collins[a], Scott S. Auerbach[a], Stephen S. Ferguson[a], James M. Harnly[b], Chris Gennings[c], Suramya Waidyanatha[a], Glenn E. Rice[d], Stephanie L. Smith-Roe[a], Kristine L. Witt[a], Cynthia V. Rider[a,*]

[a] Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
[b] U.S. Department of Agriculture, Beltsville, MD, USA
[c] Icahn School of Medicine, Mount Sinai Hospital, New York, NY, USA
[d] U.S. Environmental Protection Agency, Cincinnati, OH, USA

Assessment of Botanicals in the DNT Battery

Evidence of neurotoxicity

- Clinical reports of poisonings
- *In vivo* and *in vitro* studies
- Neurotoxicity of specific constituents
  - aconitine
  - caffeine
  - yohimbine
  - mitragynine
  - saikosaponins
  - thujone
  - oleandrin
  - linamarin

Botanical	Traditional or Common Uses
Aconite	Traditionally used in Chinese and Ayurvedic medicine for **pain, inflammation, fever, and rheumatic conditions**; historically used as an analgesic and antipyretic.
Guarana	Used as a **stimulant and energy-enhancing botanical** due to its high caffeine content; commonly marketed for **alertness, fatigue, weight management, and exercise performance**.
Yohimbe	Traditionally used as an **aphrodisiac** and for male sexual health; yohimbine-containing products have been marketed for **erectile dysfunction, sexual enhancement, and weight loss**.
Kratom	Traditionally used in Southeast Asia for **pain relief, increased energy, and endurance**; more recently used for **pain management, mood effects, and opioid withdrawal/self-management**.
Bupleurum	Commonly used in traditional Chinese medicine for **liver and digestive disorders, fever, inflammation, and respiratory conditions**; often included in multi-herb formulations.
Wormwood	Traditionally used for **digestive complaints, loss of appetite, and intestinal worms**; also historically used as a bitter tonic and in production of **absinthe**.
Oleander	Historically used in traditional medicine for various conditions including **cardiac, gastrointestinal, and inflammatory disorders**. It is **highly toxic** and has a narrow therapeutic window.
Cassava	Major **food crop and source of dietary starch/carbohydrates**, particularly in tropical regions; also used traditionally for various **medicinal and nutritional purposes**. Proper processing is important because raw cassava contains **cyanogenic compounds**.

Assessment of Botanicals in the DNT Battery

Evidence of neurotoxicity

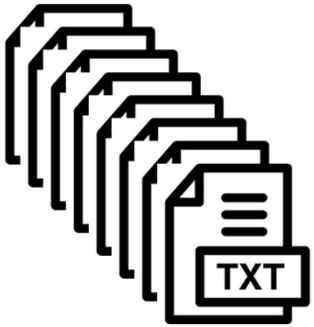
- Clinical reports of poisonings
- *In vivo* and *in vitro* studies
- Neurotoxicity of specific constituents
  - aconitine
  - caffeine
  - mitragynine
  - saikosaponins
  - thujone
  - yohimbine
  - oleandrin
  - linamarin

Procured 22 botanical products

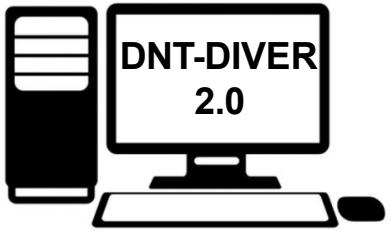
- Testing articles varied by preparation from the 8 unique botanicals:
  - powders
  - extracts
  - whole leaf
  - whole plant
- Frozen sample aliquots were shipped to testing labs (blinded)
- Bioactivity of each botanical was assessed

Data Analysis and Chemical Prioritization

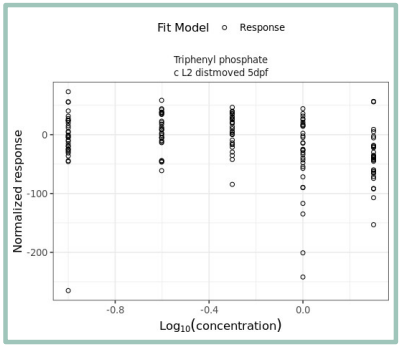
Raw data



Quality Check → Data Loading → Database Design → Data Analysis

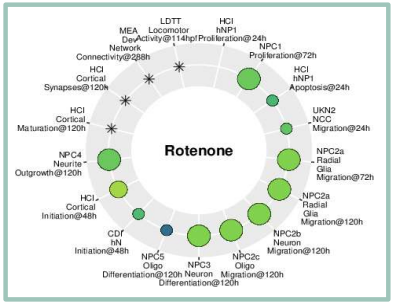


Data Analysis Pipeline

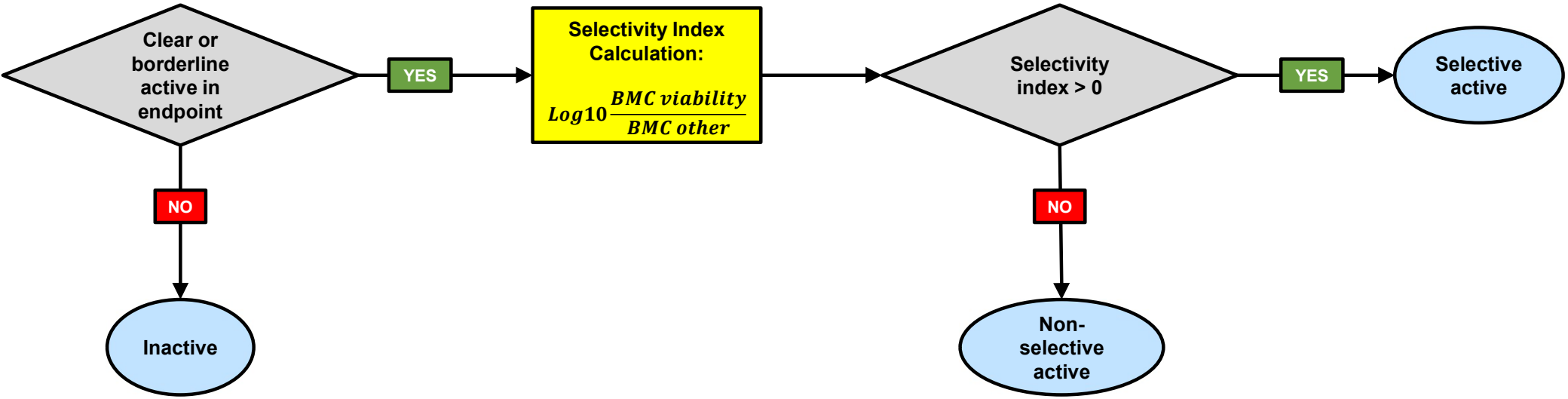


Concentration-response data

BMC Modeling → Selectivity Calculation → Activity Summary → Pareto Frontier Ranking



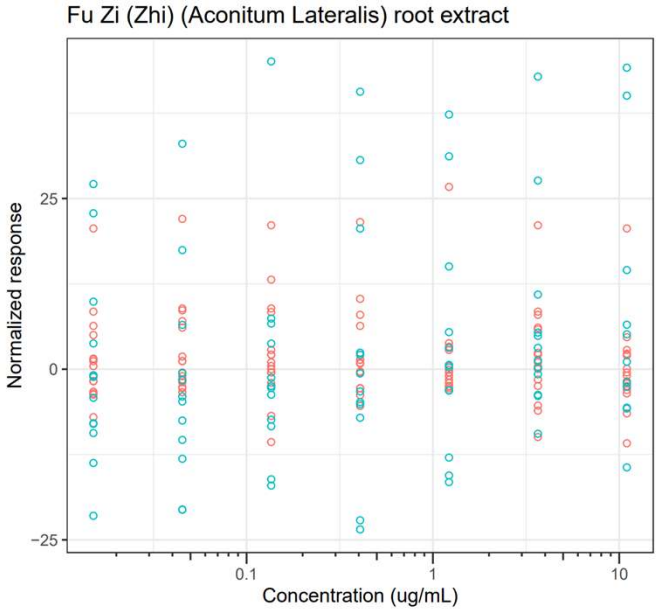
Determination of Selectivity in the Assays



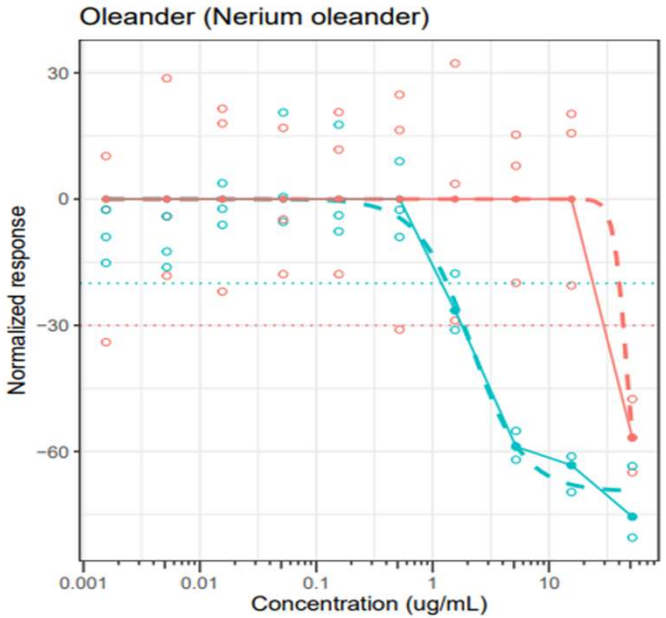
- **Inactive** = no activity detected
- **Selective active** = true DNT effect
- **Non-selective active** = cytotoxicity precedes neurotoxicity

Determination of Selectivity in the Assays

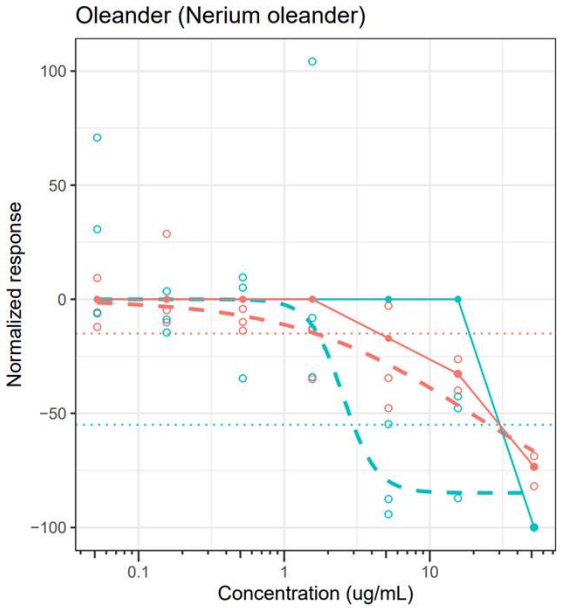
Inactive



Selective-Active



Non-selective Active



RED = cytoxicity/viability
BLUE = neuro

Fit Model
···· BMR
– – Hill
—●— CurveP

- **Inactive** = no activity detected
- **Selective active** = true DNT effect
- **Non-selective active** = cytotoxicity precedes neurotoxicity

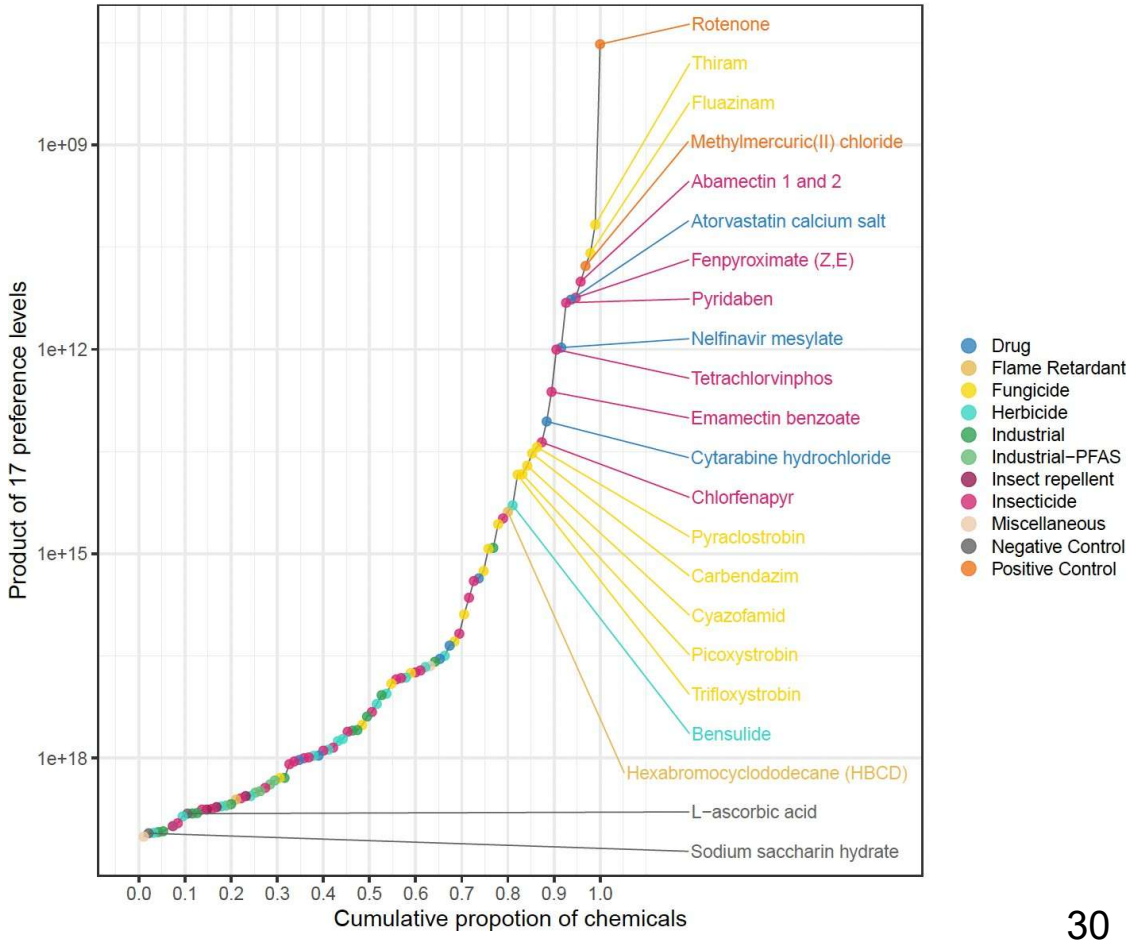
Compound Prioritization Using Pareto Frontier Analysis

Pareto ranking based on the following attributes:

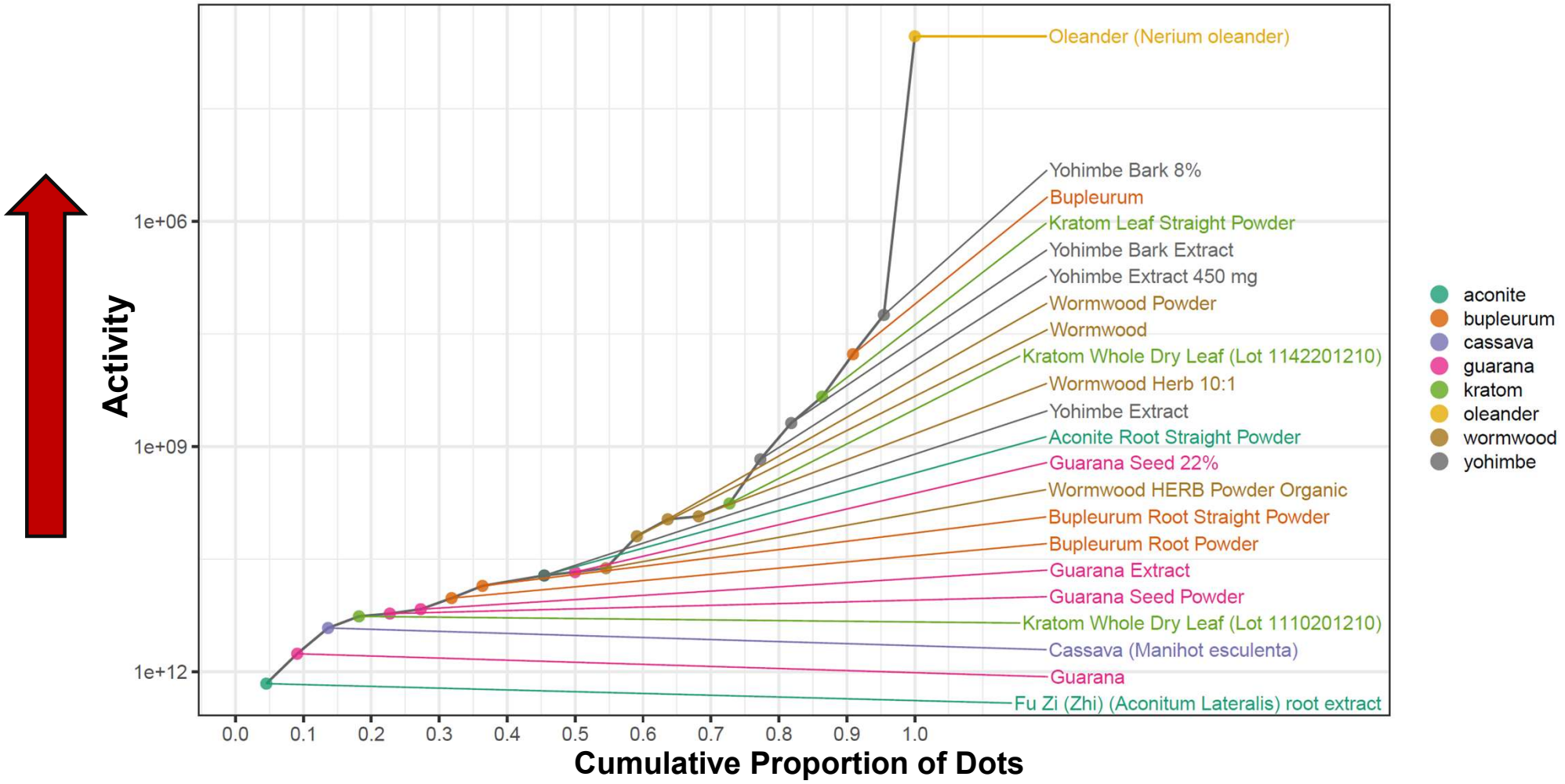
1. Mean BMC from active endpoints
2. Mean selectivity scores from active endpoints
3. Mean activity confidence scores from active endpoints
4. Fraction of active endpoints

- In this assessment, chemicals with higher potency and/or selectivity were considered to possess greater potential for developmental neurotoxicity and thus could be prioritized for further testing

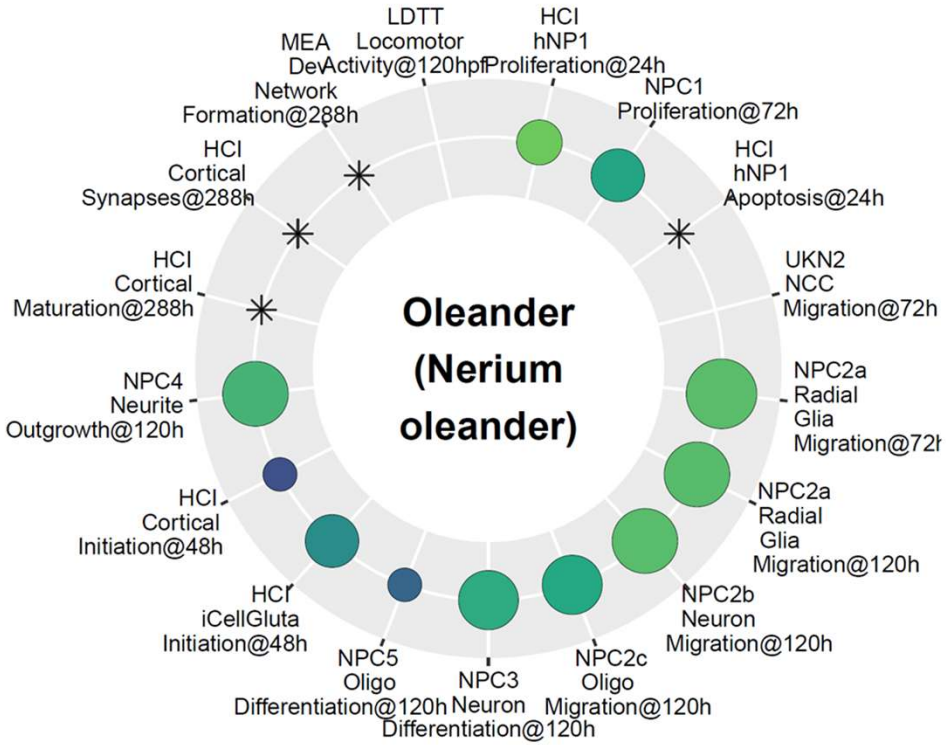
A.

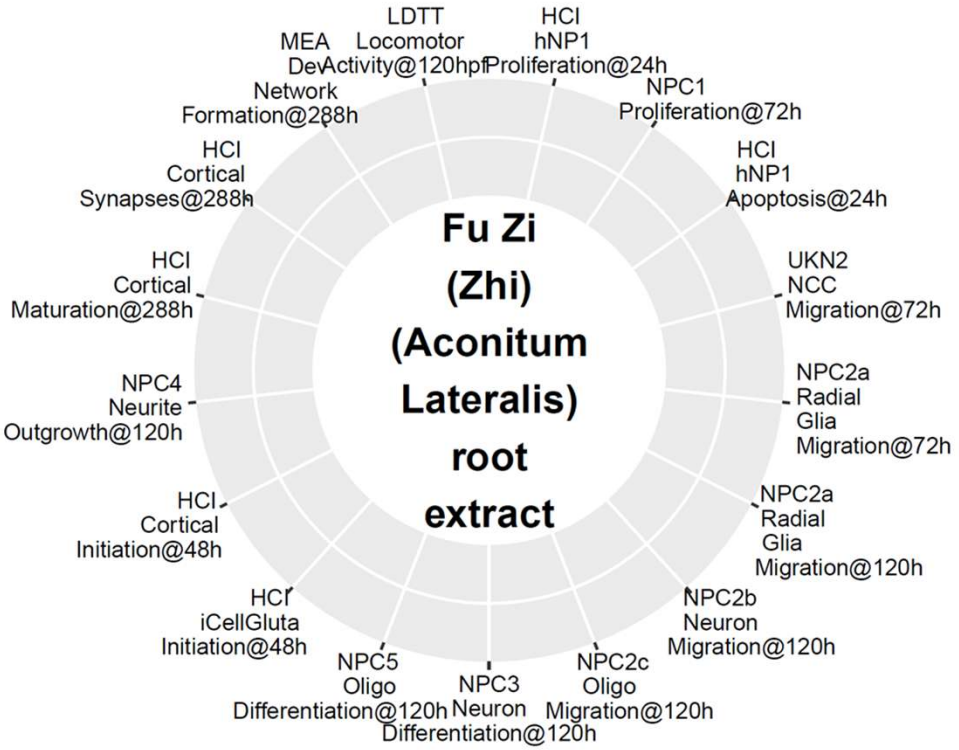


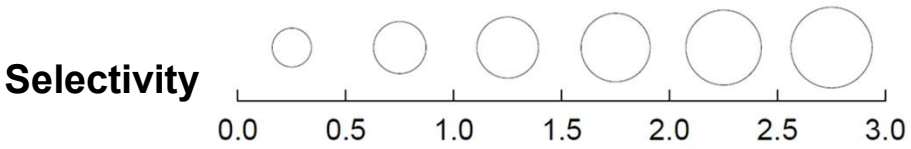
Ranked Botanicals Based on Overall Activity in Battery

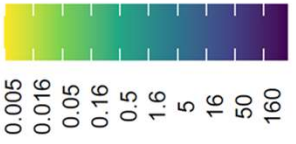


Botanicals With Most and Least DNT Potential



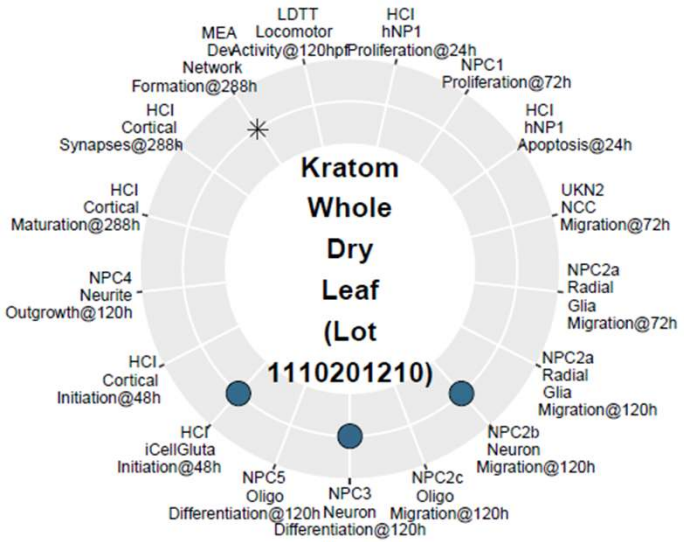


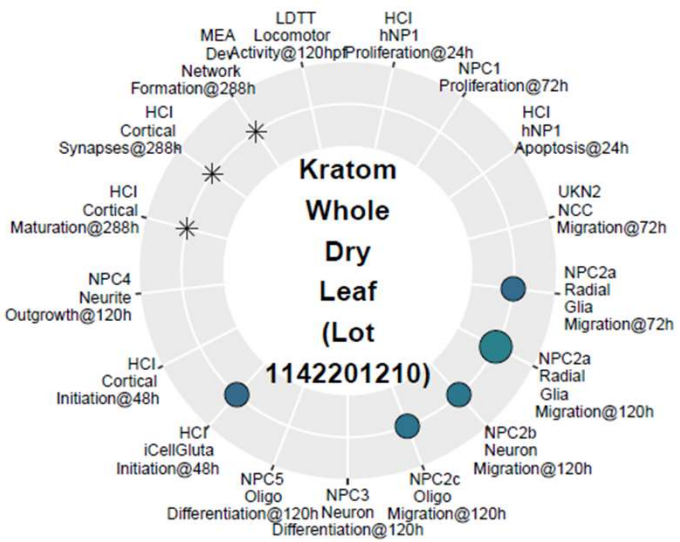


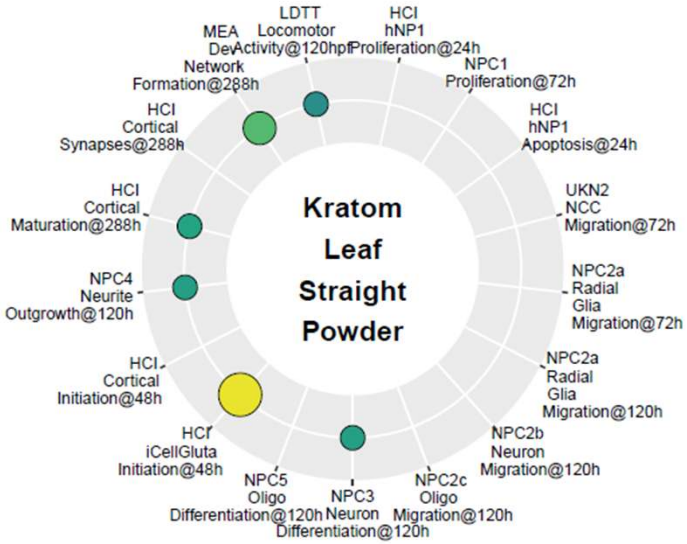


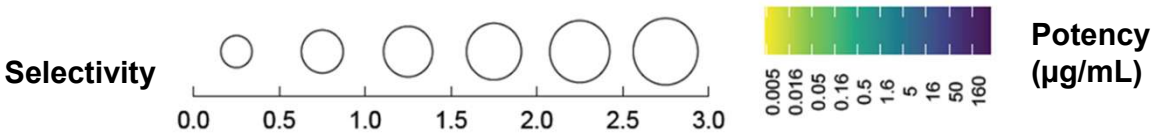
Potency (µg/mL)

Potency and Activity Differences of Same Ingredient

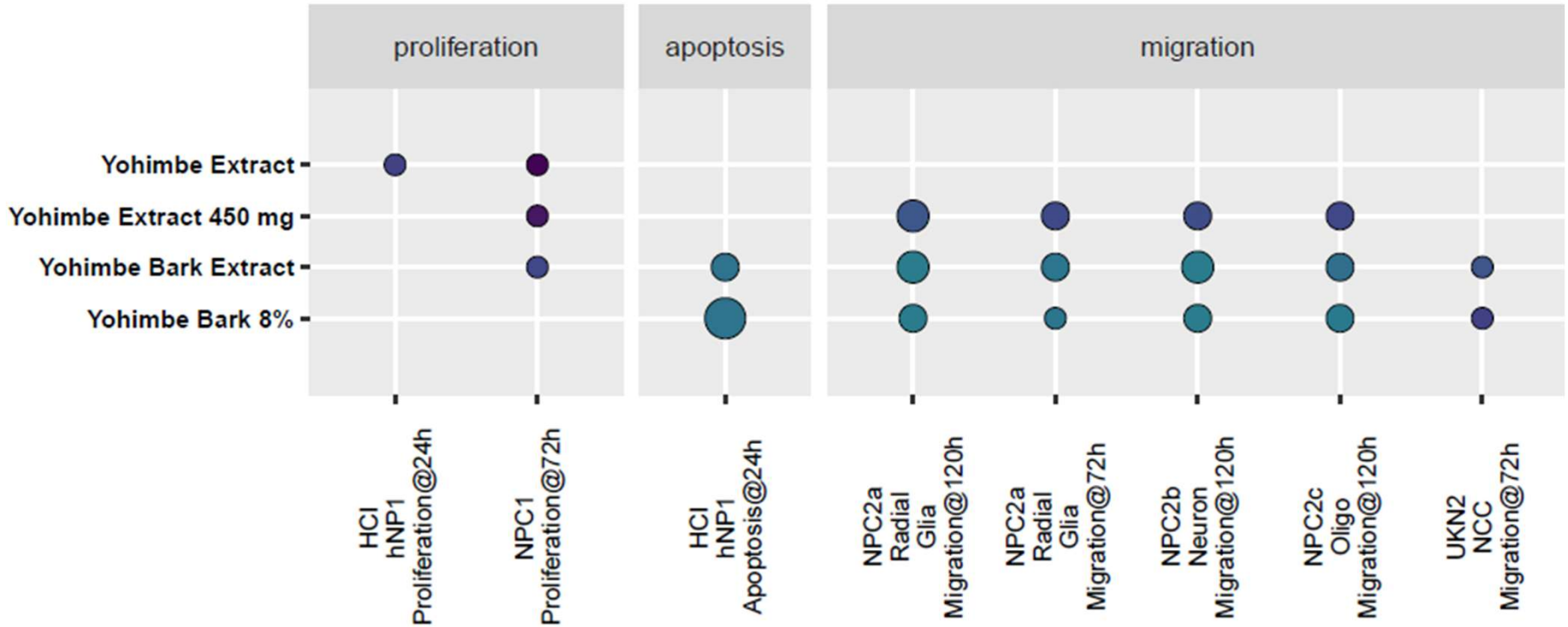




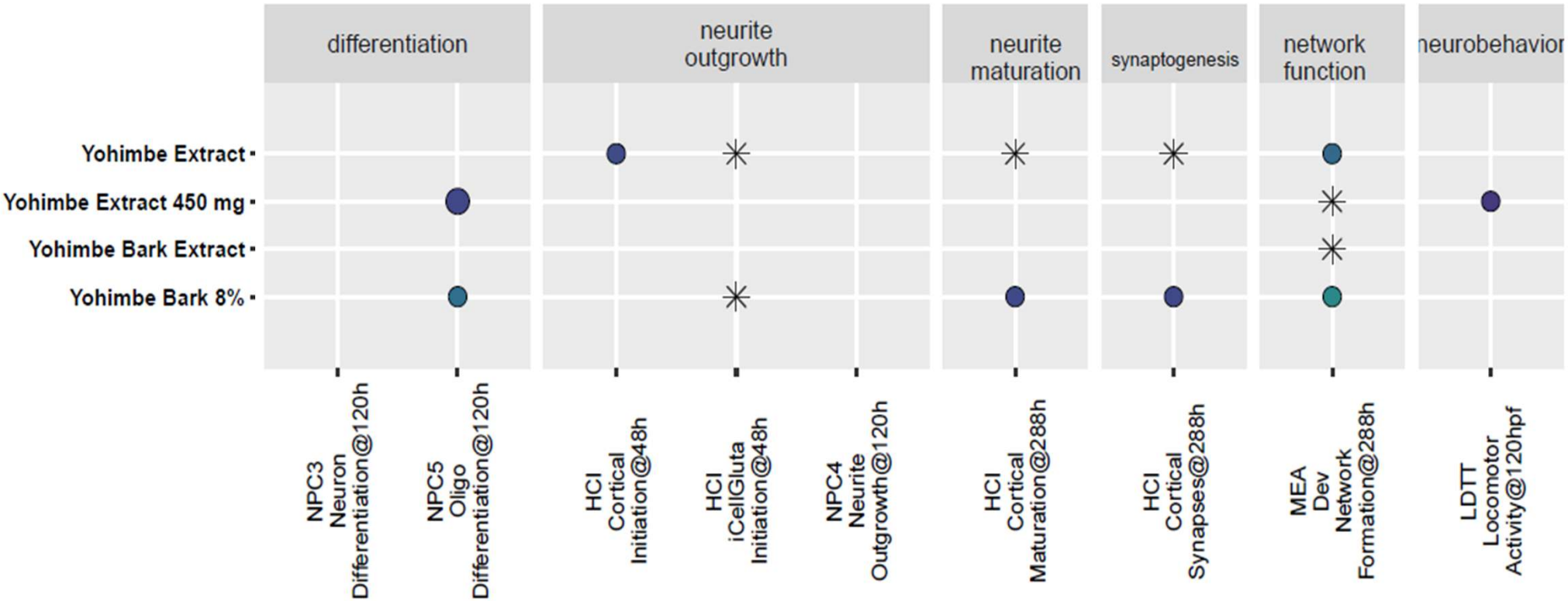




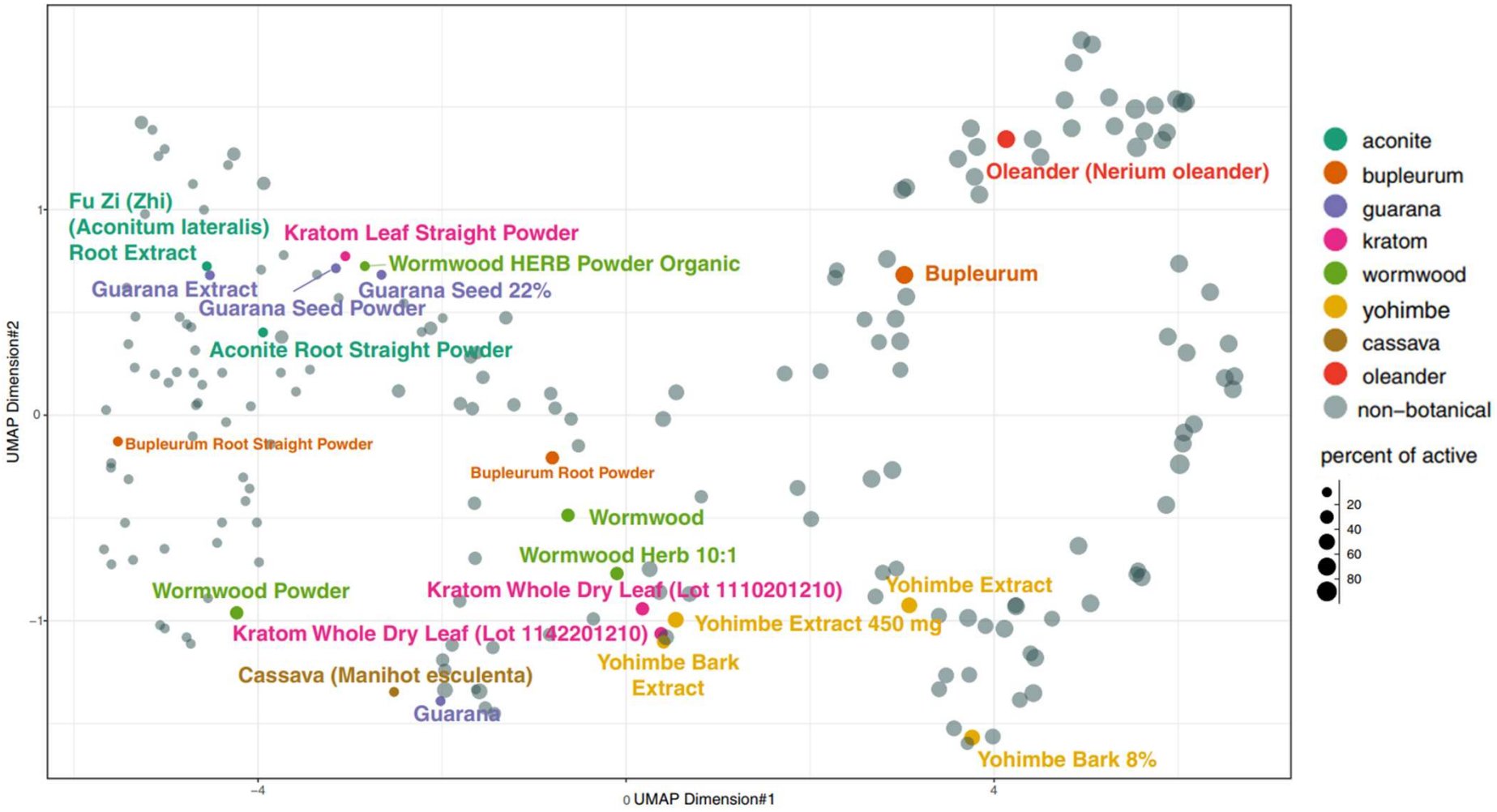
Potency and Activity Differences of Same Ingredient



Potency and Activity Differences of Same Ingredient



Cluster Analysis Shows Variability Among Botanical Samples



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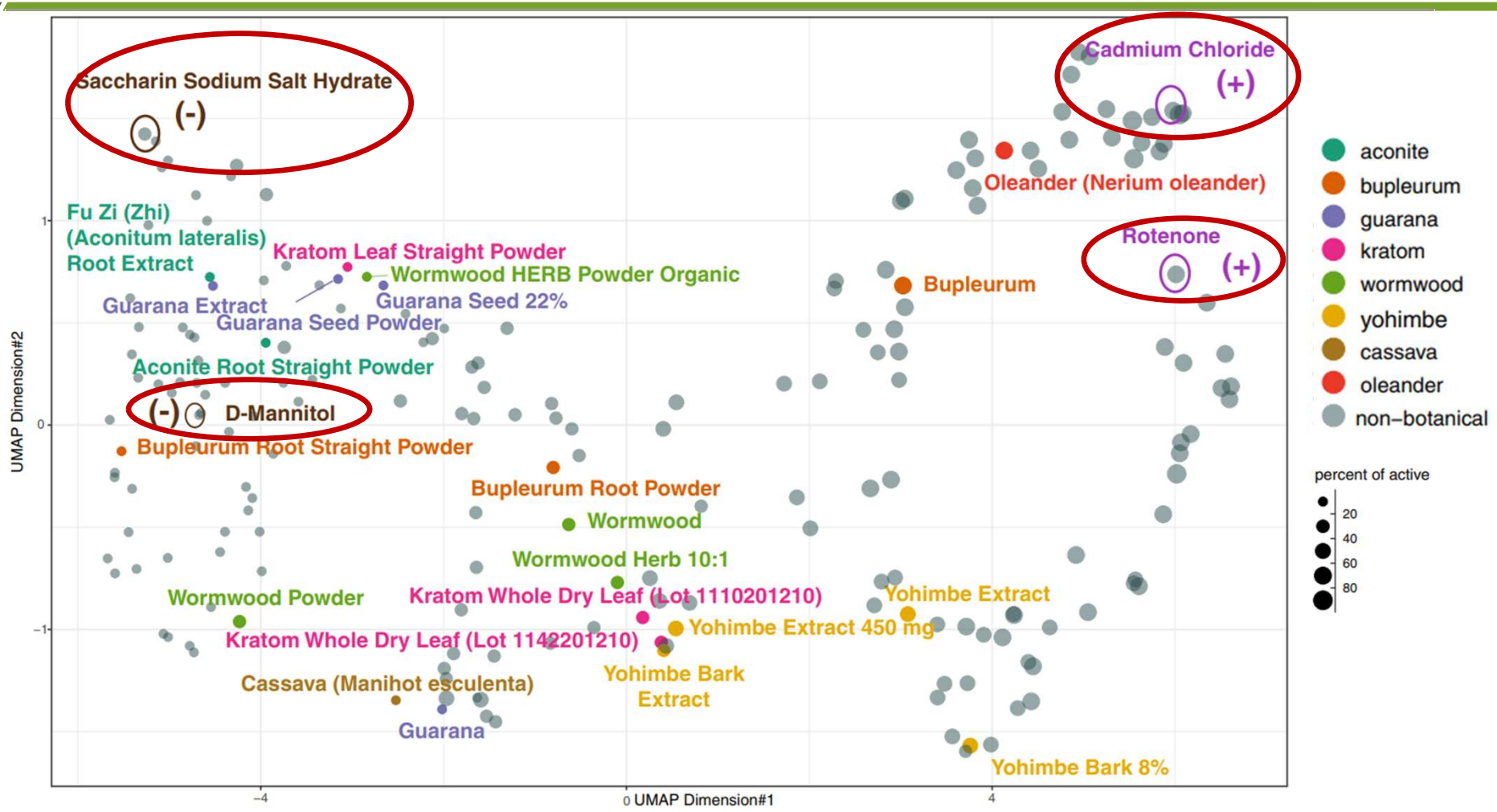

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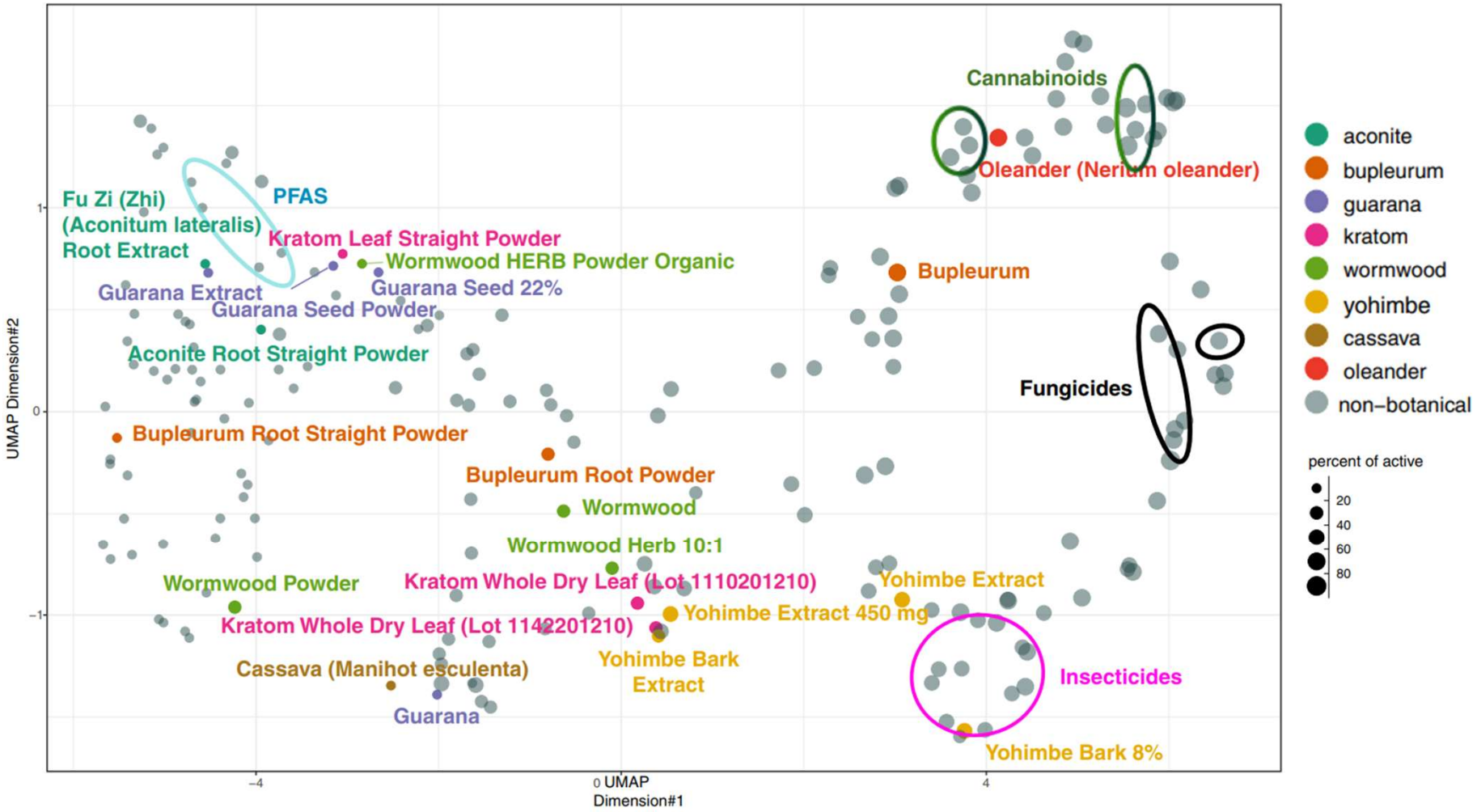
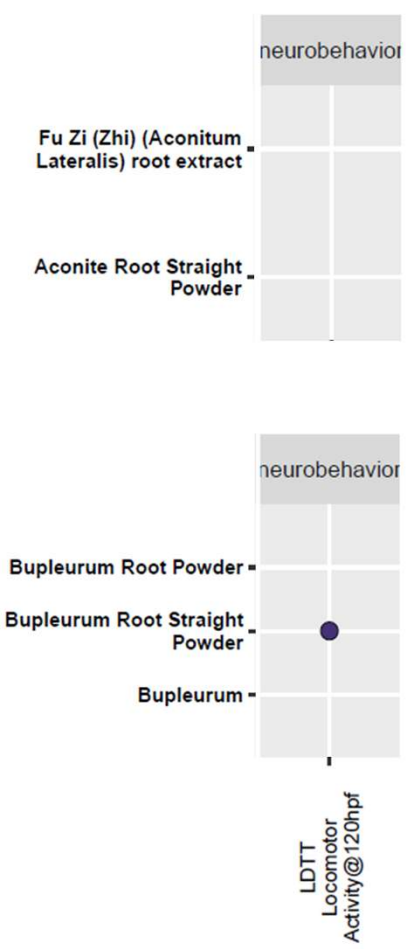
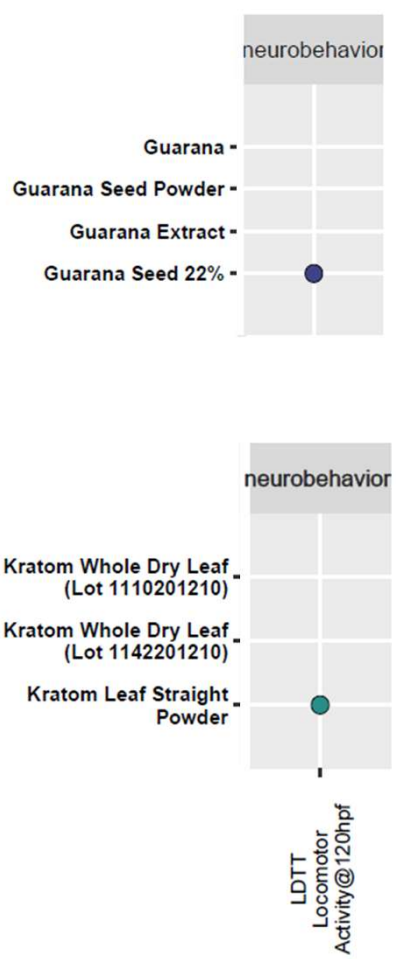
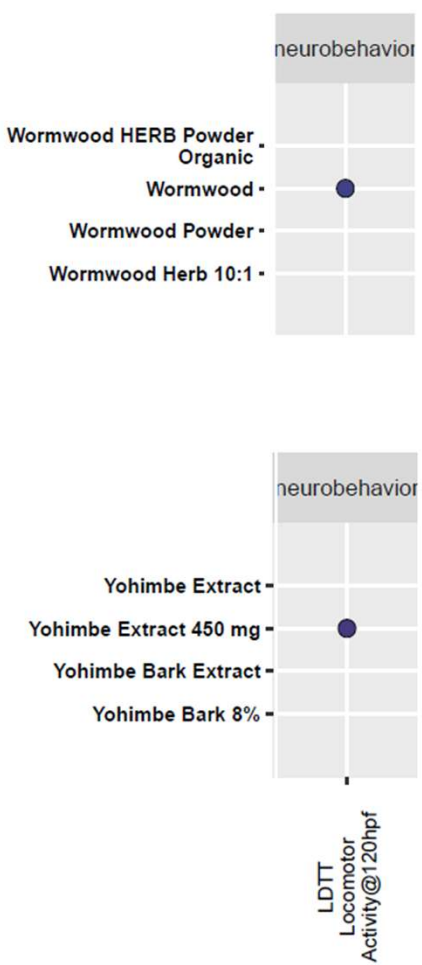


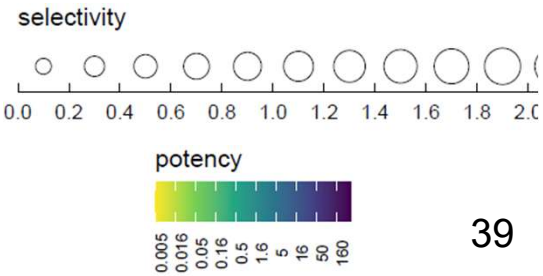
Limited Effects on Neurobehavior







- Out of 22 tested botanical samples, only 5 were active in the zebrafish neurobehavior assay.
- Oleander and cassava were also not active (data not shown)



Conclusions

- Botanical dietary supplement extracts were active in neurodevelopmental assays
- Oleander exhibited the greatest potential for DNT effects and Fu Zhi with the least
- Consistent results across individual botanicals were not observed
  - Likely due to variations in botanical processing and preparation affecting chemical composition and potency
- Limited neurobehavior activity across botanical samples
- The diverse activity phenotypes of botanicals and botanical-derived compounds further highlight the complexity of hazard characterization, including DNT evaluation.

Screening Battery Strengths

- Screening battery covers multiple endpoints, rapid, high-throughput and reproducible
- Activity was observed with varying potency across all endpoints
- The screening battery captures a wide range of potency/selectivity in the compounds we've tested.
- It is well suited for screening and prioritization.
- Can be used to assess DNT potential of complex mixtures

Screening Battery Limitations

- Mechanisms → in its current form not fit for purpose to elucidate mechanistic understanding
- Limited outcome prediction → identifies biological activity but does not directly predict human disease
- Incomplete coverage → not all neurodevelopmental processes (myelination) or cell types represented
- Limited ADME/metabolism → may not reflect real-world chemical exposure or metabolite formation

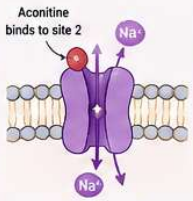
Botanical Neurotoxic Mechanisms of Action

Eight Botanicals, Distinct Mechanisms, Multiple Pathways to Neurotoxicity

ACONITE
(Monkshood / Wolfsbane)

Key Toxic Constituents
Aconitine, mesaconitine (diterpenoid alkaloids)

MECHANISM OF ACTION
Persistent activation of voltage-gated Na^+ channels



Sustained Na^+ influx
→ persistent depolarization
→ neuronal inexcitability

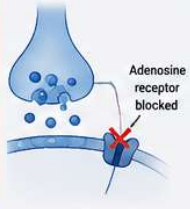
Potential Neurotoxic Outcomes
- Paresthesia, numbness
- Weakness, ataxia
- Seizures
- Severe cases: coma, respiratory failure

MECHANISM CATEGORY
1. Ion Channel Disruption (Na^+ Channel Activator)

GUARANA
(Guaraná / Brazilian Cocoa)

Key Toxic Constituent
Caffeine (methylxanthine)

MECHANISM OF ACTION
Antagonism of adenosine A_1 and A_{2A} receptors



↑ Neuronal activity
↑ cAMP / Ca^{2+} signaling
↓ Sleep pressure

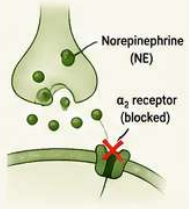
Potential Neurotoxic Outcomes
- CNS stimulation
- Anxiety, tremor, insomnia
- Seizures at high exposure

MECHANISM CATEGORY
2. Receptor Antagonism (Purinergic)

YOHIMBE
(Yohimbe Bark)

Key Toxic Constituent
Yohimbine (alkaloid)

MECHANISM OF ACTION
Antagonism of α_2-adrenergic receptors



↑ NE release
↑ Noradrenergic signaling
↕ Neural excitability

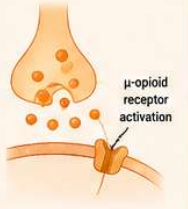
Potential Neurotoxic Outcomes
- Anxiety, agitation
- Hypertension, tremor
- Seizures at high exposure

MECHANISM CATEGORY
2. Receptor Antagonism (Adrenergic)

KRATOM
(Ketum)

Key Toxic Constituents
Mitragynine, 7-hydroxymitragynine

MECHANISM OF ACTION
Agonism of μ-opioid receptors (and others)



Modulation of neurotransmission
Analgesia, sedation or stimulation (dose-dependent)

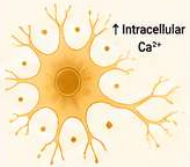
Potential Neurotoxic Outcomes
- Cognitive impairment
- Dependence / withdrawal
- Mood changes
- Potential developmental effects not fully understood

MECHANISM CATEGORY
2. Receptor Agonism (Opioid)

BUPLEURUM
(Chinese Thoroughwax / Hare's Ear)

Key Toxic Constituents
Saikosaponins (e.g., saikosaponin A, D)

MECHANISM OF ACTION
Disruption of Ca^{2+} homeostasis and induction of oxidative stress / apoptosis



Mitochondrial dysfunction
Oxidative stress
Apoptotic signaling

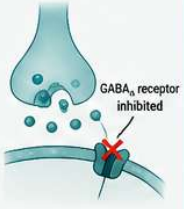
Potential Neurotoxic Outcomes
- Neurional apoptosis
- Impaired neurogenesis
- Learning and memory deficits (in models)

MECHANISM CATEGORY
5. Ca^{2+} Dysregulation / Cellular Stress

WORMWOOD
(Common Wormwood / Absinthe Wormwood)

Key Toxic Constituent
α-Thujone (monoterpene)

MECHANISM OF ACTION
Inhibition / modulation of $GABA_A$ receptors



↓ Inhibitory signaling
→ Neuronal hyperexcitability
→ Seizures

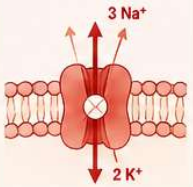
Potential Neurotoxic Outcomes
- Tremors, agitation
- Hallucinations
- Seizures at high exposure

MECHANISM CATEGORY
3. Neurotransmitter System Disruption (GABAergic)

OLEANDER
(Rosebay / Rose Laurel)

Key Toxic Constituents
Oleandrin, neriine (cardiac glycosides)

MECHANISM OF ACTION
Inhibition of Na^+/K^+-ATPase (sodium-potassium pump)



Disrupted Na^+/K^+ gradients
↑ Intracellular Ca^{2+} (secondary)
Neuronal dysfunction

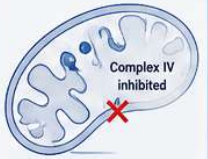
Potential Neurotoxic Outcomes
- Confusion, visual disturbances
- Weakness, dizziness
- Severe cases: arrhythmias, seizures, cardiac arrest

MECHANISM CATEGORY
4. Ion Pump Inhibition (Na^+/K^+-ATPase)

CASSAVA
(Yuca / Manioc)

Key Toxic Constituents
Linamarin, lotaustralin (cyanogenic glycosides)

MECHANISM OF ACTION
Cyanide release → inhibition of mitochondrial Complex IV (cytochrome c oxidase)



↓ ATP production
Cellular energy failure

Potential Neurotoxic Outcomes
- Headache, confusion
- Ataxia, weakness
- Seizures, coma (severe)
- Chronic exposure: neurological disease (e.g., konzo)

MECHANISM CATEGORY
5. Mitochondrial Toxicity / Energy Failure

MECHANISM CATEGORY KEY
1. Ion Channel Disruption
2. Receptor Modulation (Agonism / Antagonism)
3. Neurotransmitter System Disruption
4. Ion Pump Inhibition
5. Cellular Stress / Ca^{2+} Dysregulation / Mitochondrial Toxicity

These botanicals act through diverse molecular targets and cellular pathways, leading to a range of potential neurotoxic outcomes.

Screening Battery Limitations

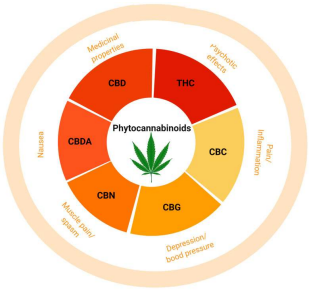
- Mechanisms → in its current form not fit for purpose to elucidate mechanistic understanding
- Limited outcome prediction → identifies biological activity but does not directly predict human disease
- Incomplete coverage → not all neurodevelopmental processes (myelination) or cell types represented
- Limited ADME/metabolism → may not reflect real-world chemical exposure or metabolite formation

Future Directions

- Manuscript forthcoming
  - Will also include planaria work conducted in collaboration by Dr. Eva-Maria Collins (Swarthmore College)
- Compare DNT battery results with some of the findings from the neurotox group of the Botanical Safety Consortium
- Cannabinoid-equivalent work ongoing by Dr. Catalina Cobos-Uribe
  - Cannabinoid safety pharmacology panel screening – could potentially put in BSC botanicals against 77 targets – elucidate mechanism

HESI

BOTANICAL SAFETY CONSORTIUM



BSC Annual Meeting

- September 28-29, 2026
- Free and open to all!
- Completely virtual

REGISTER HERE! ☺

Acknowledgements

DTT

- Mamta Behl (now at Neurocrine)
- Brad Collins (retired)
- Jeremy Erickson
- Jennifer Fostel
- Laura Hall (retired)
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- Skylar Marvel
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- **Cynthia Rider**
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- Robert Sills
- Nisha Sipes (now at US EPA)
- Suramya Waidyanatha
- Shannah Witchey (now at Inotiv)

DIR

- Jesse Cushman
- Leslie Aksu

OECD

efsa
European Food Safety Authority

Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER)

Participating Organizations

EPA United States Environmental Protection Agency

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CAAT eu
Universität Konstanz

Marcel Leist

IUF
LEIBNIZ RESEARCH INSTITUTE FOR ENVIRONMENTAL MEDICINE

Ellen Fritsche

MRIGlobal

biobide

Questions?

DNT Diver

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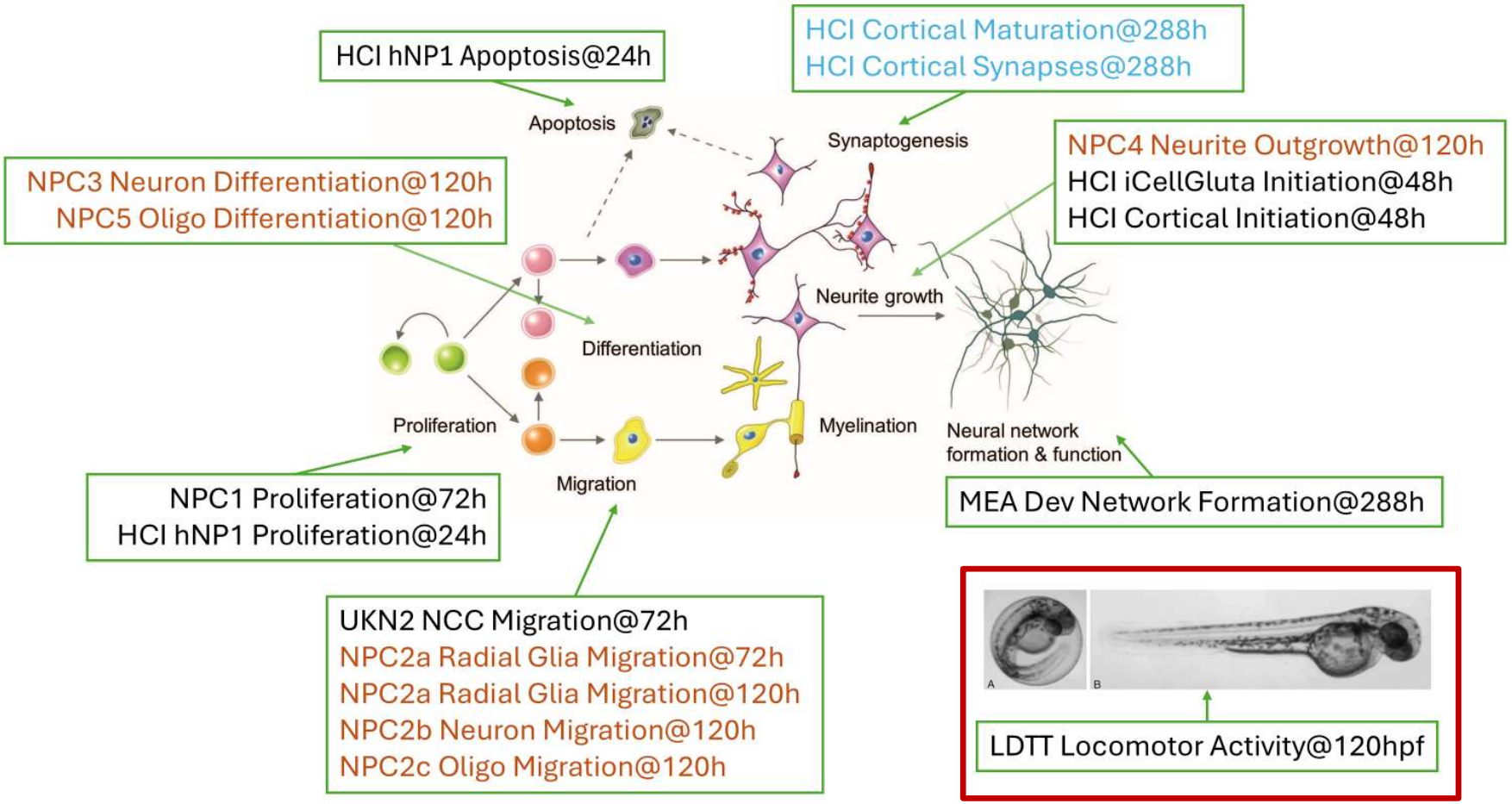
Prioritizing chemicals for developmental neurotoxicity by integrating data from a new approach methods (NAMs) battery covering key cellular events in neurodevelopment

Laura A. Hall [a], Jui-Hua Hsieh [b], Skylar W. Marvel [b], Mamta Behl [c,d], Ellen Fritsche [e], Katharina Koch [f], Anna Kreutz [g], Marcel Leist [h], Arantza Muriana [i], Timothy J. Shafer [j], Jason P. Stanko [a], Robert C. Sills [k], Helena T. Hogberg [l], Christopher A. McPherson [m,*]

Hall LA, Hsieh JH, Marvel SW, Behl M, Fritsche E, Koch K, Kreutz A, Leist M, Muriana A, Shafer TJ, Stanko JP, Sills RC, Hogberg HT, McPherson CA. Prioritizing chemicals for developmental neurotoxicity by integrating data from a new approach methods (NAMs) battery covering key cellular events in neurodevelopment. Neurotoxicology. 2026 Jul;115:103485. doi: 10.1016/j.neuro.2026.103485. Epub 2026 Jun 3. PMID: 42242322.

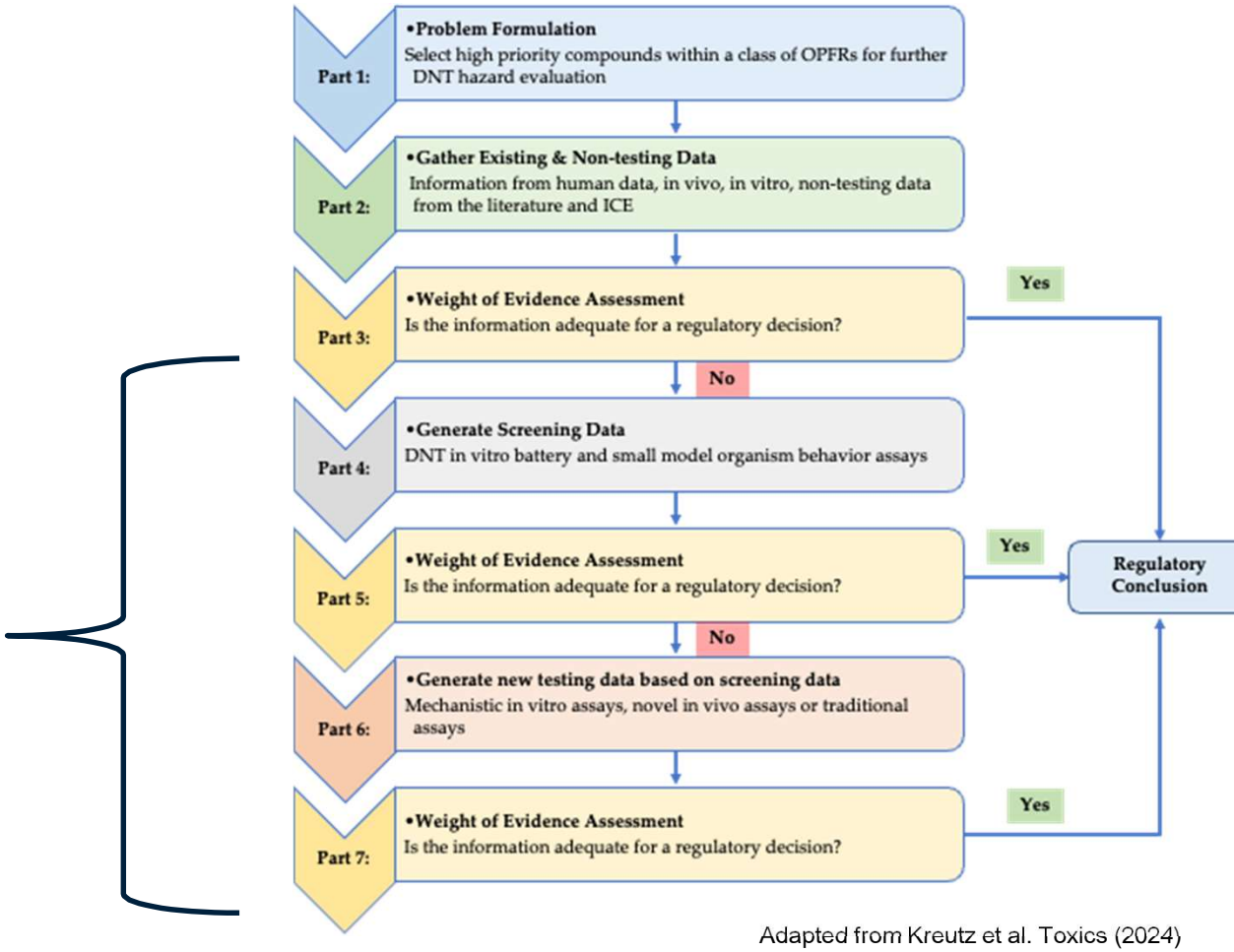
- **2-D Assays**
  - Proliferation/Apoptosis - human neuroprogitors (hNP1; USEPA)
  - Migration - human neural crest cells (UKN1; UKON)
  - Neurite outgrowth – rodent primary neurons; human iPSCs (rNOG,hNOG; USEPA)
  - Network formation - rodent primary neurons (NFA; USEPA)
- **3-D Assays - human neuroprogenitors (NPC 1-5; IUF)**
  - Proliferation/Apotposis
  - Migration
  - Oligodendendrocyte maturation
  - Neuronal differentiation
  - Neurite outgrowth

Refined DTT DNT *In Vitro* Battery



Integrated Framework in Action

Our team's approach is adding unique value to the field of developmental neurotoxicity (DNT). We are using a screening battery to prioritize compounds for further mechanistic investigation within our integrated testing strategy. Ultimately, we aim to determine whether developmental exposure to chemicals can lead to altered neurobehavioral and functional outcomes throughout the lifespan.



Adapted from Kreutz et al. Toxics (2024)